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Poster abstracts

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M001

RECOMBINANT API M 1 AND API M 2 ALLERGENS FOR MEASUREMENT OF SPECIFIC IGE TO HYMENOPTERA VENOM

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Background:IgE-mediated allergic reactions to honeybee venom (HBV) impair quality of life in affected individuals and may have fatal outcome. Phospholipase A2 (Api m 1) and hyaluronidase (Api m 2) are major allergens from HBV. The recombinant forms of these two allergens, rApi m 1, and rApi m 2, do not contain cross-reactive carbohydrate determinants (CCD) and may allow for more accurate measurement of specific IgE.
Methods: rApi m 1* and rApi m 2* allergenic proteins from HBV were expressed in Sf9 insect cells, purified, biotinylated, and used on IMMULITE® 2000 systems to measure HBV-specific IgE in 13 sera from subjects with suspected allergy to stinging insects. Tested sera were also screened for reactivity to vespid allergens rVes v 1 and rVes v 5, whole extract HBV, yellow jacket venom (YJV), bumblebee venom (BBV), and CCD markers MUXF, bromelain, and horseradish peroxidase (HRP). The same reagents were used in inhibition experiments to assess the specificity of IgE reactions to rApi m 1 and rApi m 2.
Results: Five sera reacted both to rApi m 1 and rApi m 2, and only three to rApi m 1 alone. The reaction pattern for one serum was indicative of cosensitization to HBV and YJV. The specificity of IgE binding to rApi m 1 and rApi m 2 was confirmed by >80% signal inhibition in the presence of homologous inhibitors. HBV extract was able to inhibit rApi m 1– and rApi m 2–specific IgE binding by >70%, while BBV exhibited limited antigenic cross-reactivity with three sera. As expected, YJV and recombinant vespid allergens did not inhibit IgE binding. Two anti–rApi m 1 and anti–rApi m 2 IgE–positive sera were reactive to MUXF, bromelain, and HRP, while their IgE binding to rApi m 1 and rApi m 2 could not be efficiently inhibited with homologous antigens, suggesting that they may not contain IgE antibodies specific to HBV.
Conclusions: Recombinant Api m 1 and Api m 2 antigens were used to detect and quantify IgE antibodies to HBV in sera from subjects with suspected stinging insect allergy. Due to the lack of CCD, rApi m 1 and rApi m 2 allergens may help in discriminating nonspecific IgE reactions from true HBV hypersensitivity.
*For research use only. Not for use in diagnostic procedures.

M002

SENSITIVITY AND SPECIFICITY OF LOW LEVEL IGE ANTIBODY VALUES IN PATIENTS ALLERGIC TO BETALACTAMS

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Background: Recently, in a number of analytical instruments, the analytical sensitivity for IgE antibody measurement has increased, moving from 0.35 KU/L to 0.10 KU/L. As a consequence, allergologists have to face the problem of defining the clinical significance of these low IgE concentrations. Aim of this work is to verify the significance of IgE concentrations below 0.35 KU/L in patients allergic to betalactams.
Methods: We measured IgE antibodies in 89 patients with a suspect diagnosis of allergy to betalactams on the basis of a clinical examination including skin and challenge tests. IgE against penicillin V and G, ampicillin, amoxicillin, cephalosporin (cefaclor) have been measured on UniCAP 250 instrument (Thermofisher). The IgE values have been analyzed with ROC curves to establish the diagnostic accuracy of IgE concentrations below 0.35 KU/L.
Results: In 35 patients IgE >035 KU/L for at least one antibiotic have been measured. The ROC curve analysis showed that sensitivity and specificity for the clinical diagnosis of the antibiotic allergy were 45% and 92% respectively when the value of 0.10 KU/L was considered. At the cut off value of 0.20 KU/L, sensitivity decreased to 37% and specificity increased to 98%. The analysis was performed only for penicillin G, ampicillin and amoxicillin since the other two antibiotics showed a ROC curve area <0.70
Conclusions: The improvement of the analytical sensitivity for specific IgE measurements, allows us to define IgE values useful to classify patient as allergic to betalactams with elevated specificity (>92%); however the sensitivity of the test is quite low. The ultimate diagnosis of antibiotic allergy requires the integration of biochemical and clinical tests as it was the case with lower sensitivity IgE measurements.

M003
EOTAXIN-2 (CCL24) AND EOTAXIN-3 (CCL26) LEVELS IN NASAL LAVAGE OF PATIENTS WITH EOSINOPHILIC CHRONIC INFLAMMATION

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Background: The mechanisms underlying selective eosinophil infiltration observed in some chronic inflammations of the nasal mucosa are not yet fully known. The aim of our study was to measure CCL24 (eotaxin-2)and CCL26 (eotaxin-3) in nasal lavage fluid of patients with different forms of sinonasal chronic eosinophilic inflammation.
Methods: Patients (n=73) with nasal hypereosinophilia were randomly recruited and grouped in: persistent allergic rhinitis (n=23), non-allergic rhinitis with eosinophilia syndrome (NARES) (n=25), and chronic rhinosinusitis with polyps (n=25). Non rhinitic volunteers (n=20) were recruited as controls. CCL24 and CCL26 concentrations were measured by ELISA Quantikine Human CCL24 and CCL26 Immunoassays. Differential cell counts were performed by microscopic cytological examination of nasal tissue scraped by inferior turbinate.
Results: CCL24 mean levels measured in all patients, allergic, NARES and chronic rhinosinusitis, were significantly increased ($P < 0.05$) compared to controls, and respectively 96,7 - 135,4 – 107,0 pg/mL versus 32.2 pg/mL. CCL26 mean levels were significantly higher ($P < 0.05$) in allergic and in NARES patients (132,0 and 187.63 pg/mL, respectively) than in the control group (13.5 pg/mL); instead in patients with chronic rhinosinusitis CCL26 values, although increased, did not differ significantly from those obtained in the controls (58,9 pg/mL vs 16.5 pg/mL). Moreover, in many patients independently from the type of sinonasal eosinophilic inflammation, an intraindividual inverse correlation between CCL24 and CCL26 levels was observed.
Conclusions: Our data suggest that the CCL24 and CCL26 are likely involved in the pathogenesis of the chronic nasal hypereosinophilia, with a complex cooperation and a different involvement of all members of the eotaxin family, but further studies are necessary to better understood the true physiopathologic mechanism and the possible therapeutic implications.

M004
THE GREY ZONE OF ADVERSE REACTIONS TO WHEAT NOT CELIAC DISEASE

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Background: Adverse reactions to wheat, as to any food, can be allergic (wheat or gluten allergy), intolerance (wheat intolerance, gluten intolerance, and Celiac Disease CD), or due to other mechanism (Gluten sensitivity GS). Gluten sensitivity can be defined as a non-allergic and non-autoimmune condition in which the consumption of gluten can lead to symptoms similar to those observed in Celiac Disease or wheat allergy.
Methods: 58 patients (pz) aged between 2 and 80 years, with symptoms suggestive of allergy/intolerance to wheat, sent to the laboratory by the Departments of Gastroenterology and Allergology and Immunology of the Azienda OU Policlinico of Modena. Were first tested in the following tests: tTG IgA, AGA IgG/IgA and the allergen extract f4 (wheat) using ImmunoCAP 250 and 1000 respectively (ThermoFisherScientific).Following the results of the tests mentioned above was thorough diagnostic work for wheat by the basophil activation test (BAT-Istrumentation Laboratory- Beckman).
Results: 20 pz resulted positive for auto-Ab (tTG-AGA IgG/IgA) tested for Celiac disease.(1st group:celiac disease).19 pz are found to be sensitized to the wheat (f4), between these 14 pz reported values of S-IgE >0.90 kUa/L and 5 pz values ranging between 0.10 and 0.28 kUa/L. 16 pz resulted positive to grass allergenic molecules to confirmed of the cross-sensitization. 1 pz with allergy wheat-dependent exercise-induced anaphylaxis resulted positive to ω-5 gliadin (S-IgE=57.7 kUa/L). 2 pz resulted negative for all commercially available allergenic molecules.(2nd group: allergic disease). 19 pz were negative for tTG/AGA IgA/IgG and wheat, and further investigated by the BAT (positive c.o.=>15%). 6 pz resulted positive to BAT with values >30%, 4 pz resulted to BAT with values >15%. (3rd group: GS?)
Conclusions: We have distinguished three groups and some relevant aspects related to the laboratory diagnosis of CD and allergy, more extensively, of wheat intolerance, such as the best combination of tests for early and accurate diagnosis.It's important the diagnostic role of new tests for detecting antibodies to CD and allergic diseases with cross-sensitization, the forms of non-celiac gluten intolerance (GS) also through the use of BAT.

M005
WHAT ARE THE MOST FREQUENT CAUSES OF SENSITIZATION IN CHILDREN WITH ASTHMA?

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Background: Asthma is increasing in frequency in our area. National guidelines suggest that patients with persistent asthma be evaluated for the role of allergens as contributing factors. CLA Allergen specific IgE Assays are usefull for identifying a sensitized state and may identify triggers to be eliminated and help guide immunotherapy .
Methods: Using CLA Allergen specific IgE Assays we make a semiquantitative determination of circulating allergen specific IgE concentrations in human serum. We tested 116 patients using a test that measures 36 combinant respiratory and food allergens and read the results with a luminometer that interpretate the concentration of Allergen specific IgE in classes-from 0 ,nondetectable to 4,very high concentration.
Results: The results gave us 22% of the patients have significant concentration of IgE specific for mite pterong and mite farinae,15-19% have significant concentration for grass mixt ,Bermuda grass, corn pollen ,mugwort, oranges, dog, horse,10,3-14.9% have significant concentration of IgE specific for trees mix, feather mix,Aspergillus,Penicilium,latex, ash, eng plantain, poplar mix, oak mix, cereal mix, wheat pollen, wheat, cochroash mix ,cat, cow, Candida, tomato, almond, soybean,5-10 % have significant concentration of IgE specific for peanut, milk, cod fish and only 3.4% have specific IgE concentration for egg white. We know that a positive test result does not always equate with clinical allergy, for example, 8% have positive test results for peanut, but only 1% are clinically allergic.
Conclusions: Using the CLA multiple allergen specific IgE, we found witch are the most frequent allergens that give high levels of specific IgE in children with asthma and combining this with the clinical history we can advise what triggers be avoided. We can conclude that many of our asthma patient have high levels of specific IgE for several triggers, so the usefulness of this test is for detecting the sensitization state and it is not very accurate for a specific allergen.

M006
STUDY SENSITIZATION PROFILES IN A SAMPLE OF 459 PATIENTS FROM THE DEPARTMENT OF ALLERGY BY MICROARRAY TECHNOLOGY

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Introduction: The microarray technique applied to allergy field generates great expectation because theoretically we would achieve the molecular analysis of the patients for the diagnosis of allergy. Thus it gives clinicians access to detailed information on the profiles of patient sensitization, allowing them to predict the severity and progression of allergic reactions and precise instructions to start immunotherapy. The objective was to conduct a descriptive study of the sensitization profile of patients who attended the outpatient allergy during the study period.
Patients and methods: We conducted a descriptive study of the profiles of 459 patients showed awareness that those who were asked a microarray between March 2011 and October 2012. The technology used was ImmunoCAP ISAC (Phadia). Its technology is based on biochips biotechnology and allows the study of 112 allergenic proteins simultaneously on a single biochip.
Results: We evaluated 459 polysensitized patients by isac method referred. 325 patients were positive against allergenic proteins and 134 patients were negative against allergenic proteins. Allergy to species-specific food components: 9.8% of patients were positive versus marker proteins kind of nuts and seeds (43.7% versus Jug r 2) 9.2% against specific proteins legumes (32.14% against Ara h2), 6.2% compared to egg proteins and milk-specific. 4.6% against specific proteins fruit and 3.1% against fish proteins and cereals. Allergy to aeroallergens species-specific components: 69% of patients were positive against proteins in the pollen of trees (73.21% versus Cup a1). 67.4% against grass pollen (90% vs. Phl p1) and by 36.3% compared to animal proteins (83% vs. Fel d1). Cross reactivity marker components: 31.4% of patients were positive against LTPs. 22.5 % versus profilins, versus 7.4% PR10, 5.8% vs. 3.7% tropomyosin and against serum albumin.
Conclusion: Diagnostic components through microarray allowed ascertain the specific allergen causing the allergic reaction and identified the presence of a marker indicative of allergen cross-reactivity. Knowledge of the specific allergen source enables preventive establishes clinical guidelines for the patient to avoid future sensitivities.

M007
DIAGNOSTIC UTILITY COMPONENT SEPARATION MARKERS IN THE STUDY CROSS- REACTIVITY: HYPERSENSITIVITY TO LTPS AND PR10

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Introduction: Diagnosis based on the separation of component allows us know the allergens that are involved in hypersensitivity reactions, also identifies co-sensitized patients versus cross-sensitized patients and improve the diagnosis, getting a better understanding of the patient's sensitization profile. In 325 patients from the allergy consultation with clinical complex which prompted a microarray, we performed the study of cross-reactivity markers that are more frequently associated with ODS. According to scientific literature, cross reactivity markers most associated with ODS (oral allergy syndrome) in southern Europe are the LTPs (lipid transfer proteins). Another marker of cross-reactivity is also associated with ODS and is more prevalent in northern Europe is PR10.
Material and methods: Inmunocap technology used isac biotechnology-based biochips. It microinmunoensayos platform (allergenic components immobilized purified natural or recombinant) which simultaneously enables the study of a biochip 118 allergenic proteins.
Results: Of the 325 patients studied, 185 had positive markers against cross-reactivity. LTPs: 102 patients (55%). The Protein mainly involved cause cross-reactivity Pru p3 (85.3%) Jug r3 and Cor a8 (51%) PR10: 24 patients (13%). The protein was involved mainly Mal d1 (62.5%), Pru Cor a1 and p1 (58.3%). Patients also showed reactivity to other markers cross reactivity.19 patients (10.3%) showed reactivity to tropomyosin, 12 patients (6.5%) compared to serum albumin, 73 patients (39.5%) against profilin and finally 16 (8.6%) patients versus MUXF p3.
Conclusion: According to the results we see that more than half of patients (57%) showed positive reactivity against cross markers, this underlines the importance of a technique such as microarray that allows us to identify such patients. As we have seen in our study LTPs are cross-reactive components mainly involved in allergic reactions, and this agrees with published scientific literature that proteins (LTPs) are responsible for allergic reactions to fruits and vegetables in southern Europe. The PR-10 proteins which are associated with allergic reactions in our population are not very prevalent (13%), since according to the literature, these proteins mainly prevalent in northe

M008
MARKERS OF COAGULATION AND FIBRINOLYSIS IN PATIENTS WITH C1 INHIBITOR DEFICIENCY

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Background: C1-inhibitor (C1-INH) is an important member of the serpin family, which inhibits the first component of the human complement system and controls contact activation of the coagulation and kinin system. Deficit of the C1-INH is clinically manifested as angioedema. This condition is characterized by episodic swellings of mucosa and subcutaneous tissue at various locations of the body. Swelling of the larynx can be life-threating. The aim of our study was to evaluate whether measurements of plasma D-dimer, Thrombin-antithrombin complexes (TAT) and Plasminogen activator inhibitor type 1 (PAI-1) can be useful for the diagnosis of angioedema caused by C1-INH deficiency.
Methods: We enrolled 52 patients with C1-INH deficiency (22 with acute angioedema attack, 30 in remission; 29 (56%) males and 23 (44%) females, age range 17 to 49 years and 22 healthy individuals, aged from 24 to 52 years. Plasma D-dimer, TAT and PAI-1 were measured using ELISA. Results were presented as (mean; SD).
Results: Plasma D-dimer was higher in patients with acute attacks (1237;489 ng/mL) compared to controls (257;161 ng/mL; P <0.0001) and patients in remission (793;289 ng/mL; P <0,0001). TAT complexes levels were significantly elevated in patients with acute attacks (64.15;31.43 ng/mL) compared to patients in remission and healthy subjects (24.11;9.2 ng/mL) and (19.23;9.6 ng/mL; P <0.001, respectively). Plasma PAI-1 decreased during attacks and remission (0.98;0.46 ng/mL and 1.15;0.51 ng/mL, respectively), compared to controls (4.28;1.9 ng/mL; P <0.001). We observed a statistically significant positive correlation between TAT complexes and D-dimer during acute attacks (R=0.41) and negative between PAI-1 and TAT in attacks and remission (R=-0.39 and R=-0. 33, respectively). No such correlations in the whole studied group were found.
Conclusions: Results of this study show that attacks of angioedema are associated with the activation of coagulation, kallikrein-kinin and fibrinolysis systems. Thus, D-dimer, TAT and PAI-1 can be considered markers of development and exacerbation of angioedema caused by C1-INH deficiency. Moreover, our findings may have also therapeutic implications.

M009

USE OF RECOMBINANT VES V 1 AND VES V 5 FOR DETECTION OF SPECIFIC IGE TO HYMENOPTERA VENOM

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Background: Allergy to Hymenoptera venoms may induce reactions in sensitized individuals that vary from mild local reactions to severe, life-threatening events. Phospholipase A1B (Ves v 1) and antigen 5 (Ves v 5) are major allergenic components of yellow jacket venom (YJV). Recombinant Ves v 1 (rVes v 1) and recombinant Ves v 5 (rVes v 5) may provide higher specific detection of IgE to YJV when compared to their purified native forms, due to the lack of cross-reactive carbohydrate determinants.
Methods: Recombinant Ves v 1* and recombinant Ves v 5* were expressed in insect cells, affinity-purified, and biotinylated. Thirteen sera from subjects with a presumed allergy to stinging insects were tested for specific IgE to rVes v 1 and rVes v 5, YJV, honeybee venom (HBV), bumblebee venom (BBV), and unrelated allergens from plant and animal sources, using IMMULITE® 2000 systems. Specificity of the positive reactions was confirmed with the same antigens through inhibition experiments.
Results: Out of 10 sera that were reactive to YJV, nine also had specific IgE reactivity to rVes v 1 and/or rVes v 5. Double positivity to rVes v 1 and rVes v 5 was observed in seven of the YJV-reactive sera, with three reacting solely to rVes v 5. HBV, BBV, and unrelated allergens inhibited rVes v 1 and rVes v 5 IgE reactivity up to only 15%, compared to>80% with the homologous vespid antigens. YJV was effective in inhibiting IgE binding to recombinant Ves v 1 and recombinant Ves v 5, and the degree of inhibition was serum dependent (20%–98%). Specificity of IgE reactions was confirmed for seven out of nine rVes v 5–positive and three out of five rVes v 1–positive sera. Specific IgE binding to the recombinant vespid allergens could not be confirmed in four sera that exhibited reactivity to HBV and BBV, as well as unrelated plant and animal allergens.
Conclusions: Recombinant Ves v 1 and recombinant Ves v 5 were able to detect YJV- specific IgE in sera from subjects with suspected Hymenoptera venom allergy. Agreement demonstrated between component and whole extract YJV-based IgE detection confirmed that rVes v 1 and rVes v 5 measure relevant allergenic proteins.
*For research use only. Not for use in diagnostic procedures.

M010

OXIDATIVE STRESS STATUS IN FEMALE ATHLETES WITH IGE-DEPENDENT ALLERGIC RESPONSE

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Objecitives: The aim of the present work was to assess oxidative stress biomarkers in elite athletes with known allergy and non-allergic controls.
Methods: Based on serum IgE concentration (cut-off 100 IU/L) and findings at physical examination, 66 elite athletes were divided into two groups: with some kind of IgE-dependent allergic response (n=19) and healthy controls (n=47). The following parameters were measured: White blood count (WBC), Immunoglobulin E (IgE), high sensitive C reactive protein (hs-CRP), Interleukin-6 (IL-6), oxidative stress parameters (reactive oxygen metabolites – ROMs, advanced oxidation protein products - AOPP and lipid hydroperoxides – LOOH) and biological antioxidative potential – BAP. Multivariate analysis of covariance (MANCOVA, Wilks' lambda) was performed to test the hypotheses that IgE-dependent allergic response (fixed factor) and inflammation which is not derived from type I hypersensitivity (indicated via WBC, IL-6 and hs-CRP values) (covariates) have a significant effect on the normally distributed oxidative stress parameters (dependent variables).
Results: Reactive oxygen metabolites (327±81 vs. 294±53, (Carr U); P=0.047) were significantly higher in athletes with IgE-dependent allergic response and biological antioxidative potential (2314 ± 344 vs. 2484 ± 226, (μmol/L); P=0.031) was significantly lower in this group. Multivariate analysis of covariance revealed hs-CRP and WBC to be significant covariates.
Conclusions: Increased reactive oxygen metabolites and reduced antioxidant capacity in athletes with IgE-mediated allergy lead us to suppose that free radical generation could be mediated at least in part via type I hypersensitivity response. Higher incidences of allergy or asthma in elite athletes and increased oxidative stress in these conditions emphasize the importance of augment of endogenous antioxidant protection.

M011

ASSOCIATION OF POLYMORPHISMS IN GENES ENCODING IL-4, AND ITS RECEPTOR WITH ATOPIC & NON ATOPIC DERMATITIS IN EGYPTIAN CHILDREN PATIENTS

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Background: Atopic dermatitis (AD) is caused by genetic and environmental factors that interact to determine disease susceptibility and severity. Several lines of evidence suggest that the IL-4 gene and the IL-4-receptor alpha (IL-4Ralpha) gene are involved in the development of atopic diseases. Recent breakthroughs in genetic methodology have greatly augmented our understanding of the contribution of genetics to susceptibility to AD.
Objective: To assess whether genetic variants of IL-4 and IL-4R alpha genes relate to the elevation of serum immunoglobulin E levels in patients with atopic dermatitis (AD),
Methods: We conducted an association study of genetic polymorphisms of IL-4 (-590C/T), and IL-4R alpha (Ile50Val) using PCR-RFLP assay in Atopic &non Atopic Egyptian Children (n = 100).
Results: The results revealed that there was a non significant association of IL4 -590C/T polymorphism in children with non-atopic dermatitis or those with atopic dermatitis when compared with the controls (P= 0.8, 0.4 respectively). But there was significant association between IL-4Rα I50V polymorphism and dermatitis susceptibility in atopic dermatitis (P= 0.002), whereas no such association in non-atopic dermatitis group (P=0.7). Evidence of gene interactions between both polymorphisms was found. Furthermore, there was no relation between each polymorphism and serum IL4 level (P >0.05 for each) while homozygosity for the risk alleles of IL-4 -590 C/T and IL-4Ra I50V were significantly associated with increased total IgE levels in all studied groups.
Conclusion: In Egyptian children, the IL-4Rα (I50V) may play a role in susceptibility to atopic dermatitis. In addition, gene-gene interaction between the IL-4 -590T and the IL-4Rα G allele significantly increases an individual's susceptibility to atopic dermatitis. Both polymorphisms may be involved in the control of IgE production.

M012

PREVALENCE OF MAJOR ALLERGENIC MOLECULES IN A SAMPLE OF NORTHERN ITALY, DESIGNED BY COMPONENT-RESOLVED-DIAGNOSIS (CRD)

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Background: In vitro diagnostic techniques have progressed enormously following the introduction of the advances made in proteomics and nanotechnology offering tools for the diagnosis and investigation of allergy at molecular level.
Methods: 218 patients (pts), 2-80 years, with symptoms food and/or respiratory disease studied through CRD – ISAC (Phadia) after subjects tested on the allergen extract. We studied the following molecules: rPhl p1, rPhl p5, p7 rPhl, rPhl p12, Bet v1, Bet v2, v4 Bet, nder p1, p2, p10 rder, LTP, PR-10, Vicilin-like proteins, Legumin-like proteins, 2S Albumins, Profiline, nBos d4, nBos d5, nBos d8, Gal d1,d2,d3.
Results: Grass: positive pts to Phl p 1, 5 (56%) have a good responsivity to ITS; positive pts to rPhl p 1, p rPhl 5 and 7,12 (37%) have medium responsivity to ITS; pts only positive Phl p Phl p 7 and 12 (7%) have a low responsivity to ITS; Betullacee: 65% pts has S-IgE vs Bet v 1, 48% vs Bet v 2 and 9.3% vs Bet v 4. Pts only sensitized vs Bet v 1 (44%), show good responsivity to ITS, pts (22%) with Bet v1,Bet v 2 /and Bet v 4 must be considered to be less responsive.Positive pts for Bet v 2 / Bet v 4 (34%), are not suitable for ITS. Mites: Pts with S-IgE vs Der p1, p2 = 52% they have a good responsivity to ITS; Der p1, p2, p10 (15%)medium responsivity to ITS; pts only positive vs. Der p10 (11%) have a low responsivity to ITS. For the following pan-allergens: 154 pts results symptomatic and 64 asymptomatic as follows: LTP = 16% positive pts (17% symptomatic, 7.8% asymptomatic), PR-10 = 24% positiv pts (25% symptomatic, 23% asymptomatic); Vicilin-like proteins = 15% positive pts (14% symptomatic, 16% asymptomatic); Legumin-like proteins= 2% positive pts (1.9% symptomatic, 1.6% asymptomatic), 2S Albumins = 11% positive pts (12% symptomatic, asymptomatic 8%); Profiline = 24% positive pts (20% symptomatic, 34% asymptomatic); Milk = 52 positive pts (71% symptomatic, 29% asymptomatic); Egg = 51 positive pts (80% symptomatic, 20% asymptomatic). 35% of pts with an allergen sensitization genuine to food; patients 65% pts with crossreactivity to ubiquitous proteins present in pollen and common to food.
Conclusion: This study examined new developments trough CRD as a critical element for furthering our knowledge of allergic disease

M013

PAEDIATRIC ALLERGY SCREENING USING PHADIATOP INFANT® -A REPORT SERIES

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Background: The advent of arrays to the molecular allergy diagnostic laboratory has opened new opportunities for patient management, from screening to treatment planning, as with any novel method introduced in the clinical laboratory research into its uses and limitations is forthcoming.
Methods: In 2011 our laboratory processed allergy screening test using PhadiaTop infant® for 530 paediatrics patients, with a resultant 247 positive reports, and 283 negative. With the aim of assessing if positive values discriminate for some particular clinical feature in the last year after the test results were issued, we selected a representative sample of 80 patients (P(0.5) Confidence level 95% CI 10.11%) selected using a non-repetitive random numbers table from those that had an allergy screening test in the past year (N=530), and collected their primary complain and diagnosis at their first follow up consultation after obtaining a positive test result.
Results: The output data from the clinical records is outlined in the following contingency table P N Total Asthma 6 0 6 AR 8 1 9 NA 8 14 22 None 6 15 21 other 10 12 22 Total 38 42 N=80 AR Allergic Rhinitis, NA None Available, P positive, N negative. Some records did not have a registered diagnosis and were entered as NA, other had healthy child as diagnosis and were entered as none, all other had diagnosis that were not duplicated and we compounded them under the group other If we apply Chi square's test assuming an equal occurrence probability for each item, then the critical value is 15.375 with a p value of 0.004, meaning that the differences are statistically significant at $p < 0.05$. It is also significant that the proportion of positive results is significantly greater for those diagnose with the selected conditions (Asthma 100% Allergic rhinitis 88%)
Conclusions: Allergic rhinitis and Asthma are the most prevalent diagnosis within our sample population, the possibility of considering follow up testing to positive screening results should be discussed with the clinicians that commonly order this test. Note should be taken not to interpret a correlation between positive result and a diagnosis as a true positive since the test selected doesn't evaluate the presence of the mentioned conditions.

M014

EVALUATION OF THE TOTAL SERUM IGE LEVELS IN ADULT PATIENTS IN BANJA LUKA REGION IN THE PERIOD FROM 2009-2012

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Background: The rate of alergies is constantly increasing among the world. According to the World Health Organization 30-40% of the entire world population is allergic to one or more allergens. Research have shown that in the last twenty years the significant increase of alergies is detected among the children and people under age of 35. The incidence of allergies among people under age of 35 is 48%. The continues increment in the number of alergies is considered to be one of the major health issues. Since the determination of total IgE is very important in diagnostics of alergies, the aim of this study was to determine whether there has been increased demand for the determination of the total IgE values in adults, as well as how many of the total IgE was positive, or greater than 100 kU / L in that time period between 2009 and 2012.
Methods: In this study, UniCap 100 Thermo Fisher Scientific device was used , and the determination of total IgE was determined from serum in patients who are hospitalitzed and outpatients in the Department for Laboratory Diagnostics, Clinical Center of Banja Luka, and it was based on the principle of sandwich immunofluorescence assay.
Results: The total number of processed samples N = 5414 which was taken in adults from the period between February 2009. and November 2012. Of that number (N = 5414), n = 1853 samples contained the total IgE> 100 (2009: N = 1358, n = 487 (35.86%), 2010: N = 957, n = 340 (35.53%) 2011: N = 1973, n = 636, (32.23%), 2012: N = 1126, n = 390, (34.63%). Statistically signifacant differences were not found between the curtain groups.
Conclusion: Based on the analyzed results obtained in the period of 2009-2012, it can be concluded that the number of requests for value determination of total IgE varies from year to year, which can be explained by climate changes and migration of allergens. In the last couple of years that number has increased, but the percentage of positive values ranges between 32% and 36%. In fact, the significant increse of positive total IgE values was not recorded attitudinal to the number of tested patients.

M015
EUROLINE SPAC AND EUROBLOTONE: AUTOMATED MULTI-PARAMETER COMPONENT RESOLVED ALLERGY DIAGNOSTICS

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Background: The in vitro diagnosis of allergy is generally performed by using native extracts as antigenic targets. In recent years, specific IgE detection has been improved through the use of component-resolved diagnostics, which are usually based on single purified allergen components (SPAC) instead of raw extracts. The resulting test systems are easier to standardise and provide more reliable information. EUROLINE is a multi-parameter assay platform for the simultaneous determination of antibodies, in the case of specific IgE against up to 36 different allergen parameters on one single strip. The standardisation of the test strip design allows automated processing of the strips using Westernblot incubators, such as the EUROIMMUN EUROBlotOne. The evaluation of the test strips is done by EUROLineScan software which identifies the bands, measures their intensities, calculates the EAST class results and archives the data. The objective of the study was to investigate analytical performance of a multi-parameter assay in regard to in-vitro allergy testing.
Methods: EAST class levels of specific IgE against recombinant birch pollen allergens (Bet v1, Bet v2, Bet v4, and Bet v6) and recombinant timothy grass pollen allergens (Phl p1, Phl p5, Phl p7 and Phl p12) were measured in sera from 44 birch and grass pollen double-sensitised patients using EUROIMMUN EUROLINE SPAC Pollen 1 (EU) and Phadia ImmunoCAP Allergy (CAP). All automated incubations of the EUROLINE were performed using the EUROBlotOne and the EUROLineScan system.
Results: The IgE levels correlated very well between EU and CAP, for birch pollen components (Bet v1, Bet v2, Bet v4 and Bet v6) from 95% to 100% and for timothy grass pollen components (Phl p1, Phl p5, Phl p7 and Phl p12) between 95% and 98%. Fully automated incubation was compared with manual incubation using 44 samples. The samples showed consistent EAST class results in the range of ±1 class for all 484 evaluated bands.
Conclusions: EU and CAP tests showed a very high correlation. In combination with the new EUROBlotOne having an incubation throughput of up to 1584 single specific IgE tests in 4 h, the EUROLINE SPAC is a valuable tool for the fully automated component resolved allergy diagnostic.

M016
AUTOIMMUNE DISORDERS IN PATIENT WITH NON-HODGKIN'S LYMPHOMA

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Background: The concept of an association between autoimmunity and non-Hodgkin's lymphoma (NHL) is based on reports of patients with NHL who displayed clinical or biological signs of autoimmunity.
Methods: We studied case report of a woman 53-year-old who was admitted to the rheumatological department for pain in knee and coxofemoral joints. In anamnesis she had NHL and got 4 courses of chemotherapy (Vincristine, Doxorubicin, Cyclophosphamide and Prednisolone) in combination with radiation therapy (cumulative focal dose 30 Gy). For this patient we used such examinations: complete blood count, biochemical blood count, immunological examinations (anti single-stranded DNA (anti-ssDNA), anti-double-stranded DNA (anti-dsDNA)), antinuclear antibodies (ANA)), fibrogastroduodenoscopy with obligatory biopsy and mandatory morphological examination, Rtg of knee joints.
Results: Laboratory tests revealed elevated levels of anti-ssDNA, anti-dsDNA, erythrocyte sedimentation rate (ESR) and gamma globulins. Anti-ssDNA – 1,07 (N <0,450), anti-dsDNA – 1,77 (N <0,450). ANA test was negative – 0.48 (N <1,0). On the Rtg was detected osteoarthrosis of knee joints. For patient were prescribed NSAIDs (meloxicam in dose 15 mg/date). Over 2 weeks we made control analysis. Anti-ssDNA 13 (N <20), anti-dsDNA – 2,30 (N<25), ANA – 0,3 (N <1,0). Over 1 month we controlled these antibodies again: anti-ssDNA 7,68 (N <20), anti-dsDNA – 1,24 (N <25), ANA – 0,25 (N <1,0).
Conclusions: Our findings in the present report emphasize the importance of keeping an open mind in cases of elevated levels of anti-ssDNA, anti-dsDNA and ANA. Further studies are necessary for a better understanding of the causes that lead to such autoimmune changes.

M017
REPRODUCIBILITY OF TITLES AND CONJUGATES STABILITY IN COMMERCIAL KITS FOR AUTOANTIBODIES ON HEP-2 CELLS

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Background: The “Search of Antibodies to Cellular Antigens by indirect immunofluorescence on HEp-2 cells (SACA-IIF on HEp-2), are commonly used for autoantibodies detection, due to their meaning like disease markers. This test has great clinical value, however, as any laboratory test must be considered carefully. The importance not only of the pattern found, but also the title of immunofluorescence, is debated. The fluorescent labeling of immunoglobulins, by FITC, has become very popular in the Kits of SACA-IIF on HEp-2. Since laboratories are not used to titrating the conjugate against an absolute in accordance standard, the same test performed in Kits of different brands may have different titles. Complicating to match the interlaboratory results.
Methods: This study evaluated the titers results of “reference serum” (T = 640) in SACA-IIF on HEp-2 Kits, as well as a possible decrease in fluorescence intensity of the conjugates according to the time of use. Facing up the released results by brazilian laboratories, with the value of reference serum. After was made the comparison between titers of serum and the conjugate stability test, in which the same serum was tested for thirty days mid five commercial Kits of different brands. In parallel, a reference serum was sent to 15 laboratories in Brazil to confront each other their results with the value of the title of reference serum. It might be noted, finally, that there were no differences between the results obtained in the kits and the title of the reference serum.
Results: In stability conjugate, four kits were stable and one Kit showed a decrease in fluorescence intensity. Among the 15 laboratories: 73.4% of the laboratories agreed on the result and 26.7% were divergent.
Conclusions: It follows that each commercial kit initially respond to a semi-quantitative diagnostic, however behaved differently during the time of use. Has been observed also that the intelaboratory results are shown inconsistent. Is a fact that can harm, even in the assurance of the test by requesting physicians. Is noted the need for laboratories to follow more stringent criteria for test implementation in order to standardize the system, to create harmony in the results, increasing the reliability of of this test.

M018
ELIMINATION OF INTESTINAL BIOPSY IN THE DIAGNOSIS OF CELIAC DISEASE IN PEDIATRIC POPULATION WITH GENETIC STUDY; ESPGHAN `S CRITERIA

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Introduction: ESPGHAN establishes a change in the priority sequence of tests for the diagnosis of celiac disease (CD) in pediatric population. Intestinal biopsy (IB) is the gold standard for detecting CD. However, having serological markers values 10 times higher than normal, plus a positive genetic test of susceptibility, all added to clinical symptoms, may avoid the need for IB.
Aim: To evaluate the utility of ESPGHAN´s new criteria, by evaluating HLA-DQ2 and HLA-DQ8 susceptibility halotypes in order to avoid performing an IB.
Patients and methods: 297 suspected or confirmed patients, 1-66 yrs and 38 relatives. Diagnostic tests for detecting antitransglutaminase (t-TG2-IgA) (Celikey Thermo Físher) and endomisyum antibodies. (EMA-IgA Biosystems®) Antibodies, as well as genetic studies (Innogenetics®) were performed.
Results: 170 cases were DQA1*0501, DQB1*0201 (DQ2 in cis), 33 were DQA1*0505, DQB1*0202 (DQ2 in trans), 27 were DQA1*0301, DQB1*0302 (DQ8), 8 of these 27 also expressed DQ2 in cis, while 31 expressed DQ2 in half (HLA-DQB1* 0202). 77% HLA-DQ2 positive (cis or trans) or HLA-DQ8 positive scored 1 point following ESPGHAN criteria. 10% had a single susceptibility allele and scored 0 points. Thirty eight patients had t-TG2-IgA titers >10 of normal values confirmed by EMA IgA and presented clinical symptoms of CD. All these 38 patients were HLA-DQ2 and none were HLA-DQ8.
Conclusions: The study of susceptibility HLA haplotypes for CD does not increase total costs to the diagnosis, as it’s one of the final parts of the current diagnostic process. An accurate diagnosis of CD can be established in a patient with clinical symptoms who has susceptibility alleles as well as serological markers titers 10 times higher than controls. No IB would be needed in this type of patients. IB would exclusively be performed in patients with clinical symptoms and non conclusive tests. Avoiding an IB means less discomfort for the patients, especially for children, and lower economic costs.

M019

THE PREVALENCE OF ANTI-NEURONAL ANTIBODIES IN PATIENTS WITH PARANEOPLASTIC RHEUMATIC SYNDROME

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Background: A number of studies have indicated a link between rheumatic diseases, autoimmunity and cancer. The presence of the antibodies characteristic to the rheumatic condition is frequent and makes the diagnostic process of the occult malignancy complicated and results in delayed diagnosis. Tumour antigens are recognised by the immune system, leading to the generation of antibodies. It is known that tumor cells express neuronal antigens which can induce the formation of specific autoantibodies. The aim of this study was to assess anti-neuronal antibodies in the group of patients with paraneoplastic rheumatic syndrome comparing to the control group patients with solid tumors only, that to describe their autoimmune profile.
Patients and methods: 32 patients with paraneoplastic rheumatic syndrome and 32 control group patients with solid tumors was matched for sex (male 78.1% vs. 81.2% respectively) and age (66.7±8.3 years vs. 65.0±8.5 respectively). Characteristics of solid tumors in both groups were similar – 46.9% cases with tumors of prostate, 3.1% - with seminoma, 34.4% with lung cancer and 15.6% with breast tumor. Euroline-WB test (Neuronal Antigens Profile-2, Euroimmun, Germany) was used to detect anti-neuronal antibodies (amphiphysin, Hu, Yo, Ri, PNMA2, CV2 autoantibodies) in the serum. Results were evaluated using EuroLineScan program.
Results: Anti-neuronal antibodies were detected in 13% of patients with paraneoplastic rheumatic syndrome and in 9% of control group. Antibodies were identified: 43% of all positive patients with rheumatic syndrome had anti-PNMA2, 29% – anti-Yo, 14% – anti-amphiphysin and anti-CV2 antibodies; respectively in group with solid tumors 46% of all positive patients had anti-PNMA2, 19% – anti-Yo, 35% – anti-CV2 antibodies.
Conclusion: No differences were observed comparing frequency of anti-neuronal antibodies in the group of patients with paraneoplastic rheumatic syndromes and control group patients with solid tumors. Anti-neuronal antibodies were rare in malignancies with paraneoplastic rheumatic symptoms and solid tumors without neurological syndromes. Detection of anti-neuronal antibodies is limited in assessing of paraneoplastic rheumatic syndrome.

M020

CONFIRMATION OF BIOPLEX ANTI-DSDNA ASSAY BY THE LIAISON PLATFORM

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Background: Switching platforms for ANA testing from Elisa assays to the Bioplex platform rendered a significant increase in the anti dsDNA results. The aim of the present study was to find the concordance between the Bioplex anti-dsDNA assay and other assays. The discrepant results were confirmed by the Crithidia assay and reviewed with the aid of HEp-2 ANA IFA and Bioplex ANA subsets results.
Methods: 92 samples with Bioplex positive or borderline anti-dsDNA results were tested for anti-dsDNA with two ELISA assays (Euro Diagnostica AB, Malmo, Sweden and Euroimmun AG, Lubeck, Germany) and by the Liaison (DiaSorin S.p.A, Saluggia, Italy) CLIA assay. Discrepant results were tested by the Crithidia luciliae IFA (Kallestad, BioRad Laboratories). HEp-2 ANA IFA results and Bioplex ANA subsets results were available as performed routinely.
Results: The monthly average of positive and borderline anti-dsDNA results was 12% and 22% as compared to 11% positive results with the previous Elisa assay (Euro diagnostica). 77 samples out of 92 samples (83.7%) with positive or borderline anti-dsDNA results by Bioplex were negative for anti-dsDNA with the two ELISA and the Liaison assays. One sample was positive with all the assays. In comparison to C. luciliae IFA, the Bioplex, Euro Diagnostica ,Euroimmun and Liaison assays had 13,7, 2 and 7 false positive results and 0,1,2 and 1 false negative results. Moreover, 60% of the Bioplex positive and 76% of borderline results were associated with negative both HEp-2 ANA IFA and other ANA subsets. Only 11% of the Bioplex positive and 2% of the borderline results were associated with positive HEp-2 ANA IFA results and negative results for all the other ANA subsets. 7% of the Bioplex positive and 2% of borderline results were associated with negative HEp-2 ANA IFA results but positive results to one of the other ANA subsets.
Conclusions: The high rates of positive and in special borderline results rendered by the Bioplex anti-dsDNA were poorly confirmed by other tests or platforms and should be further investigated. The Liaison anti-dsDNA assay offers a convenient random access platform for confirming the borderline Bioplex anti-dsDNA results.

M021

TRACE ELEMENT CONCENTRATION OF PROTEIN SEPARATED BLOOD SERUM OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND SYÖGREN SYNDROME

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The elemental concentration of human blood is often used for building up diagnosis in the clinical practice. Still a few studies concern the trace element and protein level of blood serum in case of autoimmune diseases and no information is available about the quantity of these essential trace elements binded to the main transport proteins. Therefore the aim of recent study was to determine the concentration of K, Ca, Mg, Zn, Cu, Fe and Mn in whole blood serum and to further investigate their quantitative distribution between high abundant antioxidant protein fractions in blood serum of patients with Systemic lupus erythematosus (SLE) and Sjögren syndrome (SS). Anion exchange chromatography was used to separate blood serum samples into protein fractions and high abundant protein components were identified by UV-VIS spectrophotometry. Samples were digested by a microwave assisted system prior to analysis. K, Ca, Mg and Zn concentration of samples were determined by flame atomic absorption spectrometry (FAAS) while Zn, Cu and Fe concentration were measured by graphite furnace atomic absorption spectrometry (GFAAS). Statistical analysis was carried out to evaluate the results. There were no significant difference ($P > 0.05$) in Mg and Fe concentration between the control, SS and SLE groups. Significantly lower concentration ($P < 0.05$) of K and Ca was measured in SS and SLE groups compared to the control. The concentration of Zn was found to be significantly decreased ($P < 0.001$) in SS and SLE groups than in the control, while the concentration of Cu significantly increased ($P < 0.001$) in both groups. Alterations were also observed in the quantitative distribution of elements among protein fractions containing Immunoglobulin G (IgG), Transferrin (Tr), Albumin (Alb) and Ceruloplasmin (Cer) of SS and SLE groups compared to the control. In control group the highest Cu concentration was determined in the protein fraction containing Cer (62.1% of total), while in SS and SLE groups in the fraction containing Alb (SS: 37.7%; SLE: 36.7 %). The concentration of Zn decreased in the Alb containing fraction (SS: 18.3%; SLE: 24.6%) and increased significantly in the IgG (SS: 33.9%; SLE: 27.4%) and Cer containing protein fractions (SS: 29.5%; SLE: 35.8%) compared to the control.

M022

EXPRESSION OF ANTINUCLEAR ANTIBODIES IN PATIENTS WITH MONOCLONAL GAMMOPATY AND WITH CRYOGLOBULINEMIA

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Background: A remarkable number of patients with Monoclonal Gammopaties (MG) exhibits unexpected autoantibody activities.This specific immunoreactivity, often consisting in antinuclear antibodies (ANA), is not attributable to the monoclonal immunoglobulin with the exception of IgM-MG associated neuropathies.Some data also suggests that the heterogeneity of immunoglobulins in Cryoglobulinemia may be related to different expression of autoimmune phenomena, such as the expression of ANA. We evaluated both the prevalence of ANA positivity and the fluoroscopic patterns in patients affected by MG and in those with Cryoglobulinemia.
Methods: Two hundred forty five sera from patients with MG and 150 from subjects with Cryoglobulinemia were assessed for expression of ANA and fluoroscopic patterns. ANA was determined by indirect immunofluorescence on Hep2 (ALPHADIA-Belgium). The characterization of immunologic subtypes of MG and cryoglobulins was assessed by immunofixation-electrophoresis (SEBIA/Paris-France). All subjects with Cryoglobulinemia were characterized for chronic hepatitis C virus (HCV).
Results: Twenty nine percent of patients with MG (71/245) were ANA positive, confirming previous literature data.Speckled was the main representative pattern (57%).It is noteworthy that the frequency of homogeneous pattern (31%) observed in MG is higher than in rheumatologic patients (15-20%).We observed similar results in patients with Cryoglobulinemia: ANA were found to be positive in 39% of patients (59/150), mainly characterized by speckled pattern (49%), whereas homogeneous pattern was present in 29% of subjects.We did not observe any significant difference as regards positivity and ANA pattern, neither among different immunological subtypes of MG or in patient group with Cryoglobulinemia as regards type II and type III. Instead we detected different percentage of ANA positivity between subjects HCV positive (29%) and HCV negative (56%).
Conclusions: These findings suggest that patients with MG and those with Cryoglobulinemia show a similar behaviour of ANA positivity and fluoroscopic pattern, with the exception of patients HCV positive, suggesting that the process leading to paraproteinemia (MG) or cryoglobulins may activate usually silent autoreactive B cells.

M023

CAN MICRORNAS MODULATE THE CYTOSKELETON NETWORK IN CELIAC PATIENTS?

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Background: Celiac disease (CD) is an immune mediated enteropathy caused by permanent sensitivity to gluten in genetically susceptible individuals. We previously demonstrated a miRNA mediated gene regulation in the small intestine of CD patients; particularly, we highlighted a correlation of miR-449a over expression with a reduced NOTCH1 pathway and a decreased differentiation of mucin-secreting surface goblet cells. We hypothesized the NOTCH pathway could be constitutively altered in the CD intestine and drive the altered differentiation of goblet cells, deranging the protective function of the mucosal barrier that interfaces with the environment. The cytoskeleton is well known for its scaffolding functions, serving to anchor and regulate membrane proteins involved in many cell signaling events; for example, in goblet cells it is necessary for granule translocation. Further, gluten challenge has been shown to cause rapid disorganisation of cytoskeleton in the intestinal mucosa from CD patients. The aim of this study is to evaluate the possible role of miRNAs in cytoskeleton's proteins regulation in CD intestinal mucosa.
Methods: Among the miRNAs previously identified by our group in CD small intestine, we selected those differently expressed between CD children and controls. The list of putative target genes of these latter miRNAs, predicted by miRecords, was analyzed using the Gene Ontology Tree Machine (GOTM) to identify the biological pathways involved in miRNAs regulation.
Results: By bioinformatics we identified many miRNAs targeting cytoskeleton proteins, such as dystrophin (DMD) and phosphatidylinositol glycan anchor biosynthesis, class M (PIGM).
Conclusions: These preliminary results suggest that miRNA mediated impairment of the cytoskeleton protein network, together to the altered cell differentiation previously shown, could take part in the CD intestinal mucosal dysfunction.

M024

CHARACTERIZATION OF THE ENTIRE CELIAC DISEASE INTESTINAL MICROBIOME BY NEXT GENERATION SEQUENCING

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Background: Celiac disease (CD) is a chronic inflammatory and multifactorial disorder that involves interactions between genetic and environmental factors. Ingestion of gluten in genetically predisposed individuals leads to abnormal intestinal immune response involving both adaptive and innate immunity, with consequent damage of the small intestinal and development of signs and symptoms. A number of studies have identified significant alterations in the composition of the gut microbiota in CD patients with respect to healthy individuals, and there is a growing research interest in understanding whether interactions between intestinal microbes and innate immunity could impair mucosal barrier function, promoting an inflammatory response, and therefore influencing CD expression. Here we report the characterization of the entire gut mucosal-associated microbiome in adult CD patients.
Methods: Ten CD active patients and ten controls were enrolled in the study. Genomic DNAs were extracted by duodenal samples. 16S rRNA next generation sequencing was carried out on the Genome Sequencer FLX instrument. Data analysis was performed using the QIIME community analysis pipeline.
Results: More than 148 Mb equivalent to 540,992 sequences were totally obtained. High quality filtered sequences allowed the identification of 10,419 operational taxonomic units (OTUs). The comparison between the two studied groups highlighted a significant different microbial composition segregating with the disease phenotype ($P < 0.05$).
Conclusions: Our data suggest the presence of a CD specific intestinal microbial signature. In addition, these results strongly reinforce the suitability of next generation sequencing technology to deeply investigate the quantitative and qualitative variability of microbiomes associated with human diseases.

M025
FECAL-CALPROTECTIN SAMPLES' ANALYTICAL STABILITY

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Background: Ideally every test requested from a clinical laboratory should be performed as soon as the samples become available, but logistics and budgetary considerations force more expensive less intensive test to accumulate until a set time has elapse or a critical number of samples is reached; it is due to this practice that the knowledge of sample stability is essential to the laboratory scientist. The calprotectin is a novel biomarker for functional bowel disorders, but even when its usefulness appears to be very promising, test standardization and our knowledge of pre-analytical considerations are still somewhat lacking.
Methods: We performed triplicates calprotectin analysis on fecal samples from 21 patients with positive clinical features, using a commercial ELISA test (EliA™ Calprotectin from Thermo Scientific®), then samples were re-tested after a 24 hour at -4 ºC (again triplicates) and then compared the results against the values obtained after extraction. Stability was defined as no more than one third of the individual replicates showing a change of 15% or greater from the observed mean at time zero, and equivalence was assessed using a linear regression and Fieller's method for two-sample equivalence. H_0:δ=0 vs.H_A:δ≠0 F=(N-2)/([SS]_24h+[SS]_0h) (Y¯_24h-λY¯_0h)^2-[(n_24h λ_2+n_0)/(n_24h n_0)]
Results: The linear regression between the zero and 24 hours groups was of 0.874, the intra group replicate variability was of 7%, 17 out of 21 subjects showed a greater than 15% drop in measured values and the Fieller critical value was of 20.999 (N-2 df in a F-distribution) which fails to reject the null hypothesis at p <5% meaning there is no equivalence as assessed by two of the three possible approaches.
Conclusions: Fecal samples of calprotectin are not stable after 24 h storage at -4 ºC, and therefore should be process immediately upon collection even when considering the cost and time implicating in performing an ELISA for the determination.

M026
PARVOVIRUS B19 AND AUTOIMMUNE DISEASE

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Abstract: Human parvovirus B19 infection, known as Erythema Infectiosum or Fifth Disease, is responsible for a wide range of human diseases. A variety of manifestations are associated with the infection such as arthralgias, arthritis, leukopenia and thrombocytopenia, anemia and vasculitis,spontaneous abortion and hydrops fetalis in pregnant women.
Case: A 44-year-old woman was admited to our hospital with fever,severe asthenia,feature symmetric wrist, knee and cervical region with superior appearance of not itchy rash member and breast. According to the patient these symptoms started 3 weeks ago. All biochemical serum parameters were normal except: C3:0.86(0.90-1.80) IgM:3.94 (0.40-2.30) ANA (anti-nuclear antibodies):title 1/160 Anti-DNA:74 (0-20.0) The clinical findings (fever, rash, and malaise) and laboratory data(presence of autoantibodies and hypocomplementemia) had initially suggested a diagnosis of SLE. But subsequently, evidence of HPV-B19 infection at the time of clinical presentation was ascertained. Serology Parvovirus B19: Ig G:+ IgM:+ The patient was diagnosed with systemic infection by Parvovirus B19 advising rest for 2weeks and control after 6 months. In the control, the patient was asymptomatic and he had an excellent clinic evolution, and the following laboratory findings were all normal: C3: 1.21 (0.90-1.80) IgM: 1.33 (0.40-2.30) ANA (anti-nuclear antibodies):negative Anti-DNA: 4.2(0-20.0) Serology Parvovirus B19: Ig G:+ IgM
Conclusions: B19 infection may simulate both clinical and laboratory features of SLE. The similarities in both clinical and serological features of parvovirus infection and SLE at presentation may hinder the differential diagnosis between these two conditions. In addition to our case, we identified others in the literature in which B19 infection produced transient SLE symptoms,some of these with manifestations persisted at least a year post infection. According with other authors the degree of autoantibody production may also predict which patients develop persistent disease. Patients with a self-limited SLE course had ANA titers of 1:320 or less (as our patient) and patients who developed persistent SLE had titers of at least 1:640 at the time of diagnosis.

M027

EXPRESSION OF MALONDIALDEHYDE AND NITRIC OXIDE IN PATIENTS WITH TAKAYASU

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Background: Takayasu arteritis (TA) is a granulomatous vasculitis of obscure etiology and pathogenesis, affecting the aorta, pulmonary artery and their main branches. The term endothelial dysfunction refers to the loss of bioavailability of nitric oxide (NO). Lipid peroxidation alters the cell membrane function; the final product is malondialdehyde (MDA), which causes inflammation.
Objectives: To demonstrate the presence of alterations in MDA and NO concentration in patients with TA, and to assess the association between ON and MDA with TA activity.
Patients and Methods: Patients were recruited from the Takayasu Clinic of a hospital of medical specialties, Centro Médico Nacional "Siglo XXI", IMSS. All patients fulfilled the classification criteria of the American College of Rheumatology 1990 for TA. We determined age, gender, duration with symptoms and disease activity based on the criteria of Kerr of the National Institutes of Health in the United States, describing them as active or inactive. The control group consisted of healthy subjects. We determined serum MDA and NO in patients and controls. Statistical analysis was performed using the T Student test and nonparametric tests.
Results: 21 patients were included in this study. Average concentrations of MDA were 148.01 uM in patients and 17.95 uM in controls (P <0.001). The average concentration of NO was 14.28 uM in patients vs. 41.08 uM in controls (P <0.001). No statistically significant difference between active and inactive status (MDA vs 200.1 uM. 10.8.8 uM and 4.20 uM vs NO. 9.6 uM) was found.
Conclusions: The high levels of MDA and low levels of NO in patients with TA, suggest inflammation and endothelial dysfunction respectively, regardless of the degree of disease activity. There may be an association between subclinical progressions of these vacuities with elevated levels of MDA. Based on our results, it is important to determine these markers in a standardized way, in a larger population and at different stages of the disease. We think that these molecules should be measured in patients with TA since the development of changes may suggest vacuity activity.

M028

ASSOCIATION OF AUTOANTIBODY PROFILES WITH DISEASE ACTIVITY IN PATIENTS WITH RHEUMATOID ARTHRITIS USING INDIRECT IMMUNOFLUORESCENCE AND LINE IMMUNOASSAY

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Background: Aims of this study were to analyze indirect immunofluorescence (IIF) patterns and autoantibodies identified by line immunoassay (LIA) in rheumatoid arthritis (RA) patients, and compare the autoantibody profiles to serological markers, determining relevance. Methods: A total of 153specimens were obtained from RA patients for diagnosis or monitoring of disease activity. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), RF, and anti-CCP were measured and autoantibody testing using IIF and LIA were performed. Frequencies of IIF patterns and autoantibodies identified by LIA were analyzed and compared to each other and other serologic markers of RF, anti-CCP, ESR and CRP.
Results: Overall positivity of IIF and the LIA was 49.7% and 34%. Most frequent IIF pattern in RA was homogenous pattern (20.9%), followed by dense fine speckled pattern (DFS, 17%). The most common autoantibody detected by LIA was anti-SS-A (22.2%), followed by anti-Ro-52 (11.1%).Anti-SS-A and/or anti-Ro-52 were the most frequently detected autoantibodies in RA irrespective of the observed IIF pattern. Positive rates and intensities of autoantibodies detected did not differ by therapeutic regimen or disease activity, as reflected by CRP or the ESR.
Conclusions: Unlike other major systemic autoimmune diseases, RA patients did not demonstrate specific or dominant IIF pattern or autoantibody identified by LIA. RA patients had lower frequencies of anti-SS-A and anti-Ro-52 compared to other systemic autoimmune diseases, serological characteristic of RA. Both IIF and LIA appear insufficient for diagnosing RA and monitoring disease activity, yet plays a role for screening and differentiating major systemic autoimmune diseases.

M029

THE DIAGNOSTIC PERFORMANCE OF ANTI-NUCLEOSOMES, ANTI-DSDNA-NCX ELISA AND ANTI-DSDNA DETECTED BY IMMUNOBLOT METHOD IN DIAGNOSING OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: The presence of anti-dsDNA is diagnostic for systemic lupus erythematosus (SLE). Anti-nucleosomes (anti-NcX) are also used for diagnosis of SLE. Wide variety of methods for detecting anti-dsDNA have been established. We aimed to study the diagnostic performance of these antibodies by two different methods to estimate their usefulness in clinical practice.
Methods: We studied 227 ANA positive patients. Clinical data were retrieved from HIS. We used ANA Profile 1 EUROLINE IgG for detecting antibodies against dsDNA (anti-dsDNA IB) and nucleosomes (anti-NcX). As second method for detecting anti-dsDNA we used anti-dsDNA-NcX ELISA IgG (EUROIMMUN, Germany).
Results: SLE was diagnosed in 48/227 patients. Diagnostic sensitivity and specificity for anti-dsDNA- ELISA were 63% and 79%, for anti-dsDNA IB 43% and 79%, for anti-NcX 50% and 86%, accordingly. In combined determination of these 3 analyses the diagnostic sensitivity increased to 73% but specificity decreased to 58%. The main diagnoses for false positive results were other systemic involvement of connective tissue and seropositive rheumatoid arthritis.
Conclusions: Anti-dsDNA-NcX ELISA had the best diagnostic performance in diagnosing SLE. It should be recommended to combine anti-dsDNA ELISA with ANA Profile 1 EUROLINE for better detection of SLE patients in our hospital.

M030

ANEMIA AS PROGNOSTIC PARAMETER OF DISEASE ACTIVITY IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease affecting multiple systems with variable activities. Hematological manifestations are frequent in SLE. Most studies in SLE have examined hemolytic anemia but not anemia in general. Little is known about the role of anemia in the course of the disease. It is possible that in SLE patients, like the general population, anemia be a marker of unfavorable outcomes.
Methods: We performed a prospective study to investigate the impact of anemia and its severity on disease activity. A cohort of 150 SLE inpatients was evaluated at baseline and after 1 year. At every visit clinical manifestations and laboratory parameters were assessed, and the SLE disease activity index (SLEDAI) was determined. The following laboratory parameters were evaluated at the start of the study and at the end: peripheral blood hemoglobin, mean corpuscular volume (MCV), erythrocyte sedimentation rate, reticulocytes, leucocytes, lymphocytes and platelets. Anemia is divided in four categories according to the hematocrit (Hct) level: no anemia (Hct > 35%), mild anemia (Hct: 30–35%), moderate anemia (Hct: 25–29%) and severe anemia (Hct < 25%). The relationship between laboratory parameters and SLEDAI was examined by multivariate generalized lineal regression models.
Results: At the start of the study all patients were in clinical remission. The average SLEDAI at the start was 4.9 and there was no statistical relationship between laboratory parameters and SLEDAI. After one year, flares were observed in 52% (78/150) of the patients. The mean SLEDAI score (SD) were: 6.43 (3.69), 10.58 (3.80), 16.12 (6.80), 20.59 (8.66) for any categories of anemia, respectively. But, only moderate and severe anemia (Hct ≤ 30%) was significantly associated with higher SLEDAI scores after adjusting for age and gender (P <0.001).
Conclusions: Mild and moderate anemia are prognostic predictors of disease activity in SLE. Different levels of anemia could be used to monitor disease activity in SLE.

M031

A PROPOSED REFERENCE CHANGE VALUE FOR AN IGA ANTI-TISSUE TRANSGLUTAMINASE IMMUNOASSAY TO DETECT GLUTEN TRANSGRESSION IN CELIAC PATIENT DIET

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Background: Celiac disease (CD) is an autoimmune disorder caused by an inappropriate immunological responsiveness to gluten ingestion in genetically susceptible individuals. IgA anti-tissue transglutaminase (tTG) antibodies have been widely employed as a biochemical marker for screening and diagnosis of CD along with small bowel biopsy. Recently, new studies have also shown the efficacy of this test in evaluating patient compliance after gluten withdrawal from their diet. Most patients with a gluten-free diet (GFD) experience a significant reduction of IgA anti-tTG values reaching a "negative" concentration one year later, which normally exhibits fluctuations inside this negative range or even near the cut-off point. This situation makes complicated to differentiate between normal physiological variation and diet transgression due to short gluten exposure.
Methods: A group of 28 celiac patients (7 men and 21 women) carrying an adequate fulfilment of the GFD during more than one year was selected for the study. IgA anti-tTG determination (EliATM CelikeyTM IgA, Thermo Scientific) was performed every two months during half year. These data were used to estimate the biological variation of IgA anti-tTG in celiac patients and to calculate the reference change value (RCV) according to the Harris and Fraser formula.
Results: Patients were between 15 and 70 years old (mean 33.8 years). IgA anti-tTG results ranged between 0.5 and 8.2 U/mL. The analytical imprecision estimated from our internal quality control was 5.7%. The within-subject and between-subject biological variation found were 19.2% and 75.6%, respectively, and the index of individuality (II) was 0.25. Using these data and the correspondent value of Z for a 95% level of significance, the RCV was 55.5%.
Conclusions: To our knowledge, we have calculated for the first time the biological variation and the RCV for an IgA anti-tTG inmunoassay in a celiac population. This test showed a strong individuality (II<0.6) which means that population-based reference intervals are of very limited use in evaluating IgA anti-tTG serial results. The RCV value and the probability curve generated from our data could be a valuable and complementary tool for clinicians to evaluate a suspicion of gluten transgression.

M032

PHOSPHATIDYLSERINE-DEPENDENT ANTI-PROTHROMBIN ANTIBODIES AND ANTIBODIES TOWARDS ANNEXIN V PLAY A ROLE IN RECURRENT MISCARRIAGES

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Background: The objective of the study was to evaluate phosphatidyserine-dependent anti-prothrombin antibodies (aPS/PT IgG and IgM) and antibodies towards annexin V (aANNXV IgG/IgA/IgM) in women with idiopathic recurrent miscarriages and negative antibodies towards phospholipids (cardiolipin, beta-2-glycoprotein-1, phosphatidylserine, phosphatidylinositol, phosphatidic acid, i.e. aPL IgG and IgM).
Methods: Fifty-seven (57) women diagnosed due to the idiopathic recurrent (>3) miscarriages, 23 healthy pregnant women in the 16th week of pregnancy and 28 healthy fertile women without poor obstetric history having at least one child, were examined. All women were screened for the presence of mentioned serum autoantibodies by standardized ELISAs.
Results: aPS/PT IgG and/or IgM were found in 16/57 (28.1%) of women with idiopathic pregnancy losses, and were almost negative in both control fertile groups (P=0.001 and P=0.004, respectively). Similarly, aANNXV IgG/IgA/IgM were found only in women with abortions (15/57, 26.3%, P=0.003 and P=0.001, respectively). Coincidental positivity of aPS/PT IgG and aANNXV IgG/IgA/IgM was found in 12/57 (21%) of miscarrying women. All aPS/PT and/or aANNXV-positive females were treated with oral corticosteroids, low-dose aspirin and low molecular weight heparin before the conception and during the pregnancy. Pregnancy rate of 11/19 (57.8%) and live birth rate of 9/19 (47.4%) were achieved in the cohort.
Conclusions: Detection of aPS/PT and aANNXV has an additional value in the diagnostics of patients with recurrent miscarriages. Based on the frequent coincidental positivity of aPS/PT and aANNXV, we have hypothesized that interference with the binding of annexin V to the PS/PT complex could be a possible mechanism of pregnancy loss in this variant of antiphospholipid syndrome.
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M033

CCL17/THYMUS AND ACTIVATION–RELATED CHEMOKINE: A BIOMARKER FOR CHURG-STRAUSS SYNDROME?

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Background: Churg-Strauss syndrome (CSS) is a Th2-mediated systemic vasculitis characterized by eosinophilic infiltration, blood eosinophilia, and high IgE levels. CCL17/thymus and activation–regulated chemokine (TARC) is a chemokine that is secreted from monocyte-derived DCs and endothelial cells and is responsible for selective recruitment and migration of activated Th2 lymphocytes to affected tissue. Although CSS belongs to the group of antineutrophil cytoplasmic antibody (ANCA)–associated vasculitides, some disease features are specific for CSS and distinguish it from Wegener's granulomatosis (WG). This study was undertaken to explore a possible role of TARC in CSS.
Methods: TARC levels in sera from patients with active (n=8) or inactive CSS (=10), active WG (n=13) and healthy controls (n=18) were determined by enzyme-linked immunosorbent assay (ELISA).
Results: Serum TARC levels were significantly elevated in CSS patients with active disease compared with controls (mean 1923.0 ± 875.7 pg/ml versus 319.6 ± 98.8 pg/mL, $P < 0.05$) and patients with inactive disease (459.6 ± 175.7 pg/mL, $P < 0.05$). These levels correlated with established laboratory markers of disease activity IgE ($r = 0.87$, $P < 0.05$) and C-reactive protein (CRP) ($r = 0.94$, $P < 0.05$). TARC levels were significantly higher in active -CSS patients than in active-WG patients (550.17 ± 105.8, $P < 0.05$).
Conclusions: We found significantly elevated serum levels of CCL17/TARC in patients with untreated CSS compared with healthy controls. Its levels correlate with established laboratory markers of disease activity IgE and CRP. To explore the specificity of our findings in CSS, we also measured levels of TARC in patients with WG. Serum TARC levels were significantly elevated in active-CSS patients compared with active-WG patients. In conclusion, serum TARC levels could reflect disease activity, and increased concentrations may precede clinical relapse of CSS in treated patients. Further studies to validate its use as a specyfic activity marker in CSS are warranted.

M034

A MULTICENTER EVALUATION OF A NEW AUTOMATED METHOD FOR MEASUREMENT OF ANTI-CYCLIC CITRULLINATED PEPTIDE

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Background: Rheumatoid arthritis (RA) is a chronic inflammatory auto-immune disease affecting approximately 1-2% of the population worldwide. RA is a potentially crippling disease since it results in malformation of the joints. RA is mostly diagnosed based on clinical manifestations but serological tests against auto-antibodies, such as rheumatoid factor and cyclic citrullinated peptides (aCCP), are available. The presence of aCCP antibodies is strongly associated with a more severe, destructive disease course. Recently, a new test for the measurement of aCCP antibodies on the IMMULITE 2000(XPi) platforms was developed by Siemens Healthcare. In this study we investigated the performance of this new anti-CCP test in three different hospital laboratories.
Methods: Samples were collected from patients presented to the hospital for aCCP measurement. Serum aCCP levels were determined by aCCP IgG assay for IMMULITE 2000(XPi) systems (Siemens Healthcare), ImmunoScan RA Elisa test (Eurodiagnostica) or aCCP IgG assay on the Modular system (Roche Diagnostics). The evaluation protocol consisted of within-run imprecision (20 sequential runs) and between-run imprecision (16 workdays) with commercial controls and control serum from a patient pool, assessment of alternative materials and a method comparison. Methods were compared by Passing and Bablok regression.
Results: Within run imprecision for aCCP IgG assay for IMMULITE 2000(XPi) was 6,82% at a level of 7,0 U/mL and between-run imprecision was 5,61% at a level of 8,4 U/mL. Method comparison according to Passing and Bablok showed good correlation of samples measured on two different Immulite analyzers (0,21 + 0,96x (n=40)). This study showed 100% agreement between positive and negative samples collected in serum and lithium-heparin (n=20) with good correlation (-0,12 + 1,08x). Comparison of the IMMULITE 2000(XPi) aCCP test with aCCP on Immunscan RA ELISA (n=112) and aCCP on the Modular system (n=140) resulted in a concordance of 90.2% and 94.8% respectively.
Conclusion: The aCCP assay on the IMMULITE 2000(XPi) has good performance characteristics and shows good concordance with the Immunoscan RA Elisa test and aCCP test on the Modular systems.

M035

PREVALENCE OF "RINGS RODS" AUTOANTIBODIES ON HEP-2 PREPARATIONS

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Background: Indirect immunofluorescence on human Hep-2 cells is the standard method for assessing antinuclear autoantibodies (ANA) as diagnostic screening for autoimmune diseases. Recently a new cytoplasmic pattern has been described as distinct "rods and rings" (RR), and Inositol-5' monophosphatdehydrogenase 2 (IMPDH2) is probably the reactive antigen.
Methods: In order to evaluate the prevalence of RR pattern, we analyzed consecutive samples tested in our laboratory for ANA screening on Hep-2 cell slides by INOVA Diagnostics (San Diego, CA) from September 2011 to October 2012.
Results: Out of 3875 routine samples 31 (0.8%) showed RR pattern, the majority of which (87%) were ANA negative. In almost all the cases RR pattern was associated with previous HCV infection: in particular, anti-RR antibodies were evidenced in patients HCV-RNA carriers without antiviral therapy, in patients treated with PEG-interferon plus ribavirin and in liver transplanted patients with mycophenolate mofetil as immunosuppresant regimen. Interestingly, anti-RR antibodies were observed in serum from a 2-year-old child affected by scleroderma and treated with methotrexate.
Conclusions: To the best of our knowledge this is the first study evaluating the occurrence of RR pattern in unselected routine samples and reporting anti-RR antibodies in HCV-infected patients at different stages of liver disease, also in organ transplantation after hepatocellular carcinoma. The expression of RR pattern in patients treated with IMPDH2 inhibitors, such as ribavirin and mycophenolic acid, seems to suggest a link between anti-RR and enzyme activity. Further investigations are needed in order to confirm the present observations, to verify if RR expression is a response to disturbances exclusively in hepatocyte synthetic pathways and to clarify possible implications of anti-RR antibodies in response to therapy.

M036

A NOVEL TECHNOLOGY FOR ANTIBODY PROFILING IN THE SEROLOGICAL DIAGNOSIS OF APS

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Background: Antiphospholipid antibody (aPLAb) profiling has been proposed to improve risk prediction in patients with antiphospholipid syndrome (APS). This study investigates the performance of aPLAb detection by a novel line immunoassay (LIA) in APS patients with differing clinical symptoms and controls in comparison with ELISA data.
Methods: IgG and IgM antibodies to phosphatidylserine (PS), phosphatidylinositol (PI), cardiolipin (CL), phosphatidylcholin, phosphatidylethanolamine, phosphatic acid (PA), phosphatidylglycerol (PG), annexin V (AV), prothrombin (PT), and beta2-glycoproteinI (β2GPI) were determined by LIA in sera of 64 APS patients (27 primary APS, 25 obstetric APS, 12 asymptomatic APS), 73 infectious disease control patients, and 50 blood donors. Anti-CL and anti-β2GPI aPLAb were assessed by in-house and commercial ELISA and compared with LIA data regarding association with the clinical phenotype.
Results: Anti-CL IgM (13/20), anti-PA IgM (11/20), anti-PG IgM (8/20), and anti-PS IgM (13/20) by LIA demonstrated a significant higher prevalence in APS patients suffering from arterial thrombosis (AT) compared to those (n=44) without ($P < 0.05$; respectively). The appearance of 5 or more aPLAb IgM assessed by LIA was significantly more prevalent in APS patients with AT (9/20 vs 4/44, $P < 0.05$). Anti-PG IgG (14/27), anti-PI IgG (17/27), anti-PS IgG (22/27), and anti-β2GPI IgG (21/27) by LIA were more prevalent in primary APS (PAPS) patients compared to obstetric APS (OAPS) ($P < 0.05$, respectively). Regarding ELISA, only the prevalence of anti-CL IgG was more prevalent in PAPS patients (24/27) compared with OAPS (11/25, $P < 0.05$). In the control groups, the LIA did not demonstrate significantly more false positive samples in comparison with ELISA. Remarkably, out of 50 patients with viral infectious disease, only 2 patients demonstrated either positive anti-AV or anti-PT IgG by LIA whereas 2 patients had positive anti-CL IgG or IgM by in-house ELISA.
Conclusions: The novel LIA is an efficient diagnostic tool for aPLAb profiling in the serology of APS. aPLAb detected by LIA appear to be more associated with the clinical APS phenotype than those detected by ELISA.

M037
PRIMARY BILIARY CIRRHOSIS-CASE PRESENTATION

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Background: Primary biliary cirrhosis (PBC) is a rare autoimmune disease, which is characterized by slow progressive destruction of the small intrahepatic bile ducts, leading eventually to cirrhosis and liver failure. PBC diagnostic criteria are: cholestatic liver biochemistry, co-existence of a ESR 1st hour >50 mm with CRP normal levels, elevated IgM levels and presence of high-titer AMA, the diagnostic hallmark of PBC. The aim of this study is the presentation of a PBC case (66 year old asymptomatic male patient).
Materials and methods: AST, ALT, ALP, γ-GT, total bilirubin, cholesterol, serum proteins, serum albumin, CRP, IgG, IgA and IgM immunoglobulin levels were measured by the OLYMPUS AU640 automated analyzer. Serum protein electrophoresis was performed with the Hydrasys SEBIA system. ANA and AMA were detected by indirect immunofluorescence on Hep-2 cells and mouse stomach/kidney tissue slides respectively (INNOVA Diagnostics Inc, San Diego CA).Complete blood count was measured on SYSMEX XT-2100 automated analyzer.
Results: AST 44.3 U/L, ALT 62.2 U/L, γ-GT 225.3 U/L, ALP 170.9 U/L, bilirubin 0.66 mg/dL, cholesterol 166.0 mg/dL, serum proteins 7.96 g/dL, albumin 4.4 g/dL, IgG 1429.5 mg/dL IgA 337.9 mg/dL, IgM 459.2 mg/d, CRP 4.88 mg/dL, neutrophils 39.5%, lymphocytes 34.1%, monocytes 7.7%, basophils 0.6% and eosinophils 18.1%. ESR 1st hour 52 mm. Protein electrophoresis: albumin 54.8%, α1-globulins 2.2%, α2-globulins 6.8%, β-globulins 10.3% and γ-globulins 25.9% (polyclonal hyper-γ-globulinaemia). ANA positive (titer 1/640, cytoplasmic pattern) and AMA positive (titer 1/160).
Conclusions: Results in accordance with PBC. Eosinophilia is a distinctive feature of early stage PBC. Clinical and experimental data suggest that mast cell activation in cholestatic liver diseases induces eosinophil chemotaxis and activation. Liver biopsy is not essential for PBC diagnosis but allows assessment of disease stage and activity.

M038
CYTOKINES IN PATIENTS WITH VARIOUS CLINICAL MANIFESTATIONS OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: This paper studies the balance between proinflammatory and antiinflammatory cytokines in serum of patients with SLE.
Methods: Complete biochemical and immunologic laboratory processing of the biomaterial, enabled classification of SLE patients (n=55), into the following groups:patients with cutaneous disease manifestation, S-SLE; patients with neurolupus, N-SLE; patients with joint changes, J-SLE; patients with blood vessel changes–vasculitis,V-SLE. Twenty healthy volunteers, comprised the control group. Concentration of proinflammatory and antiinflammatory cytokines was determined by ELISA tests.
Results: The increase was at its highest in patients with neurolupus (P <0.001) and joint disease (P <0.01), while cutaneous and vascular forms were of lesser significance (P <0.05).Comparing the groups, we noticed significant TNF-α increase in joint and neurolupus related to vascular SLE (P <0.05). IL-4,demonstrated statistically significant increase in neurolupus patients 11.96±2.91 pg/mL and vascular lupus 10.93±1.77 pg/mL compared to controll values 8.97±1.90 pg/mL for (P <0.05). The increase of the IL-10 concentration is of statistical significance in neurolupus patients 16.25±4.31 pg/mL and in vascular disease 15.23±2.18 pg/mL compared to controls 5.13±1.51, for (P <0.01) and skin disease 12.87±2.28 pg/mL, with somewhat lower significance of (P <0.05). Interleukin-13, showed the increase concentration in the whole SLE group 4.27±1.41 pg/mL related to controls 2.17±0.67 pg/mL at the level of significance of (P <0.05).
Conclusions. The results of this paper indicate that TNF-α can be of special importance in the N-SLE pathology. TNF-α released from inflammatory cells acts synergistically in the circulation, inducing peripheral vasodilatation., Increased IL-10 can be associated with neuropsychiatric menifestations of the disease. Inhibitors of cytokine production are being extensively studied as potential therapeutics in SLE.

M039
MUCOPOLYSACCHARIDOSIS I: A-L-IDURONIDASE MUTATIONS IN TWO TUNISIAN FAMILIES

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Mucopolysaccharidosis type I (MPS I) is an autosomal storage disease resulting from defective activity of the enzyme α-L-iduronidase (IDUA). This glycosidase is involved in the degradation of heparan sulfate and dermatan sulfate. MPS I has severe and milder phenotypic subtypes. The IDUA mutations in two MPS I patients from two unrelated families from central and southern Tunisia were determined by amplifying and sequencing each of the IDUA exons and intron–exon junctions.
Results: One novel IDUA mutations, c.1650+1G>T in intron 11 and one previously reported mutations p.P533R were detected. The patient in family 1 who had the Hurler phenotype was homozygous for the previously described nonsense p.P533R mutation. The patient in family 2 who also had the Hurler phenotype was homozygous for the novel splicing c.1650+1G>T mutation.
Conclusion: The identification of these mutations should facilitate prenatal diagnosis and genetic counseling for MPS I in Tunisia.

M040
HEREDITARY ANGIOEDEMA: A TH17 PATTERN DISEASE?

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Background: Hereditary Angioedema (HAE) is a rare genetic disorder characterized by acute, intermittent, and potentially life-threatening attacks of edema of the skin and mucosa, caused by C1 inhibitor (C1-INH) deficiency, which is actually described as a “non inflammatory edema”. In this study we analyzed several inflammatory cytokines (CYKS) and chemokines (CKS) in HAE affected patients, to the purpose of finding and understanding HAE inflammatory aspects.
Methods: In our study, we evaluated a group of 17 known HAE affected patients (11 females and 6 males, aged between 9 and 65). The control group consisted of 19 healthy subjects (10 female and 9 male, age between 24 and 54). For each HAE affected patient, we collected 2 to 8 observations and only 1 observation for each control healthy subject; in total 90 observations (71 for cases and 19 for controls). Samples have been tested for routine exams, C4 and C1-INH levels and for CYKS and CKS wide panel (IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-13, IL-17, IFN-γ, TNF-α).
Results: Analysis of serum samples using multiplex assay, showed that almost all pro-inflammatory CYKS are significantly increased in patients during acute attacks (IL-1b, IL-6, IL-4, IL-7, IL-9, IL-13, IL-17, TNFa) compared with basal samples and controls results. Comparison between acute attacks data and basal corresponding samples showed that IL-17 is 10,1 pg/ml higher (P=0.01) during acute attacks. Comparison between patients basal data and healthy subjects indicates that IL-17 is 14.6 pg/mL higher (P=0.016) in HAE.
Conclusions: HAE should not be described as non inflammatory disease. In particular, IL-17 is significantly higher in HAE patients suggesting a pathogenetic TH1/TH17 profile for this rare disease. IL-17 high levels in basal patients samples (compared with healthy controls) also suggest that HAE affected patients could have a “tick-over” constitutive state, ready to be sparked by acute attack inducing events. Furthermore, IL-17 increased levels might contribute to neutrophils recruitment in phlogistic area. TH17 cells involvement could be a HAE typical pattern such as is already known about some autoimmune and chronic inflammatory disease models.

M041

FIRST DIAGNOSIS OF HEREDITARY FOLATE MALABSORPTION (HFM) IN ITALY: A BIOCHEMICAL EVIDENCE FOR CLINICAL THERAPY IMPROVEMENT AFTER DIFFERENT FOLINIC ACID POSOLOGY

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Background: HFM is a rare disorder with less than 30 cases reported, characterized by folate deficiency with impaired intestinal folate absorption and impaired folate transport. Recently, mutations determining loss of function of the proton-coupled folate transporter (PCFT-SLC46A1) have been reported. Here, we describe a case of female child with symptoms suggestive to hereditary failure of folates absorption. In fact, serum folate levels were undetectable, even after oral (OS) administration of folinic acid. Herein, we describe the results of folate assays obtained on serum, whole blood and CSF after different folate treatments, in order to evaluate the optimal therapy.
Methods: Blood samples were obtained from the child and her parents. Entire SLC46A1gene was analyzed by direct sequencing. The proband underwent to folinic acid 5 mg OS, intravenous (IV) and intramuscular (IM) administrations based on different protocols. After each treatment a blood samples and CSF were collected. Samples were analyzed using ARCHITECT® Abbott Folate kit.
Results: This patient and her parents were homozygous and heterozygous for the frameshift mutation (c.194dupG) of the SLC46A1 gene, resulting in a truncated protein (p.Cys66LeufsX99). Folate IV treatment restored the normal folate levels in serum and RBCs for 22 days, and determined an increasing of the CSF folate levels. Contrastingly, 6 days after the single IM administration, serum and RBCs folate concentrations decreased, while normal levels were restored when 3 IM administrations/week were used.
Conclusions: This is the first case of HFM described in Italy, found in a small town namely Itri, where malaria was endemic. We cannot exclude a genetic drift due to selection of SLC46A1 carriers by malaria infection. Furthermore, the restoring of normal blood folate levels occured only after IV or consecutive IM doses. After consecutive IM administrations, the RBCs folate concentrations increased much more than serum folate concentration. It suggests a possible role of RBCs as folate reservoir to keep optimal serum folate concentration. As future perspective, we will organize a genetic screening in Itri community in order to establish the prevalence of this mutation and to define the imbalance of folate metabolism.

M042

SMALL AMPLICONS HIGH RESOLUTION MELTING ANALYSIS (SA-HRMA) ALLOWED SUCCESSFUL GENOTYPING OF ACID PHOSPHATASE 1 (ACP1) POLYMORPHISMS IN THE ITALIAN POPULATION

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Background: The ACP1 gene has been suggested as a genetic factor of several human diseases, including inflammatory and autoimmune diseases, favism and tumors. In Caucasian populations there are three common co-dominant ACP1 alleles: ACP1*A, ACP1*B, and ACP1*C. These alleles show single base substitutions located at three specific sites: ACP1 *A and *B alleles differ for two base substitutions [a C/T transition at codon 41 (exon 4) and a A/G transition at codon 105 (exon 6)], while ACP1 *C allele differs from the *A and *B alleles by a C/T transition at codon 43 (exon 3). From allele combination derive six genotypes: *A/*A < *A/*B < *A/*C < *B/*B < *B/*C < *C/*C. In addition, each ACP1 genotype produces two distinct isoenzymes arising through alternative splicing mechanisms, in which either exon 4 or exon 3 is excised and the other is retained. These two isoenzymes, namely 'fast' and 'slow', show different molecular and catalytic properties and strong quantitative relative variation among genotypes.
Methods: Here, we report a rapid optimized method which employs HRMA for ACP1 polymorphism identification, a molecular approach that we used to screen 80 healthy Italian subjects.
Results: HRMA proved particularly suitable for detecting ACP1 genotypes. In fact, HRMA results were 100% concordant with direct sequencing. In addition, ACP1 genotype frequency in the Italian population was in accordance with the literature [4% (*A/A), 36% (*A/B), 4% (*A/C), 50% (*B/B), 6% (*B/C)].
Conclusions: Different methods for ACP1 polymorphisms genotyping have been previously reported, including PCR/RFLP, SSCP and direct sequencing. Moreover, all these techniques need of post-amplification step procedures. Use of the HRMA in this study represents a simplification of ACP1 polymorphisms identification. In fact, HRMA is found to be a simple, rapid, sensitive and low cost method potentially useful in research and diagnostics laboratories. Finally, use of small amplicons for the set-up allowed us a better optimization of HRMA. For this reason, we present such an approach as Small Amplicons High Resolution Melting Analysis. Finally, ACP1 genotype frequency in the Italian population reported in this study may contribute to a better interpretation of ACP1 allelic frequency variation.

M043

DNA FROM BUCCAL SWAB IS SUITABLE FOR RAPID GENOTYPING OF ANGIOTENSIN-CONVERTING ENZYME (ACE) INSERTION/DELETION (I/D) POLYMORPHISM

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Background: In humans, plasma and cellular ACE levels are strongly determined genetically. Several studies have shown that approximately 50% of the variability of plasma ACE between individuals is the results of an insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene. The I and D alleles differ for the presence or absence of a 287 bp Alu sequence DNA fragment. The ACE I/D polymorphism is generally analyzed by conventional PCR technique using blood samples as source of genomic DNA.
Methods: We report the development of a new single-tube High Resolution Melting Analysis (HRMA) to determine the ACE I/D polymorphism. This method takes advantage of a presence of the 287 bp DNA fragment to distinguish three genotypes (I/I, I/D, D/D) by melting curve analysis. For primers' design (three primers: two external and one internal to 287 bp Alu sequence insertion) we took into account the different efficiency of amplification of the allele I than allele D, avoiding the I/D samples misclassification. Buccal cells were used as source of genomic DNA.
Results: HRMA was found to be particularly suitable for the identification of ACE I/D polymorphism: in fact, 50 samples previously genotyped by conventional PCR were 100% concordant with the HRMA results, showing high reproducibility, sensitivity and specificity. Three genotypes were distinguished by normalized/temperature shifted melting curves, and fluorescence difference plots. In addition, each genotype was associated with a single peak: despite of Alu sequence, there were no aspecific amplicons.
Conclusions: DNA extracted from saliva or buccal cells is becoming one of the most common source for genetics testing. This DNA can be extracted by inexpensive, easy to use, and commercially available kits and it has been proven to be a good substitute to blood-derived DNAs for individual SNP assays. In addition, buccal swabs and saliva self collection kits are less invasive and safer methods for collecting DNA. For these reasons, the use of DNA from buccal swabs for rapid ACE I/D could be method of choice for epidemiological/clinical studies concerning ACE I/D variants, specially in vulnerable populations such as children, elderly or leukopenic patients to avoid the anemization induced by several venipunctures.

M044

MULTICENTER STUDY OF FIRST-TRIMESTER SCREENING FOR FETAL ANEUPLOIDIES IN 77,914 PREGNANCIES IN CATALONIA

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Objective: To evaluate the performance of first-trimester screening for Down syndrome and the influence of demographic characteristics of pregnant women in these results.
Methods: 77,914 pregnant women underwent first trimester screening program in Catalonia during 2009 and 2010. Demographic characteristics and their influence on detection and false positive rates were evaluated, as correction factors were defined globally in all laboratories.
Results: Demographic study of pregnant women shows statistically higher age (31.2 years) and percentage of smokers (15.9%) in Caucasian women and lower weight in Chinese ones (56.3 Kg) No differences in serological parameters were observed according to gender, nor in Down syndrome fetuses neither in fetuses without aneuploidies. With false positive rate of 3.5%, detection rate was 91.4% for Down syndrome and it range from 82.4 to 94.7% for other aneuploidies.
Conclusion: Prenatal screening for aneuploidies in Catalonia shows good results in terms of coverage of pregnant women as well as in detection or false positive rates. Applying of correction factors by weight, ethnicity and smoking status are necessary in order to improve these results.

M045
COMPLEX KARYOTYPE IN A FOETUS WITH A MOSAICISM DUE TO TWO LINES CELLS WITH PARTIAL TRISOMY OF CHROMOSOME 14 (P11-Q13) AND A MONOSOMY OF CHROMOSOME 14.

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Chromosomal abnormalities are the most common cause of spontaneous miscarriage during the first trimester. A wide spectrum of aberrations, including trisomies, monosomies, polyploidies and structural chromosomal rearrangements, has been described in miscarriages, producing very heterogeneous phenotypes. Between these chromosomal anomalies, mosaicism in unbalanced rearrangements is rare and arises de-novo due to abnormal gametogenesis. The foetus reported here exhibited a complex karyotype with two line cells of female sex, one of them with 46 chromosomes with a derivative isochromosome from a 14 chromosome with a breakpoint in q13 due to a translocation 14:18 with breakpoints in q13 and q23 (31 metaphases), and the other cell line with 45 chromosomes without isochromosome 14 (17 metaphases). Resulting of aberrant chromosomal rearrangement was a partial trisomy of chromosome 14 from p11 to q13 in one line cell and in the other, a monosomy of chromosome 14. KARYOTYPE result: 46,XX,der(14;18) (q13;q23), ider(14) (q13)t(14;18) (q13;q23) [31]/45, XX, der(14;18) (q13;q23), ider(14)(q13)t(14;18) (q13;q23) [17]
Case report: The 33-year-old mother (paternal age was 35 years) underwent chorionic villous biopsy for chromosome examination during the 12th week of her second pregnancy. The first child was a healthy three-year-old male. The family did not reveal any genetic risk factor, and the only exogenous risk factor detected was that she was employed as a radiology technician. Radiation exposure was well-controlled by proper dosimeter readings and she moved to another job as soon as pregnancy was known. The indication for CVS (chorionic villous sampling) was a high risk of Down syndrome in the biochemical screening but there were not pathologic ultrasound findings. Based on the cytogenetic finding, the parents chose to terminate the pregnancy after nondirective genetic counseling. Foetus was a female with a weight of 360 grams, with rude facial features and normal palate. No external malformations in thorax and abdomen. The most significative phenotype was lymphedema in feet that has been described previously in women with alterations in chromosome 14. This is the first case described with this rare mosaicism with partial trisomy and monosomy of chromosome 14.

M046
TWO CASES OF SEVERE METHYLENETETRAHYDROFOLATE REDUCTASE DEFICIENCY

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Background: Deficiency of 5,10 methylenetetrahydrofolate reductase (MTHFR), the very rare methionine synthase reductase (CblE) and methionine synthase (CblG) defects, and the recently identified CblD-variant-1 defect are primary remethylation defects characterized by an isolated defect in methionine synthesis without methylmalonic aciduria. Severe MTHFR deficiency is the most common inborn error of folate metabolism. It is a rare autosomal recessive disorder with severe hyperhomocysteinemia, homocystinuria and hypomethioninemia. Patients show a range of neurological and vascular complications, including developmental delay, mental retardation, seizures, motor and gait abnormalities, and thrombosis.
Methods: In this study, we analyze two patients, first cousins, with marked hyperhomocysteinemia and a variety of neurological and vascular symptoms. Molecular analysis is performed using 12 specific primers for amplification of genomic DNA by PCR and for direct sequence analysis of MTHFR gene.
Results: In both patients, metabolic screening showed plasma accumulation of total homocysteine (>130 μM; reference value 5-15 μM), low plasma methionine (<10 μM; reference value 15-30 μM), no urinary metilmalonic aciduria excretion and elevate serum vitamin B12 and folate levels. By molecular analysis of the MTHFR gene, both patients are compound heterozygous for 2 mutations in the gene MTHFR: c.547C>T (p.R183X) in exon 4 and c.1013T>C (p.M338T) in exon 6, both previously described as being associated with MTHFR deficiency. In addition, polymorphisms c.665C>T (p.A222V), known as genetic risk factor for vascular disease and c.1305C>T (p.P435P), associated with preeclampsia, present in both patients.
Conclusions: Genetic test for MTHFR deficiency ensures differential diagnosis among remethylation defects and allows to close the correct therapy.

M047

GENETIC SCREENING OF FAMILIAL HYPERCHOLESTEROLEMIA IN SOUTHERN ITALY: A PROPOSED DIAGNOSTIC FLOWCHART

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Background: Familial Hypercholesterolemia (FH) is a common dyslipidemia characterized by high levels of total and LDL-cholesterol. Mutations in the LDL receptor (LDLR) gene are the main cause of FH with autosomal dominant inheritance. The prevalence of the disease is 1:500 for the heterozygotes and 1:106 for homozygotes or compound heterozygotes. Although the number of LDLR mutations is very high (more than 1100), mutation clusters have been reported in different countries. The purpose of the study was to screen FH patients in order to set up a flowchart for genetic diagnosis.
Methods: We enrolled 269 patients with clinically diagnosed FH, of whom 197 were unrelated. The promoter and 18 exons of the LDLR gene were amplified by PCR and directly sequenced. To confirm splicing alterations, we performed RT-PCR analysis on mRNA. For the detection of large rearrangements a copy number quantification of all exons of the LDLR gene was carried out using the SALSA MLPA kit.
Results: Screening of the LDLR gene revealed mutations in 136/197 unrelated patients (mutation rate : 69.0%). Of the 48 different mutations, 6 mutations (2 splicing alterations and 4 missense mutations) accounted for about 57% of cases. In addition, 13 mutations were found only in our population. Large rearrangements accounted for 5.5% of mutations. 15 compound heterozygous or homozygous patients were identified in the total population (frequency of 8%). Out of the 269 patients, the paediatric population (younger than 16 years) consisted of 40 patients showing a mutation rate of 85% (34/40 patients).
Conclusions: In agreement with the presence of genomic clusters of LDLR mutations, we were able to identify the 6 most frequent mutations in Southern Italy. Although a two-step screening (with the most frequent mutations being screened before all the others) could be suggested, we recommend to perform the complete genetic screening aimed at identifying new or rare mutations as well as patients with 2 mutations. The screening should include the detection of large rearrangements. Finally, in order to prevent fatal cardiovascular events, particular attention should be paid to the identification of compound heterozygous, homozygous and paediatric patients representing a high percentage of FH population.

M048

LONG-RANGE PCR AND NEXT GENERATION SEQUENCING FOR THE IDENTIFICATION OF PAH MUTATION STATUS IN HPA ITALIAN PATIENTS

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Background: Phenylketonuria (PKU) is the most important disorder of amino acid metabolism, resulting mainly from a deficiency of phenylalanine hydroxylase (PAH) that metabolises phenylalanine to tyrosine. Usually, defective PAH activity is caused by mutations in the PAH gene; more than 500 mutations, scattered over the entire gene length, having been reported to date. Mutation analysis is important to obtain information on expected phenotype, for family counseling, to identify PKU carriers and for prenatal diagnosis. The scanning of PAH gene by direct sequencing detected a mutation in about 90% of patients. Therefore, this strategy failed to reveal a point mutation or deletion/insertion on several alleles. In this study, we developed and tested a method for the comprehensive and sensitive detection of PAH mutations using long-range (LR) PCR and next generation sequencing.
Methods: The study was carried out on 19 PKU patients for which the conventional Sanger exons screening was able to detect only one causative mutation. DNA samples of patient's parents were also available for molecular testing and results confirmation. Sixteen LR-PCR fragments, between 3,400 and 11,700 bp, containing the promoter, all coding exons, all introns and the 3' UTR of PAH were individually obtained for each study subject. The purified amplicons of the same patient were then pooled in equimolar ratio to obtain a library/sample. The libraries were sequenced with the GS FLX System. Sequence and data analysis was performed using the Roche/454 gsMapper software.
Results: All LR-PCR fragments were completely sequenced. We detected and confirmed 100% of the mutations and SNP previously identified in the same samples by Sanger analysis. In addition, we identified some interesting possibly pathogenetic variations that segregated with the family inheritance (40% of the studied subjects).
Conclusions: Our results demonstrates that genomic LR-PCR and next generation sequencing, increasing diagnostic sensitivity, can provide comprehensive genetic information more quickly and accurately than conventional approaches, being an effective method for patient sample analysis of PAH gene.

M049

A NOVEL MUTATION IN RP1 IS A MAJOR CAUSE OF AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA IN SOUTHERN ITALY

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Background: To date, 22 different genes have been associated to autosomal dominant retinitis pigmentosa (ADRP), but they account for only 50% of cases worldwide.
Methods: We analyzed by DHPLC and sequencing the major ADRP genes, namely, rhodopsin (RHO), peripherin 2 (PRPH2), retinitis pigmentosa 1 (RP1) and con-rod homeobox containing gene (CRX), in 130 Italian families affected by ADRP. To expand our data, we analyzed by DNA sequence capture and next-generation sequencing 260 genes associated to inherited eye diseases in three ADRP patients without mutations in RHO, RP1, RDS and CRX.
Results: In 17 of the studied ADRP families (13%), we identified eleven different potentially pathogenic mutations. In our ADRP patients, the relative involvement of RHO (<7.5%) and PRPH2 (<1%) is lower than in US and UK. In contrast, about 6% of our patients have mutations in RP1. Surprisingly, a single, novel, nonsense mutation in RP1 (p.S740X) accounts for more than 4% of our cases and therefore can be considered a major cause of ADRP at least in Southern Italy. The next generation sequencing screening allowed the identification of possibly pathogenic variations in all three analyzed patients.
Conclusions: Despite the evident wide genetic heterogeneity in Italian patients, our data suggest that priority should be given to the analysis of RHO and RP1. Moreover, our extended analysis strongly indicates that advanced, high-throughput technologies for molecular screening of ADRP-associated genes are warranted for speed and cost-effective reasons.

M050

SCREENING FOR LARGE GENOMIC REARRANGEMENTS USING MLPA IN A COHORT OF 102 BRUGADA PATIENTS

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Background: Brugada syndrome (BS) is an inherited cardiac arrhythmic disorder characterized by a ST-segment elevation that predisposes to unexpected sudden death during the night or at rest, in the 3rd-4th decade of life. BS is an autosomal dominant disease with incomplete penetrance and to date several missense mutations localized in SCN5A gene, encoding the alpha-subunit of the Na+ cardiac channel, have been associated to a BS phenotype.The role of large genomic rearrangements in SCN5A is still unknown. There is only one clinical case published in literaturein which a large deletion in SCN5A is detected in a young boy while negative screenings for large rearrangements in this gene are described in other two papers in small cohorts of patients. The aim of the project is to investigate SCN5A gene usingMultiplex Ligation-dependent Probe Amplification (MLPA) technique in 102 BS patients genetically undiagnosed.
Methods: We analysed 102 BS patients with an ECG type I, spontaneous or induced by Flecainide. All of them don't have a causative missense mutation in SCN5A. We performed the analysis of the rearrangements in SCN5A using the SALSA MLPA P108 SCN5A kit (MRC Holland) and the 48-capillary ABI 3730 DNA Analyser. The fragments analysis was performed with two different softwareanalyses: the P108 SCN5Av5 MLPA software NGRL (NGRL) and the SCN5A P108 REX MLPA software (REX). When the results of these software analyses were discordant, we used also the Coffalyser.net, recently distributed by MRC-Holland.
Results: The screening of large rearrangements in SCN5A gene using MLPA technique didn't identify genomic rearrangements in our cohort. Overall the NGRL and the REX software were concordant in 77.5% of cases but they were unable to process the analysis of23 samples.In these cases the fragments analysis was also performed with the Coffalyser.netthat confirm the absence of rearrangement into the gene.
Conclusions: Our results outline that the large rearrangements in SCN5A in BS are not common using the larger cohort of BS patients analysed until now and, for the first time, using 3 different software analyses.

M051

SERUM LEVELS OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND NITRIC OXIDE IN PATIENTS WITH SCHIZOPHRENIA

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Background: Brain derived-neurotrophic factor (BDNF) plays multiple roles in regulating neuronal survival, migration, morphological and biochemical differentiation and modulation of synaptic function in the CNS. BDNF has the ability to upregulate neuronal nitric oxide synthase expression in several populations of CNS neurons in the developing and adult CNS which suggests a potential role for this neurotrophic factor. On the other side, available data on nitric oxide (NO) regulation of BDNF are conflicting. While some data show that NO downregulates both BDNF expression and release, endothelial NO knockout mice show decreased BDNF expression. On the basis of these results and the data showing NO metabolism dysregulation in schizophrenia, we studied BDNF and NO in the sera of schizophrenic patients.
Methods: This study included 38 patients with schizophrenia and 39 healthy controls. BDNF was determined by an ELISA technique while the concentration of NO was measured using the modified cadmium-reduction method based on the Griss reaction.
Results: A significantly higher NO concentration was found in patients with schizophrenia (97.5±33.3 μmol/L, P <0.001) in comparison with controls (61.4±18.9 μmol/L). However, the concentration of BDNF was significantly lower in the patient group (20.4±3.7 ng/mL, P <0.001) than in healthy controls (25.7±4.3 ng/mL).
Conclusion: These data show that none was increased and BDNF was reduced in patients with schizophrenia as compared with controls.

M052

ARE BRCA1 & BRCA2 MUTATION CARRIERS AT A GREATER RISK FOR GASTRIC CANCER? A META-ANALYSIS

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Background: The BRCA1 & BRCA2 have been linked to an increased risk of developing large panoply of cancers, greatly thanks to the used of systematic reviews to validate evidence based practice. Gastric cancer is one condition were evidence is still insufficient to base upon patient management recommendations.
Methods: We conducted a systematic review and Logit meta-analysis, using Pubmed as a search engine, restricting our search to articles publish in English, no older than 10 years by the time of the search. The search criterion introduced was “stomach cancer” “gastric cancer” “BRCA1” “BRCA2” and “Stomach Neoplasms” [Mesh]. The inclusion criterion was the study design: cohort or case and control studies. We obtained 91 articles, excluded 20 for lack of relevance, 51 were excluded after review because they did not fulfilled the inclusion criteria, leaving 19 full text studies reviewed; out of which 11 were excluded due to insufficient or inadequate study design Data was analyzed on Epi-info
Results: There was an estimated number of unpublished results of 1 for BRCA1 and 7 for BRCA2 with a Begg bias: 0.1272 for BRCA1 and 0.7515 for BRCA2; an Egger’s test: -1.9199 for BRCA1 and 1.0094 for BRCA2; data set homogeneity was assessed obtaining a critical value: 23.45 (P=0.0003) for BRCA1 and 27.34 (P <0.00001) for BRCA2 i.e. the study groups were heterogeneous, the Odds Ratio (OR) for random and fixed effects were calculated obtaining 1.464 (CI95% 0.535-4.000) and 2.273 (CI95% 1.494-3.458) respectively for BRCA1 and 0.993 (CI95% 0.427-2.309) and 0.710 (CI95% 0.533-0.944) for BRCA2
Conclusions: The lack of homogeneity of the study groups and the relatively low number of relevant appropriate studies available impacts negatively on the validity of the systematic review, nevertheless the low publication bias is worth noticing, the fixed OR indicates the results aren’t statistically significant at p <.5 for BRCA1 but are statistically significant when considering random effects (a more stringent approach) as well as for both effects in the case of BRCA2. From this data it can be drawn that the global OR for the meta-analysis indicates an statistically significant positive link between BRCA1 & BRCA2 mutant status and the risk of developing gastric cancer.

M053

NDP MUTATION –CASE REPORT AND GENETIC COUNSELING

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Background: The Norrie disease Pseudoglioma (NDP) gene is a 3 exon gene located in chromosome x at locus , that codes the protein Norrin a proponed link in the Wnt signaling pathway involved in process of proliferation, adhesion and migration, that are critical in the development of the eye and other organs particularly in the case of Norrin and its receptor frizzled-4. The expression of NDP mutations is varied, where one individual mutation can cause different clinical features and varied presentation within a family affected is the norm. Methods: We described the case of a 19 year old male patient with two NDP mutations and clinical features not strictly sufficient to fit into only one of the possible diagnoses that tend to accompany these mutations.
Results: The patient developed progressive ptisis bulbi of the right eye that started at the age of 2, and vascular malformations of the temporal retinal vessels with macular ectopia and retinal detachment of the left eye that progressed to complete left eye blindness. After DNA sequencing of the NDP gene by capillary electrophoresis using Sanger's method, the patient was found to have two mutations C.312G>T(P.K104N; EXON 3) with already ascribed deleterious character, and C.-396_-383DEL(EXON 1) a novel mutation affecting the 5' flanking region. The patient was diagnosed with an unclassified phenotype having features of Norrie disease, X-Linked Familial Exudative Vitreoretinopathy and Coats disease.
Conclusions: The Norrie disease represents a complex clinical challenge requiring a multidisciplinary approach, the range of manifestations and the varied ages of presentation that accompany this condition, translates into a diverse array of possible phenotypes, which further complicates the diagnosis. Patients are usually male-hemizygotes, but anecdotal reports of female patients can be found in the literature; which makes for a very challenging genetic counseling with little consensus as to the applicability of sex-selection preimplantation screening studies, requiring the expert advice of the experienced geneticist

M054

DIAGNOSIS OF CONRADI-HÜNERMANN-HAPPLE SYNDROME : CHOLESTEROL INTERMEDIATES MEASUREMENT, GENETIC TESTING AND HISTOLOGY NEED TO BE ASSOCIATED

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Conradi-Hünermann-Happle (CHH) syndrome is an extremely rare X-linked dominant disorder of cholesterol metabolism which causes a wide spectrum of skeletal, ocular and dermatological abnormalities. Extreme clinical variability has been reported. Diagnosis is based on both cholesterol levels measurement by Gas Chromatography-Mass Spectrometry (GC-MS) and EBP (emopamil binding protein) gene analysis in complement with the clinical examination. Histology can help the diagnosis of minor forms of CHH. We performed mutation analysis of the EBP gene on blood cells, skin or foetal biopsy. Biochemical diagnosis was based on cholesterol intermediates levels measurement by GC-MS on serum or foetal biopsy. Skin biopsies were examined by pathologists blinded for the clinical diagnosis. We studied 20 patients showing signs of CHH. The diagnosis was confirmed for 14 of them and 10 new mutations of EBP gene were identified. For 10 patients, which 7 suffered the severe form, biochemical and genetic analysis were positive. Concerning the 4 other patients the phenotype was limited to skin and some analysis could have been controversial. Biochemical analysis was uncertain for 2 patients but histology showed typical keratin calcium deposits in a hyperplasic epidermis and pathogenic mutations were found in the blood cells. And the other patient, the biochemical analysis and histology were uncertain but a somatic mosaicism was detected in the blood cells and in the skin biopsy. At last, the pathogenic mutation was not found in the blood and the pathogenic skin of a patient whereas abnormal sterol level and typical but limited skin lesions. We could add as remark the evidence of a gonadal mosaicism in the mother of 2 foetus with a typical severe phenotype. She showed no clinical or biochemical signs and the mutation of the children was undetectable in her blood cells. Biochemical, molecular analysis and histology need to be associated for the diagnosis of CHH that could be difficult in situations of mild phenotypes. These cases, due to skewed X chromosome inactivation or mosaicism, are very important to be detected especially as the disease could be more severe for the next generation.

M055

EVALUATION OF BUTYRATE EFFICACY IN TREATMENT OF CONGENITAL CHLORIDE DIARRHEA: FROM GENOTYPE TO CLINICAL RESPONSE

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Background: Congenital chloride diarrhea (CLD) is a lethal autosomal recessive disorder characterized by severe diarrhea with intestinal Cl- malabsorption resulting from a deficit of the down regulated in adenoma (DRA) exchanger activity. Disease is determined by mutations in the solute carrier family 26, member 3 (SLC26A3) gene. Currently available therapies are not able to limit the severity of fecal ion losses and dehydration in these patients. Conflicting results have been reported on the therapeutic efficacy of oral butyrate, a short chain fatty acid acting as histone deacetylase inhibitor.
Methods: We investigated the effect of butyrate (100 mg/kg/day) in seven CLD children with different SLC26A3 genotypes. Molecular analysis was performed by direct sequencing of SLC26A3 exons. Nasal epithelial cells were obtained by brushing of turbinates in order to study the effect of the different kind of mutations on DRA functionality and to assess the effect of butyrate on the expression of the two main Cl- transporters: DRA and Putative Anion Transporter-1 (PAT-1). Cells were treated with 5 mM of sodium butyrate for 24 hours. RNA was extracted and retro-transcribed in cDNA for downstream applications. Expression levels of either SLC26A3 and SLC26A6 (PAT-1) from treated and untreated cells were measured by semi-quantitative real-time PCR with Taqman chemistry. Statistical analysis was carried out by the SPSS software.
Results: Butyrate was able to significantly improve the SLC26A3 and SLC26A6 gene expression in nasal epithelial cells in 5 out of 7 patients. The best clinical response was observed in subjects with SLC26A3 missense mutations that permit to the protein to reach cell membrane, and in which butyrate induces the SLC26A3 overexpression. Subjects with other genotypes show a variable clinical response (less or no improvement of the stool pattern and of the fecal Cl- losses).
Conclusion: This study revealed that butyrate may contribute to CLD therapy in a subset of patients with well-defined genotype that permit a residual protein activity. The ex-vivo study of butyrate effect in nasal cells from CLD patients may be helpful to predict the clinical response.

M056

GENETIC TEST IN TWO PATIENTS AFFECTED BY FRUCTOSE 1,6-BISPHOSPHATASE DEFICIENCY AND MOLECULAR CHARACTERIZATION OF NOVEL MUTATIONS

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Background: Fructose 1,6-bisphosphatase (FBPase) deficiency is a rare autosomal recessive inborn error of metabolism in the gluconeogenetic pathway. FBPase converted fructose-1,6-bisphosphate into fructose 6-phosphate and inorganic phosphate. FBPase deficiency is caused by mutations in fructose 1,6-bisphosphatase 1 (FBP1) gene, which result in impaired gluconeogenesis, characterized by episodes of hypoglycemia, ketonuria, lactic and metabolic acidosis.
Methods: We recruited two patients with hypoglycemia, lactic acidosis and high plasmatic concentrations of glycerol, for which it has been suspected FBPase deficiency. We carried out mutation screening of seven exons of FBP1 gene by using direct sequence analysis. We cloned full-length cDNA of human FBP1 into the pcDNA5/FRT/V5-His-TOPO expression vector. We obtained mutant FBP1 by site-directed mutagenesis of pcDNA5/FRT/V5-His/FBP-WT. Wild-type and mutant FBPase proteins were expressed by transient transfection of COS-7 cells and analyzed by enzyme activity assay.
Results: Each patient was homozygous for a novel mutation: c. 1-50_169+5192del and c. G355A (p.D119N), respectively. The mutation c. 1-50_169+5192del is a large deletion, including ATG and most of 5'UTR of FBP1 gene, probably resulting in a lack of gene expression. Missense mutation c. G355A (p.D119N), located in exon 3, affects a highly conserved amino acid. Enzyme activity assay demonstrated that FBPase protein with D119N mutation is enzymatically less active than the wild-type (about 30% of activity compared to wild type).
Conclusions: Molecular analysis and functional studies have been essential to make a correct diagnosis and allowed us to better understand the pathogenetic role of new mutations in a rare metabolic disease.

M057
TURNER SYNDROME WITH UNUSUAL PHENOTYPE

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Background: Turner syndrome (ST) is a chromosomal disorder that is characterized for presenting a typical phenotype that includes short stature, gonadal dysgenesis, amenorrhoea, pterigium colli, low hearline and absence partially or total of the sex chromosome X. Ocurring in 10 % of the spontaneous abortions and 1/2500 of newborn babies. Our patient is a 21-year-old woman with phenotype normal, followed for 5 years in Gynaecology deparment with primary amenorrhea. She have developed secondary sex characteristics and normal external genitalia. An abdominal ultrasound scan show small uterus with linear endometrium and normal ovaries. With hormone therapy she obtains erratic spoting.
Methods: We use for the diagnosis blood investigations, hormonal test, karyotipe, Array CGH, FISH and molecular analysis.
Results: Blood investigations showed a ovarian failure. Kariotype: 45X,-15, +15 mar. Array CGH: monosomy for de X chromosome and duplication for the Y chromosome. FISH: traslocation of the Y chromosome in 15 chromosome. Molecular analysis for AZF regions: positive. Normal Karyotype in her family.
Conclusions: The first symptom that leads the patient to consulting is the absence of menstruation. The phenotype are normal so that´s why the process of diagnosis has been realized with blood investigations. The translocation of the chromosome Y to autosome it has an approximate incidence of 1 of every 2000 newborn children. The translocation Y,15 and Y,22 are the most frequent. When this happens the subjects can be of masculine or feminine sex depending on the proportion of chromosome compromised. The presence of regions of the chromosome Y increases the risk of suffering gonadoblastoma during the first decade. Is necessary the periodic follow-up of these patients. The heterogeneity of gonadoblastoma are associated with an increased risk of developing germ cell tumours. A prophylactic gonadectomy are proposed but the patient has rejected and she follows exhaustive periodic controls.

M058
DEVELOPMENT OF NOVEL METHODOLOGY FOR LOXL1 SNP GENOTYPING AND EVALUATION IN PSEUDOEXFOLIATION SYNDROME (PXFS) AND GLAUCOMA (PXFG)

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Background: Pseudoexfoliation syndrome (PXFS) is a degenerative disease which affects patients over 50 years. It is characterized by the production and progressive accumulation of a fibrillar extracellular material in ocular tissues, skin and connective tissue of various internal organs. PXFS could lead to pseudoexfoliation glaucoma (PXFG) which is accompanied by bad prognosis. The purpose of this study is to evaluate lysyl oxidase like-1 gene (LOXL1) polymorphisms as genetic risk factors for PXFS and PXFG in the Greek population of Epirus.
Methods: Genomic DNA was extracted from 77 PXFG and 69 PXFS patients, 53 patients with the more common primary open-angle glaucoma (POAG) and 107 controls. A novel methodology of real-time PCR and melting curve analysis was developed in order to genotype the LOXL1 G153D and R141L polymorphisms in two different fluorescent channels of the LightCycler instrument (Roche), which uses the format of dual hybridization probes (anchor and sensor probes labeled with fluorescein and LC640 or LC705 dyes).
Results: Our novel methodology was easy to perform, fast (within 40 min after DNA isolation) and reliable. The method was accurate when compared with DNA sequencing (n=40, 100% concordance). Amplification demonstrated good efficiency (E=1.86) and reproducibility (CV for Cq <2.5%). Peaks for the two alleles were clearly separated in melting curve analysis in both assays (ΔTm >6 °C). Reproducibility in the melting curve characteristics was very satisfactory (CV of each Tm <2.5%). A statistically significant association was found for LOXL1 gene with PXFS-PXFG in this Greek population (SNPstats/SPSS software). The association of PXFS and PXFG with G153D appeared to be less powerful in this population (PXFS: OR=2.162, p=0.039, PXFG: OR=2.794, P=0.02) than in other populations and as for R141L, the association was proved only with PXFG (OR=3.592, P <0.001). None of the two LOXL1 SNPs were significantly associated with POAG.
Conclusion: We have confirmed the association between LOXL1 and PXFS-PXFG in our population of Epirus patients. Screening of the PXFS population with R141L could potentially assist in isolating those individuals who would eventually develop PFXG. This could be proven by large prospective studies.

M059

MOLECULAR AND FUNCTIONAL ANALYSIS OF THE PROMOTER REGION OF CFTR GENE IN CF AND CFTR-RELATED DISORDERS PATIENTS

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Background: Cystic Fibrosis (CF) is the most frequent lethal autosomal recessive disorder in Caucasians and is due to mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR); despite extensive testing of coding regions, a proportion of CF alleles remain unidentified. Unknown mutations can be localized in regulatory regions of gene, not currently analyzed for diagnostic purposes and still poorly studied, such as the promoter. CFTR promoter is involved in the regulation of gene expression and CFTR is finely regulated in development stages and it shows a tissue-specific expression despite the promoter lacks of a tissue-specific gene promoter typical structure. Then mutations in this region may alter the mechanisms of CFTR expression and play a role in the pathogenesis of CF.
Methods: We studied 118 CF and CFTR related disorders (CFTR-RD) patients most with one or both unknown mutations after the scanning of CFTR coding regions and a non-CF control group (n = 75) by sequencing the 6000 bp region at the 5' of the CFTR gene. Then we expressed in vitro, in four cell systems, some of mutations identified to explore their functional effect, relating the data to clinical expression of each patient.
Results: We identified 23 mutations among which 9 novel; we expressed and tested, by Dual Luciferase Reporter Assay, 17 CFTR mutations. Some mutations reduce the expression of the gene reporter, Firefly Luciferase, in various cell lines, and may act as causing-disease mutations. Other cause an increase of Luciferase expression in some cell lines. One mutation has a different effect (i.e., increase or reduction of gene expression) in different cells lines.
Conclusions: Gene variants in the large 5' region may cause an altered regulation of CFTR gene expression, acting as causing-disease mutations or modifiers of its clinical phenotype. Studies of in vitro expression in different cell systems may help to reveal the effect of such mutations.

M060

TAQMAN MRNA QUANTITATION TEST OF LDLR GENE AND ITS CORRELATION WITH SERUM CHOLESTEROL LEVELS IN HETEROCYGOUS FAMILIAL HYPERCHOLESTEROLEMIC PATIENTS

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Familial Hypercholesterolemia (FH, OMIM # 143890), a major risk factor for coronary heart disease (CHD), is autosomal dominant disorder associated with mutations in the genes encoding the low-density lipoprotein receptor (LDLR), apolipoprotein B100 (APOB), and proprotein convertase subtilisin/kexin type 9 (PCSK9). Predominantly, the clinical phenotype of FH is caused by mutations in the LDLR or APOB genes. The frequency of heterozygotes is 1/500. The frequency of homozygotes or compound heterozygotes is 1/1 000 000. The high LDL-cholesterol level frequently gives rise to tendon and skin xanthomas, arcus corneae, and accelerated atherosclerosis resulting from cholesterol deposition in the arterial wall, thereby increasing the risk of premature CHD. The identification and treatment of FH patients and their affected relatives with effective lipid-lowering agents is important as this has been shown to significantly reduce both coronary morbidity and mortality. Our aim has been studying if the mRNA expression level of LDLR gene in leukocytes from peripheral venous blood is correlated with serum cholesterol level in patients with heterozygous FH, in order to identify in a fast way those who need genetic testing for mutations in LDLR gene.
Method: Total RNA was isolated from leukocytes of peripheral venous blood using Qiagen RNA Blood Mini kit and a Qiacube extractor from FH patients (n=80) and controls (n=40). RT-PCR was performed in order to obtain cDNA using GeneAmp RT-PCR kit (Life Technologies). Total cDNA content was measured in a Nanodrop 2000 and approximately 100 ng of cDNA was used in real time PCR TaqMan mRNA quantitation test using GADPH as control gene. Normalized mRNA level of LDLR gene was plotted versus blood serum cholesterol level.
Results: LDLR mRNA level to be highly variable in FH patient blood leukocytes ranging from 0.3 to 2.5 times respect to the medium content of LDLR mRNA of control individuals. Patients taking up high dose of statins usually demonstrate high level of LDLR mRNA. We did not found inverse correlation between LDLR mRNA level in peripheral blood FH leukocytes and plasma cholesterol level. So far, in our conditions, quantitation of LDLR mRNA in leukocytes cannot be used for identification of carriers of mutations in LDLR gene.

M061

THE NOVEL TNFRSF1A C.262T>C (S59P) MUTATION IS FUNCTIONALLY LINKED TO THE NF-KB INFLAMMATORY PATHWAY

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Background: The TNF receptor associated periodic syndrome (TRAPS) is an autosomal dominant autoinflammatory disease due to mutations within the TNFRSF1A gene which encodes the TNF receptor I (TNFR1). A novel mutation in TNFRSF1A gene(c.262T>C-S59P) was identified by studying a patient with TRAPS. Our Aims are to study the effects of S59P mutation on the signalling pathway of TNFR1 and compare the results with whose obtained from high penetrance T50M and low penetrance R92Q mutations.
Methods: Human Embryonic Kidney (HEK293) cell line was stably transfected with a construct cointaning Wild-Type(WT) TNFRSF1A gene and R92Q, S59P and T50M TNFRSF1A mutants. The control (CTL) and the new four cell lines obtained were stimulated with TNFa (60 ng/mL) for 10, 20, 30, 60 min. The expression of the TNFR1, soluble isoform (sTNFR1) and NF-kB signalling were evaluated by Western blot. The downstream targets of NF-kB signalling, IL1β, IL6 and IL8 were measured in the supernatants (ELISAs) of all five cell lines after they have been kept in culture medium alone(negative control) or stimulated for 4 h with LPS (1 μg/mL) (positive control) or TNFa (60 ng/mL).
Results: TNFR1 was basely expressed by S59P and T50M TNFR1-mutant cells, while a higher expression was found among WT and R92Q. TNFa stimulation did not affect TNFR1 expression in the TNFR1-mutants; it caused a 50% reduction in WT. The TNFR1 in supernatants did not vary after TNFa stimulation in WT while it diminished of about 20% in TNFR1 mutants. A lower phosphorylation of IkB-a was found in R92Q with respect to the other transfectants inclunding the WT. TNFa induced a significant and sustained IkB-a phosphorylation in the mutants, but not in the WT, suggesting all the three studied TNFR1 mutations favour NF-kB pathway activation in response to TNFa engagement. HEK293WT or mutants did not release IL1β and IL6 . By contrast they release IL8. The higher IL8 levels were found in R92Q (757 pg/mL), with respect to WT (519 pg/mL), T50M (385 pg/mL) or S59P TNFR1-mutant cells (317 pg/mL) after TNFa stimulus.
Conclusions: The novel c.262T>C(S59P) mutation of TNFRSF1A gene cause the TNFR1 receptor to be highly sensitive to its binding partner TNFa, which induce a significant and sustained NF-kB activation.

M062

PRENATAL DIAGNOSIS OF TYROSINEMIA TYPE I: TUNISIAN EXPERIENCE

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Background: Tyrosinemia type I (HTI) is an autosomal recessive disease caused by a deficiency in fumarylacetoacetate hydrolase, the last enzyme of the catabolic pathway of tyrosine. It leads to an increase of the level of succinylacetone (SA) which is used for diagnosis of this disorder. The phenotypic spectrum is heterogeneous, ranging from acute forms presenting with severe liver malfunction proceeding frequently to death, to chronic forms mainly with renal manifestations and slow progressive hepatic alterations. Prenatal diagnosis (PD) would be a great contribution to provide early care at birth and propose a therapeutic interruption of pregnancy (TIP) when disease recognized as incurable at diagnosis. We would like to report our experience in the PD of HTI.
Material and methods: Nine requests for PD from women with family history suggestive of high risk for HTI were received in our laboratory. For these cases, Amniotic fluids were obtained at between 16 and 18 weeks of pregnancy. The determination of SA was carried out indirectly by semi-quantitative spectrometric test using δ-aminolevulinic acid dehydratase inhibition assay.
Results: Four cases were found to be affected with levels of SA in amniotic fluid ranging from 3 to 20 times the normal values (NV) (182 to 980 nmol/L -NV: <50 nmol/L-). All negative cases were confirmed after birth. The TIP was performed in three cases. For the fourth case, the pregnancy was twin bi-amniotic bi-chorionic and the PD revealed a healthy fetus (T1) and an affected fetus (T2) with respective rates of SA 0 and 182 nmol/L (NV: < 50 nmol/L). At birth, urinary SA was investigated and revealed respective rates of 83 and 8.2 μmol/L (NV: <10 μmol/L) for T2 and T1. The newborn T2 was clinically asymptomatic. He has received early treatment at 12 days of life and the outcome was favorable.
Conclusion: HTI is a serious hereditary disease. The PD is essential to provide a TIP when the fetus is affected avoiding the recurrence of the disease in the family, it leads to early management of the child after birth when the TIP is impossible.

M063

LIQUID STABLE ENZYMATIC ASSAY FOR THE MEASUREMENT OF HOMOCYSTEINE ON THE RX IMOLA ANALYSER

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Background: Homocysteine (HCY) is a thio-containing amino acid produced by the intracellular demethylation of methionine. Severely elevated concentrations of total HCY are found in patients with homocystinuria, a rare genetic disorder of the enzymes involved in the metabolism of HCY. Patients with homocystinuria exhibit mental retardation and early arteriosclerosis.This study reports the development of a stable enzymatic assay kit with enhanced precision and assay range for the measurement of HCY in human serum and plasma applied to the fully automated RX imola. This is of value in the diagnosis of homocystinuria and also hyperhomocysteinemia.
Methods: The assay is enzymatic involving a series of steps that ends with pyruvate being converted by lactate dehydrogenase (LDH) to lactate with NADH as coenzyme. The rate of NADH conversion to NAD+ is directly proportional to the concentration of HCY (ΔA340 nm). Concentrations are calculated from two point calibration. On-board and calibration stabilities were tested by storing the reagents uncapped on the RX imola for a period of 28 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 4 replicates twice a day for 20 days. Correlation studies were conducted using a commercially available homocysteine assay.
Results: The HCY reagent presents an on-board stability of 28 days and calibration frequency of 5 days. The assay was found to be functionally sensitive to 1.65 μmol/L and be linear up to 55.62 μmol/L. The within-run and total precision for three different concentration levels typically had %C.V. of ≤8.0%. In the correlation study 60 serum patient samples were tested and the following linear regression equation was achieved: Y = 1.06x – 0.34; r = 0.99.
Conclusions: This enzymatic assay kit exhibits high sensitivity and reproducibility with the added advantage of using liquid reagents with good stability. This represents an improvement for use in the accurate and reliable determination of homocysteine in human serum and plasma.

M064

INCREASED HB A2 IN STRUCTURAL DEFECTS OF GLOBIN GENES: THE UNSTABLE HEMOGLOBINS

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Bachground. The quantification of hemoglobin A2 (Hb A2) is used in screening for hemoglobin (Hb) defects specifically to identify carriers of beta-thalassemia (Hb A2 >3.2%). High or borderline values of Hb A2 may, however, also be produced by structural defects of hemoglobin: this is the case of the unstable Hb variants. The term “unstable hemoglobins” refers to the group of Hb variants that have an instability sufficient to give appreciable intracellular precipitation with a result hemolytic anemia. These defects are considered relatively rare semi-dominant defects.
Methods. Biochemical analysis for separation and estimation of Hb fractions were done on high performance liquid chromatography (HPLC) VARIANT IITM (Bio-Rad Laboratories, Hercules,CA,USA) and capillary electrophoresis apparatus, Capyllarys 2 (Sebia, Lisses, France). The Hb isopropanol stability test was performed and inclusion bodies detected with Brilliant Cresyl Blue (BCB) after incubation at 37°C. Genomic DNA was isolated by standard methods and the entire beta-globin gene, in all cases studied, were sequenced by ABI PRISMTM 3130 DNA analyzer (PE BioSystems, Foster City,CA,USA) using the primers and the procedures previously described.
Results. We have observed in 10 families with Hb Köln and other subjects with unstable rare variants (Hb Zürich, Hb Bushwick, Hb Hope) a variable increase of Hb A2. This has also been observed in over 50% of the unstable variants reported in the literature. We were also able to document slight but significant increase of Hb A2 in carriers of Hb E, in which was not present alpha thalassemia. In Hb E the hemoglobin molecule has been shown to be unstable in addition to its associated splicing and biosyntetic defects and these factors together may contribuite to the beta thalassemia-like expression.
Conclusions. A base change resulting in either an untranslatable or unstable messenger RNA (mRNA) or the production of a highly unstable polypeptide chain would all reduce the measurable end product. They would present as a “thalassemia” if the abnormal hemoglobin was not detected by HPLC or CE. The molecular and functional characterization of the Hb defects allows diagnostic conclusions useful for specific genetic counseling.

M065

DE NOVO FMR2 (AFF2) DELETION OF EXON 6 IN A GIRL WITH A MILD INTELLECTUAL DISABILITY AND BILATERAL PERIVENTRICULAR NODULAR HETEROTOPIA

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Periventricular nodular heterotopia (PNH) is a disorder of neuronal migration in which neurons fail to migrate properly from ventricular zone to cortex during development, resulting in the formation of nodular brain tissue lining the ventricles. Most affected individuals with the X-linked form are female, while hemizygous males tend to die in utero. Affected females usually present with epilepsy, but have normal intelligence. PNH is a genetically heterogeneous condition, and mutations in FLNA and DCX genes, located in Xq28, have been described as causative in sporadic female cases. Here we report a case of a 7-years old girl with a mild developmental delay, behaviour changes, hypotonia, and discrete facial dysmorphism. Patient was in clinical follow-up since 6 months of age by cerebral ventriculomegaly observed in prenatal ultrasound exploration. In brain MRI, a nodular heterotopia in both parietal lobes with dilated lateral ventricles was detected. In EEG, epileptic discharges in atypical spike wave form in parieto-occipital lobes were observed and result in atypical absence crisis. In order to detect genetic anomalies we obtained genomic DNA from peripheral blood and a MLPA analysis for mental/developmental delay, and Lissencephaly (with FLNA and DCX probes) was performed using P-061, P-106 and P-245 SALSA MLPA kits. Patient was carrier of a deletion on exon 6 of FMR2 gene, this deletion was confirmed and it was not detected in parents. No change in gene dosage was found affecting DCX or FLNA genes. Alterations of FMR2 are related with FRAXE mental retardation (OMIM # 309548), that is a form of mild to moderate mental retardation associated with learning difficulties, communication deficits, attention problems, hyperactivity, and autistic behavior. The disorder can be caused either by silencing of the FMR2 gene as a consequence of a CCG expansion located upstream or by deletions within the gene. This finding could explain learning disabilities and mental impairment in patient but not explain PNH. FMR2, FLNA and DCX are all located in Xq28, and this chromosomic region has been related with PNH. Further studies will be necessary to establish implications of other genes of Xq28, as FMR2, in neuronal migration anomalies.

M066

A NOVEL PCR-RFLP-BASED- METHOD FOR THE DETERMINATION OF A1298C MUTATION IN METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE

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Background: Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in folic acid metabolism. Several polimorphisms in MTHFR gene are described as far, from where two are of particular clinical and diagnostic importance. A so called “thermolabile” variant of MTHFR enzyme is produced as a result of alanine to valine substitution at amino acid position 222 as a result of a single nucleotide mutation C677T. The second important polymorphism is observed at the position 429, where the glutamic acid is substituted to alanine being a result of A1298C mutation in nucleic acid chain.
Methods: Both of the MTHFR mutations described can be easily recognized by means of PCR-RFLP. However, in case of A1298C there are only a limited number of restriction endonucleases that cover the mutation site and thus allows to distinguishing between particular alleles. Moreover, resulting DNA fragments are usually relative short and need to be resolved on polyacrylamide- instead of commonly used agarose- gels. We develop a novel method for MTHFR A1298C mutation analysis, which is more convenient and may be utilized directly in high-throughput settings.
Results: Specific primer pair is designed to surround the position 1298 of MTHFR gene. By the use of mutagenic reverse primer, PCR product is altered to create an AjuI-recognizable pattern. Thus, there is no restriction site for AjuI in case of adenine position at 1298 (i.e. for glutamate residue) but there is one if cytosine is substituted instead of adenine (alanine residue).
Conclusions: Several techniques are developed allowing researchers to distinguish between particular alleles, both in C677T as well as in A1298C polymorphisms. These encompass direct sequencing, real-time fluorescent-based assays and, commonly used, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

M067

NOVEL ASSOCIATION OF FCGR2A POLYMORPHISM WITH AGE-RELATED MACULAR DEGENERATION (ARMD) AND DEVELOPMENT OF A NOVEL CFH REAL-TIME GENOTYPING METHOD

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Background: Age-related macular degeneration (ARMD) is a degenerative ocular disease, which may lead to serious loss of central vision. In Caucasian populations, a strong correlation has been established with polymorphism Y402H (rs1061170) in the complement factor H gene (CFH) in chromosome 1q32. CFH has regulatory role in innate immunity and the aforementioned SNP reduces its affinity for CRP. The H131R polymorphism (rs1801274) in FCGR2A gene in chromosome region 1q23 has been associated with many inflammatory diseases, but up till now it has not been investigated in relation to ARMD. Like CFH, the FCGR2A receptor contributes to the innate immunity and binds to CRP with different affinity depending on SNP H131R. The goal of our study was the development of a novel, fast and accurate method for Y402H (g.43097C>T), the confirmation of its association with ARMD in the Greek population and the investigation of H131R polymorphism (g.9541A>G) in ARMD.
Methods: DNAs were extracted from peripheral blood samples of 27 patients with ARMD and 34 age-matched controls, all of whom were clinically evaluated with optical acuity measurement and funduscopy. A real-time PCR and melting curve analysis method for Y402H genotyping was developed in LightCycler (Applied Roche Science, Switzerland), after in silico design of proper primes and probes (TIB MolBiol, Germany). Genotyping for H131R was performed according to an accurate real-time PCR method described previously from our group.
Results and conclusion: A fast and accurate methodology was developed for genotyping of Y402H CFH gene (Efficiency=1.79, reproducibility Ct CV=3.33%, Tm C allele 53.36 °C and T allele 61.91 °C, CV<2.5%, ΔTm=8.55). Results were confirmed with the gold standard method of DNA Sequencing. The present study confirmed the association between Y402H SNP and ARMD in the Greek population as well, in a statistically significant fashion (OR=2.68, P=0.012). FCGR2A H131R polymorphism was investigated for the first time in this present study for possible correlation with ARMD and an even stronger statistically significant association was detected (OR=3.17, P=0.004), that awaits further confirmation in a larger set of samples.

M068

FAST MICROARRAY TEST FOR DETERMINATION OF FACTOR V LEIDEN AND FACTOR II PROTHROMBIN MUTATION DIRECTLY FROM WHOLE BLOOD

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Background: Deep and superficial venous thrombosis and thromboembolism of brain, lung and coronary vessels are frequent causes of death. These conditions result from a combination of internal (genetic) and external factors (e.g. smoking, overweight, taking contraceptives). The most important genetic risk factors are the factor V Leiden mutation (rs6025) and the mutation 20210G>A (rs1799963) in the factor II (prothrombin) gene. In Europeans the prevalence of the heterozygous genotype for factor V Leiden is 3-7% and for factor II 20210G>A 1-3% (homozygous genotypes ~0.2% and <0.1%). The thrombosis risk is increased 3 to 8 fold for heterozygous factor V Leiden carriers and even up to 100 fold for homozygous carriers. Heterozygous carriers of the factor II 20210G>A mutation have an approx. 3 fold higher risk for deep venous thrombosis. The cumulative risk for heterozygous carriers of both mutations is increased approx. 20 times. Taken together, for thrombosis risk prediction determination of both mutations is of outstanding importance: a robust and easy to use microarray test was developed that can be performed directly from whole blood.
Methods: After blood samples are briefly incubated (1 minute) with extraction buffer, parts of the factor V and factor II genes are amplified by multiplex PCR and products analyzed by microarray hybridization using TITERPLANE incubation technique. The microarrays are evaluated automatically with a microarray scanner and EUROArrayScan software. Besides the genotype for factor V Leiden and factor II 20210G>A, the report documents validity of each test result based on integrated control features for sample integrity, PCR and specificity of hybridization.
Results: Validation studies of the test revealed 100% specificity and sensitivity of the microarray test for detection of the mutations factor V Leiden and factor II 20210G>A. Homozygous and heterozygous genotypes could be reliably discriminated. Each determination provided a valid result for both SNPs. The hands on time from blood sample to result was 1.5 min.
Conclusions: The direct use of whole blood as sample material, the fully automated analysis procedure and ready-to-use reagents make performance of this microarray assay fast, easy and robust.

M069

FAST MICROARRAY TEST DIRECTLY FROM WHOLE BLOOD FOR DETERMINATION OF 4 MUTATIONS IN THE HFE GENE ASSOCIATED WITH HEREDITARY HAEMOCHROMATOSIS

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Background: Hereditary (primary) haemochromatosis is a genetically caused iron-overload disorder. It is a frequent autosomal, recessive, inherited metabolic disease caused by increased resorption of iron in the upper small intestine. Since the human body does not possess the ability to excrete iron after excessive intake, the iron excess is deposited in various organs (e.g. liver, heart, spleen). In untreated patients irreversible organ damage can occur, resulting in cardiomyopathy, arthropathy, diabetes mellitus, liver cirrhosis and liver and pancreas carcinoma. Most cases of haemochromatosis are caused by mutations in the HFE gene. Two of the mutations result in amino acid substitutions (C282Y and H63D), associated with a loss or reduction of its physiological function. In addition, there are two rare mutations that cause either an additional change in amino acid sequence (S65C) or an early termination of protein synthesis (E168X).
Methods: A PCR-based microarray test system was developed to identify these 4 mutations including differentiation of heterozygous and homozygous status. The analysis can be done directly from whole blood - no initial DNA purification step is necessary. After blood samples are briefly incubated with extraction buffer, parts of the HFE gene are amplified by a one tube multiplex PCR and products hybridised on a microarray using TITERPLANE incubation technique. The microarrays are scanned and evaluated automatically by EUROArrayScan software. The report documents the genetic status of each sample as well as the correct performance for every sample based on integrated control features.
Results: Robustness of the assay was assessed by 101 samples from blood donors. Each determination provided valid results for all 4 SNP. Comparison of the results for 44 of these samples with those obtained by DNA sequencing showed 100% accordance. For every position discrimination between heterozygous and homozygous (wildtype or mutation) is ensured.
Conclusions: Analysis of 4 haemochromatosis associated mutations in the HFE gene was successfully performed directly from whole blood. Ready to use reagents and fully automated analysis make this test easy, fast and reliable.

M070

MOLECULAR DIAGNOSIS OF HEMOCHROMATOSIS BY PCR-RFLP METHOD, AND IDENTIFICATION OF C282X AND H63D ALLELES OF HFE GENE IN ALBANIAN POPULATION

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Background: Genetic analysis of hemochromatosis was not performed actually in Albania, so the analysis of HFE gene mutations C282X and H63D was a useful genetic test to the earlier diagnosis of hemochromatosis. Application of a molecular method to identify the mutations C282X and H63D of the HFE gene, should be at low costs and give unambiguous results. We aims to determine the frequencies of C282X and H63D mutations in 50 individuals from Albanian healthy population.
Methods: We used PCR – RFLP method: Specific amplification of DNA of HFE gene, restriction of PCR products with 2 RE, and fragment analysis by agarose gel electrophoresis. PCR-RFLP method was used to determine the heterozygote carriers and homozygote's for 2 most diffused mutations of the HFE gene, C282X and H63D, in a group of 50 healthy individuals selected randomly, from the Albanian healthy population.
Results: The frequencies of mutations C282X and H63D was determined and the frequence of heterozygote carriers of these mutations. Based on the genotypes and the known formula of population genetics were identified the frequencies of alleles C282Y and H63D. The frequence of C282Y allele was found 3%, and the frequence of H63D allele was found 10%, in the sample of 50 healthy individuals of Albanian population.
Conclusions: Frequencies of alleles, C282Y (3%) and H63D (10%) of HFE gene represented preliminary data that should be confirmed by a more enlarged sample.

M071

CGH ARRAY FOR THE IDENTIFICATION OF A COMPOUND HETEROZYGOUS MUTATION IN CYP1B1 GENE IN A PATIENT WITH A SUSPECT PRIMARY CONGENITAL GLAUCOMA

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Background: Primary congenital glaucoma (PCG) is the most common childhood glaucoma caused by developmental defect in the trabecular meshwork and anterior chamber angle of the eye. Presence of CYP1B1 gene mutations in PCG patients has been known for about a decade. This gene encodes an enzyme which catalyze many reactions involved in drugs metabolism and synthesis of cholesterol, steroids and other lipids. Mutation in CYP1B1 is the predominant cause of autosomal recessive inherited PCG. To date, several mutations have been identified in patients and families with PCG from numerous countries and ethnic groups. However, diagnosis can be difficult to perform in atypical cases.
Methods: A high resolution array comparative genomic hybridization (a-CGH) 4X180K was performed on the patient and his parents in order to identify potential mutation and characterize the clinical phenotype at molecular level. The a-CGH used contains 170,334 60-mer oligonucleotide probes that cover the whole genome with an average spatial resolution of 13 kb. DNA digestion, labeling and hybridization were performed according to the manufacturer's protocols. Subsequently to the identification of a mutation with a-CGH, CYP1B1 exons have been analyzed by PCR and direct sequencing.
Results: Using a-CGH analysis, we identified in the patient a heterozygous deletion in 2p22.2 of approximately 118 Kb involving CYP1B1. The mutation was inherited from the father who does not show any sign of the disease. The deletion observed was further confirmed by quantitative real time PCR. DNA sequencing was performed to detect possible mutations on the other allele and revealed the presence of a transition G>A at position g.5975 (c.182 G>A; amino acid substitution: p. Gly61Glu). This point mutation was inherited from the mother.
Conclusions: We analyzed by a-CGH a patient with an atypical PCG and identified a deletion in the CYP1B1 gene as potential causative mutation. A second mutation in this gene was identified by sequencing confirming the nature of the disease. a-CGH screening in patients with atypical clinical presentation can be therefore considered of paramount relevance to perform molecular characterization and identify genetic causes.

M072

BBS1, BBS10 AND BBS2 ARE MAJOR CAUSATIVE GENES FOR BARDET-BIEDL SYNDROME IN ITALIAN PATIENTS

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Background: Bardet-Biedl Syndrome (BBS) is a rare inherited disorder associated with obesity, retinopathy, renal defects, polydactyly, learning disabilities and hypogenitalism. Fourteen genes (BBS1–14) account for about 80% of the known cases of BBS, indicating that additional BBS genes are yet to be identified. BBS prevalence is 1 in 125.000–160.000 in Caucasian population. The wide clinical spectrum observed in BBS correlates to the high genetic heterogeneity. Usually, BBS is transmitted in autosomal recessive manner. However, in some families, a triallelic inheritance involving BBS1, BBS2 and BBS6 genes has been observed.
Methods: To design a sensitive and time-effective procedure for molecular diagnosis of BBS, we analyzed a cohort of 21 Italian patients (BBL1-BBL21). First, we used the APEX genotyping microarray (Genorama) to search for 300 known mutations in 11 BBS (BBS1-BBS10, BBS12), in PHF6, ALMS1, and GNAS1 genes. Then, we analyzed by direct sequencing the whole coding regions of the BBS1, BBS2 and BBS10 genes, in patients who resulted with one and without a mutation after APEX analysis.
Results: The genotyping microarray identified both mutated alleles in 5 patients and one mutated allele in 1 patient, for a total of 11 disease-alleles (6 in BBS1, 3 in BBS10 and 2 in BBS2), yielding a detection rate of about 26.2% (11/42). In addition, sequence analysis allowed us to identify 8 new mutations, 3 in BBS10 (1 point insertion, 1 missense and 1 nonsense mutation), 4 in BBS2 (2 missense, 1 point insertion and 1 point deletion) and 1 missense mutation in BBS1. The detection rate of the whole procedure increased to 45,2%.
Conclusions: Our study indicates that sequencing of BBS1, BBS2 and BBS10 should be chosen as first analytic step in the molecular diagnosis of BBS in Italian patients. The subsequent analytical step might be the APEX array. Obviously, in the complex framework of the molecular diagnosis of BBS, next generation sequencing analysis would be strongly recommended.

M073
GENE-DIET INTERACTIONS IN OVERWEIGHT/OBESITY RISK IN A SICILIAN POPULATION OF YOUNG WOMEN

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Background: TNF-α expression is differentially regulated by fatty acids and the TNFA-308 G/A polymorphism changes the relationship between fatty acids consumption and obesity risk. The aim of this study was to uses an integrated genomic approach to assess the risk of obesity in a Sicilian women population in order to define targets and control strategies.
Methods: A total of 336 healthy women of childbearing age were enrolled in the cross-sectional survey. Micronutrients intake was estimated by a Food Frequencies Questionnaire and adherence to the Mediterranean Diet (MD) by the Mediterranean Diet Score (MDS). DNA was extracted from blood samples for TNFA -308 G/A polymorphism genotyping using PCR-RFLP. Overweight/obesity risk analysis was performed, taking into account TNFA polymorphism.
Results: A total of women, 34.1% were overweight or obese. The most frequent genotype was the wild-type GG (79.8%), followed by the heterozygous AG (19%) and the AA (1.2%). Population was in Hardy-Weinberg equilibrium. No association was observed between TNFA genotypes and nutritional status. Mean MDS significantly increases by age. Women less adherent to the MD (MDS≤6) were shown to consume less kilocalories but also less unsaturated and more saturated fatty acids, and they were at higher risk of overweight/obesity, OR 2.9 (IC95%: 1.0–8.5). Significant interactions were found between lower unsaturated fatty acids intake (below the 75th percentile) and TNFA -308 GA/AA genotypes increasing obesity risk.
Conclusions: No evidence for an independent effect of the TNFA -308 G/A polymorphism on obesity risk was shown in our study, thus, rather the presence of the A allele may be indicative of a greater responsiveness or sensitivity of a carrier to changes in dietary intake. These diet-gene interactions which alter the signaling of TNFα, are to be considered in future intervention studies to offer opportunities to reevaluate the criteria used to determine dietary recommendations.

M074
FIRST DESCRIPTION OF S1251N MUTATION IN A TUNISIAN CBVAD PATIENT

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Background: Congenital bilateral absence of the vas deferens (CBAVD) is responsible for 2–6% of male infertility in which mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been identified. The molecular basis of CBAVD is not completely understood. Although patients with cystic fibrosis have mutations in both copies of the CFTR gene, most patients with CBAVD have mutations in only one copy of the gene. We report in this paper the first description of the S1251N mutation localized in exon 20 of the CFTR gene in a Tunisian CBVAD patient.
Methods: Diagnosis of CBAVD was initially suggested by cyto-biochemical characteristics (low semen volume and concentration and the large number of abnormal form) and confirmed by urogenital ultrasound. Molecular analysis of the 27 exons of the CFTR gene was performed by combining denaturing gradient gel electrophoresis (DGGE) and denaturing high performance liquid chromatography (DHPLC) followed by a sequencing reaction on ABI Prism 310 to identify nucleotide alteration responsible. We also analyzed a DNA variant (the 5T allele) in a noncoding region of CFTR gene that causes reduced levels of the normal CFTR protein.
Results: The complete study of the CFTR gene showed the presence of S1251N mutation identified by DGGE in exon 20 and confirmed by sequencing reaction. This mutation affects the first site of ATP binding and it has only been described that patients with classic cystic fibrosis. The mutations located in exon 20, commonly described in our population, are the W1282X and D1270N. S1251N mutation is the first description of this variation in a CBVAD patient in North African populations.
Conclusions: The second unidentified CFTR mutation may lie in introns or in regulatory regions, which are not routinely investigated, or may correspond to gene rearrangement such as large deletion at the heterozygous state which escape detection using current PCR based techniques. Other genetic or environmental factors may also be involved in CBAVD.

M075

ARE WE READY TO APPLY NEXT GENERATION SEQUENCING (NGS) ANALYSIS TO BRCA1 AND BRCA2 GENETIC TESTING?

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Background: In this study we tested a new method, provided by Multiplicom and suitable on 454-Junior machine (Roche), for detecting BRCA1/2 genes mutations. The 454 Genome Sequencer employs sequencing-by-synthesis technology through pyrosequencing.
Methods: We analyzed the following 24 samples: 8 samples were previously genotyped with Sanger sequencing method (6 carrying mutations and/or polymorphisms in either BRCA1 or BRCA2 genes, and 2 samples completely negative) and 16 unknown. All samples belonged to individuals with a personal and/or family history of breast and/or ovarian cancer, previously screened by geneticist consultant. DNA quantity and quality took particular attention, in fact DNA samples with 260/280 < 1.8 and 260/230 < 2.1 were excluded and subsequently re-extracted. The total sequences obtained after the sequencing were analyzed for the variant detection by both 454 Amplicon Variant Analyzer Software and custom scripts in perl language.
Results: All mutations/polymorphisms detected in the 8 previously analyzed samples, were correctly identified by NGS: of these, six samples presented single nucleotide substitutions and two samples had small deletions (2 and 4 bp respectively). Moreover NGS results for the remaining 16 unknown samples were subsequently confirmed by Sanger, showing that results of two methods overlapped perfectly. Nevertheless, in the analysis of all these samples we noticed that different results were found when the homo-polymers insertion-deletions were considered. In this case, Sanger method performed better than NGS one.
Discussion: This study represents one of the first NGS applications to the study of BRCA1/2 genes analysis in breast cancer. The strict superimposability of results between Sanger and NGS confirms the raw base accuracy reported by Roche (over 99.9%). However, the reported relatively error-prone raw data sequence, especially insertion-deletion associated with homo-polymers, is a major concern. In this regard, the use of a dedicated kit (already commercial available), will really improve the troubleshooting. A true expertise is necessary for the correct managing of all NGS steps, above all for the post-analysis PCR and bioinformatics data elaboration.

M076

MUTATION SPECTRUM OF THE ASS1 GENE IN KOREAN PATIENTS WITH CITRULLINEMIA TYPE I

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Background: Citrullinemia type I is a rare metabolic disorder and the distribution of mutations in the ASS1 gene varies among ethnic groups. We aimed to determine the molecular characteristics of citrullinemia type I in Korean patients.
Methods: We identified five Korean patients with citrullinemia type I. All patients had high citrulline levels (>1,000 μmol/L) and no significant problems at birth. Biochemical and clinical findings were investigated and mutations in the ASS1 gene were identified using direct sequencing method. We also reviewed previous genotypes reported for Korean patients with citrullinemia type I.
Results: We identified five mutations in 10 mutant alleles from the five patients. The most common mutation was the Gly324Ser mutation, which was present in 40% of the mutant alleles, followed by the c.421-2A>G mutation (30% of the mutant alleles). The other mutations (c.1128-6_1188dup67, Arg127Gln, and Arg279Gln) were identified in one mutant allele each. A comprehensive review of previous Korean reports revealed that Gly324Ser, c.421-2A>G, and c.1128-6_1188dup67 mutations accounted for 80.8% of the total mutations reported to date. In terms of genotype-phenotype correlations, a patient homozygous for the c.421-2A>G mutation had fatal clinical manifestations and two patients who were compound heterozygous for the Gly324Ser and c.1128-6_1188dup67 mutations presented with a mild clinical course.
Conclusions: We provided important information about the mutational spectrum of ASS1 gene in Korean patients with citrullinemia type I and demonstrated a difference in common mutations in the ASS1 gene according to ethnic and geographic backgrounds.

M077

GENETIC APPROACH OF MLL2 GENE SCREENING MUTATIONS ASSOCIATED WITH KABUKI SYNDROME IN A SPANISH PATIENT

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Kabuki syndrome (KS, Kabuki makeup syndrome, Niikawa-Kuroki syndrome, OMIM #147290) is an autosomal dominant disorder characterized by distinctive facial features, fetal pads, intellectual disability and postnatal growth deficiency. Cardiac, renal and skeletal defects are sometimes associated. Most cases are sporadic, but a few familial cases have been reported. It is caused by mutations in MLL2 gene in most of cases but it is also suggested a genetic heterogeneity. MLL2 is composed by 54 coding exons and mutations associated to KS are distributed along these exons. Our goal has been to start up for the first time in Spain, the screening of mutations in MLL2 gene to confirm clinical diagnosis of pediatric patients with KS. Here we report a Spanish patient with evident clinical features compatible with KS which has been subjected to sequencing analysis of MLL2 gene finding a de novo dominant mutation in heterozygosis confirming the clinical diagnosis of KS in this patient. This Spanish patient has been subjected first to routine cytogenetic evaluation presenting normal karyotype and then genomic DNA was extracted and was subjected to mutation screening by direct sequencing in both directions using the 3130xl Genetic Analyzer ABI PRISM (Applied Byosistems). PCR amplification of MLL2 coding exons and intronic flanking sequences was performed using primer sequences and PCR conditions previously described in literature. The mutation found was confirmed on a second PCR reaction and double sequencing. The pathogenicity of the mutation was based on de novo occurrence, in silico prediction of non-functionality of the mutated protein using PolyPhen 2 (http://genetics.bwh.harvard.edu/pph2), location, type and conservation of mutated amino acids. We detected one heterozygous mutation in our patient. This mutation was not inherited and it was located in exon 48. This de novo mutation produces a change of amino acid in the protein (p.R5048P; c.28502G>C) and it was previously described in Hannibal et al. (Am J Hum Gen 2011). In silico prediction using PolyPhen 2 indicates that this mutation can potentially affect to the protein function. This is the first description in a KS Spanish patient of a pathogenic missense mutation located in exon 48 of MLL2 gene.

M078

PREVALENCE OF HFE GENE MUTATIONS IN PATIENTS WITH IRON OVERLOAD IN ARGENTINA

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Background: Hereditary hemochromatosis (HH) is an inherited autosomal recessive disorder of iron metabolism. Due to excessive intestinal absorption, iron accumulates in parenchymal cells of the liver and other organs with resultant damage to their structure and impairment of their function. The discovery of the responsible gene HFE enabled molecular analysis to be included in the diagnostic strategy for HH. A number of different mutations have been identified in this gene worldwide. Three mutations are particularly frequent among patients with HH: C282Y mutation within exon 4, H63D and S65C mutation within the exon 2. The aim of this study was to estimate the frequencies of C282Y, H63D and S65C mutations in a population of patients with iron overload from Argentina.
Methods: In the present study, we used 228 patients. DNA was extracted from blood samples. The presence of three mutations of HFE gene was tested with the LightCycler 2.0 instrument (Roche) using LightMix IVD kit HFE H63D S65C C282Y (Tib MolBiol).
Results: Of the 228 samples tested, 126 (55.26%) did not carry any of the three mutations. For the C282Y mutation we found 22 (9.65%) heterozygous samples and 6 (2.63%) mutated homozygous, for the H63D mutation H63D 76 (33.33%) were heterozygous and 5 were homozygous mutations (2.19%), and for the S65C mutation, 8 (3.51%) samples were heterozygous and we found no homozygous mutant samples. In addition, we found 13 (5.7%) samples heterozygous for C282Y mutation and H63D; 1 (0.44%) sample heterozygous for C282Y and S65C mutations; y 1 (0.44%) sample heterozygous for H63D y S65C mutations.
Conclusions: The results obtained agree with previous work in Argentina and with frequencies obtained from Spanish population, were the C282Y mutation is not as frequent as the H63D mutation. This is probably due to the large number of people with Spanish ancestry in Argentinian population. According to the large number of samples from patients with iron overload, which do not possess any of the three mutations studied, we suggest analyzing other genes related to alterations in iron metabolism or also to check less frequent mutations in the HFE gene (V53M, V59M, G93R, I105T, V272L y E277K) in order to find the molecular cause of this disease.

M079

DEVELOPMENT OF NOVEL PTT METHODOLOGY FOR RAPID SCREENING OF LARGE EXONS OF PALB2 BREAST CANCER GENE

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Background: Beyond BRCA1 and BRCA2 genes, numerous other genes have also been implicated to hereditary breast cancer due to their role in the same pathway of DNA repair, homologous recombination (HR). Among them PALB2 (Partner and localizer of BRCA2) emerges as the third breast cancer susceptibility gene. PALB2 is located in chromosome region 16p12.1 and consists of 13 exons transcribing approximately 3.5Kb mRNA, which encodes a protein of 1186 amino acids. Exons 4 and 5 are much larger than all others. This study focuses on the development of a method for quick mutation screening of these two large exons and therefore provides an alternative cost-effective strategy for those health systems not affording Next Generation Sequencing methods.
Methods: Genomic DNA was extracted from 15 samples of BRCA (-) Greek patients with hereditary breast cancer. These samples were submitted to PTT (Protein Truncation Test) for large exons 4 and 5 After PCR with appropriate primers designed by our group, amplified products were translated (should encode 50 and 30 KDa peptides respectively, TNT, Promega). SDS-PAGE electrophoresis was performed and then the peptides were transferred to a PVDF membrane and detected with a colorimetric kit (Promega). The rest of small exons were screened with High-Resolution Melting (HRM) curve analysis in LS32 in the presence of LCGreenPlus dye (Idaho Technology). Large DNA rearrangements were screened with MLPA technique (MRC Holland) and fragments were analyzed in ABI310 DNA Sequencer.
Results: Although no mutant sample were found in this group of patients, positive control samples were run alongside and the truncated peptides were easy to identify in the newly developed PTT test. The method was accurate when compared with DNA sequencing. HRM and MLPA were negative.
Conclusion: A novel accurate method that can easily identify truncating deleterious mutations in the two large exons of PALB2 gene was developed. No mutant sample was detected till now, but this research needs to be extended to more samples since mutations in this gene usually account for 1-4% of BRCA negative breast cancer families, in most populations studied so far.

M080

ASSOCIATION OF GENE POLYMORPHISM OF CLUSTER OF IL-1 AND RHEUMATOID ARTHRITIS WITH POSITIVE ACPA IN ETHNIC ALGERIAN PATIENTS

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Background: Rheumatoid arthritis (RA) is an inflammatory degenerative rheumatic chronic frequent pathology which affects 1% of the world's adult population. RA is more prevalent in women with frequent beginning between the ages of 30 and 50. The exact cause of RA is still unknown. It is believed that it results from genetic as well as environmental triggers. Among the identified susceptibility genes to RA HLA, class II genes are the most studied. Furthermore, other genes are found associated in RA, in populations of various ethnic groups and geographical locations. Among them are the genes of proinflammatory cytokines such cluster of IL-1 (IL-1α, IL-1β and IL-1Ra). Many polymorphisms are found in this cluster. Three (the most studied) seem to affect the rate of production of the cytokine: Two of these polymorphisms affect the gene encoding IL-1β (IL1-B +3953C/T and IL-1B-511C/T); the third concerns 86pb forVNTR of IL1RN gene encoding IL-1Ra. Several studies have shown an association between at least one of these polymorphisms and susceptibility to RA. Serologically RA is characterized by the presence of autoantibodies: rheumatoid factor (RF) and anti-citrullinated peptides antibodies (ACPA), In addition to their predictive value, the ACPA is an indication of severe course of the disease.
Material and methods: A case / control study was performed on 100 RA cases filling at least 4 of 7 criteria of the ACR and 127 controls without any inflammatory rheumatic pathology. The Immunochemical assays of RF and ACPA was measured by néphelometry.IL-1 Cluster genotypes were assessed by PCR (l'IL-1RN) and PCR-RFLP (IL1-B +3953C/T and IL-1B-511C/T).
Results and conclusion: No association was observed between the three polymorphism mentioned above and RA among Algerian population. However, there is a positive correlation of the haplotype IL-1RN*1/IL-1B-511T/IL-1B+3953C with the synthesis of ACPA, which is probably related to a strong synthesis of IL-1 to cause bone destruction.

M081

ATYPICAL CYSTIC FIBROSIS: DEVELOPED A NEW GENETIC TEST FOR IDENTIFICATION OF ENAC MUTATIONS

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Background: Cystic fibrosis (CF) is an autosomal recessive disease of sodium and chloride ion transport that is caused by loss-of-function mutations in both copies of the CFTR gene.Some patients may have all the classical symptoms of CF, while others have milder or even atypical disease manifestations. The large variability of CF symptoms suggests the existence of CF modifier genes; one of these could be the gene coding for the Epithelial sodium Channel (ENaC). Since increased sodium absorption through ENaC is part of the basic CF pathophysiology,mutations in this gene are prime candidates for causing CF-like disease.The aim of this study is to set up a new strategy, based on reverse line blot, for the evaluation of cases of atypical CF through the identification of mutations in the ENaC gene.
Methods: We have developed a genetic test for identification of mutations in the subunits β and β of ENaC, that were previously described in literature by several research groups. The procedure involves a single multiplex PCR for the amplification of subunits α and β of the ENaC gene and its hybridization with 18 selected probes. Nine probes are specific for wild-type alleles and the others for the mutants.The test was developed using wild-type samples and synthetic construct for homozygous mutations.
Results: In order to evaluate the performance of the method, we have analysed a series of patients with atypical CF; among them we have found 7 samples with one or two mutations in the ENaC subunits. In particular four patients were heterozygous for R181W,W493R,G294S and for S82C respectively. Two other patients were compound heterozygous for P267L and 1670-2A>G and for G294S and E539K respectively.Finally one patient carried a single-copy mutation (P267L). All the results were confirmed by sequencing of the ENaC subunits α and β.
Conclusions: Our ENaC test can be used for the identification of those mutations that cause CF-like phenotypes, not included in commercial tests for CF. This test, in combination with the detection of CFTR gene mutations, could be a powerful integrated system for the identification of both classical CF and the CF-like conditions.Currently, the CF-like disease caused by mutations in the ENaC gene can not be detected by any kits on market.

M082

MOLECULAR ANALYSIS OF PATIENTS WITH ELEVATED LONG-CHAIN 3-OH-ACILCARNITINES ALLOWS DIFFERENTIAL DIAGNOSIS BETWEEN LCHAD AND MTP DEFICIENCY

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Background: The mitochondrial trifunctional protein (MTP) is a multienzyme complex of the fatty acid beta-oxidation cycle. It is composed of 4 a-subunits (encoded by HADHA) harboring long-chain 2,3 enoyl-CoA hydratase (LCEH) and long-chain L-3-hydroxyacyl-CoA dehydrogenase (LCHAD) activity, and 4 β-subunits (encoded by HADHB) harboring long-chain 3-ketoacyl-CoA thiolase (LCKT) activity. MTP deficiency is an autosomal recessive disorder distinguished in two diseases: isolated LCHAD deficiency (LCHADD), characterized by reduced LCHAD activity, and general MTP deficiency (MTPD), characterized by reduced activity of all three MTP enzymes.
Methods: We analyzed three patients for which plasma acylcarnitines profile analysis, performed by tandem mass spectrometry (LC-MS/MS), showed an increase of long-chain 3-hydroxy species: 3-OH-palmitoylcarnitine (C16OH) and 3-OH-oleylcarnitine (C18:1OH). These findings suggested either long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHADD) or complete MTP deficiency. Coding region, intron/exon boundaries, and 5' and 3' untranslated regions of the HADHA and HADHB genes were amplified by polymerase chain reaction (PCR) and screened for mutations by automated sequencing. We searched new sequence variation in 600 control chromosomes of healthy individuals by dHPLC. mRNA analysis was performed by RT-PCR of total HADHB cDNA.
Results: Molecular analysis of the HADHA and HADHB genes revealed patients 1 and 2 were homozygous for the common a-subunit mutation c.G1528C (p.E510Q), that is located directly within the catalytic region of the LCHAD domain. These results are consistent with LCHADD diagnosis. Patient 3 was found to be a compound heterozygous for 2 novel mutations in HADHB gene: c.184A>G (p.T62A), absent in 600 normal chromosomes, and c.354+1 G>A, resulting, by mRNA analysis, in the skipping of exon 5 with an in-frame 45-bp deletion. These data suggest MTP deficiency.
Conclusions: In conclusion, we emphasize the importance of genetic analysis for a correct differential diagnosis of diseases that show similar biochemical findings, such as LCHADD and MTPD.

M083

SHORT BRANCHED CHAIN ACYL-COA DEHYDROGENASE DEFICIENCY: FROM METABOLIC STUDIES TO CHARACTERIZATION OF A NOVEL MUTATION

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Background: Short/branched-chain acyl-CoA dehydrogenase (SBCAD), also known as 2-methylbutyryl-CoA dehydrogenase, is a member of the acyl-CoA dehydrogenase family, involved in the isoleucine pathway. The SBCAD deficiency (SBCADD) has been known for little more than a decade and only about twenty patients have been described. Here we report an asymptomatic case of SBCADD, diagnosed by metabolic neonatal screening and we show results of molecular and functional studies.
Methods: Analysis of amino acids and acylcarnitines was performed by tandem mass spectrometry (LC-MS/MS) on blood spots. Acylglycines were assayed by GC/MS with negative chemical ionisation and stable isotope dilution. Molecular analysis of ACADSB gene is performed by PCR and automated sequencing. We cloned full-length cDNA of human ACADSB into the pcDNA5/FRT/V5-His-TOPO expression vector. The mutants of SBCAD were obtained by site-directed mutagenesis of pcDNA5/FRT/V5-His-SBCAD/WT. WT and mutant SBCAD mRNA levels were measured by real-time RT-PCR. WT and mutant recombinant SBCAD proteins were expressed by transient transfection of COS-7 cells and analyzed by enzyme activity assay.
Results: LC-MS/MS revealed a high concentration of pentanoylcarnitine (C5) and the analysis of urinary organic acids revealed increased levels of 2-methylbutyrylglycine (2-MBG), which was consistent with SBCADD diagnosis. Genetic analysis revealed a novel variant in the ACADSB gene among the two found: c. 439A>T (p.N147Y) and the c.443C>T (p.T148I) mutations, both in exon 4. mRNA levels in COS-7 cells transfected with normal or variant constructs indicate much lower expression levels in altered mRNAs. Each recombinant protein variant showed lower enzyme activity, however when both altered proteins were mixed a partial recovery of the activity occurred, indicating possibility of chimeric tetramer formation and/or protein crosstalk recovery mechanism.
Conclusions: These data show the presence of a compensatory effect between the two mutant proteins, suggesting an unusual pathogenetic mechanism that may influence genotype-phenotype simple correlation, and may explain the leak of symptoms in our patient.

M084

ASSOCIATION STUDY BETWEEN DRD2 GENE AND COCAINE DEPENDENCE IN A SPANISH SAMPLE

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Background: Drug addiction arises from an interaction between genetic and environmental factors. Genetic and physiological evidences suggest that dopaminergic system may play an important role in cocaine dependence. Analysis of genetic polymorphisms allows the genes that confer susceptibility to diseases to be analyzed. This study seeks links between TaqIA (A1 allele) single nucleotide polymorphism (SNP, rs1800497), located near the D2 dopamine receptor gene (DRD2; chromosome 11q22–q23) and cocaine dependence.
Methods: Genetic association between TaqIA and cocaine dependence was investigated using a case-control approach. The case group is made up of 70 individuals addicted to cocaine collected from detoxification centres of Aragon, Spain. Cocaine dependence was determined by DSM-IV criteria. The control group is made up of 70 volunteers that do not have any kind of dependence. Then, genomic DNA was extracted from venous blood then DNA fragment containing TaqIA polymorphism was amplified by PCR reaction. The genotyping was performed by RFLP using restriction enzyme TaqIA and displayed by electrophoresis. The results are compared using SPSS 15.0 software in order to find association between the TaqIA polymorphism and cocaine dependence.
Results: Average age of patients is 35 ± 8,81 years. Three genotypes were obtained from size in base pairs: A1A1 (5,7%), A1A2 (40%) and A2A2 (54,3%). This distribution remains in Hardy-Weinberg equilibrium. The control group was A1A1 (1,4%), A1A2 (38,6%) and A2A2 (60%). Patients with A1 allele had A1A1 or A1A2 genotype. Patients without A1 allele had A2A2. Therefore, we found 32 individuals with allele A1 (45,7%) and 38 without allele A1 (54,3%). Significant differences in genotypes were not found ($\chi 2$ =1,085, P=0,297) between patients to study and control group.
Conclusions: Initially TaqIA SNP does not seem to influence susceptibility to cocaine dependence. The findings from this study are consistent with multifactorial inheritance of addiction. So it is necessary to study more polymorphisms, for the simultaneous analysis and haplotype frequency estimation, a more accurate method to estimate the association with the quantitative character studied.

M085
GENE METHYLATION: A NOVEL REGULATORY MECHANISM OF CFTR EXPRESSION

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Background: The CFTR gene shows a clear temporal and tissue-specific regulation of its expression but the molecular mechanisms underlining the transcriptional control are still poorly understood. DNA methylation profile are complex and dynamic, and can vary with developmental stage, tissue type, age, and also disease state and this is compatible with the different clinical expression of cystic fibrosis (CF). DNA methylation play an important role in the regulation of gene expression: in many cases it occurs within CpG islands that overlap promoters and this correlates with transcriptional silencing of the gene. Interestingly, high levels of promoter region CpG methylation were observed in cell lines with very low CFTR expression. However, the role of methylation pattern of CpG island of CFTR gene has been poorly studied.
Methods: We investigated the methylation status and the expression of the CFTR promoter in different primary cells, following two strategies: i) we analyzed methylation of the promoter by bisulphite sequencing in twenty samples from peripheral blood and from IMR-90 cells; ii) we investigated the CFTR reactivation in primary cells after of 5-Aza-2′-deoxycytidine treatment, which causes DNA demethylation. Cells were treated with different concentrations of DNA-demethylating for seventy-two hours and expression levels of CFTR from treated and untreated cells were compared by semi-quantitative real-time PCR.
Results: Sequencing analysis of thirty-two CG sites revealed that some sites result methylated in all samples deriving from blood, and this data was confirmed also in IMR-90 cells suggesting that these sites undergo to a tissue specific pattern of methylation. The semi-quantitative analysis demonstrated that 5-Aza-2′-deoxycytidine was able to significantly improve the CFTR transcript in all analyzed cell lines, suggesting that the DNA methylation could downregulate the CFTR gene expression.
Conclusions: The CFTR methylation may have a relevant role in the regulation of gene expression at tissue level. This mechanism may help to explain the strongly discordant genotype-phenotype correlation observed in CF patients.

M086
A SYSTEMATIC REVIEW OF TNFα -308 G>A POLYMORPHISM AND COLORECTAL CANCER

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Background: The TNFα is a well characterized pleiotropic inflammatory cytokine, considered a mediator in a large array of pathologies and of growing significance in current scientific literature. The human TNFα gene is located in the short arm of chromosome 6 (6p21.3), and several polymorphisms at this locus and its promoters have been linked to an increased risk for several pathologies. The TNFα -308 G>A is a candidate proposed risk factor for several conditions ranging from septic shock to cancer, we decided to assess the link between it and colorectal cancer in current medical literature as an explanation for what we have empirically observed in our patient population
Methods: We conducted a systematic review and Logit meta-analysis, using Pubmed as search engine, and restricting our search to articles published in English and no older than 10 years by the time of the search. (TNF or “tumoral necrosis factor”) and (polymorphisms or variant) and (colorectal or colon or recto and cancer). The inclusion criterion was the study design: cohort or case and control studies, using odds ratio as a measure of effect, grouping the individual cases according to their genotype into AA, AG and GG, this with the aim of identifying the roll of being a carrier for the allele A. 40 articles were obtained; those considering other polymorphisms were excluded leaving 11, 2 of which were not available due to pending publication status. The data was processed using Epi-info.
Results: There were an estimated maximum number of unpublished results of 12, a Begg bias of 2.4 and Egger’s test of 1.7884, showing a small negative publication bias. The data set homogeneity was assessed with a critical value of 36.86 (P=0.00012) i.e. the study groups were heterogeneous, the Odds Ratio for random and fixed effects were 1.12 (CI95% 0.88-1.41) and 1.04 (CI95% 0.92-1.17) respectively.
Conclusions: The lack of homogeneity of the study groups lowers the validity of the systematic review, nevertheless the low publication bias is worth noticing, the random and fixed OR indicates the results aren’t statistically significant at p <.5 , therefore given the available data at the time this systematic review failed to be determined a link between this polymorphism and colorectal cancer.

M087
DO CFTR MUTATIONS PREDISPOSE TO THE DEVELOPMENT OF ASTHMA? A META-ANALYSIS

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Background: Mutations in the CRTR gene are a relatively common finding in the day to day practice of the genetics laboratory, and as with any other condition understanding its associations helps orient the multidisciplinary approach that is patient management. The association between asthma and CF has been proposed in the relevant literature but so far evidence has been scant, and evidence based management recommendations are lacking.
Methods: We conducted a systematic review followed by Logit meta-analysis using Pubmed as a search engine, and restricting our search to articles publish at least partially in English and no older than 10 years by the time of the search. The criteria were “Asthma”[Mesh] OR bronchial asthma AND (cftr[All Fields] AND (“mutation”[MeSH Terms] OR “mutation”[All Fields])). The inclusion criteria were the study design, focusing on cohort or case and control studies. We obtained 41 articles, excluded 13 for lack of relevancy, other 13 were excluded because they did not fulfilled the inclusion criteria, an additional article was added after reviewing the references of the articles included for a final number of 7+1. We then used the Epi-info software to process the data from the selected publications
Results: There was an estimated number of unpublished results of 5 with a Begg bias of 0.3712 and an Egger’s test of -0.148, the data set homogeneity was assessed obtaining a critical value of 36.86 with P=0.00012 that is the study groups were heterogeneous, the Odds Ratio (OR) for random and fixed effects were calculated obtaining 1.105 (CI95% 0.661-1.847) and 1.135 (CI95% 0.918-1.402) respectively.
Conclusions: The lack of homogeneity of the study groups and the reduced number of available relevant publications lightens the validity of the study, the random and fixed OR indicates the results aren’t statistically significant at p<.5; but the forest plot suggest that there might a positive link between CF and Asthma that warrants for renewed systematics reviews as the literature on the subjects increases with the aim of clarifying if such link is real - thus requiring a different management approach of this patient population- or just an anecdotal finding.

M088
ALTERATION OF BIOCHEMICAL MARKERS IN A SEVERE CHOLESTEROL BIOSYNTHESIS DISORDER

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Background: Smith-Lemli-Opitz Syndrome (SLO) is a severe, relatively frequent (~1:40,000) inherited disease with somatomental retardation caused by decreased activity of 7-dehydrocholesterol-reductase (7DHCR).
Methods: Fifteen Hungarian patients were diagnosed with SLO since 2002 on the basis of clinical symptoms, serum cholesterol, 7DHC and molecular genetic testing. Serum cholesterol was determined by enzymatic method (CHOD-POD), serum 7-dehydrosholesterol (7DHC) was measured by UV spectrophotometry, and lipoproteins were visualized by gel electrophoresis.
Results: Serum cholesterol in mild type SLO (n=4, clinical score <20) was 2.4±0.8 mmol/L and 7DHC was 147±55 mg/L. In typical SLO (n=7, clinical score 20-50) cholesterol and =DHC levels were 1.5±0.7 mmol/L and 202±77 mg/L respectively. Patients with severe SLO (n=4, clinical score >50) died as newborn, showed the lowest cholesterol (0.64±0.2 mmol/L) with 7DHC 180±52 mg/L. Initial serum cholesterol correlated with clinical severity (r=0.86) and initial Cho/7DHC showed similar prognostic value (r=0.82). Significant difference was observed between the initial cholesterol level of mild and severe SLO (P=0.01) and between the Cho/7DHC ratio of the three groups (P=0.004). In severe SLO, the α-lipoprotein ratio (7±7%) was significantly lower than in typical (32±9%) and mild SLO (33±6%). High cholesterol diet and statin therapy generally improves Cho/7DHC ratio and clinical condition, however liver function damage was observed in two statin resistant patients. Transaminase enzyme activity was twice as much in severe and typical SLO (AST:50 U/L, ALT:47 U/L, n=5) than in mild type (AST:23 U/L, ALT:21 U/L, n=5).
Conclusions: Laboratory specialists should be aware of the diagnostic importance of low cholesterol level. When it is below ~2.8 mmol/L in a child with clinical symptoms of SLO syndrome, 7DHC level should be checked. Life expectancy is fundamentally determined by the initial cholesterol, but dehydrocholesterol and α-lipoprotein levels have additional prognostic value. During statin therapy we suggest to monitor lipid parameters and liver function, since liver damage occurs frequently in typical and severe SLO.

M089
IMPACT OF ILLICIT DRUG USE IN THE COUNT OF CD4 + T LYMPHOCYTES WITH HIV

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Background: Considering the main epidemiologic aspects related to HIV/AIDS which have assumed increasing impact on people kept out of society with low partner-economic level, abuse drugs dependent, femaletion, low schooling and the illness advance in individuals above fifty years of age. This study objectified to investigate the correlation among the hard drugs use in the CD4+ T-lymphocyte counting in HIV seropositive.
Methods: A life style questionnaire was applied in 176 HIV seropositive, test (N=68) and control (N=108) groups. An active search in medical registers in the Tropical Diseases Hospital from interviewed seropositives was done to verify the last results of CD4+ T-lymphocyte counting that had been done in the Central Public Health Laboratory – Goiás, Brazil (LACEN-GO).
Results: The test group showed: 63 marijuana users, 43 of cocaine, 25 of methadone, 22 of crack and 6 of ecstasy. In which, 25.4% of marijuana users, 25.6% cocaine, 28,0% of methadone, 40.9% of crack and 33.3% of ecstasy had their CD4+ T cells counts less than 200 cells/mm³ (indicating to individuals in need of therapy); 22.2% of marijuana users, 20.9% of cocaine, 20,0% of methadone, 18.2% of crack and 50.0% of ecstasy had their CD4+ T cells count between 201 and 349 cells/mm³ (indicating the need for observation) and 52.4% of marijuana users, 53.5% of cocaine, 52,0% of methadone, 40.9% of crack and 16.7% of ecstasy had their CD4+ T cells count exceeding 350 cells/mm³ (indicating no need for therapy).
Conclusions: In the test not generate, directly, influence in the CD4+ T-lymphocyte dosage. However, other studies had demonstrated that the illicit drugs use is, many times, associated to the information lack and a risk social life (with violence and promiscuity) which can induce, indirectly, to the contamination by HIV and the probable AIDS dissemination.

M090
CHARACTERIZATION OF HOSPITAL INFECTIONS IN INTENSIVE CARE UNITS

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Background: Hospital acquired infections is one of the major causes of increased mortality and morbidity in intensive care units (ICU), increasing also the length of staying and their costs. The objective of this study was the characterization of nosocomial infections in ICU 1 and 2, of Hospital of Urgencies from Goiânia (Brazil), unit linked to the Goiás Public Health Department, which has 221 operating blocks. The study occurred during the period from December 2008 to December 2009.
Methods: The data presented on this study was obtained by the survey of medical records. The bacterial identification and susceptibility testing were performed according to the conventional methods established by the National Committee for Clinical Laboratory Standards (NCCLS), Clinical and Laboratory Standards Institute (CLSI) and "National Health Surveillance Agency" (ANVISA) of the Brazilian Ministry of Health.
Results: In the results were evaluated 889 biological samples from 337 patients. In this, 36,3% had been positive for bacterial isolation. The most frequent microorganisms were the following ones, Staphylococcus aureus (20,4%), Klebsiella spp (18,0%), Pseudomonas aeruginosa (17,0%), Gram negative glucose non fermenting (13,9%). These results corroborate with other ICU infections findings in Brazil, in which between the most commonly found bacterias are the Pseudomonas aeruginosa and Staphylococcus aureus. As well as a medium percentage of infections in the ICU, observed at the same period of a year in others researches, has been pointing to around 30% positivity.
Conclusions: In conclusion, the results reinforce the need of epidemiological studies of nosocomial infections in the ICU in order to promote effective development of preventive strategies. Once the patients on ICU are exposed to factors that potentiate nosocomial infections, as his compromised immunological system and the use of broad-spectrum antimicrobials at which are submitted during hospitalization.

M091
SYPHILIS IN WOMEN PRISONERS

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Background: Syphilis is a treatable sexually transmitted disease that is becoming a health problem in prisons. Once the living conditions of the prisoners bring them into risk factors such as: the fact that they are women (congenital syphilis), possibility of increasing exposure ace drugs (licit and illicit one) and poor hygiene conditions. Making the early diagnosis of syphilis in these population an important role in the treatment determination. This study aimed to estimate the prevalence of syphilis infection in women prisoners from the Goiás Prison Agency, in Brazil.
Methods: A questionnaire was carried to the transmission of sexually transmitted disease infections and blood samples were also taken to be analysis by the technique of flocculation - VDRL, where the syphilis positivity samples were confirmed by fluorescence, with the FTA-ABS.
Results: Of the 31 women prisoners, the average age was 31.4 years old. Positive reactions for syphilis were observed in 9 women, where five had been confirmed by FTA-ABS. Noting the total prevalence of 16.1% of infection by Treponema pallidum in this population.
Conclusions: It is concluded that syphilis is a serious public health problem in the prison system justifying the need of urgent preventive measures. Given that, women prisoners are a group particularly vulnerable to infections. And the lack of public programs for early diagnosis, treatment and prevention contribute to the increased incidence and prevalence of diseases, especially sexually transmitted diseases.

M092
PSEUDOMONAS SPP. IN SKIN BURNS

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Background: The local inflammation after a burning is essential for the wound healing and response of the host against infection. Among the related factors that favor the development of infection in the burned area, is the destruction of the skin, followed by the immune response depression. Other factors, such as age, depth and extension of injury, microorganisms involved in the infection, enzymes and toxins produced by them are also determinants for the degree aggression of invasion in the lesion and its complications. The burned skin surface is a nutrient-rich environment, which favor the installation and also the proliferation of infectious microorganisms into the injured area. Which can be endogenous or exogenous traced, wherein the most involved microorganisms in these infections are bacterias, mainly the Pseudomonas spp. This is a Gram-negative rod, non-fermenter carbohydrates, resident in the intestinal microbiota and in areas of high humidity of the skin, it can present an intrinsic and acquired resistance to certain antibiotics, making the lesions treatment more complex.
Methods:This retrospective study included 360 samples of burn injuries of patients attended in the Emergency Department of Burns Goiânia (PSQ). The samples have had microbiological analyzes performed in the clinical microbiology laboratory of the Goiano Institute of Oncology and Hematology (INGOH), through the overnight System Automation in Microbiology, AutoScan - 4, and their specific panels MicroScan ® Dade Berhing / Siemens.
Results and Conclusions: Among the 360 evaluated results, it was found 276 (76.7%) showing infectious process confirmed by clinical and laboratory diagnosis. In this found infectious processes had been observed that the population of Pseudomonas spp. was the second most frequent in these lesions with 13.8%, after the presence of Staphylococcus spp with 41.8% of incidence. The analysis of the susceptibility patterns performed on these Pseudomonas spp strains isolated, showed a resistance level from: 37.9% to Ceftazidime, 31% for Imipenem, 36.2% for Meropenem, 12.2% for Piperacillin/Tazobactam and 0% for Polymyxin B, which although toxic side effect was the most effective antibiotic against Pseudomonas spp. this research.

M093

TORCH ON THE LIAISON® XL – PERFORMANCE OF THE NEW ANALYZER

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Background: The aim of the present study was to evaluate the performance of the DiaSorin TORCH assays on the LIAISON® XL, DiaSorin's next generation CLIA analyzer, in comparison to the performance of these kits on the LIAISON® analyzer in an outpatient setting laboratory.
Methods: CMV IgM, CMV IgG, Toxoplasma gondii IgM, Toxoplasma gondii IgG, Rubella IgM, Rubella IgG, and HSV-2 IgG were tested simultaneously on both analyzers.The concordance between the analyzers by interpretation of the results was calculated. The quantitative assays results were correlated as well. Discrepant results were further tested by a third method and compared to clinical relevant data. Repeatability and reproducibility were tested with the kit controls as well as sera samples.
Results: Interpretation concordances of 92%, 99%, 98%, 100%, 98%, 100%, 99% were found between the analyzers for the CMV IgM, CMV IgG, Toxoplasma gondii IgM, Toxoplasma gondii IgG, Rubella IgM, Rubella IgG, and HSV-2 IgG assays, respectively. Correlations of 0.994 and 0.956 were obtained between the results of the two analyzers for CMV IgG and Toxoplasma gondii IgG, respectively. The single test that the concordance between analyzers was less than 98% was the CMV IgM. When testing this results against both Enzyme Immuno Florescent Assay (ELFA, Vidas) and against relevant clinical data, the LIAISON® XL results were found more suitable. Repeatability ranged from 1.3% to 4.0% for the IgM various assays and from 1.2% to 3.6% for the IgG various assays. Reproducibility ranged from 2.7% to 9.4% for the IgM various assays and from 2.9% to 7.7% for the IgG various assays.
Conclusions: The performance of the LIAISON® XL for the TORCH assays was highly comparable to the performance of the LIAISON® analyzer. The LIAISON® XL offers secure traceability of all processes, status of reagents and consumables as well as all the useful information for work monitoring and achieving higher effectiveness and safety quality.

M094

COMPARISON OF THREE COMMERCIAL METHODS FOR HUMAN PAPILLOMAVIRUS DETECTION IN CONSECUTIVE CLINICAL LABORATORY SAMPLES INCLUDING MEN AND WOMEN

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Background: Human Papillomavirus (HPV) is the most common sexually transmitted virus. Persistent infection by this virus is a necessary but not sufficient cause of cancers or genital lesion in men and, mostly in women. The aim of this work was to compare the analytical performance of three commercial methods, Hybrid Capture 2 (HC2) (QIAGEN), Papillocheck (Greiner bio-one), Clart-HPV 2 (bioMérieux) in consecutive samples of a clinical laboratory routine.
Materials and Methods: Ninety two (61 women and 31 men) consecutive samples from HPV detection routine were submitted simultaneously to the three methods covered in this study. All samples were collected in the Specimen Transport Medium (Quigen). HC2 is a signal amplified hybridization microplate-based assay designated to detect 18 HPV genotypes. Pappilocheck and Clart-HPV 2 are PCR amplified hybridization microarray-based methods that simultaneously detects and identify 24 and 32 different HPV genotypes, respectively. Chi-square analysis was used to test whether the methods differed for each other.
Results: In women, HPV positivity was 19.7%, 24.6%, and 17 27.9% for HC2, Papillocheck, Clart-HPV 2, respectively (P=0.56). In men, HPV positivity was 25.8% for HC2, 56.7% for Papillocheck, and 70% for Clart-HPV 2 (P=0.0019). The overall concordance was 50% for men and 85.2% for women. Detailed analysis in men shows that HC2 results proportion significantly differed from Papillocheck (P=0.0143) and Clart-HPV 2 (0.0026). Papillocheck and Clart-HPV 2 results proportion did not differed from each other (P=0.56). Moreover, the numbers of genotypes covered by each test were responsible for divergence in only 3 samples (all men). The exclusion of these samples change only the HC2 and Papillocheck comparison (P=0.078).
Conclusion: For women, the three commercial assays evaluated were statistically similar. For men, the PCR based methods show superior performance than HC2, probably due the high sensibility associated to the DNA amplification. The number of genotypes detected by each method was not the main responsible results divergences, but inability to detect a genotype included in the assay. PCR based methods should be used for HPV detection in men.

M095

GENOTYPIC HIV-CORECEPTOR TROPISM TESTING WITH GENO2PHENO[CORECEPTOR]: DIFFERENCES IN PREDICTION DEPENDING ON HIV-1 SUBTYPE

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Background: Determination of HIV-1 coreceptor tropism and detection of CCR5-tropic virus is a major prerequisite before starting treatment with a CCR5-blocker like Maraviroc. While most of the patients currently under treatment with Maraviroc are probably infected with HIV-1 subtype B viruses, recently published data show differences in the distribution of coreceptor tropism in different HIV-1 subtypes. Material and Methods: In a Germany-wide project within the HIV-GRADE society, V3-loop sequences of 2466 patients were analysed with geno2pheno[coreceptor] for coreceptor tropism using a FPR cut-off of 10%. HIV-1 subtype was determined by using the COMET HIV subtyping tool. The ratio of CCR5 vs CXCR4 tropic virus was calculated for each subtype. On the observed distribution in each subtype statistical analysis was performed using the chi2 test.
Results: Most of the samples were classified by the COMET subtyping tool as belonging to HIV-1 subtype B (79%, n=1952). Other subtypes present in at least 23 samples were A1 (9.5%, n=234), C (4.8%, n=118), CRF01_AE(2,2%, n=55), G(1,6%, n=39), D(1,1%, n=27), F(0.9%, n=23). The calculated normalized mean distribution over all subtypes was 71% CCR5-tropic virus vs. 29% CXCR4-tropic virus.. A higher rate of CXCR4 tropic virus could be detected in HIV-1 subtypes D (52% CXCR4, 48% CCR5, P=0.01) and CRF01_AE (49% CXCR4, 51% CCR5, P=0.001), while in HIV-1 subtypes A1 (22% CXCR4, 78% CCR5, P=0.02) and G (13% CXCR4, 87% CCR5, P=0.02), a higher rate of CCR5-tropic virus was observed.
Conclusions: Our analysis shows a different distribution of CCR5 and CXCR4 tropic virus in some of the analysed subtypes. Without further data on treatment success of patients with non-B subtypes under treatment with Maraviroc, it remains unclear if subtype-specific differences in the distribution of tropism are biased by differences in clinical variables before test or if there is a bias in the tropism interpretation system. In the latter case, individual interpretation cut-offs for different subtypes may be necessary.

M096

REAL-TIME PCR AND TURN AROUND TIME: CLINICAL RELAPSE IN "TRUE" MICROBIOLOGICAL EMERGENCIES MANAGEMENT

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Background: Real Time PCR (RT-PCR) reduces Turn Around Time (TAT) and increase meningoencephalitis and sepsis diagnosis sensibility, while for malaria its efficacy is still to be demonstrated. Aim of this study was to analyze the impact of RT-PCR in terms of diagnostic efficacy in the management of microbiological "emergencies".
Methods: Nucleic acids from 160 cerebrospinal fluids (CSFs), positive to physical-chemical analysis, and from 14 cases of suspected malaria, were extracted using the BioRobot EZ1 Advanced with Virus DNA kit (Qiagen). Qualitative/quantitative RT-PCR for bacteria (EuSepScreen lattanti/adulti, Eurospital) and viruses (Herpes Simplex Virus and Enterovirus, Biomerieux) were performed for the detection of most important pathogens causing meningoencephalitis. Quantitative RT-PCR Artus (Qiagen) and Liferiver (DID) kits were used for detecting and typing Plasmodium species.
Results: 16 CSFs analyzed were positive for bacteria (6 to S. pneumoniae: 3 with negative bacterioscopic analysis and 4 with negative culture test) and 17 for viruses (including 1 to Human Herpes Virus 7). All positive bacterial cases showed an increased procalcitonin (PCT) blood level. There were no cases with negative molecular test and positive culture test. The malaria RT-PCR has confirmed all positive cases (n = 8) of thin blood smears (TBS), including the only case with gametocytes alone. RT-PCR parasitemia was comparable to BST one.
Conclusions: In meningoencephalitis management evaluation of biochemical test results, such as PCT and blood cells count, allows to decide which RT-PCR panel is more appropriated; 2) a fully automated platform for RT-PCR methods can provide a more effective response in terms of treatment. In fact, RT-PCR methods are faster (TAT <2-3 h), more sensitive (>99%) and negative predictive value is 100%. In addition, RT-PCR allows many viral pathogens detection, reducing the cases in which therapy remains on empirical basis. Limits? The reduced number of pathogens, especially bacteria, that can be detected. For malaria diagnosis RT-PCR improves identification of Plasmodium species and allows standardization of parasitemia calculation, making it suitable for its insertion in urgency/routines, with important clinical relapses for the patients.

M097

CORRELATION OF PRESEPSIN (SCD14-ST) WITH PCT IN CRITICALLY ILL PATIENTS: DIAGNOSTIC USEFULNESS IN SEPSIS

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Background: Differentiating sepsis from non-infectious triggers of the systemic inflammatory response syndrome is difficult, especially in critically ill patients. Sepsis is a major cause of mortality in patients admitted in the intensive care unit and early detection and specific clinical intervention are crucial for favorable outcomes. Procalcitonin (PCT) has emerged as the most used diagnostic and prognostic marker of sepsis in clinical practice in intensive care units. However, a meta-analysis showed that has poor diagnostic utility for differentiating sepsis from other non-infectious causes such as pancreatitis, burns, paraneoplasic syndromes. Recent studies suggested that measuring of Presepsin (sCD14-ST) levels would be valuable for the diagnosis of sepsis. The purpose of this study was to evaluate the correlation of Presepsin with PCT and its diagnostic usefulness in critically ill patients with sepsis.
Methods: We selected 102 samples from 68 patients admitted to Intensive Care Unit and Reanimation Unit, in which existed the clinical suspicion of sepsis and to which had been requested PCT. 30 of these patients were requested a second determination of PCT, and 4 of them third. In plasma of all patients was determined, besides PCT, Presepsin. Presepsin and PCT measurements were determined in Pathfast analyzer (Mitsubishi Chemical ®) and in Cobas 6000 analyzer (Roche Diagnostics®) respectively. Statistical analysis was performed using SPSS software v15.0. (Chicago, Illinois, USA).
Results: We found a good correlation between PCT and Presepsin (r=0.518, P <0.000). Levels of Presepsin also were significantly correlated with blood culture positivity (r= 0.311, P < 0.05). To evaluate clinical effectiveness of Presepsin for the diagnosis of sepsis we elaborated Receiver operator characteristics (ROC) curves. The areas under the ROC curves for PCT and Presepsin were 0.712 P <0.005 (95% CI, 0.582-0.842) and 0.775 P <0.001 (95% CI, 0.654 to 0.897), respectively.
Conclusions: In these patients, the diagnostic usefulness of Presepsin is higher to that of PCT, emphasizing a greater area under the curve. However, we consider that further studies are needed for his incorporation to the clinical daily practice.

M098

EVALUATION OF PRESEPSIN (SCD14-ST) IN CORD BLOOD AS A MARKER FOR EARLY-ONSET NEONATAL SEPSIS

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Background: Presepsin (sCD14-ST) is the N-terminal 13 kDa fragment of the CD14 protein. Is involved in the recognition of bacterial products and is considered a novel diagnostic marker for sepsis. Our aim was to determine if Presepsin measurements in cord blood were a useful parameter to recognize newborns with early-onset neonatal sepsis (EONS) and analyze its correlation with Procalcitonin (PCT), C-reactive protein (CRP) and Interleukin 6 (IL6).
Methods: The study population was divided in 4 groups: group 1 (G1) was used as a control and it included 40 neonates who were studied to present any risk factors and who were discharged without requiring hospitalization; group 2 (G2) consisted of 40 newborns who present suspected infection; group 3 (G3) included 40 preterm neonates and group 4 (G4) were composed of 10 neonates diagnosed of EONS. In cord blood of all neonates were determined Presepsin, CRP, PCT and IL6. Presepsin measurements were determined in Pathfast analyzer (Mitsubishi Chemical®). PCT, CRP and IL6 were assessed in Cobas 6000 analyzer (Roche Diagnostics®). Data are expressed as median [interquartile range p25-p75].
Results: In the descriptive study were obtained the following results of Presepsin (pg/mL): G1 = 786 [527-1104], G2 = 767 [580.75-1230], G3 = 625 [476-948], G4 = 628 [539-718]. We observed that G4 had a significantly lowers levels of Presepsin compared to G1 (P <0.05) and compared to G2 (P <0.05). Presepsin were significantly correlated with PCT, in the total population (r=0,278; P <0.05) and in G1 (r=0,536; P <0.05) and G2 (r=0,342; P <0.05). We elaborated Receiver operator characteristics curves considering Presepsin's levels of G1 and G4. The area under the curve was 0,705 (95% CI, 0.562 to 0.848).
Conclusions: We found lowers cord blood levels of Presepsin in neonates with sepsis compared to those without sepsis. Newborns with extreme immature innate immunity have defects in bacterial recognition and in the capacity to mount a proper inflammatory response, and this increase susceptibility of EONS. There are studies which have suggested that newborns with EONS had significantly reduced levels of CD14 and this could explain our decreased levels

M099
PANDORAEA PULMONICOLA ISOLATION IN A YOUNG PATIENT WITH CYSTIC FIBROSIS

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Background: Cystic fibrosis (CF) is an autosomal recessive genetic disorder that affects most critically the lungs and is caused by a mutation in the gene for the protein CF transmembrane conductance regulator. As a result, an abnormal transport of chloride across epithelial membrane is produced, leading to thick, viscous secretions. The recently described Pandoraea pulmonicola has mainly been recovered from the respiratory tracts of CF patients. Its identification by routine microbiology methods is difficult, and differentiation from B.cepacia complex may be especially problematic.This can have important consequences for the management of patients
Methods: A 18-year-old male was diagnosed with CF (homozygous for the F508 mutation) at 8 months of age and was admitted to the Hospital in 2011 for pulmonary exacerbation.At this time, he was chronically colonized with P.aeruginosa and had history of Chronic Respiratory Insufficiency. During the course of his hospital stay, his sputum was sent to Microbiology Laboratory and a nonfermenting gram-negative bacteria was identified as P. pulmonicola by mass spectrometry (MALDITOFF). The susceptibility profile of the organism was determined by automated system Wider I and demonstrated susceptibility to trimethoprim-sulfamethoxazole (SXT), minocycline and piperacillin-tazobactam. His clinical course was favourable and his respiratory specimens were negative after to be treated with SXT, linezolid and colistin. He underwent a lung transplant in 2012.
Results: Due to the correct diagnosis of P.pulmonicola by classical phenotypic techniques is difficult, it is crucial to use proteomic methods, such as MALDITOFF. It is a fast and reliable method for bacterial identification. Recently used in Microbiology Laboratory, is our best diagnostic tool, allowing a rapid and accurate identification of our isolates, mainly in those species which identification with other procedures, may be especially problematic
Conclusions: P.pulmonicola may determinate the evolution and prognosis of the CF.Its isolated is a fatal prognostic factor, therefore it is necessary its correct identification. The MALDITOFF system is suitable to the identification this bacteria, on the Microbiology Laboratory, because the routine clinical microbiology methods are not reliable

M100
MOLLARET MENINGITIS: A CASE REPORT

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Background: The Mollaret's meningitis is a rare form of meningitis that is recurrent,aseptic and self-limiting. The etiology is unknown, although different infectious agents have been isolated. Less than a hundred cases have been reported in the world and this disease is rarely seen in clinical practice
Methods: A 43 year old male was admitted to the Neurology Service in 2010 with a 12 day history of sudden holocranial headaches, feverishness, photophobia and nausea. He reported a history of postcoital migraine refractory to treatment. Physical examination: 37.7 °C, no neck stiffness and focal neurological signs. Results of CT scan brain and MRI brain were both normal.The cerebrospinal fluid (CSF) white blood cell count was 428 cells/mm^3 (94.4% mononuclear cells),41 mg/dl glucose (serum glucose:90 mg/dL) and 101.7 mg/dL protein. Gram's stain did not show microorganisms and the culture was sterile. Results of the MANTOUX and hemoculture were normal. Serology and polymerase chain reaction (PCR) of CSF bacterial and neurotropic virus usual were also negative. Accordingly to those results, he was discharged home with a diagnosis of viral lymphocytic meningitis. However the patient was still showing 3-4 day episodes of fever and migraines. All the analytical tests were repeated and the results showed positive seroconversion to herpes simplex virus (HSV) and the presence of large mononuclear cells of irregular nuclei suggestive of Mollaret´s meningitis on the CFS. The patient was treated with valacyclovir and immunosuppressive therapy. The fever subsided completely and the headache episodes were less intense and sometimes self-limiting after suspending the treatment. Subsequent analytical tests were normal and his symptoms and signs resolved without sequelae
Results: The diagnosis was proposed for the following reasons: recurrent episodes of severe headache and fever,development of episodes after symptom-free periods of weeks to months, spontaneous remission of symptoms and signs observed during the episodes, pleocytosis in the CSF composed mainly of mononuclear cells and HSV seroconversion although the PCR was negative for the virus
Conclusions: We present a case that meets the clinical criteria necessary for the diagnosis of Mollaret's meningitis, confirming the discovery of classic Mollaret.

M101
PROCALCITONIN - MARKER OF CHOICE IN INFECTION AND SEPSIS DIAGNOSIS

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Background: Procalcitonin (PCT) is an established marker for severe systemic bacterial infection and sepsis. It is prohormone, peptide precursor of the hormone calcitonin. PCT, synthesized in monocyte and macrophage cells, does not suffer of proteolysis and can be found in circulation as a stable, intact molecule. In physiological conditions PCT is not released into the bloodstream and its concentration in the blood is low (<0.05 ng/mL), but it raised in severe infections (above 100 ng/mL). The most potent stimulator of PCT synthesis are bacterial endotoxines, and also TNF-α, IL-6, IL-1β, IL-2. Levels of PCT are elevated in many conditions such as: bacterial infection, pancreatitis, polytrauma, newborns infection. Autoimmune and viral diseases, locally bacterial infections and chronic inflammation do not induce a rise in PCT. The aim of this investigation was to compare concentrations of PCT with the C-reactive protein (CRP) and Serum Amyloid A (SAA) as well known inflammatory markers.
Methods: Sera of 30 patients, 17 males and 13 females, from different clinics: Intensive Care Unit, nephrology, rheumatology, surgery, transplantation, etc., were examined by automated immunofluorescent method (Brahms Kryptor). SAA and CRP were measured by immunonephelometry (SIEMENS DADE BN II) method.
Results: Mean average values for PCT 1.44±2.76; SAA 103.69±59.96 and CRP 118.68±76.94 were all highly elevated in comparison to their reference intervals. Student's t-test among all tested parameters was statistically significant at the level P< 0.05. Results of ROC analyze showed that PCT was the best parameter for the specificity and sensitivity in comparison to the CRP and SAA for the confirmation of sepsis.
Conclusion: Obtained results showed that PCT is better parameter for detection complications of systemic bacterial infections (sepsis, severe sepsis, septic shock) than standard parameters (Le, concentrations of CRP, SAA, cytokines) and is in concordance with the other authors statements. It is recommended that the concentrations of this marker should be considered together with other biochemical parameters, microbiology results and patients' signs and symptoms.

M102
PROCALCITONIN LEVEL ASSOCIATED WITH BACTERIEMIA ETIOLOGY IN SEVERE SEPSIS AND SEPTIC SHOCK

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Introduction: The etiology of bacteremia determines the choice of adequate therapy for severe infections. The clinical manifestations of gram-negative and gram-positive bacterial infections are similar while biological markers may serve as a guide for the early diagnosis of the nature of a pathogen.
Objective: The purpose of the study was to assess an association between the level of procalcitonin (PCT) and the etiology of bacteremia.
Methods: We analyze clinical data, biomarkers and etiology of blood culture in 245 patients with severe sepsis or septic shock hospitalized in Intensive Care Unit (ICU) over a period of 2 years (2009-2011).The PCT was analyzed by immunoassay (Vidas, Brahms) and was measured in the first 24 hours from severe sepsis or septic shock onset. The program used for the data processing and statistical analysis was Statistica 7.1. Soft Inc®
Results: Forty three patients (17,6%) were severe sepsis and 202 (82,4%) septic shock. The median age of the study sample was 64 years old [inter-quartile range (IQR): 50,5-72], 60% were men, APACHE II was 24,6±6,69 and SOFA 9,57±3,14. The median length of stay in ICU was 6 days [IQR: 4-12] and 28- days mortality was of 26,9% (n=66). Blood cultures were realized in 226 patients, 111 were negative (81 cases received antibiotics before the blood culture) and 6 cases the result was fungi. In the gram-negative bacteremia group (n=54), plasma PCT median were statistically significantly higher than in the gram-positive bacteremia group (n=58): 39,16 ng/mL [IQR: 16,12-88,65] vs 17,07 ng/mL [IQR: 5,17-30,87], P=0,0008 (U-Mann Witney). Escherichia coli (n=31) had the highest value into the gram-negative group (66,68 ng/mL [IQR: 24-134,3]; P=0.0009) and Streptococcus pneumonia (n=26) in the gram-positive group (25,2 ng/mL [IQR: 21,45-61,6], P=0,004; U-Mann Witney).
Conclusions: Patients with severe infections and plasma PCT levels, may be supposed the etiology before obtaining blood culture results. In abdominal or urinary sepsis E. coli and S. Pneumoniae in the case of pneumonia could be the responsible microorganism with high values PCT.

M103
NEW STRATEGIES FOR THE IDENTIFICATION OF ACTIVE HEPATITIS C VIRUS (HCV) INFECTIONS

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Background: The resolution of the World Health Organization (WHO) issued in May 2010 encourages to realize measures of epidemiological surveillance and to put in place an integrated, efficient and cost-effective approach for prevention, control and management of viral hepatitis B and C. Testing strategies for the identification of active HCV infections are needed.
Methods: Literature search (Medline) on HCV epidemiology and chronic liver disease in Italy and on HCV core antigen (HCVAg) compared to HCV-RNA in treated and untreated subjects.
Results: In Italy more than 65% of chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) cases are linked to a chronic HCV infection. Open population studies have shown positivity rates for anti-HCV ranging from less than 3% to more than 15%. The bulk of infection occurred over 2-3 decades after 1950 by parenteral routes of contagion and a sharp drop in incidence rates was recorded in the last 20 years due to the availability of screening assays and the adoption of disposable medical devices. The rate of active infection (positivity for HCV-RNA) in anti-HCV positives ranges from 60% to 80% and usually decreases with age. While HCV persists in more than 80% of newly infected individuals, a spontaneous clearance of HCV is achieved in 10-30% of cases. The sensitivity of the HCVAg assay corresponds to about 1,000 IU/mL of HCV-RNA and viremia levels are usually much higher than that in untreated subjects.
Conclusions: The current diagnostic algorithm is based on a screening for anti-HCV followed by a confirmation of the serological reactivity, necessary in low-prevalence setting due to a poor positive predictive value. The presence of an active infection requires a second blood draw in order to carry out testing for HCV-RNA. This two-step approach increases the direct and indirect costs and thence limits the implementation and efficacy of surveillance programs. In order to identify ongoing, asymptomatic HCV infections, a sound approach may include targeting the age groups at higher risk and test people directly for HCVAg, that can be determined by a fully automated serological method in all routine settings and whose sensitivity is adequate to identify the majority of infected, untreated individuals.

M104
PROGNOSTIC VALUE OF THYROID FUNCTION IN PATIENTS WITH SEVERE SEPSIS OR SEPSIS SHOCK

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Background: Alterations in serum thyroid hormone levels under critical illness or stressful conditions are referred to as non-thyroidal illness syndrome. Several studies have reported that the magnitude of the alteration correlates with the severity of the illness suggesting that thyroid function could play an important role in predicting outcome in these pathologic situations. The aim of the study was to assess the prognostic value of thyroid hormone levels in patients with severe sepsis or septic shock.
Methods: Prospective observational study of 50 patients hospitalized in Reanimation Unit with diagnosis of severe sepsis or septic shock. Serum levels of TSH, FT3 and FT4 were measured by ECLIA (modular analytics E170, Roche Diagnostics) in samples which were collected at the following time points: In the first 72 hours after the diagnosis (TSH-1, FT3-1 and FT4-1), in the next 72 hours (TSH-2, FT3-2 and FT4-2) and between 7th and 9th days (TSH-3, FT3-3 and FT4-3). The primary outcome of this analysis was death in Reanimation Unit due to any cause. Hormonal parameters were compared with the Mann-Whitney U test and prognostic accuracy in predicting mortality was determined by ROC curve analysis. All calculations were performed using SPSS version 19.0.
Results: The median age of the 50 patients included was 75.5 (P25-P75: 59.7-80.0), 31 (62%) of them were men, and 15 (30%) died during their Reanimation Unit stay. When comparing thyroid hormone levels of survivors with non-survivors, we found statistically significant differences in FT3-2 (1.70 ± 0.64 pg/mL vs. 1.23 ± 0.28 pg/mL; P=0.013), FT4-2 (1.07 ± 0.37 ng/dL vs. 0.73 ± 0.35 ng/dL; P=0.007) and FT4-3 (1.28 ± 0.39 ng/dL vs. 0.98 ± 0.35 ng/dL; P=0.036). The areas under the ROC curves of FT3-2, FT4-2 and FT4-3 for predicting mortality were 0.781 (P=0.011), 0.693 (P=0.083) and 0.733 (P=0.036) respectively.
Conclusions: Our results showed that in patients with severe sepsis or septic shock worse outcomes are associated with lower levels of FT3-2, FT4-2 and FT4-3. Among the parameters studied, FT3-2 was the best predictor of mortality.

M105

METHOD COMPARISONS BETWEEN THE VERSANT HIV-1 RNA, HCV RNA, AND HBV DNA 1.0 ASSAYS (KPCR), ON THE VERSANT KPCR MOLECULAR SYSTEM AND OTHER APPROVED METHODS

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Background: VERSANT® HIV-1, HCV and HBV 1.0 Assays (kPCR) IVD assays are available for use with the VERSANT® kPCR Molecular System* (VERSANT kPCR; Siemens Healthcare Diagnostics, Tarrytown, NY, U.S.). Method comparisons were conducted between VERSANT HIV-1 and HCV RNA 1.0 Assays (kPCR) and Abbott RealTime HIV-1 and HCV (ART; Abbott Molecular, Inc., Des Plaines, IL, U.S.); between VERSANT HBV DNA (kPCR) and Roche COBAS AmpliPrep/COBAS TaqMan HBV Test v2.0 (CAP/CTM; Roche Molecular Systems, Pleasanton, CA, U.S.); and with VERSANT HIV-1 RNA, HCV RNA and HBV DNA 3.0 Assays (bDNA); (bDNA; Siemens).
Methods: HIV-1 (n=87), HCV (n=155), or HBV (n=279) positive sera or K2EDTA plasma specimens were collected with IRB-approved protocols or commercially available panels, and processed according to manufacturer's instructions for each method. Testing was performed for VERSANT kPCR at Siemens, Berkeley, CA; for ART at BioCollections Worldwide; for CAP/CTM at Biomnis, Lyon, France; and for VERSANT bDNA at Siemens Clinical Laboratory, Berkeley, CA. Testing was done in singlicate by each method, and results compared for all samples with paired quantitations within the reporting ranges of each pair of methods. Deming regression was used to determine if methods had a linear relationship. Average log difference was used for quantitative equivalence.
Results: Deming regression slopes for all paired comparisons were between 0.97 and 1.04, indicating that VERSANT kPCR has a linear relationship with all the comparator methods. The average log difference (comparator method: VERSANT kPCR) was 0.26 log IU/mL for ART HCV; 0.19 log IU/mL bDNA HCV; –0.05 log IU/mL for CAP/CTM HBV; –0.10 IU/mL for bDNA HBV; –0.13 log copies/mL for ART HIV; and 0.11 log copies/mL for bDNA HIV.
Conclusions: The results demonstrate a linear relationship and quantitative equivalence between VERSANT kPCR and VERSANT bDNA (HIV-1, HCV and HBV), VERSANT HIV-1 RNA 1.0 Assay (kPCR) and Abbott RealTime HIV-1, VERSANT HCV RNA 1.0 Assay (kPCR) and Abbott RealTime HCV, and VERSANT HBV RNA 1.0 Assay (kPCR) and Roche CAP/CTM HBV.The VERSANT kPCR Assays and kPCR Molecular System are CE marked and are commercially available in EU countries. They are not for sale in the U.S.

M106

CLOSTRIDIUM DIFFICILE IS STILL RARE DESPITE INCREASING ANTIBIOTIC USE IN KENYA

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Background: Clostridium difficile is an important pathogen that causes complications in hospitalized patients.Unfortunately clostridium difficile is a growing challenge globally and is threatening the safety and lives of patients. There has however been a dearth of information in the African continent on the prevalence of Clostridium Difficile associated disease (CDAD).In a study done in Nairobi in 1996 amongst HIV positive patients the routine culture of stool specimens for C. difficile was stopped prematurely during the study due to the total lack of recovery rates. In the background of increasing incidence of CDI (Clostridium difficile associated infections) during the past 5 years that has been observed in America and Europe it has become evident that an epidemic of CDIs that are associated with increased clinical severity and that were more refractory to conventional therapy is being experienced. It is in view of this that the audit was carried out to determine if there has been a change since 1996 in the prevalence of patients with CDI amongst in patients in Nairobi. With the increasing availability and antibiotic use the audit also set out to determine what proportion of patients had prior exposure to antibiotics and if this might have an effect on our prevalence rates
Methods: This is a retrospective audit that looked at tests done in our facility during the period June 2011 to July 2012. The tests were done using a rapid enzyme immunoassay with ability to detect Toxin A and B. A file review was done to determine who was on antibiotics and the type of antibiotics they were on.
Results: A total of 305 results were analyzed. There were only 2 cases with a positive serology (0.65%) 303 cases had a negative serology (99.35%). A multiracial population of patients was profiled as shown: 58% were African 34% were Asian 8% were Caucasian. 78% of the patients had documented use of antibiotics prior to having the test done with majority of these patients (78%) being on beta lactams. Of the patients on antibiotics 69% were on more than one antibiotic
Conclusion: The prevalence of CDI is very low amongst inpatients in Aga Khan Hospital, Nairobi and these cuts across the various races. The prevalence rate of 6.5 per 10000 is lower than rates reported in North America of 13.5 per 10000 amongst inpatients. It is also possible that the exposure to antibiotics was higher than 78% since our institution is a referral hospital, there is a possibility that patients may have been exposed to antimicrobial agents prior to being seen in our institution, or may have self-medicated on antimicrobials prior to being seen at our institution. Due to the widespread and unregulated use of antibiotics which unfortunately are frequently available over the counter without doctors' prescriptions we had hypothesized that we would get higher rates of CDI.

M107

CONTINUAL GRAM-NEGATIVE BACTERIAL CHALLENGE ACCELERATES STROKE ONSET VIA INDUCTION OF OXIDATIVE STRESS IN STROKE-PRONE SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Infection and inflammation are considered additional risk factors of stroke. We hypothesized that continual gram-negative bacterial challenge caused by chronic infectious diseases may influence stroke onset in combination with conventional stroke risk factors such as hypertension. To test this hypothesis, we investigated the effects of continual injection of gram-negative bacterial cells and/or lipopolysaccharide (LPS) on the blood pressure, serum levels of NO2/NO3 (NOx), thiobarbituric acid reactive substance (TBARS) and 8-hydroxydeoxyguanosine (8-OHdG), symptoms of stroke onset, rates of stroke onset and survival in stroke-prone spontaneously hypertensive rats (SHRSP).
Methods: Male SHRSP were continually injected with a bacterial cell suspension of Escherichia coli or Porphyromonas gingivalis, or LPS every third day until stroke onset was observed. Systolic blood pressure was determined using a tail-pulse pick-up method with a photoelectric detector. Stroke onset was assessed by the appearance of neurological symptoms (hyper/hypokinetic behavior, paralysis of limbs, and piloerection) and changes in body weight. Peripheral blood samples were withdrawn from the caudal vein at 8 weeks of age to determine the serum levels of NOx, TBARS, and 8-OHdG.
Results: Systolic blood pressure was moderately elevated in SHRSP prior to stroke onset following the continual injection of either bacterial suspension. Stroke onset occurred significantly earlier in SHRSP injected with either bacteria compared to uninjected controls. Furthermore, the injection of LPS displayed similar effects. Decreased body weight and paralysis in the forelimb were observed during stroke onset in all SHRSP, excluding cases of sudden death. In contrast, paralysis in the hindlimb, piloerection, hypokinesis, and hyperkinesis were observed only in SHRSP injected with LPS during stroke onset and SHRSP without injection, during the late survival period, but not during stroke onset. The serum levels of NOx, TBARS, and 8-OHdG significantly increased in LPS-injected SHRSP compared to the uninjected group.
Conclusions: These results suggest that continual gram-negative bacterial challenge induces accelerated stroke onset in SHRSP, probably caused by oxidative stress responses derived from LPS.

M108

VANCOMYCIN-RESISTANT ENTEROCOCCUS AVIUM FROM WOUND OF A PATIENT WITH DIABETES MELLITUS

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Background: Enterococcus avium (E. avium), frequently found in birds, is rarely reported as a pathogen in humans. Although there are several studies of infection due to E. avium, little is known about its clinical features and antimicrobial susceptibility. We isolated vanA-producing vancomycin-resistant E. avium from the wound specimen of a patient with diabetes mellitus.
Case & result: A 79 year-old Korean male who presented with fever, cough, yellowish sputum was admitted in a tertiary university hospital in Korea. He had diabetes mellitus, hypertension, end-stage renal disease on hemodialysis, and he had left hemiparesis due to cerebral infarction for 2 years. He was treated with vancomycin and other antibiotics for the pneumonia due to isolation of methicillin-resistant Staphylococcus aureus (MRSA). After 3 months of admission, an ulcer with discharge occurred on his 2nd toe of left foot. The wound culture revealed the growth of three distinct gram positive cocci, which were dominant Enterococcus faecalis (E. faecalis), E. avium and some MRSA. The isolated E. avium identified by the Vitek 2 GPI and API 20 Strep systems (bioMérieux, USA) and this organism resistant to ampicillin, tetracycline, vancomycin and teicoplanin and intermediate to quinupristin/dalfopristin. The E-test was also performed for vancomycin and teicoplanin in the E. avium isolates and showed that the minimal inhibitory concentrations (MIC) for vancomycin and teicoplanin were >256 mg/L and 48 mg/L, respectively. The 16s rRNA sequences of the isolates showed 98% homology with those of E.avium reported in GenBank. Real-time PCR for the detection of the vanA gene was performed Seeplex® VRE ACE Detection kit (Seegene Inc., Seoul, Korea) and vanA gene was detected in E. avium isolate. The wound was healed after treatment, and he was transmitted to other long-term care facility.
Conclusion: Although vancomycin-resistant E. avium was rarely observed among intestinal colonizing vancomycin resistant enterococci, there are very few case reports of vancomycin-resistant E. avium from clinical specimen. Further research on the clinical features of E. avium and its susceptibility to antimicrobial agents in the isolates from clinical specimens is needed.

M109
IDENTIFYING DENV-1 B-CELL EPITOPES USING PHAGE DISPLAY TECHNIQUE

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Background: Dengue is a mosquito-borne viral disease prevalent mostly in tropical countries. Nearly half the global population is at risk of dengue infection. The symptoms range from mild fever to possibly fatal haemorrhagic fever or shock syndrome. There is neither a vaccine nor an effective antiviral therapy against the disease, but the fatality can be minimized by symptomatic treatment. Early and accurate diagnosis is essential for effective treatment and for surveillance and outbreak investigations. There are four dengue serotypes (DENV-1,2,3 and 4), which also resemble other flaviviruses such as West Nile virus and tick-borne encephalitis virus. This leads to cross-reactivity of antibodies, which complicates the development of a reliable serodiagnostic method. Our aim is to use phage display to identify the linear epitopes, which could be utilized to develop a dengue-specific diagnostic tool.
Methods: We have constructed gene fragment phage display libraries (GFPDL) comprising different fragment sizes, covering several DENV-1 genes (C, M, E and NS1). The genes were randomly enzymatically fragmented and ligated to phage display vector, which was cloned to E. coli cells. The diversity and quality of the library was estimated by sequencing randomly selected phage clones. The functionality of the concept was demonstrated with a known DENV-specific monoclonal antibody (mAb).
Results: The diversity of the libraries is ~3E6. Out of 228 sequenced clones, 89 encode DENV-1 peptide. Out of 39 sequenced, mAb selected clones, 38 contain the correct 7 amino acid epitope.
Conclusions: All the GFPDLs have sufficient diversity for identifying linear epitopes. Sequencing indicates that significant part (~40%) of phage clones displays a DENV-1 peptide. GFPDL technique enables the identification of minimum linear epitopes as demonstrated with mAb. The results show that the display of linear DENV-1 peptides on phage has been successful and that the concept of selection is working.

M110
FACILITATING CO-INFECTION DETECTION IN RESPIRATORY TRACT INFECTIONS (RTIS) WITH THE USE OF A MULTIPLEX ARRAY FOR THE SIMULTANEOUS DETECTION OF BACTERIAL AND VIRAL RESPIRATORY PATHOGENS

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Introduction: RTIs are a leading cause of mortality and morbidity worldwide amongst infectious diseases. The clinical manifestation of RTIs are highly similar but can be caused by a heterogeneous range of both viral and bacterial pathogens.Traditionally, treatment for such infections involved antibiotics on the assumption of bacterial infection.However, the repeated and improper use of antibiotics is the primary cause of the rise in bacterial resistance and decreased antibiotic effectiveness. The use of a methodology, which enables the simultaneous detection of multiple bacterial and viral respiratory pathogens, increases the screening capacity facilitating treatment efficiency and minimising unnecessary antibiotic consumption.This study reports the utility of a multiplex array assay to identify co-infections.This enables simultaneous detection of 22 respiratory pathogens from a single patient sample.
Methods: The assay is based on a combination of multiplex PCR coupled with biochip array technology applied to the Evidence Investigator analyser. Pre-screened respiratory samples (n=399) using the Taqman assay were obtained from a regional viral laboratory.These samples were further tested with the multiplex array assay for the presence of Influenza A/B, Respiratory Syncytial Virus A/B, Parainfluenza 1/2/3/4, Bocavirus, Coronavirus OC43/NL63/229E, HKU1, Adenovirus, Rhinovirus, Enterovirus, Metapneumovirus, Mycoplasma pneumoniae, Haemophilus influenzae, Moraxella catarrhalis, Legionella pneumophila, Streptococcus pneumoniae and Staphylococcus aureus.
Results: A high level of agreement was found between the methodologies.In addition,the multiplex array assay detected further pathogens (n=69) in a number of samples not previously reported by the Taqman assay.These samples were sent for confirmatory testing,where it was found that 94% of re-tested for co-infections were positive.
Conclusions: The data shows that this multiplex array facilitates the identification of co-infections.The capacity of simultaneously detecting 22 common respiratory pathogens from a single patient sample enhances diagnostic capabilities which in turn, may allow treatment to be tailored avoiding the inappropriate use of antibiotics and a reduction in the emergence of resistant bacteria.

M111
N-TERMINAL-PRO-B-TYPE NATRIURETIC PEPTIDE IN PATIENTS WITH INFECTIOUS DISEASES

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Background: N-terminal-pro-B-type natriuretic peptide (NT-proBNP) is a biomarker for help in diagnosis of congestive heart failure (CHF) and for differentiation of patients with acute dyspnea between conditions like CHF and lung diseases. NT-proBNP in sepsis can also serve for prognosis and for recognizing patients with heart problems.
Aim: Determine the usefullness of NT-proBNP measurement and evaluate the decision threshold of 125 ng/L for excluding cardiac dysfunction in our patient population with infectious diseases.
Methods: NT-proBNP was measured in serum of patients with infectious diseases (n=47), with electrochemiluminiscence on a Elecsys 2010, Hitachi, Roche Diagnostics analyzer. Patients were divided into four groups based on diagnosis: sepsis, pneumonia, other acute infectious diseases without CHF and acute infectious diseases with CHF. Statistical analysis was done in MedCalc.
Results: NT-proBNP concentration in groups were as follows (median, 25-75th percentile): sepsis (n=18) 4692,0 ng/L (2722,0-9317,0); pneumonia (n=6) 396,7 ng/L (79,1-1042,0); other acute infectious diseases without CHF (n=16) 217,5 ng/L (82,5-1689,5); acute infectious diseases with CHF (n=7) 16278,0 ng/L (6753,0-53834,8). NT-proBNP values showed statistically significant difference between all four groups (ANOVA), but for pairwise comparisons (Student-Newman-Keuls test) the only significant difference was found for the group with acute infectious diseases with CHF and the other three groups.
Conclusions: Altough we found very high values and a statistically significant difference of NT-proBNP in the group of acute infectious diseases with CHF there is an overlap between these values and the sepsis group. Also the recommended cut-off value of 125 ng/L should always be used in conjuction with medical history and clinical findings, especially in patients with infectious diseases.

M112
HPV PREVALENCE AMONG HEALTHY ITALIAN MALE SEXUAL PARTNERS OF WOMEN WITH HPV INFECTION

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Background: Genital human papillomavirus (HPV) is the causative agent of cervical cancer and is responsible for one of the most common sexually transmitted infections. The main risk factor for infection of the female population is the heterosexual transmission with HPV infected partners. While procedures to be applied to HPV infected women are well-established, there is often less focus and less sensibility in terms of both diagnostic and therapeutic strategies with regard to the male population. The aims of the present study were: 1. to evaluate what type of specimen collection is more suitable to investigate HPV infection in male 2. to determine the prevalence and the genotype distribution of HPV in the male partners of HPV infected women.
Methods: 146 asymptomatic men whose partners had presented cervical HPV infection were enrolled. HPV infection was investigated in urethral swab and/or urine and sperm samples. HPV presence was determined by detection of HPV DNA by a commercial real-time PCR; positive samples were genotyped by pyrosequencing (HPV sign; Diatech, Jesi, Italy).
Results: A total of 69 subjects collected both urine and sperm specimens, 55 subjects only urine, 2 subjects only sperm, 17 subjects underwent urethral swab collection and 3 subjects collected urine sperm and swab. The overall prevalence of HPV infection was 39% in urine, 43% in sperm and 50% in swab samples. With regards to 69 subjects with urine and sperm samples it was found that 19 subjects had both urine and sperm positive, 10 had urine-positive/sperm-negative, 9 had urine-negative/sperm-positive and finally 31 had both urine and sperm negative. Therefore, the HPV prevalence was 42% in urine, 40.5% in sperm; when the positivity of at least one test was considered, the prevalence was 55%. The prevalence of high risk HPV was 58% and HPV-16 proved to be the most prevalent viral type.
Conclusions: Our data indicate that men have a better compliance in collection of urine and sperm samples rather than urethral swabs. The analysis of both urine and sperm improves HPV detection. HPV related disease is a clinical infection of the couple and the high prevalence of HPV infection in males shown in our study warrants a much higher attention to the couple than the one currently afforded.

M113
EVALUATION OF A NEW SYPHILIS ASSAY ON A BECKMAN COULTER AU 480 ANALYZER

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Background: Syphilis is a sexually transmitted disease caused by the spirochetal bacterium Treponema pallidum subspecies pallidum. The route of transmission of syphilis is almost always through sexual contact, although there are examples of congenital syphilis via transmission from mother to child in utero. The serologic tests for syphilis infection have been performed manually, but the procedures are time-consuming and interpretations may be subjective. Recently, automated assays were developed for rapid and efficient testing for syphilis infection. The purpose of this study is to validate the analytical and clinical performances of Syphilis TP Latex on Beckman Coulter AU 480 analyzer.
Methods: The Syphilis TP Latex is an immunoturbidimetric assay, using microparticles coated with Treponema pallidum fixed on the surface of polystyrene latex particle which agglutinates by an antigen-antibody reaction when anti-TP exists in the specimen. Beckman Coulter AU 480 analyzer is a random-access analyzer. Modified CLSI protocols were adopted. Acceptance criteria as total imprecision were ≤5% for negative samples and ≤4% for positive samples. In comparison to commercial methods, sensitivity must be ≥99.5% and specificity ≥99.5%.
Results: Total imprecision (36 days) gave CV% at 6 U/L lower than 5%, and CV% at 11U/L and 48 U/L lower than 4%. LOD was 1.2 U/L. LOQ was 4.5 U/L. The test was linear from 0 U/L up to 80 U/L. No prozone up to 9120 U/L. On board calibration stability and reagent on board stability were up to 36 days. Compared vs a commercial immunoassay method (n=301), specificity was 100%, sensitivity was 81%. Compared vs reference methods (TPHA, RPR, EIA, VDRL) (n=301), specificity was 100%, sensitivity was 100%. Bilirubin (70 mg/dL), Hemoglobin (500 mg/dL) and Triglycerides (1000 mg/dL) did not interfere.
Conclusions: Analytical and clinical performances of Syphilis TP Latex on Beckman Coulter AU 480 analyzer meets the requirements for its use as screening assay. It shows clinical sensitivity as good as the predicate device and a better specificity. This assay can be used on a single high volume chemistry analyzer, which is highly valuable for optimizing workflow and efficiency in today's laboratory.

M114
FREQUENCY OF MOST IMPORTANT SEXUALLY TRANSMITTED DISEASES AND METHODOLOGICAL IMPLICATIONS

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Background: Persisting High Risk (HR) HPV infection is the most important risk factor to develop cervical cancer. Infections due to C. trachomatis (CT), N. gonorrhoeae (NG), U. urealyticum (UU), U. parvum (UP), M. hominis (MH) and M. genitalium (MG) are responsible of cervicitis that can cause infertility. Several studies identify Mycoplasma and Ureaplasma as pathogens causing infections during pregnancy and involved in pulmonary complications in pre-term infants. Aim of this study was: 1) to estimate CT, NG, MH, MG, UU, UP and HPV prevalence to select appropriate age group that should be screened; 2) to compare Real Time (RT) PCR and endpoint PCR sensitivity; 3) to check sample suitability and its influence on analytical sensitivity.
Methods: DNA from 933 samples (135 urine, 110 seminal fluids, 135 urethral swabs and 553 cervical swabs) was amplified with RT-PCR to detect CT (Artus, Qiagen), NG, MH, MG, UU and UP (Nuclear Laser). 300 cervical swabs were analyzed also with PCR endpoint to detect CT, NG, MH, UU/UP and HPV (using L1 as gene target and reverse hybridization technology for genotyping; Innogenetics). HPV results were reconfirmed with PCR endpoint (primers towards L1 and E6-E7 genes) and genotyped with microarray technology (Biotech).
Results: With RT-PCR overall positivity for CT, MH, MG, UU and UP was respectively 8.6%, 8.8%, 2.25%, 9.1% and 27.8%. PCR endpoint positivity (only females), compared to RT-PCR positivity, for CT, NG, MH and UU/UP was respectively: 4.3% (vs 5.7%), 7,3% (vs 4%), 7.3% (vs 11.3%) and 29.3% (vs 41.7%). HPV resulted in 17.33% of women. Age groups with higher positivity are: 23-32 years old (10.9%) for CT, < 23 years old for UU (21.6%) and 18-27 years old (35.7%) for HPV. More frequent co-infections were found between CT and MG; UU, UP and MH; HPV and UU/UP.
Conclusions: Compared to PCR endpoint, RT-PCR is more sensitive, faster and it allows a better laboratory organization. RT-PCR and microarrays are preferred diagnostic methods for the future in view of an increase of examinations. Data show that women up to 27 years old have to be screened for CT. HPV test and genotyping should be performed in women aged between 35 and 50 years, because infection persistence with HR genotypes cause lesions leading to cervical cancer.

M115

KINETICS OF TOXOPLASMA IGG SEROCONVERSION IN A SWISS PREGNANT WOMEN POPULATION

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Background: Whereas a high Toxo IgG avidity result excludes an acute toxoplasmosis a low avidity result does not diagnose a recent toxoplasmosis. The goal of this evaluation was to determine if Abbott ARCHITECT Toxo IgG antibody titer ratios, using paired sera, can assist in the detection of a recent toxoplasmosis in patient samples containing Toxo IgM antibody and low avidity Toxo IgG.
Methods: Archived patient samples (n=284) were selected from pregnant women (n=53) with documented recent seroconversion for toxoplasmosis and tested by the ARCHITECT Toxo IgG, IgM and IgG avidity assays. ARCHITECT IgG titer ratios from paired sera were calculated as follows: ARCHITECT Toxo IgG titer sample 2/ARCHITECT Toxo IgG titer sample 1, where sample 2 is ideally drawn 2-4 weeks after sample 1. Both samples were tested on the same instrument, run and day. A significant increase in ARCHITECT Toxo IgG antibody titer was defined as a Toxo IgG titer ratio > 2.0 and was considered the titer ratio cutoff.
Results: In samples from pregnant women undergoing seroconversion for toxoplasmosis, detection of Toxo-specific IgM before IgG occurred in 6/53 patients (11%). Analysis of the ARCHITECT Toxo IgG titer ratios was possible in 36 patient cases: 18 cases were untreated and 18 cases were treated with Rovamycine (spiramycin). In untreated cases, sensitivity of detection of a recent toxoplasmosis, defined as Toxo IgG seroconversion within the past 3 months, was 94% (17/18) by the ARCHITECT Toxo IgG titer ratio. In treated cases, the sensitivity of detection of a recent toxoplasmosis drops to 61% (11/18).
Conclusions: In cases of pregnant women not treated with Rovamycine, where Toxo IgM and low avidity IgG were present, if the ARCHITECT Toxo IgG titer ratio was > 2.0 in paired sera ideally drawn 2-4 weeks apart, the infection likely occurred within the past 3 months from the first serial bleed draw. In untreated cases, where the ARCHITECT Toxo IgG ratio was <2.0, no conclusion can be drawn. The sensitivity of detection of a recent toxoplasmosis in treated cases is presumably less due to the likely attenuation of the Toxo IgG response by Rovamycine (spiramycin) therapy.

M116

HIGH POST-TEST PROBABILITY OF PROCALCITONIN AND MID-REGIONAL PRO-ADRENOMEDULLIN PLASMA LEVELS IN THE DIAGNOSIS OF SEPSIS

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Background: The early diagnosis of sepsis plays a central role in patient management. Many biomarkers have been proposed at this purpose. In the present study the combined measurement of procalcitonin (PCT) and mid-regional pro-adrenomedullin (MR-proADM) and their appropriate cut-off values in sepsis patients were evaluated.
Methods: Plasma samples from 404 consecutive sepsis patients, 258 patients with SIRS and 30 healthy individuals, admitted at the University Hospital Campus Bio-Medico of Rome, were collected. PCT and MR-proADM were measured with commercially available immunoluminometric assays (Brahms, Hennigsdorf,Germany). Receiver Operating Characteristic (ROC) curves. Areas Under the Curve (AUCs) and post-test probability after the combination of both test were calculated (MedCalc 11.6.1.0. Software; Mariakerke, Belgium)
Results: PCT and MR-proADM mean values were 0.05 ng/mL (0.02- 0.26) and 0.58 nmol/L (0.30-1.42) in the healthy controls, 0.17 ng/mL (0.01-0.81) and 0.62 nmol/L (0.22-2.48) in SIRS patients without blood culture positivity and 12.29 ng/mL (0.02-413) and 3.52 nmol/L (0.6-38.68) in sepsis. In the 404 septic patients the areas under the curve (AUCs) for PCT and MR-proADM were 0.939 and 0.962 respectively, with statistically significant difference between the two areas of 0.0229 (P=0.0177). Healthy controls and non–infectious SIRS were clearly distinguished from sepsis patients using the following cut-off values: 0.50 ng/ml for PCT and 1 nmol/L for ADM. The combined use of PCT and MR-proADM gave a post-test probability of 0.996 in the cohort of all septic patients.
Conclusions: Data from this study demonstrates that the combined use of PCT and MR-proADM, may improve the early diagnosis of sepsis, while till now the plasma level of MR-proADM has been proposed only as a prognostic marker in sepsis or septic shock.

M117

DIAGNOSTIC AND PROGNOSTIC VALUE OF PROCALCITONIN: MEASUREMENT IN SEPTIC CONDITION

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Systemic inflammatory response syndrome (SIRS) as a specific metabolic and immunological reaction was determined by the activity of many bacteria in conditions such as sepsis as well as septic shock. Fever or fall in temperature, tachycardia, shortness of breath as well as increasing or decreasing of polymorphonuclear leukocytes (PmN WBC) with onset of their non-differentiated forms in the peripheral blood are clinical manifestations of SIRS. However, this reaction can be caused by other conditions of non-infectious nature such as a malignant disease, metabolic disorders, trauma etc. It is crucial to determine clinical and laboratory parameters to distinguish the systemic inflammatory response syndrome in infectious diseases from non-infectious conditions. According to the data collected recently, there is not relevant either clinical or laboratory indicator of sepsis although there are many interesting clinical trials where the correlation between blood cytokines and sepsis was examined. Procalcitonin (PCT) is one of the newest potent useful markers of diagnostic value for sepsis. Many authors described in clinical trials diagnostic as well as prognostic value of PCT blood levels depending on disease severity and involving infection in the study population. In many clinical trials, PCT was examined as a valid diagnostic measure in the group of patients with acute, severe inflammation in addition to IL-6 as well as IL-8. PCT was assessed by many authors in clinical trials as a biochemical parameter superior to others named ‘’gold standard” such as C-reactive protein (CRP), fibrinogen (Fib.), D-Dimer (DD2), SE or ESR and leukocyte count (WBC).

M118

AVERAGE LEVEL OF PCT (PROCALCITONIN) IN BLOOD OF PATIENTS WITH GRAM-POSITIVE AND GRAM-NEGATIVE ETIOLOGY OF SEPSIS

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Background: Sepsis is a systemic inflammatory response in the presence of a bacterial infection. PCT (procalcitonin) is a marker of the inflammatory response. Inflammatory process is induced by gram-negative and gram-positive bacteria in different ways depending on the differences of outer membrane composition of the bacterial cell. Therefore both, inflammatory reaction and its marker PCT, can be different. Aim of the present research is to determine whether there is a difference in the average level of PCT in patients blood depending on whether sepsis is caused by gram-negative or gram-positive bacteria.
Materials and methods: Measuring of PCT was performed using electrochemiluminiscence immunoassay (Roche Diagnostics, Cobas e411). The reference range was <0,5 ng/mL. Sepsis pathogens were isolated and identified using standard microbiologic laboratory protocols (BactALERT, Beckton Dickinson). WHONET software was used for retrospective identification of septic patients.
Results: The data was obtained from HIS for 2010-2012. The study sample consisted of 90 patients with confirmed sepsis and positive blood cultures. Among investigated patients: 51 (56.7%) were with sepsis caused by gram-negative bacteria, 39 (43,3%) were with sepsis caused by gram-positive bacteria. Patients average value of PCT: with gram-negative sepsis – 43,8 ng/mL, with gram-positive sepsis – 16,1 ng/mL. Average value of PCT in the most frequently occurred gram- negative and gram- positive sepsis: klebsella pneumoniae (G-) - 80,6 ng/mL, escherichia coli(G-) – 38,4 ng/mL, streptococcus pneumoniae(G+) – 33,2 ng/ml, pseudomonas aeruginosa (G-) 23,5 ng/mL, staphylococcus aureus (G+) – 13,6 ng/mL.
Conclusion: Concentration of PCT in blood of patients with sepsis caused by gram-negative bacteria is 2,7 times higher than in blood of patients with sepsis caused by gram-positive bacteria. The highest average values of PCT were detected in sepsis caused by gram-negative bacteria: klebsella pneumoniae - 80,6 ng/mL and escherichia coli – 38,4 ng/mL.

M119
INTERFERENCE OF AUTOMATED CEREBROSPINAL FLUID CELL COUNTS PERFORMED ON THE SYSMEX XT-4000I BY YEAST AND BACTERIA

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Background: Traditionally, cerebrospinal fluid (CSF) cell counts have been performed using manual methods which are time-consuming, labour intensive, require experience and are wrought with significant imprecision. Analyzers with fluid channels have been developed to automate fluid cell counts. However, it has been reported that yeast can interfere with white blood (WBC) cell counts performed on haematology analyzers using similar technoogy. This study investigated the potential interference of CSF cell counts by bacteria and yeast in an analyzer using fuorescent flow cytometry.
Methods: Several aliquots of sterile molecular grade water were spiked with different standard microorganisms to 0.5 McFarland and analyzed using the fluid channel of the Sysmex XT 4000-i. This was repeated using pooled CSF spiked with the microorganisms that showed significant interference in the initial experiment.
Results: Pseudomonas aeroginosa (P.aeroginosa) and Candida krusei (C.krusei) resulted in a marked increase in WBC count when added to molecular grade water and pooled CSF. Majority of what was counted as WBCs were identified as mononuclear and polymorphonuclear cells in the samples spiked with P.aeroginosa and C.krusei respectively. There was no effect on red blood cell count.
Conclusions: P.aeroginosa and C.krusei can falsely elevate CSF WBC counts and give wrong differential counts in analyzers using fluorescent flow cytometry. We hypothesize that the nucleic acid binding dyes used in these analyzers bind nucleic acid in some bacteria and affect the lateral fluorescent scatter used in enumerating and differentiating WBC counts. This has the potential to mislead clinicians when investigating possible causes of central nervous system infections. Laboratories using automated analyzers for routine CSF cell counts should be aware of any potential interference by bacteria and yeast.

M120
NOVEL TREATMENT FOR INFLAMMATION AMONG SUBJECTS IN RURAL COMMUNITY IN NIGERIA

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Background: Accidental cuts, wounds, abrasion and bruises are common features in developing countries with law standard of hygiene and lack of first aid treatment. Such infections eventually lead to inflammation. The purpose of this study was to determine the effect a new lotion in treating inflammations arising from wounds, cuts and abrasions.
Methods: Two hundred and fifty subjects (comprising 80 males and 70 females all young adults), encountered during rural community during health campaign who had inflammations were treated with the lotion, composed of salicylic acid 4 percent WT/VOL, glycerin 3 percent VOL/VOL and absolute ethanol 100 mls vol. the lotion was applied to the affected sites using cotton wool bud. Subjects gave their approval verbally and strict confidentiality was assured them before commencement of study.
Results: Satisfactory clinical response was achieved within 7 days of treatment, as the inflammation disappeared and the wounds were healed.
Conclusion: This study presents, the effect of treating inflammation using this novel lotion.

M121

PERFORMANCE EVALUATION OF A PROTOTYPE CMVG ASSAY ON THE ADVIA CENTAUR SYSTEM

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Objective: Cytomegalovirus (CMV) is a member of the herpesvirus family. Diagnosing CMV infection is aided by serological testing. We report the evaluation of exposure to CMV using a fully automated CMVG assay* on the ADVIA Centaur® system. The assay uses viral lysate in a chemiluminescent immunoassay format for the qualitative detection of CMV-specific IgG antibodies in serum or plasma.
Methods: The diagnostic sensitivity and specificity of the assay were evaluated by testing a commercially available seroconversion panel and approximately 1100 blood bank serum samples. The results were reported in index values as reactive (≥1.00), equivocal (≥0.75, <1.00), and nonreactive (<0.75). All samples were run against the VIDAS CMV IgG assay as well. Discordant samples were tested on the IMMULITE® 2000 CMV IgG assay (Siemens). Precision was determined using a 10-day protocol on two systems, two runs/day.
Results: The ADVIA Centaur CMVG assay detected IgG reactivity on the same bleed as the VIDAS CMV IgG assay on the Boston Biomedica, Inc., panel. Among the blood bank serum samples, Lot 1 showed negative agreement of 99.41%, positive agreement of 98.70%, and the percent of samples in the equivocal zone was 0.18%. After resolution testing of 12 samples, sensitivity was 100% and specificity was 99.71%. For Lot 2, negative agreement was 98.53%, positive agreement was 98.45%, and the percent of samples in the equivocal zone was 0.18%. After resolution testing of 17 samples, sensitivity was 100% and specificity was 99.14%. For Lot 3, negative agreement was 98.52%, positive agreement was 98.57%, and the percent of samples in the equivocal zone was 0.09%. After resolution testing of 16 samples, sensitivity was 100% and specificity was 99.13%. The ADVIA Centaur CMVG assay had a total %CV of <11% over a 10-day period with three lots of reagents.
Conclusion: The results of these studies show good performance of the fully automated ADVIA Centaur prototype CMVG assay in comparison with the VIDAS CMV IgG assay.
For investigational use only. The performance characteristics of this product have not been established. Not available for sale.

M122

INCIDENCE OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES IN AN AREA OF BUENOS AIRES AREA (ARGENTINA)

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Background: More than 100 fully characterized human papillomavirus (HPV) types are distributed in five genera of the Papillomaviridae family. Types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66 are considered to be carcinogenic to humans and are classified as high risk type. HPV testing seems to be the best tool for cervical cancer screening as the negative predictive value of the methods currently employer is near 100%, which allows the screening to be performed at longer intervals of time.
The aim of the study was to describe the genotype incidence of HPV infection in area of Buenos Aires in order to provide useful information for the selection of diagnostics tests to be performed as well as to carry out prophylactic strategies through vaccination and sexual health education programs.
Methods: The study include sample of 427 women suspected of HPV genital infection in Buenos Aires area. HPV DNA in samples was analyzed by PCR amplification (primers MY09 and MY11) followed of DNA sequencing using the same primers. DNA sequences were analyzed using the ChromasPro and BLAST programs. Both, cell samples or tissue samples were employed.
Results: Among these 427 samples 108 were HPV positive. Genotypes detected were: high risk genotypes: 70 (65%), low risk genotypes 34 (31%) and co-infections and indeterminate genotype: 4 (4%). The most prevalent high risk genotypes were by descending order of frequency HPV 16 (11.11 %), HPV 31 (11.11%), HPV 58 (10.19%) and HPV 18 (6.48%). Most prevalent low risk genotypes were HPV 6 (15.74%), HPV 11 (10.19%) and HPV61 (3.7%).
Conclusions: High risk genotypes were found to be widely distributed in the selected population including the low frequency genotypes. Unlike other works HPV 16 and HPV 18 which are commonly associated with high risk were not the most prevalent. The incidence of HPV 58 was unexpectedly high. This study provides data about the HPV incidence of genital associated genotypes demonstrating the importance of the employment of diagnostic tests to detect all high risk genotypes and to select those with good sensitivity for high risk HPV of low frequency. Current bivalent and tetravalent vaccines should thus be improved in order to provide protection against all circulating genotypes.

M123

HUMAN PAPILLOMA VIRUS (HPV) IN MAN: DOES INFECTED SPERM PLAYS A ROLE IN MALE INFERTILITY?

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Background: Human Papillomavirus (HPV) are the most common infectious agents of sexually transmitted infections (STIs). They are the cause of genital warts and cancerous processes in men and women. There are more than 40 HPV "high risk" involved in STIs .
Genotypes 16 and 18 are involved in more than 90% of the carcinogenic process. In men, the virus is found in the glans and the sheath but also in the vas deferens, epididymis, testis and the seminal fluid. The presence of HPV in the semen is frequently associated with some impairment of sperm parameters. However no real correlation with male infertility has been demonstrated.
Objectives: Investigate the existence of a relationship between the presence of azoospermia and HPV DNA in the testicular tissue biopsies. Investigate the prevalence of HPV in the semen of patients sperms alterations.
Methods: Prospective study to investigate the presence of HPV (genotyping and patients supported through an infertility of the couple.) HPV research on two types of cell matrix: semen and testicular tissue. Different sperm profiles studied : Azoospermia original excretory and secretory OligoAsthénoTératozoospermie (OAT), isolated asthenozoospermia. The principle of genotyping is based on the extraction and PCR amplification of one DNA fragment in presence of specific primers placed in wells on a DNA microarray. The biochip allows the detection of 24 genotypes (18 oncogenic HPV "high risk" and 6 "low risk")
Results: Human papillomavirus have been detected in two samples (16%) containing sperm (N = 12). The HPV virus is not found in testicular samples. HPV 16 was the only genotype detected.
Conclusion: Our results show the presence of the HPV virus in the sperm sample Our small series shows the presence of HPV in patients with multiple sperm alterations. HPV is one of the rare virus tumor for which there is prevention. Vaccination is usually not recommended in man. There are no precise data on the use of sperm carrying HPV virus in In Vitro Fertilisation technics, espacially the possibility of micro-injecting this sperm into the oocyte. The presence of HPV in sperm and its possible involvement in the origin of abnormal sperm and/or abnormal development (embryonic miscarriage) should be evaluated.

M124

MYCOPLASMA GENITALIUM FREQUENCY IN SPERM SAMPLES OF A MEN POPULATION CONSULTING FOR INFERTILITY. NEW MULTIPLEX PCR METHOD FOR THE SIMULTANEOUS DETECTION OF CHLAMYDIAE TRACHOMATIS (C.T), NEISSERIA GONORRHOEAE (N.G) AND MYCOPLASMA GENITALIUM (M.G)

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Background: 3 species of mycoplasmas could be isolated in urogenital tractus with pathogenic potential: Mycoplasma genitalium (M.g), Mycoplasma hominis (M.h) and Ureaplasma urealyticum (U.u). Mycoplasmas are recognized as causative agent of sexually transmitted infections (STI). These bacteria could adhere to the spermatozoa involving a mobility decreased or a partial immobility by agglutination. M.g was not considered in infectious assessment for several years. it is very difficult to culture. So, Molecular diagnostic is a method of choice to detect M.g in routine.
Objectives •To determine frequency of M.g in a population of infertile men and to study relationship between the presence of this bacteria and the spermogram values.•Validate the clinical performances of a molecular diagnostic assay on a new sample type (sperm). •Comparison between frequency of M.g and of Mycoplasmas causing male genital infections.
Methods: •Screening : M.h , U.u and M.g. M.h and U.u by culture in 2 to 4 days •The Dx CT/NG/MG assay is a multiplex nucleic acid in vitro amplification testing used to detect M.g : The procedure takes: a non automated DNA extraction followed by an amplification/detection of target DNA by real-time PCR •M.g probe, targets a sequence in the MgPa gene. When the target DNA is present, fluorescence intensity increases and is measured by the optical module of the thermocycler Dx Real-Time System.
Results: •Frequency of bacteria in sperm of infertile men:9,3% for U.u 1,4% for M.h 0,6% for M.g 0,6% for U.u + M.h •No correlation found between positive specimens and spermogram features.
Conclusion: •This study allows to validate the use of sperm samples with the Dx CT/NG/MG assay •Preliminary results seem to show that M.h et U.u are more present than M.g in men consulting for infertility. •The frequency of M.g has no impact on the spermogram quality in this study. •The presence of M.g is associated with high risk transmission of tubular pathology that may cause female infertility. •The detection of M.g in routine should be proposed in the microbiology assessment of symptomatic patients. •Even the most difficult bacteria to culture with traditional techniques, would be detected, including in specimens considered as difficult.

M125
EVALUATION OF A SEXUALLY TRANMITTED INFECTIONS (STI) MULTIPLEX ARRAY FOR THE SIMULTANEOUS DETECTION OF BACTERIAL, VIRAL AND PROTOZOAN STI PATHOGENS

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Introduction: Most STIs can be treated successfully, however many are asymptomatic and can go untreated leading to further transmission of infection or complications in later life. Although many different pathogens cause STIs, some display similar or overlapping symptoms, thus co-infections may remain undiagnosed. This study reports the evaluation of an assay - based on innovative multiplex PCR coupled with biochip array technology- which enables simultaneous detection of 10 STI pathogens from a single sample. This methodology increases detection capacity and has the potential to improve patient outcomes, therefore reducing the economic burden posed by STIs.
Methods: DNA extracted from a urine and urogenital swab sample cohort (n=300), obtained blind from a regional viral laboratory (RVL) for assay validation, were tested for the presence of Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Herpes simplex 1 and 2 (HSV1, HSV2), Treponema pallidum (TP), Trichomonas vaginalis (TV), Haemophilus ducreyi (HD), Mycoplasma genitalium (MG), Mycoplasma hominis (MH) and Ureaplasma urealyticum (UU) using Randox's STI Multiplex Array on the Evidence Investigator analyser. The protocol involves amplification of DNA using highly sensitive primers, followed by spatial separation and detection using biochip array technology. Controls are included for each step to ensure result reliability.
Results: Results correlated with those obtained by RVL. Of the 300 clinical samples, 203 tested positive for an infection and of these, 20% harboured at least one additional infection (15.5% double, 4% triple and 0.5% quadruple infections). The most prevalent co-infections were for CT+MH (10/203, 5%), HSV1+UU (9/203, 4.5%), MH+UU (8/203, 4%) and CT+UU (6/203, 3%). CT and NG were co-infected in 2% of samples (4/203). All of the MH positive samples were co-infections.
Conclusions: The data indicate that this STI Multiplex Array facilitates the identification of co-infections. With the capacity of testing simultaneously 10 common STI pathogens from a single sample, diagnostic capabilities are increased, which may allow treatment to be tailored, reducing broad spectrum antibiotic use and, in turn, the build-up of antibiotic resistance.

M126
EVALUATION OF A NEW EUROIMMUN ANTI-BORRELIA SELECT ELISA (IGM/IGG) TEST KIT

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Background: The diagnosis of Borrelia infections in Estonia have increased very rapidly. For screening anti-Borrelia antibodies MIKROGEN (Germany) recomWell Borrelia IgM/IgG ELISA is used routinely, the test kit contains recombinant Borrelia burgdorferi antigens (IgM: OspC, p41/intern, VlsE; IgG: p100, OspC, VlsE, p18). The new ELISA test kit from EUROIMMUN (Germany) Anti-Borrelia Select ELISA (IgM or IgG) is based on a mixture of very specific recombinant antigens of different human pathogenic Borrelia stains.The aim of this study was to compare the new ELISA test kit from EUROIMMUN with the routinely used ELISA screening test (MIKROGEN recomWell Borrelia IgM/IgG) and a confirmatory test (EUROIMMUN Anti-Borrelia EUROLINE-RN-AT (IgM/IgG)).
Methods: 67 preselected samples from patients, which were positive (anti-Borrelia IgM or IgG or both) with the routinely used ELISA screening or confirmatory test were tested in parallel with the new Anti-Borrelia Select ELISA IgM and IgG.
Results: Borrelia burgdorferi IgM antibodies: Both ELISA test kits gave the same positive results for 11, negative results for 23 and borderline results for 2 samples. MIKROGEN recomWell Borrelia ELISA gave 32 and EUROIMMUN Anti-Borrelia Select ELISA 13 positive results. The confirmatory test gave 26 positive results. The confirmatory test and recomWell ELISA gave the same positive results for 18 samples (69%), and with Select ELISA for 9 samples (35%). The sensitivity for EUROIMMUN kit was 43% and for MIKROGEN kit 75%. The specificity was 95% and 65% accordingly. Borrelia burgdorferi IgG antibodies: Both ELISA test kits gave the same positive results for 18, negative results for 37 samples. MIKROGEN recomWell Borrelia ELISA gave 29 and EUROIMMUN Anti-Borrelia Select ELISA 18 positive results. The confirmatory test gave 32 positive results. The confirmatory test and recomWell ELISA gave the same positive results for 25 samples (78%), and with Select ELISA for 18 samples (56%). The sensitivity for EUROIMMUN kit was 58% and for MIKROGEN kit 78%. The specificity was 100% and 96% accordingly.
Conclusion: The new ELISA test kit from EUROIMMUN (Germany) Anti-Borrelia Select ELISA (IgM or IgG) is less sensitive compared with MIKROGEN recomWell Borrelia IgM/IgG ELISA.

M127

SWITCHABLE LANTHANIDE LUMINESCENCE LABEL TECHNOLOGY IN RAPID AND SIMPLE PCR ASSAYS FOR BROAD-RANGE AND PSEUDOMONAS BACTERIA DETECTION FOR SEPSIS DIAGNOSTICS

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Background: Sepsis is caused by pathogens invading the blood circulation and it constitutes a world-wide health concern. Urgent diagnosis and treatment by antibiotics are essential as severe sepsis may rapidly lead to organ dysfunctions and death. As a part of the EU FP7 funded ACUSEP project, real-time PCR methods were developed for rapid broad-range and Pseudomonas bacteria detection for approaches in sepsis diagnostics.
Methods: PCR primer and probe pairs were designed for a part of the highly conserved bacterial gene 16S rRNA thus enabling broad-range detection of bacteria. Another probe pair was designed to detect Pseudomonas genus bacteria using a less conserved region of the same amplicon. State-of-the-art switchable lanthanide luminescence label technology was utilized to monitor the probe hybridization to the amplification product. For each probe pair, one probe was conjugated at 3' to a non-luminescent chelate carrying Eu3+ and the other probe at 5' to a light harvesting antenna ligand molecule. The probes were designed to hybridize next to each other to the PCR amplification product thus enabling the assembly of a highly luminescent lanthanide complex. Luminescence signal was measured at time-resolved mode to follow the increase of the target DNA. Amplification reactions were performed either on traditional PCR plates using a separate reader or with dry-reagent chips in an automated instrument integrating amplification and signal measurement (GenomEraTM analyzer, Abacus Diagnostica).
Results: The broad-range bacteria assay detected extracted DNA from 21 sepsis causing target bacteria in the PCR plate format. The pseudomonas assay was specific for the Pseudomonas genus bacteria. On the dry-reagent chips, the detection limit was 50 CFU of Escherichia coli with the broad-range assay and 10 CFU of Pseudomonas sp. bacteria with the pseudomonas assay.
Conclusions: The current assays utilizing switchable lanthanide luminescence label technology can be used for simple and rapid bacteria detection for example in approaches for sepsis diagnostics. The possibility to use the same amplicon for the broad-range and Pseudomonas bacteria detection serves as a starting platform for high level of multiplexing required in sepsis diagnostics.

M128

IMPORTED MIXED MALARIA INFECTION DUE P. FALCIPARUM AND P. OVALE

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Introduction: Malaria is a public health problem affecting 200 million people worldwide. In the last decades the incidence of imported malaria have been increased in many European countries, been P. falciparum responsible for more than 70% of all cases".Infection by P.ovale has been considered a disease with a low prevalence and benign course.
Objective: Review clinical cases of mixed malaria parasitation due P.ovale and P. falciparum.
Methods and results: Cases report: 1) Male, 31 years old, native from Costa de Marfil , resident in Spain for 4 years, who travel in the past month to Africa and return; he was seen in emergency department presenting headache, fever and vomit. Physical examination normal. Hemogram: WBC normal, RBC 5,38 x 106/ul, Hb 15,1 gr/dL, PLT 177 x 103/uL; in biochemical analysis : PCR 2,64, total bilirubin 2,01 mg/dL (direct 0,32 mg/dL, indirect 1,69 mg/dL) , LDH 238 UI/L. Coagulation TP 67,4%, INR 1,2, APTT 26 seg. Plasmodium antigen was positive. Blood smear examination detected intraerythrocytic parasites (ring forms, some with double parasitation), and matures schizonts. Some of the parasitized cells were oval and had fimbriated edges. The parasitation was 2% approximately. Multiplex PCR confirm the mixed parasitation. 2) Male, 42 years old, native from Mali, resident in Spain for 11 years. Last time in Africa 3 years ago. No personal history of any disease. He came to the emergency department referring epigastric pain, fever, malaise, and brown-yellow urine. Physical examination: dehydration, jaundice and hepatomegaly. Hemogram: WBC 4,07x103/uL, RBC 4,67 x 106/uL, Hb 12 gr/dL, PLT 20 x 103/uL; Biochemical analysis : PCR 22,04, total bilirubin 22,73 mg/dL (direct 12,71 mg/dL, indirect 10,02 mg/dL), LDH 690 UI/L. Coagulation TP 85,6%, INR 1, APTT 28,1 seg. Blood smear examination detected excessive break down of red blood cells, intraerythrocytic parasites. The parasitation was 5% approximately. Multiplex PCR confirm the mixed parasitation. Both patients were treated and progressed well.
Conclusions: This type of mixed parasitation it's not common in developed countries. It is important to know because of the increasing number of refugees from malaria endemic countries in Europe.

M129

THE NEW SEPSIS MARKER PRESEPSIN IS SUPERIOR FOR PROGNOSIS AND DISEASE MONITORING COMPARED TO PROCALCITONIN

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Background: CD14 is expressed on the membrane of monocytes and activates the TLR4-specific inflammatory reaction against infectious agents whereby soluble CD14 is released yielding presepsin (soluble CD14-subtype). The objective of our study was to examine the suitability of presepsin for prognosis and disease monitoring in patients with sepsis.
Methods: Presepsin and procalcitonin (PCT) were determined using commercial available methods at admission in the emergency room (ER), after 24 and 72 h in 140 patients with sepsis. APACHE II score was extracted from the clinical files. 119 healthy individuals served as control group. Primary endpoint was death within 30 days. The combined endpoint “major adverse event” (MAE) consisted of at least either the primary or at least one of the secondary endpoints (intensive care, mechanical ventilation or dialysis).
Results: Mean presepsin concentrations of the control group and the patient group were 159 (90% CI: 148-171) pg/mL and 2563 (90% CI: 1458-3669) pg/ml, respectively. Presepsin and APACHE II score but not PCT differed highly significant between patients with sepsis and patients with severe sepsis or septic shock (P <0.0001). The 30-day mortality was 16.4%, ranging from 2.7% to 39.4% between the 1st and the 4th quartile of presepsin concentration. Presepsin and APACHE II score but not PCT demonstrated a strong relationship with 30-day mortality. Receiver operating curve analysis (ROC) of presepsin, APACHE II score and procalcitonin revealed AUCs of 0.878 (95% CI: 0.801-0.934), 0.815 (95% CI: 0.709-0.895) and 0.668 (95% CI: 0.570-0.757), respectively. The course of presepsin concentration during the first 72 hours was strongly associated with effectiveness of treatment and patient`s outcome. Presepsin increased significantly during the first 72 hours in patients with worse outcome (occurring of MAEs) whereas the concentration decreased in patients without MAEs.
Conclusion: Presepsin demonstrated a strong relationship with disease severity and outcome. The prognostic accuracy was superior compared to PCT. Presepsin provided more reliable prognosis and early prediction of 30-day mortality already at admission in the ER. The course of presepsin during the first 72 hours was related to patient`s outcome

M130

SMALL-DENSE LDL CHOLESTEROL/LARGE-BUOYANT LDL CHOLESTEROL RATIO AS AN EXCELLENT MARKER FOR INDICATING LIPODYSTROPHY IN HIV-INFECTED PATIENT

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Background: Advance of HIV treatment with the highly active antiretroviral therapy displays HIV-associated lipodystrophy, abnormal body-fat distribution. The aim of this study was to examine whether the lipids, lipoproteins and theirs ratio were predicting factors for lipodystrophy.
Methods: Whole-body fat compositions of total 79 HIV-positive patients receiving stavudine containing antiretroviral regimens (34 males and 45 females) were determined by using dual-energy X-ray absorptiometry scan. Lipodystrophy in HIV-infected patient was defined as the ratio between the trunk fat mass to the lower limb fat mass greater than 2.28. Blood samples were analyzed for total cholesterol (TC), triglycerides, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), small-dense LDL-C (sdLDL-C), apolipoproteinAI (apoAI), apolipoproteinB (apoB), lipoprotein(a), and CD4 cell counts. We calculated the large-buoyant LDL-C (lbLDL-C) by subtracting sdLDL-C from LDL-C concentrations. Ratios of TC/ HDL-C, apoB/apoAI and sdLDL-C/lbLDL-C were calculated.
Results: Twenty-six HIV-infected patients were classified into lipodystrophy. Using univariable analysis, the mean concentrations of triglycerides, HDL-C, sdLDL-C, apoB, TC/ HDL-C, apoB/apoAI and sdLDL-C/lbLDL-C ratios showed the significant differences between patients with and without lipodystrophy (P <0.02). However, only sdLDL-C/lbLDL-C ratio was identified the significant predictor of lipodystrophy (P <0.001) by using backward stepwise logistic regression analysis. The area under the ROC curve for sdLDL-C/lbLDL-C ratio was 0.817 (95% CI: 0.713 – 0.921, P <0.001). At a ratio of 0.554, the odds ratio is 17.8 (95% CI: 5-57) with a likelihood ratio of 5.5.
Conclusions: The results of this study show that the ratio of sdLDL-C and lbLDL-C is an excellent marker for indicating lipodystrophy in HIV-infected patients. The early detection of lipodystrophy in patients would be performed for taking into account interruptions and changes of therapy.

M131

PREVALENCE AND ANTIFUNGAL SUSCEPTIBILITY OF CANDIDA SPECIES ISOLATED FROM PATIENTS IN TWO SICILIAN HOSPITALS: A RETROSPECTIVE PILOT STUDY

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Background: Although Candida albicans remains the most pathogenic species among those belonging to the genus Candida, in recent years, we have witnessed a dramatic increase in fungal infections caused by other species which are commonly referred to as "non-albicans" Candida species. Among these species, C. parapsilosis, has emerged as one of the most important opportunistic pathogen of candidemie and represents often the second most commonly isolated Candida species from blood cultures in many areas of the world. Between January 2010 and July 2012 we conducted a pilot surveillance study to determine the occurrence of Candida species in clinical samples and to elucidate the present epidemiological situation in Papardo/Piemonte Hospitals, Messina, Italy.
Methods: All yeasts were isolated from clinical samples using standard mycological methods and identified by VITEK 2 system (YST-2143 card). Antifungal susceptibility tests were performed with the VITEK 2 system using AST-YS01 cards, according to the manufacturer's instructions.
Results: Among biological samples examined we isolated a total of 921 pathogenic fungi. Of these 917 (99,6%) were yeasts belonging to Candida genus and C. albicans was the most encountered species with incidences of 92,4% in 2010, 89,9% in 2011 and 96,9% in the first six months of 2012. Overall this species represented 92,2% of all Candida species isolated in the period 2010-2012 followed by C. parapsilosis. This latter species was isolated only from blood and CVC samples and its incidence raised from 4,6% in 2010 to 6,8% in 2011. A prevalence of 2,1% was detected from January to July 2012. In addition we also recovered C. krusei with a global incidence of 2,2% (1,6%-2010; 3%-2011; 1%-2012). Global resistance to voriconazole was detected for C. albicans (16%) and for C. parapsilosis (23%).
Conclusions: Based on the results of our pilot study we reported an increase C. parapsilosis isolations, a well-known nosocomial pathogen. Resistance to voricanozole in this species increased from 14,3% in 2010, to 27,6% in 2011 and 25% in the first six months of 2012 highlighting the need to type these isolates genetically to show possible cross-transmissions between hospital staff and patients of particular voriconazole-resistant isolates.

M132

THE HIV AVIDITY INDEX FOR THE IDENTIFICATION OF RECENT HIV INFECTION: SELECTION OF THE BEST CUTOFF

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Background: The identification of recent HIV infections (RHI, 6 months or less from seroconversion) is important to accurately estimate HIV incidence, and to monitor HIV spread. The Avidity Index (AI) of anti-HIV antibodies has been shown to be useful in identifying RHI. Previous studies were conducted using 3rd generation immunoassays (EIA). The objective of the present study is to analyze the accuracy of different cutoffs for AI values, using a 4th generation assay.
Methods: We collected 231 serial serum samples from 68 HIV-positive individuals for whom the date of seroconversion was estimated as the midpoint of the interval between the last negative and the first positive HIV test. Specimens positive for the p24 antigen and specimens collected from individuals under anti-retroviral treatment were excluded from the analysis. Moreover, 190 serum samples from as many patients with established infection (AIDS or CD4<200 cells/uL) were assayed. The AI assay was performed using Architect HIV Ag/Ab Combo (Abbott). We studied sensitivity and specificity, and overall accuracy by ROC curve analysis, using four AI cutoff values (0.70; 0.75; 0.80; 0.85). HIV subtyping was available for 325 samples.
Results: A total of 163 specimens were collected within 6 months after seroconversion. The following results were obtained: cutoff 0.70= sensitivity 81.0%, specificity 97.7%, ROC area 89.3; cutoff 0.75 = sensitivity 84.7%, specificity 95.3%, ROC area 90.0%; cutoff 0.80 = sensitivity 90.8%, specificity 92.6%, ROC area 91.7%; cutoff 0.85 = sensitivity 93.9%, specificity 87.2%, ROC area 90.5%. The proportion of samples with established infection misclassified as recent was similar among samples with B (8.2%) and non-B subtypes (8.1%).
Conclusions: The choice of the cutoff value would depend on the specific objectives (epidemiological or clinical purposes). This study shows that a cutoff of 0.80 yields the best overall accuracy and should be employed for epidemiological purposes (incidence estimates and surveillance).

M133
SOME BIOCHEMICAL PARAMETERS AT THE PATIENTS WITH MENINGITIS IN KOSOVO

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Background: Meningitis still remains a life threatening problem worldwide (especially for developing countries). In Kosovo, meningitis is still frequent, but special tests are still unavailable. The aim of this study is to evaluate the diagnostic importance some of biochemical parameters, glucose, proteins, C-reactive protein, lactate dehydrogenase, in cerebrospinal fluid and serum and determining of ratios CSF/serum glucose, CSF/serum LDH like early indicators for the diagnosis of acute meningitis.
Methods: In this study were examined 50 CSF and serum samples of patients with meningitis (1-75 years of age), which were hospitalized and diagnosed in Clinic of Infectious Diseases of UCCK Pristina, during period October 2010-Februar 2011. Patients are divided into 3 groups: BM (n=20), AM (n=17), and control group (n=13), who presented with fever, dehydration or electrolyte disturbances and had undergone lumbar puncture, to exclude the presence of meningitis. The results of this study have been elaborated with the statistical program VASARSTATS.
Results: CSF glucose values were significantly decreased (P <0.001) in patients with bacterial meningitis (1.64) compared to aseptic meningitis(3.21 mmol/L) and control group (3.25 mmol/L). Mean value of CSF /serum glucose ratio (0.33) in patients with BM was significantly lower (P <0.001) than patients with AM (0.61) and patients without meningitis (0.64) .Mean concentration of CSF protein in group with BM (1.58) was increased significantly (P <0.001) compared to the group with AM (0.72 g/L) and non meningitis group (0.24 g/L). LDH activity in CSF was significantly higher (P <0.001) in the BM group (146U/L) compared with the AM (45U/L) and control group (14U/L) and mean value of LCS/serum LDH ratio was significantly higher (P <0.001) in patients with MB (0.4) compared with AM group (0.13) and control group (0.04)
Conclusion: Simultaneous determination of glucose, proteins, CRP, LDH in CSF and serum and their ratios will increase diagnostic efficacy and could be utilized to distinguish between BM and AM.

M134
HS-CRP, IL-1β AND PGE2 LEVELS IN CHRONIC PERIODONTITIS PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Large scale studies have been performed to observe the effects of oral condition on chronic obstructive pulmonary disease (COPD). However, the data on the effect of COPD to periodontal tissues via systemic inflammation is limited.
Methods: In this case-control study, the effects of COPD on periodontal tissues were evaluated by measuring the clinical parameters and gingival crevicular fluid (GCF) interleukin-1 beta (IL-lβ), prostaglandin-E2 (PGE2), serum and GCF high-sensitive C-reactive protein (hs-CRP) levels. 60 COPD patients with periodontitis and 60 systemically healthy periodontitis patients fulfilling the inclusion and exclusion criteria participated in the study.
Results: There was not any significant difference in plaque index, gingival index and bleeding on probing between the patients and controls. Even though clinical attachment level was not different between patients and controls, probing depth (PD) was significantly higher in the controls. Both the PD ≥4 mm and % PD ≥4 mm were significantly higher in the controls than the patients. There was not any significant difference in the GCF IL-1β and PGE2 levels between patients and controls. The hs-CRP levels, both in GCF and serum were significantly higher in patients than the controls and there was a correlation between GCF and serum hs-CRP levels.
Conclusion: Based on the results of clinical and biochemical findings we may conclude that having COPD and/or the medication used in COPD may change the course of periodondal disease as manifested by lower PD and higher GCF hs-CRP levels.

M135
EPIDEMIOLOGICAL PROFILE OF INFECTIOUS DISEASES IN THE REGION OF BEN GUERDANE (SOUTHERN TUNISIA)

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Infectious diseases remain a major health problem due to an increased risk of transmission and the imposed cost. This work intended to assess the epidemiology of infectious diseases such as brucellosis, typhoid fever, B viral hepatitis, syphilis, and acquired immunodeficiency syndrome (AIDS) in the region of Ben Guerdane (Southern Tunisia). For this purpose, we conducted a retrospective study during 12 months (April 2011 until March 2012) using the biological diagnosis data of medical biology laboratory of regional hospital of Ben Guerdane that provides care equally for local population as well as for refugees. Brucellosis and salmonellosis infections were diagnosed using different antigen suspension that agglutinates in the presence of the corresponding antibodies. Syphilis, B viral hepatitis, and AIDS were qualitatively diagnosed by rapid test-cassette that detects specific antibody or antigen. The diagnosis of parasitic intestinal infections was carried out by direct stool examination. Clinical data were also considered. Generally, these are different infectious diseases with different prevalence in our region. 6 cases (among 90 tested serums) of AIDS and 5 cases (among 670 tested serums) of syphilis detection were positive. For both pathologies, all patients were among the refugees with different African origins. In fact, we will describe here one of syphilis cases. During the period of our study, 1181 serum were tested for hepatitis B virus antigen (HBsAg) detection. 3.5% of patients were HBsAg positive and were mainly among local population. Stool examinations were positive for 68% of requested tests. This study showed that the prevalence of amebiasis was 5.2%. Endolimax nanus was the most frequently identified parasite (48.9 %). Most brucellosis and salmonellosis positive cases were detected in July and August. Considering the existence of some cases of cutaneous leishmaniasis each year and very rare cases of tuberculosis, we can conclude that hepatitis B, salmonellosis, and amebiasis were respectively the common viral, bacterial, and parasitic infections diseases in our region. For successful control of these infections, health education about hygiene measures should be enhanced and further studies must be conducted to evaluate the risk factors.

M136
ENZYMATIC STATUS IN PATIENTS WITH HEPATITIS C VIRUS INFECTION

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Background: The outcomes of hepatitis C virus (HCV) infection range from asymptomatic chronic infection, with normal or nearly normal liver functions, to severe chronic hepatitis, evolving rapidly to cirrhosis and hepatocellular carcinoma. The aim of this study was to analyse the changes in the concentration of serum levels of enzymes: alanine transferase (ALT), aspatrate transaminase (AST), gamma-glutamyltransferase (γGT) and total bilirubin level in patients with HCV infection.
Methods: Serum samples were taken from patients with HCV infection (n=42) before the treatment with antiviral drugs. Control samples (n=18) were collected from healthy individuals, and were used for establishing accurate referent values of enzymes levels. Serum levels of ALT, AST, γGT and total bilirubin were measured by biochemical analyzer COBAS Integra 700.
Results: According obtained results: concentration of ALT in patients with HCV infection (75 U/L) was 63,73% higher than in healthy individuals (27,2 U/L), concentration of AST was 90,06% (169 U/L) higher than in healthy individuals (16,8 U/L), while concentration of γGT (52 U/L) was in referent range (11-61 U/L). Significant correlation (r=0,46, P <0.01) between serum level of ALT and duration of HCV infection was noted. Concentration of total bilirubin 26,3 mmol/L was 34,98% higher than in healthy individuals (17,1 mmol/L).
Conclusions: Establishing of accurate referent values of enzymes levels is very important because half of untreated patients with chronic HCV infections have normal or minimally elevated serum levels of enzymes. Obtained results have shown that changes of enzyme levels are in correlation with duration of HCV infection. Serum levels of enzymes are crucial for screening and follow-up of hepatitis C infection. Biochemical examinations play important role in evaluation of HCV infection, and they are in correlation with clinical, ultrasonographic and histological characteristics of patients.

M137

COMPARISON OF SOME ACUTEPHASE PROTEIN LEVELS IN RATS WITH EXPERIMENTALLY INDUCED INFECTIOUS AND NONINFECTIOUS INFLAMMATION

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Acute phase reaction is a nonspesific reaction that occurs during infectious, immunulogic, neoplastic, traumatic or parasitic causes. Most important features of acute phase reaction is the production of some acute phase proteins such as CRP, SAA, haptoglobin, ceruloplasmin, fibrinogen in the liver. In this study, the levels of acute phase proteins in rats with experimentally induced different inflammation models were investigated. In first group rats were treated with 0,5 mg/kg turbentin oil (singledose, ip) for non infectious inflammation. For infectious inflammation model, 21 rats involved in second group were treated within 106 CFU S. aureus subcutaneously, and other 21 rats comprising control group were given serum physiological in same route. In addition as initial values, blood samples were collected from 7 healty animals on day 0. On days 1., 4. ve 7., blood samples were withdrawn via intracardiac route among randomly selected 7 rats under ether anesthesia and afterwards euthenasia was performed within cervical dislocation. Complete blood samples were used for fibrinogen analyses and blood counting; serum samples were analysed for CRP, SAA, haptoglobin within ELISA reader, ceruloplasmin, total protein and albumin analyses were measured within spectrophotometric methods.
In conclusion haptoglobin, fibrinogen and ceruloplasmin levels were significantly increased in rats with acute infectious and noninfectious inflammation group when compared among blood sampling days and in day before induction. CRP and SAA levels were elevated, however no statistically significant differences were found. Besides haptoglobin and fibrinogen concentrations were detected elevated in infectious group.

M138

BLOOD BIOMARKERS IN BACTEREMIC AND NON-BACTEREMIC PATIENTS IN EARLY FEBRILE NEUTROPENIA

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Background: After intensive chemotherapy neutropenic fever is common. Neutropenic infections, especially gram-negative bacteremia, are notorious and can progress towards lethal outcome rapidly. Physicians and laboratory tools currently in routine use are not able to identify reliably the cause of neutropenic fever at an early stage, thus quick solutions are needed to help targeting adequate antimicrobial and supportive treatment to right patients.
Methods: Our study involves ca. 100 patients treated with intensive chemotherapy for hematological malignancies. Patients were monitored and treated according to local policies at a hospital ward. Blood samples were taken at the onset of febrile neutropenia (d0) and two following mornings (d1 and d2). Blood cultures were taken at the beginning of the febrile neutropenia and later if seen necessary. We measured the levels of C-reactive protein (CRP), procalcitonin (PCT), pentraxin 3 (PTX3), soluble urokinase-type plasminogen activator receptor (sUPAR) and interleukins 6 and 10 (IL6, IL10) at the above mentioned times and eventually compared them to final blood culture findings. In respect of each studied biomarker, we included only cases where all three (d0-d2) measurements were available. Means of biomarker levels were compared between groups defined by blood culture findings, statistical significance was explored by independent samples t-test ($P < 0.05$ significant).
Results: Number of cases varied between 84 and 93 and blood culture findings were gram-positive (G+) in 11-13, gram-negative (G-) in 5 and with no bacteria (NB) in 68-77 cases. Mean bi-omarker concentrations (d0-d1-d2) were following: CRP G+78.4-131.7-132.9, G- 66.8-146.4-196.8 NB 46.8-81.2-109.1. PCT G+0.30-0.77-0.61, G-0.58-1.62-1.92, NB 0.11-0.30-0.38. PTX3 G+26.2-46.2-111, G-26.0-91.8-16.8, NB 21.5-30.8-38.9. SUPAR G+3.54-3.56-3.68, G-7.47-7.68-7.71, NB 3.65-3.70-3.78. IL6 G+ 1035-132-98.1, G- 569-608-190, NB 94.3-107-106 group. IL10 G+42.5-27.8-19.5, G-146-126-27.6, NB 29.7-33.4-24.1. Statistically significant correlation to blood cultures was detected in all biomarkers except sUPAR, at all or some of the time points examined here.
Conclusions: More rapid diagnostic means for bacteremia can be developed if these results recur in larger studies.

M139

EVALUATION OF PROMETHEUS INDEX IN PATIENTS COINFECTED WITH HEPATITIS C VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS

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Background: The rate of liver fibrosis progression and the development of hepatocellular carcinoma in hepatitis C virus and human immunodeficiency virus (HCV-HIV) coinfected patients are faster than HCV monoinfected individuals. The treatment of HCV with pegylated interferon plus ribavirin is controversial, especially HCV-HIV coinfected individuals whom show suboptimal response. Recently, Prometheus index (PI) has been proposed as a predictive index of sustained virological response (SVR) in patients coinfected with HCV and HIV whom received this treatment. Therefore, it could be clinically relevant to evaluate the diagnostic efficacy of PI as a screening of patients affected of HCV-HIV in our population.
Methods: From a total population of 103 HCV infected patients, a group of 32 HCV-HIV coinfected individuals were included in this prospective study. These patients had completed a course of pegylated interferon plus ribavirin therapy. PI was calculated using the variables liver stiffness (in kPa), HCV genotype (1 and 4 versus 2 and 3), HCV RNA level (in log IU/mL) and the rs12979860 polymorphism of IL28B (CT or TT versus CC). The liver stiffness was measured using FibroScan. The diagnostic accuracy of PI was evaluated by ROC curve analysis. The cut-off points used from ROC curves were: 0.25, 0.50 and 0.75. Statistical analysis was performed using SPSS 17.0.
Results: The mean age was 37.4 (SD: 6.9) and 65.6% of the population were men (n=21). The area under the ROC curve (AUC) for predictive index of SVR was 0.847 [95% confidence interval(CI): 0.696-0.998] which was statistically significant (P=0.001). The AUC and predictive values for the cut-off points 0.25, 0.50 and 0.75 were: AUC=0.567 (P >0.05), positive predictive value (PPV)=56.7%, negative predictive value (NPV)=100%; AUC=0.700 (P=0.05), PPV=65.4%, NPV=100% and AUC=0.782 (P=0.002), PPV=81.3%, NPV=75%, respectively. We improved the predictive SVR establishing a cut-off point of 0.80 or PI=80% [AUC=0.816 (P=0.002), PPV=86.7%, NPV=76.5%, OR=18.5 (95% CI: 3.2-165.9)].
Conclusions: Having elected the convenient cut-off points, this study concludes that Prometheus index represents a useful and inexpensive non-invasive tool for the prediction of sustained virological response in patients coinfected.

M140

PROGNOSTIC IMPACT OF CD68 EXPRESSION IN SUPERFICIAL BLADDER CANCER

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Background: Bladder cancer is the second most common malignancy of the urogenital region. The majority of bladder cancer deaths occur as a consequence of metastatic disease. Tumour-associated macrophages (TAMs) has been reported to correlate with prognosis in various types of cancer. The aim of this study was to evaluate the predictive value and prognostic significance of TAM in human non muscle invasive bladder cancer (NMIBC) treated by BCG immunotherapy.
Material and methods: The frozen sections of 27 non muscle invasive bladder cancer specimens were stained with CD68 antibody using the standard streptavidin-biotin immunoperoxidase method. TAM was measured using macrophages count determined by the expression of CD68 and stained macrophages were scored on high-power field (hpf). The univariate impact of factors on recurrence was tested by log-rank test and quantified by univariate Cox analysis. Multivariate survival analysis was performed for recurrence.
Results: Univariate Cox regression analysis of baseline characteristics showed that there were no associations between clinical characteristics and the expression of CD68. Similar results were showed by Kaplan Meir survival curves. However, Multivariate Cox regression analysis selected TAM as assessed by CD68, stage, CIS and multiplicity as an independent factor of tumor recurrence after BCG immunotherapy.
Conclusion: This study suggests that determination of TAM count in bladder cancer tissues is of value to predict the clinical outcome or prognostic and to select appropriate treatment strategies in patients with NMIBC. These findings require further investigations on larger cohort in order to ascertain new molecular markers of the response to BCG immunotherapy.

M141

EVALUATE THE CORRELATION OF THE EXPRESSION OF AR AND AMACR TRANSCRIPTS IN PROSTATE CANCER

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Background: Androgen receptor (AR) plays a key role in prostate cancer (PCa). Prostate cells are androgen dependent for growth and survival. Overexpression of the metabolic enzyme α-methylacyl coenzyme A racemase (AMACR) in PCa has been previously shown. This study aimed to investigate the expression level of AR and AMACR transcripts in cancerous and non-cancerous prostatic tissues.
Materials and methods: The expression level of AR and AMACR transcripts were determined by specific time-resolved fluorescence-based quantitative, internally standardized, reverse transcription PCR assays. A panel of 145 tissue specimens consisting of 7 histologically benign tissues from PCa-free prostate (BPCF), 69 histologically benign tissue samples from prostates with PCa (BPC) and 69 samples from cancerous prostate tissues (PC) was studied.
Results: AR transcripts were found in all of the samples while the expression of AMACR was not detected in 5 of the histologically benign tissues from PCa-free prostate samples. AR and AMACR were significantly over-expressed in BPC and PC samples compared to BPCF samples and there was no significant difference for the expression levels of AMACR and AR between BPC and PC samples. There was a highly significant correlation ($P < 0.0001$) between AR and AMACR expression levels in all samples.
Conclusions: The study showed that the expression of AR and AMACR correlate and associate with each other. AMACR and AR showed significant overexpression in PC and BPC sample groups compared to BPCF samples. The difference in mRNA levels of AR and AMACR between BPCF and BPC samples is remarkable. The similar expression level of AR and AMACR in BPC samples compared to PC samples could be due to field effect from the cancer site. Additional studies such as the evaluation of the correlation of AR and AMACR protein expression and the correlation between gene and protein expression should be considered in future studies.

M142

BRMS1 PROMOTER METHYLATION IN CELL-FREE DNA CIRCULATING IN PLASMA OF PATIENTS WITH NON-SMALL CELL LUNG CANCER

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Background: Breast Cancer Metastasis Suppressor 1 (BRMS1) was originally identified to suppress metastasis of highly metastatic human breast cancer cell lines. Reduced expression of the BRMS1, caused by aberrant promoter methylation, is correlated with poor prognosis in breast cancer. Limited data exist on the role of BRMS1 in non-small cell lung cancer (NSCLC). The aim of the present work was to study the methylation status of BRMS1 promoter in tumor tissues and circulating cell-free DNA in patients with non-small cell lung cancer (NSCLC).
Methods: Lung carcinoma tissues and corresponding serum samples were obtained sequentially from 57 patients with NSCLC. Cell-free circulating DNA was isolated from 200 μL plasma from 48 of these patients. Plasma from 24 healthy individuals was collected and used as a control group. The extracted DNAs were subjected to a sodium bisulfite conversion reaction. Methylation specific polymerase reaction (MSP) for BRMS1 promoter methylation was performed.
Results: BRMS1 promoter was found to be methylated in 34/57 (59.6%) of NSCLC tumor samples and in 31/57 (54.3%) of adjacent non-cancerous tissues. We evaluated the presence of BRMS1 methylation in cell-free DNA circulating in plasma. BRMS1 promoter was found methylated in 23/48 (47.9%) of plasma samples from NSCLC patients but not in any of the control plasma samples. Kaplan Meier analysis showed that the detection of BRMS1 promoter methylation in plasma has prognostic implication, since it was associated with decreased disease free interval (DFI) ($P = 0.029$, log rank test) and decreased overall survival (OS) ($P = 0.008$, log rank test).
Conclusions: The detection of BRMS1 promoter methylation in circulating cell-free DNA should be further evaluated as a biomarker in NSCLC in a larger number of patients.

M143

PCA3 AND PROSTATE HEALTH INDEX (PHI) DO NOT CHALLENGE F/TPSA IN PROSTATE CANCER (PCA) DIAGNOSIS

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Background: PCa biochemical diagnosis remains a challenge. Serum PSA is limited in specificity;the reflex determination of free/total PSA (f/tPSA) for 4-10 ng/mL PSA grey zone is widely used to enhance the detection rate of PCa but many cancers continue to be missed. Among the wide number of new biochemical and molecular biomarkers, the most promising are the urinary measurements of PCA3 mRNA transcript and the serum determination of %p2PSA and the derived PHI. The aim of this study was to compare the performances of PSA, f/tPSA, PHI and PCA3 in PCa diagnosis.
Methods: A total of 100 men were consecutively enrolled in the study. All underwent blood sampling and urinary collection after DRE before prostate biopsy for suspected PCa. Serum PSA and f/tPSA (Immulite® 2000 System), %p2PSA (Beckman-Coulter) and urinary PCA3 (Gen Probe Inc., San Diego, CA, USA) were measured. PCa was histologically diagnosed in 40 men, while benign prostatic lesions were identified in 60 cases.
Results: At univariate analysis (Mann-Whitney test) a significant difference between PCa and reference patients was found for f/tPSA (P <0.0001), PHI (P=0.001) and PCA3 (P=0.002), not for PSA (P=0.250). The areas under the ROC curves were 0.713±0.052 for f/tPSA, 0.696±0.054 for PHI and 0.682±0.054 for PCA3, and they did not significantly differ each other. The best threshold for any biomarker was identified (ROC curve analysis). Sensitivity and specificity were: 55% and 83% for f/tPSA (<=9%), 70% and 68% for PHI (>36), 45% and 87% for PCA3 (>52). None of the studied biomarkers was correlated with a positive or negative DRE finding, while f/tPSA correlated with prostate volume (r=0.476, P <0.0001). Binary logistic regression analysis corrected for age which included f/tPSA, PHI and PCA3 was found to significantly include in the model only f/tPSA (ExpB=0.856, 95%CI=0.78-0.94, P=0.001) and allowed to correctly classify 69% of the patients. Binary logistic regression analysis made including biomarkers and DRE included only f/tPSA and DRE as significant predictors and allowed to correctly classify 74% of the patients.
Conclusion: PHI and PCA3 are less sensitive and specific than f/tPSA and a strategy based on f/tPSA and DRE findings actually appears the most reliable approach for PCa diagnosis.

M144

THE INFLUENCE OF INFLAMMATION IN THE SEARCH OF DISCRIMINATORY BIOMARKERS FOR PROSTATE CANCER: A PROTEOMIC STUDY

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Background: Despite the improvements in clinical and surgical practice, prostate cancer (PCa) remains one of the most widespread cancer in male. The serum marker currently used for the diagnosis of PCa is the prostate-specific antigen (PSA), but its increase does not discriminate benign prostatic hyperplasia (BPH) from PCa. In our study, we investigated the serum protein expression of BPH compared to PCa, in order to identify by Surface Enhanced Laser Desorption/Ionization - Time of Flight - Mass Spectrometry (SELDI-ToF-MS) analysis distinctive protein profiles able to unquestionably discriminate patients with a benign prostate condition from those with a malignant situation. Moreover, we considered these conditions focusing on the co-existence of inflammation.
Methods: Patients with clinical suspect of PCa (PSA elevation and/or palpable mass at digital rectal exploration) and candidates for trans-rectal ultrasound guided prostate biopsy were enrolled. The analysis of protein profile of 30 patients with PCa and 30 subjects with BPH was carried out. All histological specimens were examined in order to graduate and classify the tumor and to recognize the BPH condition and presence of inflammation, that was classed in chronic and acute and then graduated in mild, moderate and severe. Serum was depleted of the 6 high-abundance proteins by immunoaffinity chromatography prior to SELDI-ToF-MS analysis.
Results: The comparison between protein spectra from PCa and BPH considering the inflammation parameter and excluding samples with moderate and/or severe inflammation, identified 17 differentially expressed protein peaks using H50 ProteinChip Array.The analysis of protein profile in presence of inflammation showed different protein peaks in the two groups, some of which overlapped with those found also in the comparison between PCa and BPH in absence of inflammation.
Conclusions: The inflammation seems to lead a crucial contribution in the protein profile assessments of these conditions. On the basis of our results, we believe that certain different protein peaks could be reasonably associated to inflammation rather than to cancer. Therefore, inflammation might be a confounding parameter in the search of specific biomarkers to discriminate PCa from BPH.

M145

[-2]proPSA AND PROSTATE HEALTH INDEX (PHI) IMPROVE DETECTION OF PROSTATE CANCER AT INITIAL AND REPEATED BIOPSIES IN YOUNG MEN (≤ 60 YEAR OLD) PREFERENTIALLY DETECTING CLINICALLY SIGNIFICANT CANCER

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The prostate-specific antigen (PSA) screening reduced prostate cancer (PCa) mortality but due to overdiagnosis and overtreatment of indolent PCa, PSA based screening is not recommended. Detailed analysis of large randomised studies has shown that the benefit of PSA based screening is maximal for young men. A molecular isoform of free PSA, [2]proPSA, demonstrated improved clinical specificity for the detection of PCa compared with total PSA (tPSA) or percent free PSA (%fPSA). The [-2]proPSA results can be combined with tPSA and fPSA in an innovative "prostate health index" (phi). The clinical performance of phi for PCa detection was evaluated in a multicenter study. A total of 1362 patients scheduled for initial or repeated prostate biopsy (668 with, 694 without PCa all confirmed with >10 cores biopsy) were recruited in 4 different sites based on PSA level 1.6 – 8.0 ng/mL WHO calibration (ie 2 – 10 ng/mL with classical calibration). Serum samples were taken before DRE examination. The serum concentrations of tPSA, fPSA and [-2]proPSA were measured with Beckman Coulter immunoassays on Access2 or DxI800 instruments. Univariate analysis showed that [-2]proPSA/fPSA (%p2PSA) and phi were the best predictors or PCa detection in patients at initial biopsy (AUC: 0.72 and 0.73) and repeated biopsy (AUC: 0.74 and 0.74). In multivariate analysis %p2PSA and phi significantly improved the prediction of a model based on age, prostate volume, DRE, tPSA and %fPSA at initial (AUC from 0.69 to 0.73) or repeated biopsy (AUC from 0.74 to 0.80). Analysis of the data for men ≤60 years old (n=472) showed that phi and %p2PSA significantly improved PCa detection (AUC: 0.72) as compared with tPSA (AUC: 0.53) or %fPSA (AUC: 0.62). When the detection of significant cancer (based on the PRIAS criteria) was assessed, %p2PSA and phi also demonstrated the best performance for detection of clinically relevant cancer in the whole cohort of patients but also in younger men aged ≤ 60 (AUC: 0.70 and 0.73 respectively). This multicenter study demonstrated that %p2PSA or phi have a superior clinical performance in detecting PCa in the tPSA range of 2 – 10 ng/mL compared with tPSA or %fPSA at initial or repeated biopsy but also improve detection of clinically relevant PCa in young men.

M146

COST EVALUATION OF INTRODUCING THE PROSTATE HEALTH INDEX (PHI) IN THE MANAGEMENT OF PROSTATE CANCER DIAGNOSTIC

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Recently the [-2]proPSA, a PSA isoform, integrated into the Prostate Health Index (phi) have been proposed to improve clinical specificity over tPSA for early detection of prostate cancer (PCa). At comparable sensitivity, the use of the phi index may reduce significantly the number of unnecessary biopsies as compare to tPSA. The objective of this study is to evaluate the cost of introducing the phi index in the management of PCa detection. A total of 177 patients were enrolled between June 2009 and October 2010 in a university hospital. Patients were followed for at least 6 months and all diagnostic tests, from the first contact with the urologist to the therapeutic decision, were collected. All direct medical costs were considered for this analysis and were valued using the current pricing in 2012. The methodology of decision trees was used to describe the patient's pathway. For each branch of the decision tree, a probability and a cost have been calculated. "Monte-Carlo" simulations have yielded an average cost of each strategy. A sensitivity analysis was performed to validate the robustness of the results. The PPV (Positive Predictive Value) increased from 55.8% to 69.7% with phi and NPV (Negative Predictive Value) of 39.1% to 70.7%. The average cost of the strategy "PSA" is € 484 as compared with € 479 for the phi strategy. Modification of the PPV and NPV parameters for the phi index demonstrated that the phi strategy is largely dominant. The cost differential observed between the two strategies is ranging from -40 € to -100 € per patient. If we apply this differential to the 60,000 new cases of prostate cancer detected every year in France, the gain becomes very significant. In this study the most conservative approach was followed as the phi was offered only at initial biopsy in university hospital. Even more favourable cost results could be expected if the phi index would have been evaluated also at repeated biopsy and if the evaluation would have been performed in private hospital where the cost of care is higher than in university hospital. The introduction of the phi index in PCa detection strategy could avoids unnecessary biopsies without increasing the cost and in most of the cases reducing the cost of care.

M147

THE DIAGNOSTIC VALUE OF THE [-2]proPSA AND PHI MARKERS IN THE DIAGNOSIS OF PROSTATE CANCER

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Background: Despite its low specificity and low positive predictive value, serum prostate-specific antigen (PSA) is the most widely used serum biomarker for the differential diagnosis of prostatic diseases, early diagnosis and screening of prostate cancer (PCa), tumor volume evaluation, management, etc. In the current study we examined the usefulness of p2PSA tumor marker and the phi index in the detection of PCa patients, and the associated of the aggressive forms of tumor (Gleason score ≤7 or greater than 7).
Methods: Between 2010 and 2012, the total PSA (tPSA), free PSA (fPSA) and [-2]proPSA (p2PSA) concentrations of 173 serum samples of patients with PCa (mean ages was 67.2 years [from 53 years to 89 years]), and 518 samples of healthy subjects (mean ages was 66.9 years [from 49 years to 87 years]) were measured. The PCa was diagnosed histologically by transrectal ultrasound-guided octant prostate biopsy, if the tPSA levels were >4.0 ng/mL. Blood samples were taken before any diagnostic or therapeutic procedures involving the prostate. Serum specimens was determined by Hybritech Tandem Access (Beckman Coulter, Inc., USA) PSA assay using Uni Cel DxI 800 Access Immunoassay System (Beckman Coulter, Inc., USA). The prostate health index (phi, Beckman Coulter) a mathematical formula combining tPSA, fPSA and p2PSA. Statistical calculations were performed with SPSS 13.0 for Windows. Diagnostic usefulness was assessed using ROC analysis.
Results: The use of phi and p2PSA provides better discrimination between PCa and the control group than tPSA level (1.0 to 10.0 ng/mL), and are more associated with aggressive pathological stage of the tumor (Gleason score ≤7 vs >7). The results of the ROC AUC of phi (AUC=0.847) was greater than the fPSA (AUC=0.737), tPSA (AUC=0.734) and p2PSA (AUC=0.823).
Conclusion: The study demonstrate that use of phi and p2PSA is more reasonable in the diagnosis of PCa, and it could reduce unnecessary prostate biopsies of men with tPSA level between 1.0-10.0 ng/mL.

M148

THE INFLAMMATORY CALCIUM BINDING PROTEIN S100A8 AND ITS N-TERMINAL PROTEOLYTIC FRAGMENT INTERACT WITH TRANSFORMING GROWTH FACTOR-BETA1 (TGF-B1) AND ALTER AKT, MTOR AND NF-KB CANCER CELL SIGNALLING

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Background: In pancreatic cancer S100A8 is highly expressed by stromal cells when SMAD4 is not mutated or by cancer cells when SMAD4 is mutated, suggesting a link between TGF-b1 and S100A8 pathways. The proteolytic fragment of S100A8, NT-S100A8, highly abundant in pancreatic cancer, is involved in altering insulin secretion and action. We ascertained whether S100A8 and NT-S100A8 interacts with TGF-b1 in altering intracellular calcium, NF-kB, Akt and mTOR signalling.
Methods: BxPC3 cells were stimulated with S100A8 (10 nM), NT-S100A8 (50 nM) alone or combined with TGF-b1 (0.02 ng/mL). Intracellular calcium was monitored by Fluo4 (epifluroescence). Akt (Ser473,Thr308), mTOR (Ser2448), NF-kB (p-IkB-a) were WB analyzed.
Results: NT-S100A8 evoked a train of intracellular calcium fluxes after 150 seconds lag time, which was reduced to few seconds in the presence of TGF-b1. S100A8 or TGF-b1 alone did not alter intracellular calcium. NF-kB signalling was activated in a calcium-dependent manner by S100A8 and by NT-S100A8 in the presence of TGF-b1. Akt Ser473 phosphorylation was reduced by NT-S100A8, TGF-b1 but mainly by their combination. AktThr308 was not affected by the studied molecules. mTOR phosphorylation (Ser2448) was induced by S100A8 and, at a lesser degree, by TGF-b1 and NT-S100A8. The phosphorylation (Ser235/236) of the downstream effector of mTORC1, S6RB, was reduced by TGF-b1 and NT-S100A8 independently, not by S100A8.
Conclusions: NT-S100A8 mimics TGF-b1 inhibitory effects on Akt and mTOR signalling. These two molecules co-operate in inhibiting Akt probably by altering intracellular calcium, while they co-operate in activating NF-kB in a calcium-independent manner mimicking the entire S100A8 molecule effect.

M149

THE INFLAMMATORY MOLECULES S100A8 AND TGF-B1 ACTIVATE THE EPITHELIAL-MESENCHYMAL TRANSITION (EMT) PROCESS IN PANCREATIC CANCER (PaCa) CELLS

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Background: Chronic inflammation is suggested to play a role in cancer initiation and progression. EMT is an essential developmental program that becomes reactivated in adult tissues to promote the progression of cancer. EMT is characterized by an enhanced cell motility, the loss of the epithelial marker E-Cadherin (CDH1), and the overexpression of the mesenchimal marker N-cadherin (CDH2). Master regulators of EMT are Snail, Slug, ZEB and Twist. We ascertained whether the inflammatory molecules S100A8, S100A9 and TGF-b1, overexpressed in PaCa, might start the EMT program.
Methods: BxPC3 cells were treated with 10nM S100A8 or S100A9, with or without 0.02 ng/mL TGF-b1 for 72 h. The expression (mRNA) of Snail, Slug, ZEB1, ZEB2, Twist, CDH1 and CDH2 were quantified by RT-PCR (Light Cycler). N-Cadherin protein expression was evaluated by immunocytochemistry (IICC).
Results: TGF-b1 enhanced the expression of all the EMT markers (P=0.037 for CDH1, CDH2, Slug, ZEB1 and ZEB2; P=0.005 for Snail and Twist). S100A8 enhanced the expression levels of Twist (median fold increase=4, range=2.6-22.3, P=0.007) while S100A9 did not (median=2, range=0.8-9.3, P=0.10). Both molecules reverted TGF-b1 effects on Twist expression (TGF-b1: median=6, range=3.6-42.6; TGF-b1 and S100A8: median=3, range=2.3-3.8; TGF-b1 and S100A9: median=3.3, range=3.1-4.2). By ICC, control cells showed a dysomogeneous non-continuous membranous N-Cadherin immunostain. The treatments with both TGF-b1 and S100A8 resulted in an enforced moderate complete membranous immunostain. The combined treatment with TGF-b1 and S100A8 was less effective on protein visualization in comparison to any single treatments.
Conclusion: TGF-b1 activate the EMT program in PaCa cells. A dual role for S100A8 in the EMT was shown: it promotes the EMT by inducing Twist mRNA and N-Cadherin protein expression, but it antagonizes TGF-b1 effects on both targets. S100A9 exerts inhibitory effects on TGF-b1.

M150

BRCA1 LOSS IN PROSTATE CANCER IS ASSOCIATED WITH METASTATIC SPREAD: NOVEL PREDICTOR OF PARP-INHIBITORY THERAPY?

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Purpose: A preliminary study performed on a small cohort of multifocal prostate cancer (PCa) detected BRCA1 allelic imbalances among circulating tumor cells (CTC). The present analysis was aimed to elucidate the biological and clinical roles of BRCA1 losses in metastatic spread and tumor progression in PCa patients.
Experimental Design: To map molecular progression in PCa outgrowth, we used fluorescence in situ hybridization analysis of primary tumors and lymph node sections, and CTCs from peripheral blood.
Results: We found that 14% of 133 tested patients carried monoallelic BRCA1 loss in at least one tumor focus. Extended molecular analysis of chr17q revealed that this aberration was often a part of larger cytogenetic rearrangement involving chr17q21 accompanied by allelic imbalance of the tumor suppressor gene PTEN and lack of BRCA1 promoter methylation. The BRCA1 losses correlated with invasion to pelvic lymph nodes ($P < 0.05$), as well as biochemical recurrence ($P < 0.01$). The analysis of 11 matched primary PCa-LNM pairs confirmed the suspected transmission of genetic abnormalities between these two sites. In four of seven patients with metastatic disease, BRCA1 losses appeared in a minute fraction of cytokeratin- and vimentin-positive CTCs.
Conclusions: Previous research has shown that PARP-inhibitors prevent repair of cells with a BRCA1 inactivation condition, thus leading to apoptosis of these aggressive cancer cells. PCa cells bearing BRCA1 losses might be one confounding factor initiating tumor dissemination and might provide an early predicator of PARP-inhibitor therapy.

M151
THE ASSOCIATION OF PLASMA D-DIMER LEVELS WITH THE TUMOR STAGE IN COLORECTAL CANCER PATIENTS

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Background: The process of blood coagulation results in the conversion of fibrinogen into fibrin by thrombin and subsequent fibrin polymerization, which is followed by fibrin degradation. D-dimer is the final product of fibrin degradation. Increased levels of D-dimer have been detected in patients with disseminated intravascular coagulation, thromboembolism and hemorrhage. Disseminated intravascular coagulation represents the second largest cause of death in cancer patients. Plasma D-dimer levels have been shown to be elevated in patients with various solid tumors including lung, prostate, cervical, ovarian, breast and colon cancers. We aimed to examine the relationship between plasma D-dimer levels and tumor stage in colorectal cancer patients.
Methods: 34 patients (23 men and 11 women) with different stages of primary colorectal cancer who had applied to Private Tansan Oncology Clinic, Istanbul/Turkey for the systemic chemotherapy were included in this study. Tumor stages were evaluated according to the TNM classification. Plasma levels of D-dimer, CEA and Ca-19.9 were measured using a two-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA, Vidas Biomerieux). For the statistics, SPSS 15.1 version was used.
Results: A significant positive correlation was found between plasma D-dimer and clinical stage of the disease by the Spearman ranking procedure ($r=0.55$; $P<0.001$). Clinical stage was also positively correlated with both CEA ($r=0.44$; $P=0.01$) and Ca-19.9 ($r=0.50$; $P=0.009$) concentrations in colorectal cancer patients.
Conclusion: Advance cancer stage with high tumor burden and high proliferation rate is associated with high coagulation activation, as evidenced with increased levels of plasma D-dimer. In colorectal cancer patients, determination of D-dimer levels may contribute to other parameters in evaluating the prognostic outcome.

M152
EVALUATION OF THE THIRD-GENERATION TO SECOND-GENERATION PTH RATIO ON THE LIAISON XL (DIASORIN) AS A MARKER FOR PARATHYROID CARCINOMA

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Background: Patients suffering from parathyroid carcinoma (PCa), a very rare but difficult to diagnose disease, overproduce a special form of PTH (amino-PTH; N-PTH). As the N-PTH is only recognized by 3rd generation (and not by 2nd generation (or “intact” PTH) assays), it has recently been shown that this disease could lead to an inversion of the 3rd to 2nd generation PTH ratio. This feature can not physiologically occur otherwise as, in normal patients, N-PTH is expressed in limited amounts. Thus, an inverted ratio could be of diagnostic use as a marker for PCa. This has already been reported in PCa patients using the Scantibodies duo, a kit that combines 2nd and 3rd generation PTH IRMA assays. However, this technique uses radioisotopes, is cumbersome, difficult to automate and long to proceed. Recently, DiaSorin launched 2nd and 3rd generation automated PTH assays on the Liaison XL. Like the Scantibodies assays, these 2 kits are calibrated against the same source. The aim of our study was to see if the same results could be obtained with this fully automated chemiluminescent method.
Methods: The 3rd to 2nd generation PTH ratio was compared between PCa patients (n=10) and control groups including elderly healthy subjects (n=50), hemodialyzed (HD; n=80), renal transplanted (n=41) and patients suffering from severe primary hyperparathyroidism (PHP; n=144). The determination of 2nd and 3rd generation PTH was performed on the Liaison XL.
Results: We reported 1 inverted ratio among the HD patients, 1 in the renal transplant group, none in the elderly healthy or in the PHP patients. In contrast, among the PCa patients, 8 out of 10 presented an inversion of the ratio. As a tumor marker for PCa, a 3rd to 2nd generation PTH ratio higher than 1 has a sensitivity of 80% and a specificity of 99%.
Conclusion: As with the Scantibodies Duo kit, we observed an inverted 3rd to 2nd generation PTH ratio in PCa patients with the Liaison XL. It is thus possible to obtain a reliable result of the ratio with the same level of performance on a totally automated, simple and rapid platform. Therefore, this method could be used prospectively to identify patients with PCa and detect patients either at risk of developing PCa or those in whom recurrence is taking place.

M153

DEFINITION OF THE LIMIT OF DETECTION (LOD) OF [-2]proPSA FOR A POSSIBLE APPLICATION OF THE TEST AS EARLY MARKER OF BIOCHEMICAL RECURRENCE OF PROSTATE CANCER

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Introduction. [-2]proPSA (p2PSA) is a serum precursor of prostate-specific antigen (PSA) proved to be more specific than PSA in prostate cancer (PCa) detection. Being a precursor of PSA detectable in serum at very low concentration levels, its measurement might be useful in early detection of biochemical recurrence of PCa after radical prostatectomy (RP). The aim of this study was to test the Limit of Detection (LoD) for p2PSA using sera from patients with proved recurrent prostate cancer after RP.
Method. The blood sample of 10 patients with histological proved PCa recurrence after RP with a tPSA >0.2 ng/mL and <5 ng/mL were collected before starting a secondary treatment. The samples were stored at -80 °C (5 of them) and at -20° C for the other 5. One aliquot of the sample was measured to confirm the tPSA values at entry and each sample was diluted with a PSA free serum to obtain a tPSA result of about 0.2 ng/mL. Diluted sera were aliquoted and frozen again at -80 °C. Subsequently each day one aliquot per sample was thawed and p2PSA was measured in duplicate. The primary end point was to detect the LoD according to the CLSI EP17-A protocol (LoD = LoB+Cβ * cumulative SD (where Cβ is 95th percentile of the standard Gaussian distribution corrected = 1.645/(1-1(4 x f) [f = degrees of freedom =90])).
Results. The p2PSA values obtained in the 10 diluted samples were in the range 0.73 - 3.67 pg/mL, with a cumulative SD of 0.1979 pg/mL. Using the limit of blank (LoB) indicated by Beckman Coulter (0.5 pg/mL) the LoD resulted =0.8 pg/mL. No difference was observed for samples stored at -80 °C or -20 ° C.
Conclusions. In conclusion the system is able to detect reliably p2PSA starting from a concentration as low as 0.8 pg/mL and this concentration might be considered the cut-off sufficient to detect and monitor biochemical recurrence of PCa. Further work is now needed to verify if measuring p2PSA will provide clinical advantages over tPSA measurement.

M154

EFFECTIVE VEGFA SHRNA INHIBITION OF IN VITRO HUMAN HEPATOMA CELLS MIGRATION

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Background: The aim of study is to investigate the ability of the functional short hairpin RNA (shRNA) of vascular endothelial growth factor (VEGFA) to arrest cell migration in HepG2 cells.
Methods: The secondary structure of VEGFA mRNA was predicted by minimum free energy (MFE) and base pair probabilities. MFE was computed using a loop-based energy model and dynamic programming algorithm to identify and evaluate target sites for shRNA. The intrinsic VEGFA level was determined in three human hepatocellular carcinoma (HCC) cell lines (HepG2, Hep3B, and HuH7 cells). We constructed five VEGFA-expressing shRNAs in lentivector (pLKO.1-puro) and stably transfected VEGFA shRNAs into human HepG2 HCC cells. The efficiency of VEGFA shRNAs in knocking down VEGFA protein was examined by western blotting. The effect of VEGF shRNAs on cell migration was studied using wound-healing cell migration assays.
Results: The free energy of VEGFA in the thermodynamic ensemble is -1465.38 kcal/mol. After processing, the VEGFA shRNA converts to short interfering RNA (siRNA). The interaction of VEGFA siRNA and its target region was calculated as the total free energy of binding, the energy from duplex formation, and the opening energy for the longer and shorter sequences. The endogenous VEGFA protein level was 2- and 6-fold higher in HepG2 cells compared with Hep3B and HuH7 cells, respectively. Five stable lentiviral VEGFA shRNAs (sh1 to sh5) were stably transfected into HepG2 cells. Two shRNAs (sh3 and sh5) strongly decreased VEGFA protein expression in HepG2 cells, whereas three shRNAs (sh1, sh2 and sh4) partially decreased VEGFA expression. In a wound-healing cell migration assay, three lentiviral VEGFA shRNAs (sh3 to sh5) inhibited cell migration.
Conclusions: This study provided two optimal VEGFA shRNAs (sh3 and sh5) that can strongly knockdown VEGFA protein and inhibit cancer cell migration. Blocking the VEGF signaling pathway may provide a novel therapeutic target for the treatment of HCC.

M155

ROLES OF ESTROGEN RECEPTOR ALPHA AND ITS ISOFORM 36 IN THE PROLIFERATION AND AUTOPHAGY OF THYROID CANCER CELLS

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Background: Epidemiological and animal experimental data have indicated that the female sex hormones such as estrogen (E2) contribute to the development of thyroid cancer. Although estrogen receptor (ER)-α has been supposed to mediate the proliferation-promoting effect of E2 on thyroid cancer cells, the exact molecular mechanism underlying is unclear.
Methods: We employed RT-PCR and Western blot to measure full-length ER-α (ER-α66) and its truncated isoform ER-α36 in a number of human normal thyroid cells and thyroid cancer cells. Cell proliferation and autophagy were measured. Relevant molecules were determined to reveal the possible mechanism.
Results: Our results found that ER-α66 was not expressed in normal thyroid cells. In thyroid cancer cells, ER-α66 was weakly expressed or not expressed. However, thyroid cancer cells expressed a significant amount of ER-α36, a novel isoform of ER-α66, at both mRNA and protein levels while this isoform was absent in normal thyroid cells. Over expression of ER-α66 by ER-α66DNA transfection enhanced the proliferation and autophagy in thyroid cancer cells. Inhibition of ER-α36 could attenuate the proliferation and autophagy in thyroid cancer cells. We also demonstrated the increase of phosphorylated ERK but the decrease of Bcl-xL contributed to ER-α-related proliferation and autophagy in thyroid cancer cells.
Conclusions: Human normal thyroid cells do not express ER-α66 but thyroid cancer cells express can express ER-α66. A significant amount of ER-α36 is present in thyroid cancer cells but not in normal thyroid cells. ER-α66 promotes the proliferation and autophagy and the inhibition of ER-α36 attenuates both in thyroid cancer cells. The increase of phosphorylated ERK but the decrease of Bcl-xL contributed to ER-α-related proliferation and autophagy. These findings may help to design some target therapies towards ER-α36 in thyroid cancer cells.

M156

URINARY ALTERATIONS OF MALIGNANT KIDNEY TUMORS REVEALED BY PEPTIDOMIC APPROACHES

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Background: Renal Cell Carcinoma (RCC) is typically asymptomatic and surgery usually increases patient's life only for early stage tumors. Moreover, some cystic and solid renal lesions cannot be confidently differentiated from RCC. Therefore robust markers to distinguish malignant kidney tumors and for their early detection are needed. Urine is an important source of biomarkers, with several advantages, such as its accessibility and stability of polypeptides. Renal diseases, as RCC, are thus ideally appropriate for such a research, given that urine should contain an higher amount of altered molecules directly deriving from kidney. Therefore the present work was focused on the application of magnetic beads (MB) purification coupled with mass spectrometry (MS)-based profiling techniques to explore possible urinary peptide signatures of patients affected by clear cell RCC (ccRCC), other kidney tumors (non-ccRCC) and controls (Ctrls).
Methods: Urinary proteome of 85 Ctrls, 103 ccRCC and 35 non-ccRCC patients were fractionated by MB and their spectra profiles were obtained by MALDI-MS and elaborated with statistical softwares. RapidMiner was used for feature selection (cluster of signals with discriminant capability), cross-validation and performance evaluation. Peptides in MB enriched fractions of urine pools from Ctrls (n=80) and ccRCC (n=80) were also identified and quantified by label-free (LF) approach using nLC-ESI-MS/MS. Peptide abundance in pools was evaluated by IDEAL-Q.
Results: A cluster of four signals could significantly differentiate malignant tumors from benign renal masses and ctrls (sens.=89%; spec.=84%). Two different clusters of six ions efficiently distinguish ccRCC (sens.=96%; spec.=93%) and non-ccRCC (sens.=95%; spec.=98%) from ctrls. A library containing identified endogenous peptides, including almost all selected peaks, was built. Peptide ratios determined by MALDI were compared with those achieved by LF strategy after an accurate mass alignment and no statistical difference was revealed strengthening outcome validity.
Conclusions: Our results suggest the possibility to distinguish malignant kidney tumors based on specific urinary peptide clusters, encouraging a better understanding of molecular mechanisms underlying malignant transformation.

M157

SERUM HER2 ECD LEVELS IN TWO DIFFERENT ADIUVANT CHEMOTHERAPY REGIMENS OF TRASTUZUMAB IN PRIMARY BREAST CANCER

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Background: The clinical utility of serum HER2 ECD measurements for monitoring HER2/neu targeted therapy in the primary breast cancer (PBC) is currently matter of debate. Aim of this work is to investigate the possible influence of two different adjuvant chemotherapy regimens of Trastuzumab on the serum levels of HER2 ECD.
Methods: 142 serum samples from 71 patients included into the ShortHER study has been investigated; this is a multicentric randomized study conducted in HER2 positive, surgically resected breast patients in order to evaluate two different adjuvant chemotherapy regimens of Trastuzumab. Serum levels of HER2 ECD at the beginning (pre) and at the end (post) of therapy in patients randomized for the two regimens have been measured: long arm (A) in which chemotherapy plus Trastuzumab was administred for 12 months (n= 24 patients) and short arm (B) in which therapy was administrated for 3 months (n= 47 patients). The assays were performed by ADVIA Centaur XP (Siemens). The currently approved cut-off value for increased serum HER2 ECD is 15 ng/mL; 20% or more decrease in serum HER2 ECD concentration from baseline it was reported to predict a significant therapeutic response.
Results: Pre and post samples in the two arms showed median values <15 ng/mL. The differences between pre and post samples in both arms were not statistically significant as it was the difference between post samples in the two arms. We found only one patient per arm with a decrease >20%.
Conclusions: On the basis of the present data we can conclude that the two therapeutic regimens did not show any difference in the post treatment HER2 ECD serum concentrations. In the clinical setting of PBC we cannot confirm the correlation between a post treatment decrease >20%, and response to treatment. A possible limitation of this study is that the great majority of enrolled patients present serum levels <15 ng/mL.

M158

ASSESSMENT OF SERUM TUMOR MARKER HE4 CONCENTRATIONS IN PATIENTS WITH OVARIAN CANCER

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Background: The human epididymal protein 4 (HE4) belongs to the family of whey acidic four-disulfide core (WFDC) proteins. The aim of this study was assessment of serum HE4 concentrations in patients with epithelial ovarian cancer (EOC).
Materials and Methods: Serum samples were acquired from female patients with epithelial ovarian cancer (N=56). Control samples (N=20) were collected from healthy females. Concentrations of HE 4 were measured by the immunochemical analyzer Elecsys 2010 with enhanced chemiluminescence. For quality control we used Elecsys PreciControl HE4 1 and 2.
Results: The Roche HE4 assays showed a good linearity (r=0.99) and precision (intra assayed total CV <5%). The median HE4 serum concentrations was significantly higher among EOC patients than healthy females (P <0,05). As a single marker, HE4 had a sensitivity of 78,4 % with a specificity of 95 %.
Conclusions: HE4 is the tumor marker of choice in ovarian cancer with a higher sensitivity. The presented results of the analytical evaluation methods for the determination of HE4 on the Elecsys 2010 analyzer showed an acceptable accuracy and precision.

M159
INTRATHECAL SYNTHESIS OF TUMOR MARKERS AND TREATMENT MONITORING OF CANCER PATIENTS

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Background: Leptomeningeal metastasis (LM), caused by the entry of neoplastic cells into the subarachnoid space, is a neurological complication of systemic cancer occurring in 5-10% of patients affected by solid tumors. An early diagnosis is crucial to start aggressive therapies and to prevent progressive neurological deterioration. The methods currently used to diagnose LM are clinical presentation, MRI examination of CNS and CSF cytology, but the latter is considered the gold standard. However, since CSF cytology has an unsatisfactory sensitivity, considerable efforts have been made to identify alternative diagnostic markers. In a previous study (Corsini et al, 2009), we demonstrated that the evaluation of intrathecal synthesis of the tumor markers (TM) CEA, CA125, CA15.3 and CA19.9 is a reliable diagnostic tool for the identification of LM. Aim of the present study is to compare the sensitivity of intrathecal synthesis of TM with CSF cytology and the CSF biochemical parameters glucose and QAlb for monitoring the therapeutical effectiveness in 10 patients with diagnosed LM.
Methods: The patients (6F, 4M), affected by breast cancer, lung cancer and esophageal cancer, underwent intrathecal treatment with Depocyte and were monitored at least 3 times. TM concentrations were evaluated by Modular Analytics SWA (Roche), both in serum and in CSF. The lower detection limit was validated on 20 CSF samples of patients with normal biochemical parameters. Intrathecal synthesis was calculated using a mathematical approach aimed to correctly identify the amount of TM detected in CSF due to filtration from blood and/or barrier dysfunction.
Results: Qalb showed a better correlation with LM as compared to glucose, but both are aspecific parameters. Although CSF cytology had a strong correlation with intrathecal synthesis of TM, in one case intrathecal synthesis was even more sensitive. Among TM, CEA was the most sensitive analyte (there was intrathecal synthesis in 8 patients out of 10).
Conclusion: Intrathecal synthesis of TM, calculated according to Reiber formula, is a specific and sensitive parameter, obtained by reproducible and standardized methods, which can be easily used in laboratories of clinical investigations for monitoring therapy efficacy.

M160
THE PLASMA LEVEL OF MYELOPEROXIDASE (MPO), URIC ACID AND TOTAL ANTIOXIDANTS STATUS (TAS) IN GASTRIC CANCER PATIENTS

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Background: Reactive oxygen species (ROS) are produced as a normal product of cellular metabolism and have important roles in cell signaling and homeostasis. Uncontrolled increase however is known as oxidative stress and may have serious consequences. The neutrophils are the main phagocytic cells in the blood and it is accepted that their number and functional status determines the rate of ROS generation The purpose of this investigation was to evaluate in the plasma of gastric carcinoma patients the level of MPO as source of cellular production ROS and uric acid - important plasma antioxidant with TAS (total antioxidant status).
Methods: MPO concentration was measured using chemiluminescent immunoassay technology (CMIA) (Abbott, Wiesbaden, Germany) and uric acid was also measured using a spectrophotometric method. TAS concentration was measured using the colorimetric method with RANDOX reagents (TAS, Randox, Crumlin, United Kingdom). All of these parameters are adapted to Architect ci8200 (Abbott, USA)
Results: The study included 32 patients (12 females - group A, and 20 males - group B) with III and IV stage cancer (TNM/UICC classification). Group AB ages 51-80 years (mean 65 years) treated at the Department of General and Gastrointestinal Surgery of the Medical University of Bialystok, Poland. Plasma samples were drawn before operation. Twenty subjects ages 44-65 years (mean 54 years) were in the control group and they submitted one blood sample. In group A, MPO concentration was 698,95 pmol/L and group B and AB was statistically higher (871,65 and 785,30 pmol/L) in comparison to the control group (256,45 pmol/L). Concentration uric acid in group A was 3,89 mg/dL (statistically significant to the control group), group B 4,27 mg/dL, and group AB 4,23 mg/dL. Plasma concentration uric acid in control group was 4,50 mg/dL. TAS concentration was 1,88 mmol/ in group A, 1,86 mmol/L in group B and 1,87 mmol/L in group AB. TAS results was statistically significant decreased comparison to the control group (1,93 mmol/L)
Conclusion: High plasma concentration of MPO, low level of uric acid, and TAS in patients with advanced stomach cancer before treatment may indicate prolonged oxidative stress in malignant disease.

M161

BRCA1 AND BRCA2 RAPID GERMLINE MUTATIONS SCREENING BY NEXT GENERATION SEQUENCING APPROACH

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Background: Hereditary breast and ovarian cancer (HBOCs) accounts for about 10% of all breast cancers and BRCA1 and BRCA2 are the most prevalent genes causing this pathology. Inactivating BRCA1/BRCA2 germline mutations dramatically escalates the risk of developing HBOCs by up to 20 fold. Due to their highly penetrant nature, testing for BRCA gene mutations is important to improve the clinical management of the high-risk patients and of their family members. Here, we report the use of a next generation sequencing screening for the identification of BRCA1 and BRCA2 germline mutations.
Methods: The study was performed on 150 patients with early-onset breast cancer ("under forty"), and/or with positive family history, and/or clinical features suggestive for BRCA mutations. BRCA1 and BRCA2 coding regions (including their flanking sites) were amplified using the BRCA MASTR v2.1 Assay kit (Multiplicom) to obtain a library/sample. The subsequent sequencing reactions were performed with the GS FLX System (Roche) allowing the simultaneous analysis of 50 patients/run. Finally, the downstream data analysis was carried out through the SeqNext tool (JSI Medical Systems). All the identified mutations were validated by standard Sanger sequencing.
Results: About 10% of the analyzed patients carried a mutation in the BRCA1/2 genes mostly known to be causative, except one that is under in vitro assay to be tested for its effect on cell physiology. The subsequent analysis of the families of the mutation carriers, allowed the identification of the at risk subjects that have been involved in surveillance program of preventing health care. In addition, 16 variants with unknown clinical significance were also totally detected.
Conclusion: Our results assess the feasibility of a next generation sequencing approach for BRCA1/BRCA2 mutation detection to be included in a routine diagnostic workflow, because of speed and cost-sparing features of this recently developed methodology.

M162

THE CYTOTOXIC T-CELL LYMPHOCYTE ANTIGEN 4 GENE ALLELE POLYMORPHISMS AMONG CANCER PATIENTS FROM UKRAINE

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Background: The host immunogenetic background plays an important role in the development of cancer. Cytotoxic T-cell lymphocyte antigen 4 (CTLA-4) is a member of the superfamily of immunoglobulins that are mainly expressed by activated T cells. The polymorphisms in CTLA-4 gene, which enhance the CTLA-4 pathway and thus interfere with T-cell proliferation and/or function, might be a genetic susceptibility factor for common human cancers. To evaluate the potential influences of CTLA-4 gene polymorphisms on breast cancer, colon cancer and leukemia risk, a case control study was conducted among patients with cancer from Ukraine.
Methods: Genotyping of -318 T/C and +49 G/A polymorphism of CTLA4 gene was performed in 69 patients with oncological diseases (colorectal cancer, breast cancer, leukemia) and in 50 healthy persons without cancer pathology in anamnesis. The molecular- genetic analysis was performed by PCR (Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism). Statistical analysis was conducted by Chi-square tests and odds ratio (OR) was calculated.
Results: The 318 TT genotype frequency of CTLA-4 gene was significant higher in patients with colorectal cancer vs control (0.13 vs 0.02, P <0.05) and the 318 TT genotype associated with increased risk of breast cancer development (OR=2.04), colorectal cancer (OR=2.33) and increased risk of leukemia development (OR=7.74). It was established significant higher frequency of CTLA4 49 GG genotype among patients with breast cancer vs control (0.44 vs 0.20, P <0.05), significant higher frequency of CTLA4 49 AA genotype in patients with leukemia vs control (0.55 vs 0.28, P <0.05) and significant lower frequency of CTLA4 49 GA genotype in group with colorectal cancer compared to control (0.23 vs 0.52, P <0.05) The presence of 49AA genotype was associated with increased risk of colorectal cancer (OR=2.14) and leukemia (OR=3.04).
Conclusions: The results suggest that the polymorphic variant of CTLA4 genes may play a role in the susceptibility to cancer development.

M163

RETINOIC ACID RECEPTOR β2 (RARβ2), A PROMISING NONINVASIVE BIOMARKER TO DISTINGUISH BETWEEN MALIGNANT AND BENIGN PROSTATE LESIONS IN URINE SAMPLES

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Background: Alterations in the methylation patterns of promoter CpG islands have been associated with the transcriptional inhibition of genes in many human cancers, including prostate cancer (PCa).The aim of our study was to explore the diagnostic value of aberrant promoter hypermethylation of retinoic acid receptor β2 (RARβ2) gene in urine DNA samples from patients with the diagnostic of PCa and benign prostatic hyperplasia (BPH), as a new epigenetic biomarker in distinguishing between malignant and non-malignant lesions.
Methods: Aberrant promoter hypermethylation was investigated in DNA isolated from the urine of 91 patients with diagnostic of PCa and 94 with BPH (control subjects). To evaluate the methylation status of the RARβ2 gene we used the quantitative methylation-specific PCR (QMSP) method.
Results: Promoter hypermethylation of RARβ2 gene was detected in the urine samples from 89 of 91 (92.86%) patients with PCa, and in 10 of the 94 (10.7%) patients with BPH.
Conclusions: This quantitative assay represents a promising molecular biomarker which may be used in discriminating between malignant and benign prostatic diseases by noninvasive methods. Keywords: prostate cancer (PCa); benign prostatic hyperplasia (BPH); quantitative methylation-specific PCR (MSP); retinoic acid receptor β2 (RARβ2)

M164

PROGNOSTIC VALUE OF ADHESION MOLECULES (sVCAM-1, sICAM-1) AND VEGF IN PATIENTS WITH COLORECTAL CANCER

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Background: Adhesion molecules take part in the interaction between host cells and cancer cells. In the current study the relationship between the soluble adhesion molecules sICAM-1 and sVCAM-1 and proangiogenic factor VEGF in colorectal cancer progression were measured.
Methods: The study group consisted of 46 patients with colorectal carcinoma (classified due to TNM classification) and 40 controls. sVCAM-1, sICAM-1 and VEGF plasma levels were measured by enzyme-linked immunosorbent assay (ELISA).
Results: All measured parameters levels were increased significantly in patients with colorectal cancer in comparison to controls ($P < 0.001$). sICAM-1, sVCAM-1 and VEGF increased significantly due to colorectal cancer progression. There was a positive correlation between sICAM-1 and sVCAM-1 in all study groups. VEGF had the highest diagnostic power (AUC=0.991, $P < 0.009$).
Conclusions: Our results demonstrated in CRC patients significantly increased levels of soluble adhesion molecules (VCAM-1 and sICAM-1) and angiogenic factor (VEGF) as compared to control group. The dynamics of these molecules showed the growing tendency along with tumor size and metastasis formation.

M165
ANALYSIS OF METHOTREXATE IN SERUM USING UPLC/MS/MS

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Background: Methotrexate is used for the treatment of proliferative diseases, including acute lymphoblastic leukaemia, osteosarcoma, non-Hodgkins lymphoma and breast cancer. The dosage of methotrexate is varied, ranging from low-dose oral therapy to high-dose infusion therapy. After administration of high-dosage methotrexate, therapeutic drug monitoring is important to monitor the patient's plasma or serum levels so that the patient can be rescued, if necessary, with the correct dose of leucovorin, a folic acid antagonist that bypasses the enzymes inhibited by methotrexate. Here we evaluate the potential of Ultra Performance liquid chromatography tandem mass spectrometry (UPLC/MS/MS) for the analysis of methotrexate in serum.
Methods: For this initial study, calibrators and QCs were created by spiking a certified reference solution of methotrexate into pooled serum. Calibrators, QCs and samples were treated with methanol containing 2H3-methotrexate and vortex mixed. Following centrifugation, the supernatant was diluted with water. Samples outside the calibration range were diluted with blank serum prior to extraction. Analysis was performed using a Waters ACQUITY UPLC coupled to a Waters TQD mass spectrometer. The analysis time per sample was approximately 2.5 minutes injection-to-injection.
Results: Using Design of Experiment techniques, the assay was shown to be robust when extraction parameters were modified +/-10%. Following CLSI-EP6-A the calibration range was shown to be linear from 0.1–10 μM/L, with no detectable carryover. Inter- and intra-assay imprecision for low, mid, high QCs were all <10% (n=5 for each QC level). Samples used for the method comparison were obtained from The Christie Hospital, Manchester, UK and had previously been quantified using the Abbott TDx Immunoassay (n=50). Mean recovery from pooled serum was 105% and extracted samples were shown to be stable for at least seven days when stored at 4°C. Ion suppression was <1% when determined using the peak area response ratio for methotrexate and 2H3-methotrexate.
Conclusions: We have successfully quantified methotrexate in serum using a rapid UPLC/MS/MS method. The assay demonstrates good linearity, precision and accuracy with minimal ion suppression.
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M166
SERUM MALDI PROFILING FOR PANCREATIC DUCTAL ADENOCARCINOMA BIOMARKERS DISCOVERY: A PILOT STUDY

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Background: Pancreatic cancer is one of the most lethal pathology causing patients'death within 6 months after diagnosis (5-year survival rate lower than 5%). The available biomarkers like CA19-9, lacks in sensitivity and specificity for an early detection requiring additional efforts to better understand the molecular basis of this pathology and to find novel strategies for a more accurate patient's screening. Recent advances in quantitative proteomics based on non-invasive approaches has stimulated its clinical applications founded on the analysis of biological fluids like serum. Despite the high biological variation and the protein dynamic range of serum, improvements in MS and fractionation techniques could contribute to new biomarkers discovery being a helpful tool to stratify specimens.
Methods: 10 sera from histologically proven pancreatic ductal adenocarcinoma patients and 10 from healthy controls were analyzed in order to evaluate the small proteins and peptides which could discriminate the two classes. In order to reduce the dynamic range, the high abundant protein components of serum were removed and the MALDI Profiling was adopted for the detection of differentially changed species possibly related to the tumor onset. After acquisition, spectra were processed by ClinProTools for statistics (Wilcoxon test $p < 0.05$, PCA analysis and AUC >0.800).
Results: MALDI Profiling allowed to detect 82 peaks in the acquisition range of 1.5-35 kDa which underwent statistical analysis. The comparison between pathological and control samples revealed a high discrimination power as indicated by the presence of 35 significantly changed peaks (10 over and 25 underexpressed in cancer) with AUC not lower than 0.872. In addition, several peaks were found strongly represented exclusively in one of the classes suggesting the presence of proteins and peptides which characterize one of the two states, only.
Conclusions: These preliminary results suggest the potentiality of this approach to discriminate pancreatic cancer patients and controls. The next step will consist on their validation, by increasing the number of analyzed samples, and identification of molecules characterizing the changed peaks associating them to their hystological pattern.

M167
A NOVEL PSA GUIDED APPROACH FOR A BETTER DIAGNOSIS OF PROSTATIC ADENOCARCINOMA BASED ON MALDI PROFILING

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Background: Prostate adenocarcinoma (PCa ADK) is the most frequently occurring cancer in men and its diagnosis and management is currently based on PSA test which is often unable to discriminate different cancer forms providing, in some cases, false negative and positive outcomes which require prostate biopsy resulting in overtreatment and overcosts with little benefits for patients. Moreover, PSA concentration in serum seems to be linked more to the inflammatory status rather than to the real presence of ADK. Thus the development of more specific and sensitive tests is required. In particular, the idea of protein species able to discriminate between patients and controls perfectly match with the proteomic approach based on the analysis of the serum protein patterns to improve the predictive power of clinical tests.
Methods: The goal of this study is the detection of proteins able to discriminate controls from ADK patients by adopting a PSA guided samples' pooling (threshold=4 ng/mL) to generate two distinct comparisons: ADK vs Controls with low and high PSA values respectively. Sera were depleted from high abundant proteins and the resulting fractions were profiled by MALDI-MS. Statistics was performed by ClinProTools (Wilcoxon test p-value <0.05, evaluation of PCA, CV% and ROC curves) which generated lists of changed peaks whose identification was performed following a multi fractionation approach.
Results: Low PSA samples were characterized by a MALDI Profiling in which 13 changed peaks in the range 1-10kDa were detected, whereas the high PSA samples were characterized by 24 changed peaks. Interestingly, the low PSA group analysis showed that in PCa patients a fragment of a complement factor was overexpressed, as further confirmed in sera by western blot considering both the levels of the intact protein and of its fragments.
Conclusions: We demonstrated that combining the PSA test with MALDI profiling can be helpful for samples classification decreasing their heterogeneity. In addition, MALDI profiling allowed to identify proteins specifically related to the cancer rather than to the inflammation. Further efforts will regard the identification of a higher number of altered molecules that could be used for PCa diagnosis and monitoring in a not-invasive way.

M168
REFERENCE INTERVALS FOR THE NEW BRAHMS CHROMOGRANIN A (CGA) ASSAY

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Background: CGA measurement is used in the evaluation of neuroendocrine tumours. To improve analytical performance and decrease test turnaround time, we introduced the new fully automated Brahms CGA assay performed on Kryptor platform. As CGA results from different assays are not comparable, we performed a study for establishing reference intervals for the new assay.
Methods: Fresh serum samples were obtained from 200 healthy blood donors and immediately measured for CGA. Shapiro-Wilk and Wilcoxon rank sum tests were employed to assess distribution of CGA values and compare groups, respectively. Multiple regression models were used to evaluate the influence of age and sex on CGA concentrations, including the interaction between the two factors.
Results: 4 elevated CGA values were statistical outliers and in 3 of those individuals an interfering condition possibly increasing CGA was identified (hyperthyroidism, vitamin D supplementation, use of hormonal contraceptives). After their exclusion the remaining values from 196 subjects [99 males and 97 females; median age (range) 44 years (19-67)] were analyzed. Median (range) CGA concentration was 41.6 μg/L (15.9-146.2), with no gender-related difference. Although deviating from normal frequency distribution, the visual examination of data did not suggest log transformation. Regression analysis confirmed the lack of gender influence, showing however that CGA concentrations increased with age (P <0.001). The lack of biological interaction between age and sex excluded the hypothesis that menopausal status may influence CGA release. Aiming to decide if reference values should be partitioned by age, we compared CGA concentrations in subjects <45 (n=99) and ≥45 years (n=97). Higher CGA concentrations were found in older people (mean±SD: 49.1±18.6 μg/L vs. 41.8±19.4 μg/L, P=0.0006). Accounting for manufacturer's declared imprecision at CGA range of 80-120 μg/L (CV <7%) and the estimated upper reference limit (97.5th percentile - URL) for subjects ≥45 (98.5 μg/L) and <45 (87.0 μg/L), we however decided to adopt a single URL for overall population (93.7 μg/L; 90%CI: 79.0-114.1).
Conclusions: In healthy subjects age but not gender may affect CGA release. However this does not appear to require age-related reference limits.

M169

COMPARISON OF FOUR COMMERCIAL ASSAYS FOR TOTAL AND FREE PROSTATE SPECIFIC ANTIGEN

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Background: Historically, different assays for the measurement of total PSA and fPSA provide discordant results. However, the comparability of total and free PSA serum levels has been improved since the introduction of WHO standards in their measurements.
Methods: We have measured 155 serum samples submitted to our laboratory for PSA testing with total PSA between 0 and 20 μg/L. PSA and fPSA were measured using 4 frequently used commercial assays, including Architect (Abbott), Cobas e 411 (Roche), Advia Centaur XP (Siemens), and Immulite 2000 (Siemens). All the assays use the WHO standards in the measurement of total and free PSA. Regression analysis was performed for PSA and %fPSA with the Cobas e 411 as comparison method. The κ statistics for PSA was used to calculate the concordance with the comparison method at the critical cut-offs of 0.4, 3, 4 and 10 μg/L.
Results: For patients with PSA <2 μg/L the regression slopes varied from 0.921 to 1.104 (with r from 0.984 to 0.997) and the κ value varied from 0.93 to 1. For patients with PSA between 2 and 20 μg/L regression slopes varied from 0.861 to 1.081 (with r from 0.979 to 0.985), and κ values varied from 0.58 to 0.81 for cut-off of 3 μg/L; from 0.67 to 0.86 for cut-off of 4 μg/L; and from 0.76 to 0.88 for cut-off of 10 μg/L. Particularly, we found statistically differences in concordance (κ < 0.75) for the cut-off of 4 μg/L between Cobas e 411 and Advia Centaur (0.67) and between Cobas e 411 and Architect (0.74). Regression analysis was performed for %fPSA for patients with total PSA between 2 and 20 μg/L and κ statistics was used to calculate the concordance with the comparison method at the critical cut-offs of 10% and 25%. Slopes ranged from 0.918 to 1.216 (with r from 0.930 to 0.955), and κ values varied from 0.45 to 0.78 for the cut-off of 10% and from 0.62 to 0.74 for the cut-off of 25%.
Conclusions: Differences in the measurement of total and free PSA persist despite the introduction of WHO standards in their measurements. It suggests that specific cut-offs could be necessary for each assay. Additional harmonization efforts are required, especially in reference to %fPSA.

M170

NOVEL STRATEGIES TO DELIVER MELATONIN (IN SLN AND BY CRYO-LASER THERAPY) TO PROSTATE CANCER CELLS

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Background: Melatonin (MT) is a chemical signal of dark/light, and serves as a bioclock and bio-calendar to mediate many receptor- or non receptor-mediated functions. MT also has a relevant oncostatic activity, especially with respect to prostate cancers, recently related to hypoxia and sphingolipids signaling pathways. The aims of the investigation are: to confirm that MT is active in the cure of prostate cancer, to speculate on the underlying mechanisms, to investigate the signaling pathways involved and to assess whether alternative and novel ways to deliver the drug may be competitive.
Methods: We used an in vivo model of human prostate tumor LNCaP cells xenografted into nude athymic mice. MT has been administered i.p. as saline (n=13) and by SLN (solid lipid nanoparticles) (n=13) or transdermally by Cryopass therapy (n=14). For each treatment controls were also included. Each group received the same administration schedule: 3 treatments per week, for 6 week. At the end the animals were sacrificed and along the treatment period the mice weight were recorded as well as the tumor volume was measured. MT concentration was assessed in plasma and tissues by ELISA test and tumors were evaluated for morphology, MT content and HIF-1a expression.
Results: Tumors developed slowly in all the MT-treated (topical and i.p.) groups and at the end of the treatment, the mean volume was significantly lower vs control. Both tumor and plasma levels were significantly higher in treated vs not-treated animals. Harvested tumor showed a strong inflammatory reaction which seemed to surround and infiltrate the tumor cells. In SLN-MT treated animals, in addition to a strong lymphocyte infiltration, the tumor appeared limited also by the presence of fibroblast type cells. Preliminary results showed HIF-1α expression increased in both treatment groups vs control.
Conclusions: We have confirmed the positive effects of MT on tumor growth and we have focused on its effect on hypoxia. The possible role as anti-tumor drug candidate deserves to be further investigated. We demonstrated that different alternative and novel ways to deliver MT are effective as well. This would accelerate the transferability of obtained data towards a therapy. on MT oncostatic activity.

M171

CARCINOEMBRYONIC ANTIGEN (CEA), ALPHA-FETOPROTEIN (AFP) AND CANCER ANTIGEN 19-9 (CA 19-9) IN FOLLOW-UP OF COLON CANCER

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Background: Alpha-Fetoprotein (AFP), an albumin-like glycoprotein with a molecular weight of 70.000 Dalton, is formed in the yolk sac, non-differentiated liver cells and the fetal gastro-intestinal tract. 70-95% of patients with primary hepatocellular carcinoma have elevated AFP values. Carcinoembryonic Antigen (CEA), a monomeric glycoprotein with an approximately molecular weight of 180.000 Dalton, belongs to the group of carcinofetal antigens that are produced during the embryonic and fetal period. High CEA concentrations are frequently found in cases of colorectal adenocarcinomas. Cancer Antigen 19-9 (CA 19-9), defined by the use of the monoclonal antibody 1116-NS-19-9 with a molecular weight of 10.000 Dalton, corresponds to a hapten of Lewis-a blood group determinants and is a component of a number of mucous membrane cells.
Methods: CEA, AFP and CA 19-9 were measured in 128 sera of colon cancer patients who underwent surgery of colon or rectum cancer and in 198 sera of healthy men and women. CEA, AFP and CA 19-9 with a electro-chemiluminometric assay [ECLIA].
Results: The assays showed within-run coefficients of variation (CV) from 0,7-1,3% for AFP; 1,0-1,4% for CEA and 1,5-2,6% for CA 19-9 and between day CVs from 4,0-6,0% for AFP; 5,2-6,0% for CEA and 3,1-5,2% for CA 19-9. The colon cancer patients were staged according to the ICD10 [C18.0 – C20]. Median and range were calculated for CEA to 1,4 (0,2-10,1) ng/ml for the control group and to 3,5 (0,3-4115) ng/mL for patient group, for AFP to 2,0 (0,5-84) ng/mL for the control group and to 3,4 (0,9-16,7) ng/mL for the patient group and for CA 19-9 to 8,9 (0,6-62,8) U/mL for the control group and to 19,3 (0,6-19554) U/ml for the patient group. The cut-off points were calculated for CEA to 5,0 ng/mL (95th percentile) and 6,3 ng/mL (97,5th percentile); AFP 4,7 ng/mL (95th percentile) and 4,9 ng/mL /(97,5th percentile) and for CA 19-9 to 44,1 U/mL (95th percentile) and 52,4 U/mL (97,5th percentile). In the ROC curve analysis AFP has the biggest area under the ROC curve (0.76) followed by CEA (0.74) and CA 19.9 (0.69).The differences between control group and patient group were shown as significant for CEA, AFP and CA 19-9. The sensitivities were calculated for the tumor markers and for all combinations of the markers. The p-values between the tumor group and the control group were calculated with the Man-Whithney-Wilcoxon test. The combination of AFP and CEA has the highest sensitivities to the tumor group.
Conclusions: The sensitivity of the combination of AFP and CEA could give additional information for selecting patients to systemic adjuvant therapy.

M172

14-3-3 THETA, A DIRECT INTERACTOR OF AF4, INFLUENCES HOXA9 EXPRESSION IN RS4;11 LEUKEMIA CELL LINE

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Background: AF4 is the most common fusion partner of the MLL gene in t(4;11) infant acute lymphoblastic leukemia (ALL). The AF4 protein is a transcriptional activator with a central role in transcriptional elongation and chromatin remodeling. A hallmark of MLL leukemia is aberrant H3K79 hyper-methylation and over-expression of HOXA9, a target gene of the MLL-AF4 fusion protein. The MLL-AF4 chimera recruits AF4 and transactivates HOXA9. We recently demonstrated that AF4 interacts with the scaffold protein 14-3-3 theta. We studied the molecular features of such interaction in the context of the molecular pathogenesis of the t(4;11) ALL.
Methods: To evaluate the interaction between AF4 and 14-3-3 theta, we performed in vitro binding assay, western blot analysis and fluorescence resonance energy transfer (FRET) experiments. We transiently expressed 14-3-3 theta in RS4;11 leukemia cells and analyzed HOXA9 transcript levels by real time RT-PCR.
Results: Purified recombinant HA-14-3-3 theta and Flag-AF4 interact in vitro; FRET analysis showed that the recombinant proteins GFP-AF4 and Cherry-14-3-3 θ actually interact into the nucleus. Real time RT-PCR showed that transcript levels of HOXA9 increase in RS4;11 leukemia cells that ectopically express 14-3-3 theta. Notably, we found that HOXA9 expression level varies in direct proportion to the amount of ectopic 14-3-3 theta.
Conclusions: Our data indicate that 14-3-3 theta directly interacts with AF4 in the nucleus and probably contributes to trigger the epigenetic modifications that results in transcription of MLL-AF4 chimera target genes. Recently, small nonpeptidic molecules that inhibit 14-3-3 protein-protein interactions have been discovered. Therefore, 14-3-3 theta might be considered a promising molecular target in therapy of t(4;11) ALL.

M173

PANCREATIC CANCER-DERIVED SOLUBLE MEDIATORS INDUCE DENDRITIC CELLS TO ACQUIRE AN IMMUNESUPPRESSIVE PHENOTYPE BY DOWNREGULATING CTLA4

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Background: An altered function of lymphocytes, dendritic cells (DC) and immature myeloid cells appears to be an hallmark of tumor-mediated immune suppression and the two inhibitory co-stimulatory receptors PDL-1 and CTLA4 might have a role in this context. The aim of the present in vitro study was to assess whether Pancreatic cancer (PaCa) cells cross-talk with normal mononuclear circulating cells (PBMC) causing them to acquire an immunesuppressive phenotype and to evaluate whether PDL1 and CTLA4 are involved.
Methods: PBMC from blood donors were cultured for 4 days in Control (CTL) and in the PaCa cancer cell line Capan1 conditioned media (CM). Lymphocytes subsets (CD4+, CD8+, CD4+CD25+) and CD33+ immature myeloid cells subsets (CD14+/-; HLA-DR+/-) expressing or not PDL1 and/or CTLA4 were analysed by flow cytometry. To assess immunesuppressive function, myeloid cells were FACS sorted and co-coltured with allogenic total T lymphocytes in 1:20 and 1:40 ratio. Total T lymphocytes proliferation was determined by 3H-Thymidine uptake.
Results: Capan1 CM caused an expansion of CD4+CD25+ (P=0.01) and a reduction of CD33+CD14-HLA-DR+ (P=0.03) cells. In this latter cellular subset, CM caused also an increase of PDL1 (P=0.046) and a decrease of CTLA4 (P=0.05) positive cells. FACS sorted CTL and CM CD33+CD14-HLA-DR+ cells did not significantly affect the proliferation of allogenic total T lymphocytes at 1:20 (P=0,54) or at 1:40 ratio (P=0,81). The CD33+CD14-HLA-DR+ PDL-1+ cells did not significantly modify allogenic T cells proliferation with respect to PDL- cells (P=0,11), while those cells which were CTLA4 negative caused a significant inhibition of T cell proliferation in comparison of CTLA4 positive cells (P=0,008).
Conclusions: PaCa-derived soluble factors induce the expansion of the inhibitory lymphocytes subset CD4+CD25+ and a reduction of the immature CD33+CD14-DR+ dendritic cells. The tumor associated reduced expression of the inhibitory molecule CTLA4 in this cell population was demonstrated to characterize an immunosuppressive phenotype and this study suggests to take care in the use of anti-CTLA4 therapies.

M174

INTERLEUKINE-1B PROMOTER POLYMORPHISM AND PROSTATE CANCER SUSCEPTIBILITY IN EASTERN CROATIAN POPULATION

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Background: Single nucleotide polymorphisms (SNPs) in promoter regions of cytokine genes are associated with differential levels of cytokine expression. We hypothesized that C→T polymorphism at +3954 position in the promoter region of the Interleukine-1β (IL-1β) gene might influence prostate tumour development and progression by affecting the efficiency of the antitumor immune response and/or pathways of angiogenesis.
Methods: A total of 275 subjects from Eastern Croatia were included in the study, 120 Prostate cancer patients (CaP), 120 benign prostate hyperplasia (BHP) patients and 35 controls. They were genotyped for IL-1β C+3945T polymorphism using real-time PCR (LightCycler Instrument, Roche Diagnostics) and melting curve analysis method. CaP patients were classified in two groups according to Gleason score (≥4+3 and ≤3+4). All groups were age-matched (mean age 67,5 years). $\chi 2$ test was used to compare distribution of IL-1β polymorphism genotypes. Relative risk was estimated by the Odds ratio (OR).
Results: There was significant statistical difference ($\chi 2$=13,92, P=0,000, OR=4,48, 95%CI=2,0139-1,8059) between controls and patients with CaP and BHP related to low (CC) and high (CT and TT) producer genotypes of IL-1β +3945 polymorphism. There was no significant statistical difference ($\chi 2$=0,86, p=0,355, OR=1,32, 95%CI=0,787-2,2259) between BHP and CaP patients and between two groups of CaP patients according to Gleason score ($\chi 2$=0,00, P=0,946, OR=1,05, 95%CI=0,4775-2,3354).
Conclusions: Our data suggest that, although, there is a tendency to high producer (CT and TT) genotypes in BHP and CaP patients vs. control patients, IL-1β 3945 polymorphism is not associated with prostate cancer development and/or progression in Eastern Croatian population. These data taken together with contradictory results of other studies imply ethnical dependency of the IL-1β 3945 polymorphism.

M175

ISCHEMIA-MODIFIED ALBUMIN: A HELPFUL MARKER IN THE DIFFERENTIAL DIAGNOSIS OF MALIGNANT OVARIAN CYSTS

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Background: To evaluate whether serum and peritoneal fluid ischemia-modified albumin (IMA) levels have benefit in the differential diagnosis of benign and malignant ovarian cysts and to investigate correlation with CA-125.
Methods: The study consisted of 15 patients with ovarian malignancy and 25 patients with benign ovarian cysts which were confirmed pathologically. Blood samples were taken preoperatively to evaluate serum IMA and CA-125 levels and peritoneal fluid samples were picked up intraoperatively in order to evaluate IMA in peritoneal lavage.
Results: The mean age of patients in the malignancy group was 46,6±7,2 (36-66) and was 43,4±5.2 (35-56) in benign ovarian cyst group. There was no statistically significant difference between groups regarding age, gravidity, parity. The calculated IMA level in malignancy group was 0,773±0,087 in serum and 0,867±0,110 in peritoneal fluid; on the other hand the calculated IMA level in benign ovarian cyst group was 0,667±0,154 in serum and 0,745±0,156 in peritoneal fluid.When groups are compared, there was statistically significant difference regarding serum IMA levels (P= 0,01) and peritoneal fluid IMA levels (P=0.022) respectively. CA-125 levels were significantly increased in malignancy group (P=0,00), but the correlations between serum IMA and CA-125, between peritoneal IMA and CA-125 were not statistically significant (P=0,164 and P=0,057) respectively.
Conclusions: Our study results support that serum and peritoneal IMA levels are increased in case of malignant ovarian cysts, so we propose that serum IMA levels can be assessed preoperatively in patients with ovarian cysts in order to confirm malignancy.

M176

ARE CIRCULATING TUMOR CELLS A FUTURE WEAPON IN THE FIGHT AGAINST CASTRATION RESISTANT PROSTATE CANCER?

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Background: Prostate cancer is currently the second most frequent oncologic disease and the most frequent urological tumor in men in the Czech Republic. Almost 20% of patients develop castration resistant prostate cancer (CRPC) which has bad prognosis and 85% of them die from bone metastases. The aim of the project is to measure the presence of the circulating tumor cells (CTCs) in the peripheral blood of CRPC patients and subsequently use these results to evaluate the effect of the treatment and to estimate the prognosis of a patient.
Methods: Immunomagnetic separation of CTCs by ProstateCancerSelect kit (Adnagen) from 5 mL of peripheral blood of patient with CRPC or benign prostatic hyperplasia (BPH) was done. mRNA was isolated from enriched fraction of CTC using ProstateCancerDetect kit (Adnagen). By reverse transcription cDNA was prepared and used as a template for mulitiplex-PCR. Amplification of at least one of three tumor-associated antigens proved presence of CTC in samples. Peripheral blood was drawn at the time of CRPC diagnosis and after the 4th cycle of systemic therapy.
Results: During the year 2012 18 patients were tested (16 with CRPC, 2 with BPH) so far. 10 patients were already tested before and after therapy, for remaining ones only the first measurement was done. CTCs were detected in blood of 15 patients with CRPC before chemotherapy. All of these patients had a high level of prostatic specific antigen (PSA) expression in their CTCs. The patients with BPH were CTCs negative. In measurement after the 4th cycle of chemotherapy 30% of the patients became CTCs negative. The patients who remained CTCs positive are further monitored.
Conclusions: Our results show that almost all CRPC patients are CTCs positive at the time of diagnosis. Moreover, CTCs are highly heterogeneous in gene expression. By comparing the results of the CRPC patients with the results of the patients with BPH we can conclude that the high PSA expression in CTCs is not connected to the high PSA level in serum. From the first results it seems probable that CTCs could serve as a powerful biomarker for monitoring of CRPC progress and therapy efficiency.

M177

ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) ACTIVITY IN THE SERA OF PATIENTS WITH BRAIN CANCER

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Background. Various alcohol dehydrogenase (ADH) isoenzymes and aldehyde dehydrogenase (ALDH) exist in the human brain tissue and cancer cells of brain. Moreover the activities of total ADH and class I isoenzymes are significantly higher in cancer than in healthy tissue . From this reason we investigated alcohol dehydrogenase and its isoenzymes activity and the total activity of aldehyde dehydrogenase in the sera of patients with brain cancer because these changes may be reflected by enzymes activities in the serum.
Methods. Serum samples were taken from 52 patients (27 males, 23 females 36-82 years) with glioblastoma. Class I and II ADH isoenzymes and ALDH were measured by fluometric method using the specific substrates (4-metoxy-1naphtaldehyde and 6-matoxy-2-naphtaldehyde respectively). The activity of class III was measured by photometric method with n-octanol and class IV with m-nitrobenzaldehyde as a substrate. Total ADH activity was estimated by the photometric method with p-nitrosodimethylaniline.
Results. A statistically significant increase (30.8%) of class I ADH isoenzymes was found in the sera of cancer patients (2.33 mU/l vs 1.61 mU/l in control group). The total ADH activity was also significantly higher (about 38.7 %) in patients with cancer (1025 mU/l vs 628 mU/l in control group). The activities of other tested ADH isoenzymes and total ALDH were unchanged.
Conclusions. The increased total activity of ADH and class I isoenzyme in the sera of patients with brain tumor (glioblastoma) probably can be caused by release of this isoenzyme from cancer cells and may be useful for diagnostics of this cancer.

M178

THE RELATIONSHIP BETWEEN INSULIN-LIKE GROWTH FACTOR-I AND CONCENTRATION OF SERUM TUMOUR MARKERS IN PATIENTS WITH CANCER

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Background. The insulin-like growth factor (IGF-1) is produced by cells in the liver, kidney, pituitary gland, gastrointestinal tract, muscle and cartilage. IGF-1 system is known for regulating cell growth and apoptosis. Recently published data suggest an association between higher serum levels of IGF-1 and increased risk of cancer. The aim of this study was to compare the serum concentration of IGF-1 with significantly elevated serum concentrations of cancer antigens CA19-9, CA125, total prostate specific antigen (TPSA) and carcinoembryonic antigen (CEA).
Methods. We analysed 98 patients with malignant diseases: 48 – colorectal cancer (34 female and 14 male, age range 40-70 years), 22 – prostate cancer (age range 60-86 years), 28 – ovary cancer (age range 33-85 years). Blood samples were collected into BD Vacutainer® SST™ II Advance plastic tubes (BD, USA). CA19-9, CEA, CA125, TPSA tests were performed on architect ci8200 system (Abbott Lab, USA) using Chemiluminescent Microparticle Immunoassay (CMIA) technology. Radioimmunoassay (DIAsource ImmunoAssays S.A.) was used for measurement IFG-1. SPSS software was used for statistical data analysis.
Results. TPSA and IGF-1 serum concentrations ranged from 6.01 to 247.0 ng/L (mean value 48.880) and from 3.4 to 224.6 ng/mL (mean value 81.429) respectively. A weak negative correlation ($r = -0.355$) between the TPSA and IGF-1 groups was found. Patients with ovary cancer showed a weak negative correlation ($r = -0.299$). CA125 and IGF-1 serum concentrations ranged from 46.5 to 7275.7 mIU/L (mean value 1189.641) and from 6.9 to 212.0 ng/mL (mean value 112.322) respectively. A relationship between CA19-9, CEA and IGF-1 was controversial. CA19-9 and IGF-1 serum concentrations ranged from 14.6 to 63115.2 mUI/L and from 8.4 to 216.2 ng/mL respectively. Weak negative correlation ($r = -0.256$) between CA19-9 and IGF-1 serum values and moderate positive correlation ($r = 0.496$) between CEA and IFG-1 were found.
Conclusions. IFG-1 and CA125, CA19-9, TPSA results showed a weak reverse relationship between these subgroups without statistical significance. We found a moderate positive correlation with no statistical significance (P=0.07) between CEA and IGF-1 values.

M179

DOES THROMBOPOIETIN CONCENTRATION AND PLATELET COUNT COULD BE CONSIDERED AS A NEGATIVE MARKER OF SEVERITY OF THROMBOCYTOPENIA AND ADVANCEMENT OF MULTIPLE MYELOMA?

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Background: In physiological state platelet count is inversely proportional to TPO concentration. In multiple myeloma (MM) infiltration of the bone marrow by neoplastic plasmocytes might impair megakaryopoiesis. The aim of the study was the evaluation of TPO concentration and platelet count (PLT) depending on the stage of the MM advancement. Additionally we would like to evaluate if TPO, similarly to the albumin concentration, can be considered as a marker of severity of thrombocytopenia and advancement of MM.
Methods: The study group consisted of 41 patients (mean age 67.7) with newly diagnosed MM prior to treatment and categorized depending on the Durie and Salmon staging system. PLT was measured on the hematological analyser, TPO with the use of ELISA and albumin with the use of immunonephelometry method. Differences were considered statistically significant for $P < 0.05$. ANOVA rank Kruskal- Wallis test was used for the comparison of three samples. Correlation coefficients were obtained by applying Spearman's rank method. The usefulness of TPO diagnostics was determined with the use of ROC curve and AUC (Area under the ROC curve).
Results: Median TPO was significantly increasing with the disease advancement (I-64.34, II–164.46, III–282.56 [pg/mL];P=0.0001 respectively for I vs. II and I vs. III;P=0.05 for II vs. III). Median platelet count was significantly decreasing with the stage of the disease advancement (I–277, II–212, III-142 [x103/μL]; P=0.0001 respectively for I vs. II, I vs. III and II vs. III). Thrombocytopenia (139 x103/μL) and the highest concentration of TPO (288.58 pg/mL) was observed only in the III stage. TPO concentration was negatively correlated with albumin concentration (r=-0.64; P=0.0001) and with the platelet count (r=-0.39; P=0.013). AUC for TPO was 0.976, sensitivity equaled 92.68% and specificity equaled 96.67%.
Conclusion: TPO concentration and platelet count depend on the stage of the multiple myeloma advancement. The elevated concentration of TPO in III stage MM patient can be considered as an unfavourable marker of thrombocytopenia and advancement of the disease. J. Kamińska and OM. Koper were supported by a scholarship studying, researching, commercialization-PhD students of the Medical University of Bialystok support program.

M180

CAN HE4 AND ROMA SCORE BE MORE HELPFUL THAN CA125 ALONE AS DIAGNOSTIC TOOLS IN PATIENTS WITH PELVIC MASS?

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Objective: The human epidydimis proitein 4 (HE4) has emerged in the last few years as one of the most promising biomarkers in gynaeco - oncology. The purpose of this study was to evaluate the potential of HE4 and the calculated Risk of Ovarian Malignancy Algorithm (ROMA) in comparison with CA125 for distinguishing between benign and malignant pelvic masses in the Bulgarian female population.
Methods: The patients included in this study, had a pelvic mass proven with diagnostic imaging techniques and were scheduled for surgery. All diagnoses were histologically verifeid. Serum samples were obtained preoperatively from 376 women undergoing surgery for pelvic mass: 61 of them had ovarian carcinoma; 20 had endometrial cancer, and 295 had a nonmalignant ovarian disease (benign ovarian cyst and endometriosis). The values of the markers were compared with the results, obtained from 85 female controls (46 premenopausal and 39 menopausal).The samples were tested for HE4 and CA125, using the Architect System (CMIA method) by Abbott Diagnostics. The diagnostics specificity and sensitivity for HE4, CA125 and ROMA score were estimated using ROC curves and areas under the curves.
Results: The levels of HE4 and CA 125 were significantly higher among the patients with malignant tumors, compared with patients with nonmalignant disease and controls . The diagnostic sensitivity in distinguishing ovarian cancer was heighest for ROMA score -90.16% (73.8% for HE4 and 88.2% for CA125). The best specificity has been shown for HE4-97.6% (85.5% for ROMA and 81.09% for CA125). Importantly, the level of HE4 in the premenopausal group did not differ significantly between the patients with endometriosis and with other nonmalignant diseases (this is not valid for CA125 level). Additionally we found HE4 to be useful in patients with endometrial cancer – it has higher specificity and sensitivity than CA 125 in this group.
Conclusions: HE4 is a heplful biomarker for gynaecological cancer diagnosis. HE4 could be the preferred marker for distinguishing endometriosis from ovarian cancer, where CA125 is non-specific. ROMA score proved to be useful as a Negative Predictive Marker in excluding malignant diagnosis in premenopausal women.

M181

DIAGNOSTIC UTILITY OF PSEUDOCHOLINESTERASE AND CHOLESTEROL FOR THE SEPARATION OF MALIGNANT FROM BENIGN PLEURAL EFFUSIONS

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Background: Most patients who were admitted to a hospital with a pleural effusion(PE) undergo extensive diagnostic procedures in an attempt to identify the cause of the effusion. Among the lot of diagnostic procedures, there is a thoracentesis too, with multiple chemical determination. It was proved that patients with malignancy had a significant higher serum LDH, CEA and tissue polypeptide level than did those with benign diseases, while those with benign diseases had a significantly higher serum protein level. There was no laboratory tests about cholinesterase (PCHE) and cholesterol (CHOL) diagnostic utility in separation of malignant from benign pleural effusions.
Methods: Prospective study of 200 patients with PE of known etiology, who underwent a diagnostic thoracentesis, was done. Pleural fluid (pf) and serum (s) value, and pleural fluid and serum ratio (p/s) of PCHE and CHOL were measured on AU 680 Beckman Coulter analyzer. According to receiver operating characteristics (ROC) curve we chose the best cut-off values for PCHE and CHOL, and determined area under curve (AUC) values.
Results: Biochemical type of pleural effusion was determined according to its pathophisyological mechanism of origin. There were 44 (22%) transudates and 156 (78%) exudates.The patients with malignant pleural effusions had a significantly higher pfPCHE and also pfCHOL level (P <0.01), while dose with benign pleural effusions had significantly higher sPCHE (P <0.01) and p/sCHOL ratio (P <0.05).
Conclusion: Our studies have shown that malignant pleural effusions have a significant higher pfPCHE and pfCHOL. Benign pleural effusions have a significant higer sPCHE and p/sCHOL ratio. It would be important for the diagnosis and treatment.

M182

EVIDENCE-BASED EARLY BIOLOGICAL DETECTION OF PROSTATE CANCER: WHICH TEST TO USE AND WHY?

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Background: Despite conflicting data for the benefit of prostate cancer (PCa) screening, prostate specific antigen (PSA) is currently widely used for early detection of PCa. New biomarkers aiming to improve the predictive value of PSA are also used. We evaluated the clinical validity of PSA, PCA3, [-2]proPSA and fusion oncogenes for the early detection of PCa.
Methods: We searched PubMed for studies published between 2000 and 2011 that included >200 subjects. Data describing the study design, patients' characteristics, sample handling, methods used and results were extracted. The level of evidence (LOE) for clinical utility was evaluated using the tumor marker utility grading system.
Results: 6 randomised controlled trials (RCTs) on PSA. Largest trial (182,160 subjects from 8 European countries with a median follow-up 9.8 years) showed 29% reduction in PCa mortality in the screened group. Relative risk in a meta-analysis of all six trials was 0.90 (95% CI: 0.81-0.99). Although these trials included a large number of subjects and long-term follow-up, their limitations include contamination in the control group (opportunistic screening) and lower quality follow-up in this group. Also great variability was reported for pre-analytic treatment, PSA assay conditions and cut-off values (between trials and over time). Despite these limitations, we attributed an LOE of IA to PSA for early PCa detection, but, since its benefit is small in the overall population we do not recommend its use in mass screening. Emerging biomarkers have been assessed in prospective case-control and cohort studies: PCA3 (n=3); kallikreins (n=3); [-2]proPSA n=5); fusion oncogenes (n=2). These studies, including from 262 to 2914 patients, used biopsy result (PCa) to determine specificity and sensitivity, but they did not assess the effect on PCa mortality. Also they often used 'in-house' assays and were monocentric. The LOE is low (III-C).
Conclusions: PSA can be used for early PCa detection but mass PSA screening is not recommended as it fails to select aggressive PCa. Studies on other biomarkers suggest that they could be used, individually or in combination, to improve the selection of patients with elevated PSA levels for biopsy, but RCTs with a PCa mortality outcome are needed.

M183

PLASMA LEVELS OF M-CSF, HE-4 AND CA 125 AS THE NEW LABORATORY PANEL IN DIAGNOSTIC OF OVARIAN CANCER

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Background: M–CSF is one of the glycoproteins called hematopoietic growth factors (HGFs). Some clinical investigations have shown an autologous production of M–CSF in various human cell lines in vitro and by tumors in vivo, for example in ovarian cancer. In this study, we investigated plasma levels of M-CSF in comparison with the tumor markers (HE-4 and CA125) in ovarian cancer patients and in relation to the control groups: benign ovarian tumor patients and healthy subjects.
Methods: The group tested included 50 epithelial ovarian cancer patients (serous sub-types). The control groups consisted of 40 benign ovarian tumor patients and 40 healthy volunteers. Plasma levels of M-CSF were determined using immunoenzyme assay (ELISA), HE-4 and CA125 concentrations with the use of chemiluminescent microparticle immunoassay (CMIA).
Results. Plasma levels of M-CSF and the tumor markers (HE-4, CA125) were significantly higher in ovarian cancer patients as compared to healthy controls. M-CSF, HE-4 and CA 125 diagnostic specificities received high values (equal to 93%). The diagnostic sensitivity, positive and negative predictive values were higher for M-CSF than for HE-4 and CA 125 in the ovarian cancer group. A higher area under the ROC curve (AUC) was observed for M-CSF (0,8964) and HE-4 (0,8320), and was slightly lower than the AUC of CA 125 (0,9207). The combined use of the parameters tested resulted in an increase in the sensitivity range and AUC, but the highest values were obtained analyzing all tested parameters (95%; 0,9484, respectively).
Conclusions: These results suggest a potential usefulness of the laboratory panel (M-CSF, HE-4, CA 125) in the diagnosis of ovarian cancer.

M184

PLASMA LEVELS AND DIAGNOSTIC UTILITY OF METALLOPROTEINASE 9 (MMP-9) IN OVARIAN CANCER PATIENTS

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Background: Matrix metalloproteinases (MMPs) play an important role in cancer cell invasion and metastasis by degrading the extracellular matrix. In this study, we investigated plasma levels of metalloproteinase 9 (MMP-9) in comparison with the classical tumor marker (CA125) in ovarian cancer patients and in relation to the control groups: benign ovarian tumor patients and healthy subjects.
Methods: The group tested included 50 epithelial ovarian cancer patients (serous sub-types). The control groups consisted of 40 benign ovarian tumor patients and 40 healthy volunteers. Plasma levels of MMP-9 were determined using immunoenzyme assay (ELISA), CA 125 concentrations by chemiluminescent microparticle immunoassay (CMIA).
Results: Plasma levels of MMP-9 and CA125 were significantly higher in ovarian cancer patients as compared to healthy controls or benign ovarian tumor patients. MMP-9 and CA 125 diagnostic specificities received high values (93%). The diagnostic sensitivity, positive and negative predictive values were higher for MMP-9 than for CA 125 in the ovarian cancer group. The combined use of the parameters tested resulted in an increase in the sensitivity range. A higher area under the ROC curve (AUC) was observed for MMP-9 (0,8804) and was slightly lower than the AUC of CA 125 (0,9016).
Conclusions: These results suggest a potential usefulness of MMP-9 in the diagnosis of ovarian cancer, particularly in combination with CA125.

M185

PERITUMORAL OEDEMA IN HIGH-GRADE GLIOBLASTOMAS WITH VEGF(+), AND NEURONAL NOS(+) IMMUNOPHENOTYPE AND TP53 GENE AMPLIFICATIONS - CASE REPORTS

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Background: Authors demonstrated in low-grade gliomas the significance of VEGF and number of microvessels for undergoing malignant transformation to high-grade glioblastomas. Patients with VEGF immunopositivity of gliomas had shorter median survival time. Recent studies reported data suggesting meaning of peritumoral edema on MRI at initial diagnosis as an independent prognostic factor for glioblastomas. Methods: Cases of high-grade glioblastoma multiforme (GBM) diagnosed patients (n=5) with preoperative CT scan, surgery, histology, and receiving standard treatment. Oedema on CT-scans was classified as minor (<1 cm), and major (>1 cm). 1 patient was diagnosed as secondary GBM. The primary GBM patients survived from 21 up to 24 months. Patient with secondary type of GBM survided 11 months after neurosurgery. Immunohistochistry was performed with primary antibody anti-VEGF, anti-neuronal NOS (NOS I), anti-Nestin, anti-Ki67, and anti-p53 (Chemikon, Lifespan Biosciences, Abcam), and the color was developed with di-amino-benzidin (DAB), and in situ hybridisation (FISH) with Vysis LSI HER-2/neu/CEP 17 Probe (Abbot Molecular); Vysis LSI TP53 SpectrumOrange/ CEP 17 SpectrumGreen Probe (Abbot Molecular). Informed consent: The ethics committee at each institution approved this study, and written informed consent was obtained from patients. Results: In our study, we detected lower immunohistochemical expresion of VEGF (<5%), overexpression of p53, and neuronal NOS I in primary GBM with peritumoral oedema before neurosurgery less than 1 cm in comparison with higher expression of VEGF (20%), and NOS I in patient with pre-neurosurgery peritumoral oedema more than 1 cm. Immunohistochemical positivity of Nestin labeling progenitor oligodendroglioma cells was revealed in secondary GBM. FISH investigations were focused on proved detection of TP53 amplification, and rare amplification of HER-2/neu in primary GBMs. We did not prove polysomi of CEP 17 in primary GBM in comparison with detection of CEP17 polysomi in secondary GBM.
Conclusions: Immunohistochemical overexpression of VEGF, preoperative peritumoral oedema, and TP53 amplification can play a prognostic role in patients with GBMs.

M186

AFFINITY IMPROVEMENT OF A UNIQUE PSA ANTIBODY USING PHAGE DISPLAY TECHNOLOGY

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Background: Prostate specific antigen (PSA) is a marker that is commonly used for the detection of prostate cancer. A panel of immunoassays of kallikrein forms, including intact free PSA (fPSA-I), is able to predict the biopsy outcome in men with elevated PSA in the circulation. The fPSA-I assay uses a unique monoclonal antibody (Mab) called 4D4, which is specific for fPSA not internally cleavage at Lys145-Lys146. Due to the low binding affinity and fast dissociation-rate of the 4D4 Mab, it cannot capture the target antigen (fPSA-I) tightly. In this study, we cloned the 4D4 Mab into a recombinant fragment (Fab) and constructed three mutant libraries with the aim to increase its binding affinity and to secure the sensitive and accurate assay of fPSA-I.
Methods: fPSA-I specific antibodies were enriched from three mutant libraries by phage display technology. Single clones were screened for the identification of high affinity clones. The binding properties (association rate, dissociation rate, dissociation constant and specificity) of the mutants were determined by immunoassay. L3-2 Fab mutant was purified and labeled with europium(III) [(Eu)(III)] chelate and fPSA-I assay was performed for the determination of analytical sensitivity.
Results: After three rounds of panning from enriched phage stocks, the signal-to-background ratios were increased 1.5-3 times from the first round to the third round. In order to find the highest affinity clones, Eu(III)-labeled PSA was used as tracer to detect the immobilized Fabs and Eu(III) signals were measured. In the screening of 930 clones, the signal of 31 clones was 2-5 times higher than the wild type (wt) 4D4 Fab. Fourteen of the 31 clones were unique when sequenced, and in these clones 2-5 amino acids were mutated. In the dissociation-rate assay, the dissociation of the PSA conjugate from the five mutants was slower than the wt-4D4 Fab. The affinity of these five mutants was increased 3-6 times compared with the wt-4D4 Fab. The analytical sensitivity of intact PSA assay using mutant L3-2 Fab was 0.6 ng/ml, and wt-4D4 Fab was 2.4 ng/mL.
Conclusions: The high affinity of these mutants has potential to provide more sensitive and robust detection of fPSA-I from patient samples.

M187

FREE CIRCULATING DNA SERUM PROMOTER GLUTATIONE-S-TRANSFERASI (GSTP1): MOLECULAR BIOMARKER FOR PROSTATE CANCER

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Prostate cancer (PC) is the most common cancer in men. Actually, a widely used marker for the diagnosis and follow-up is the PSA (Prostate Specific Antigen). Molecular analysis of neoplastic prostate tissues shown the inactivation of the Glutatione-S-Transferase gene (GSTP1), due to the hypermethylation. This features could be a potential biomarker for PC. The aim of this study is the specific and sensitive detection of the methylation status of GSTP1 gene in serum. The diagnostic efficacy of the test were evaluated on the 20 donors healthy subject, 57 benign prostatic hypertrophy (BPH), and 57 PC patients. In this way, specific evaluation of methylation status of GSTP1 gene may be an useful tool for the prediction of patients at risk of PC. A case-control epidemiological study has been performed on samples of 3 groups of patients: patients with PC, with benign prostatic hypertrophy (BPH) and healthy subjects. Circulating cell-free DNA was extracted by serum. After sodium-bisulfite treatment, extracted DNA was analyzed for GSTP1 promoter hyper-methylation. MSP: methylated Specific Real-Time PCR . The experiments were performed following the protcol of “Ampli GSTP1 kits®” manufactured by Dia-Chem Italy.GSTP1 promoter gene hypermethylation was detected in 0% of healthy subjects (20/20, median age 32,7 years), in 43,9% of patients with BPH (25/57 mean age 60,5 years) and in 57,6% of patients with PC (34/57 mean age 67,8 years). Significantly, the 81,8% of patients with PC, age >65 years and total PSA≤4 ng/ml were positive for the hyper-methylation status within promoter of GSTP1 gene. Osmatici silencing of GSTP1 gene is an early epigenetic event in the carcinogenesis of prostate . The absence of hypermethylation of healthy subjects and the presence in 57,6% of PC patients are in according with literature. Our Results, confirming a good sensitivity and specificity of the test based on circalating cell-free DNA (isolated by serum), in comparison of data regarding bioptic tissue . It should be emphasized that the mean age of healthy subjects was significantly lower than patients with BPH or PC. This seems to indicate a correlation between age and carcinogenesis process of prostate tissue, of the criteria assessment in subjects potentially at risk of PC.

M188

CLINICAL SIGNIFICANCE OF TISSUE INHIBITOR OF MATRIX METALLOPROTEINASE-1 (TIMP-1) IN PANCREATIC CANCER

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Background: Matrix metalloproteinases (MMPs) are able to degrade basement membrane and extracellular matrix. MMPs and their tissue inhibitors (TIMPs) play an important role in progression of cancer, thus they are associated with growth, migration and invasion of tumor cells. Pancreatic cancer is very aggressive malignancy that is characterized by late stage of diagnosis and poor prognosis of patients' survival. Therefore, the aim of this study was to assessed the usefulness of tissue inhibitor of matrix metalloproteinase 1 (TIMP-1) as well as classic tumor markers – carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) in the diagnosis of pancreatic cancer patients.
Methods: The study included 97 subjects: 30 patients with pancreatic cancer, 37 patients with chronic pancreatitis and 30 healthy subjects. Serum MMP-9, CEA and CA 19-9 levels were determined using immunoenzyme assays. The diagnostic criteria, such as diagnostic sensitivity and specificity, predictive value for positive and negative results as well as accuracy of analyzed proteins were calculated.
Results: The serum TIMP-1 and classic tumor markers – CEA and CA 19-9 concentrations were statistically higher in pancreatic cancer patients in comparison to healthy subjects. Moreover, the TIMP-1 levels were higher in more advanced stage of disease, in patients with lymph node and distant metastases when compared to patients in early stage of cancer, patients without nodal involvement and distant metastasis, similarly as classic tumor markers. The diagnostic sensitivity, predictive value for negative results for TIMP-1 levels were higher than for classic tumor markers. The diagnostic sensitivity increased for combined used of TIMP-1 with CEA (91%) and CA 19-9 (91%) and these values were higher than those of classic tumor markers (79%). The TIMP-1 area under ROC curve (0.8913) was larger than AUC for CEA (0.8458) and CA 19-9 (0.8836).
Conclusions. This study suggest higher usefulness of TIMP-1 than classic tumor markers (CEA and CA 19-9) in the diagnosis of patients with pancreatic cancer, especially in combine measurement with classic tumor markers.

M189
CA 242 A NOVEL MARKER FOR GASTRO-INTESTINAL CARCINOMAS

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Background. Tumour marker CA 242 is defined by the monoclonal antibody, which was obtained by immunising mice with a human colorectal carcinoma cell line. The structure of the antigenic determinant is a sialylated carbohydrate; it's related, although not identical, to the antigenic epitopes of CA 19-9 and CA 50. In the serum, the CA 242 epitope has shown to be coexpressed with CA 50 and with sialylated Lewisa, i.e. CA 19-9, on the same macromolecular complex.
Methods. The CanAg CA242 is a solid-phase, non-competitive immunoassay based on the direct sandwich technique. During the incubation is using biotinylated anti-CA242 monoclonal antibody (MAb) C241 in Streptavidin coated microstrips. After washing, buffered Substrate / Chromogen reagent is added to each well; during the enzyme reaction a blue colour is develop, where the intensity is proportional to the amount of CA242 antigen present in the samples.
Results. For a period of 6 months we studied 25 patients suspected for gastro-intestinal carcinomas. 17 were males, 8 females. The age was between 35 and 65 years. The results were compared to a control group of 25 with no evidence of gastro-intestinal carcinoma. The serum CA 242 levels in healthy group were average 4.48 U/mL (0.25 – 20.1) for males and average 3.16 U/mL (0.58 – 6.12) for females. In 25 patients we determined elevated CA242 levels, correlating to CEA and CA 19-9 values. The elevation of CA 242 levels was average of 88.26 U/mL (65.9 – 125.4) for males and average of 71.25 U/mL (44.78 – 101.20) in females.
Conclusions. The CA242 marker may be used as an aid in the diagnosis and management of patients with known or suspected gastro-intestinal carcinomas. It should not be used as a substitute for any established clinical examination of malignancy, but as a complement to existing clinical and laboratory methods.

M190
TRANSLATING GENE EXPRESSION PROFILING OF BREAST CANCER INTO CLINICAL PRACTICE: EXPERIENCE IN AN ITALIAN BREAST CANCER HOSPITAL UNIT

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Background: it is increasingly clear that cancers belonging to different molecular classes show differences in disease outcome and treatment efficacy that affect management selection. Thanks to financial support from Regione Lombardia, we performed in 2011-2012 a multidisciplinary study to evaluate the impact of molecular classification of breast cancer into clinical practice.
Methods: 73 patients treated at the Breast Unit of Desenzano General Hospital with breast cancer with previously defined criteria were selected for the study. Tissue samples were analyzed by DNA microarray-based technology at Agendia (Amsterdam) for a 70-gene expression profile. This classified patient as high/low risk for metastasis development. Additional 80-gene profile characterized the tumour as luminal, basal or c erbB2 (HER2) molecular subtype; quantitative expression of ER (Estrogen receptor), PR (Progesterone Receptor) and HER2 was also performed. Finally, 56 target genes, involved in drug response or resistance, were tested.
Results: 63% of patients were classified as high risk. In the high risk group: 83.3% of breast cancers were luminal-type, 14.3% basal like and 2.4% Her2-type. In the low risk group all samples were luminal-type. Immunohistochemistry and gene expression for the status of ER, PR and HER2 showed correlations respectively of 100%, 86.6% and 98.5%. The correlation with cell replication index Ki67 has shown that 3% of high risk tumors had a low index (<5%) and 4.5% of low risk tumors had a index >20%. 49% of the patients had a Ki67 between 5% and 20%, with 54.5% of these classified as low risk-luminal type, suggesting that for this class of patients chemiotherapy could be avoided.
Conclusions: microarray-based high/low risk and molecular subtype classification can provide independent prognostic additional information. It can be efficiently used for treatment optimization of selected breast cancer patients, with metastasis development risk classified as "indeterminate" by current clinical-pathologic criteria (according to St. Gallen recommendations, 2011). Furthermore, quantitative and more objective gene expression for ER, PR and HER2 provides additional information to conventional immunohistochemistry regarding response to hormonal therapy.

M191

LUMIPULSE™ G1200 AND TUMOUR MARKERS: ANALYTICAL PERFORMANCES AND COMPARISON WITH THE KRYPTOR® AND THE MODULAR® E170

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Background: The analysis of specific tumor markers is playing an increasing role in the assessment of cancer prognosis and treatment. Reliable, reproducible and comparable assays are required to meet clinician expectations. The LUMIPULSE™ G1200 (Innogenetics®) is a new fully automated analyser based on chemiluminescent enzyme immunoassay (CLEIA) technology
Goal: The goal of this study was to assess the analytical performances on the LUMIPULSETM G1200 for the following tumour markers : CA 125, CA 15-3, CA 19-9, AFP, ACE, PSA, CYFRA 21-1 and to compare the results versus our reference methods Roche Modular® Analytics E 170 for the AFP and BRAHMS Kryptor® for the six other markers.
Materials: 524 samples were selected among our biobank for which at least one of the studied markers was above the normal ranges of our reference methodology. Additionally 100 samples not affected by any malignant pathology were provided from donors coming from the French blood bank. Three levels of sera controls were utilised for the precision study (Seronorm™ Immunoassay), however for the CYFRA 21-1 only 2 internal controls from Thermo Scientific were used.
Results: The coefficients of variation (CV) of repeatability (n=10) are all excellent and less than 2,5%. The results obtained with blood donors confirmed the normal values established by the manufacturer within a healthy population. The correlation study versus our reference methodology was excellent and was done after a selection of concentrations within our current linearity range. The correlation coefficient (r) was between 0.907- 0.997.
Conclusion: In this study the results were precise (CV < 2,5%) and well correlated with our methods. The LUMIPULSETM G1200 is easy to use and has a customer friendly interface. We delivered fast results thanks to its constant time for sample result of 25 minutes.

M192

CONFIRMING AND MONITORING FORMS OF PROSTATE CANCER BY SELECTIVE TUMOR MARKERS PSA OR PAP?

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Background: The past decade has witnessed a marked increase in the interest in prostate cancer, reflecting the desire for improvements in treatment of all forms of cancer. Prostate cancer is the most common visceral cancer with second leading cause of cancer deaths and major cause of morbidity and health care expenditure. Both the incidence and age-adjusted mortality rates of prostate cancer have increased substantially in resent years. Despite significant advances in the diagnosis, staging and treatment the optimal management of this disease remains undefined. Prostatic specific antigen (PSA) and prostatic acid phosphatase (PAP) are the tumor markers used for confirming and monitoring prostate cancer. The clinical utility of PSA and PAP for early detection of prostate cancer is hampered by elevation of serum PSA levels in men with prostate nodular hyperplasia.
Method: To evaluate possible discrimination of prostate adenocarcinoma from prostate nodular hyperplasia, the serum PSA and PAP concentrations were measured in 50 patients with prostate adenocarcinoma and 50 patients with nodular hyperplasia.
Results and Conclusions: 95% of patients with prostate carcinoma having serum PSA level >4 ng/mL indicate higher sensitivity for detecting prostate cancer, however 36% patients with prostate hyperplasia has PSA levels >4 ng/mL. Results indicate that serum PSA can discriminate prostate adenocarcinoma from Prostate nodular hyperplasia better than PAP.

M193
THE UTILITY OF OSTEOPONTIN AND CANCER ANTIGEN 125 IN THE DETECTION OF OVARIAN CANCER

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Background: In women with pelvic mass, cancer antigen 125 (CA125) had not achieved satisfactory sensitivity and specificity in the detection of ovarian cancer. The objective of this study was to determine the potential of the osteopontin (OPN) and OPN+CA125 combination in differential diagnosis of the ovarian cancers and nonmalignant ovarian disease.
Methods: A prospective cross-sectional study was conducted at the Clinic for Gynecology and Obstetrics, and at the Center of medical biochemistry, Clinical Center of Serbia. Serum samples were obtained preoperatively from 79 women undergoing surgery for pelvic mass; 48 of them had ovarian carcinoma, and 31 had benign cyst. The samples were analyzed for the levels of OPN and CA125 (using ELISA and CMIA methods) and then compared with the final pathologic results. The diagnostic performance of OPN and CA125 was estimated using receiver operating characteristic curve and area under the receiver operating characteristic curve.
Results: The mediana plasma level of OPN in patients with benign and malignant cysts were 356.33 ng/mL and 865.15 ng/mL, respectively (P <0,001). Receiver operating characteristic (ROC) analysis for plasma OPN revealed the area under the curve of 0.838. At the predefined specificity of 90%, OPN showed sensitivity of 62.5%, whereas the combination of OPN+CA125 reached 74.9% at the same specificity.
Conclusion: OPN showed satisfactory capability of distinguishing benign from malignant ovarian cyst, particularly in combination with CA125.

M194
HELICOBACTER PYLORI SERO-PREVALENCE IN DIFFERENT LIVER DISEASES

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Aim: To investigate the seroprevalence of Helicobacter pylori infection in patients with different liver diseases and determine the association and correlation between the seroprevalence of Helicobacter pylori infection and the liver diseases. METHODS: The presence of a Helicobacter pylori antigen was investigated in serum samples from 274 individuals with liver diseases as well as 120 healthy individuals. H. Pylori antigen was detected using ELISA and western blot based on specific anti-H. Pylori antibody. The results was analyzed using the chi-square test.
Results: H. Pylori was detected in sera samples of 31.7% (20/63) (X2=3.7) of non-cirrhotic, 50% (11/22) (X2=3.9) of cirrhotic & 56.1% (106/189) (X2=5.2) of HCC individuals, compared by 8.4% (10/120) of healthy individuals. The levels of H. Pylori antigen were significantly higher (P <0.05) in sera of different stages of liver diseases compared by healthy individuals. We found a good correlation between H. Pylori antigen levels and the severity of the liver diseases(Pathology) (r =0.368, P <0.001). Also, there is a correlation between age and H. Pylori antigen levels (r =0.25, P <0.001).
Conclusion: Helicobacter pylori infection is correlated ans associated with occurance and development of different stages of liver diseases.

M195

CLINICAL UTILITY OF ADIPONECTIN AND RESISTIN IN ACUTE LYMPHOBLASTIC LEUKEMIA AMONG EGYPTIAN CHILDREN

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Background: White adipose tissue secretes a number of peptide hormones, including leptin, several cytokines, adiponectin, resistin etc., and also produces steroids hormones. The aim of our work is to study the disturbance of secretion of adiponectin and resistin in de novo and relapsed acute lymphoblastic leukemia (ALL) children patients.
Methods: Measurements of adiponectin and resistin were performed at diagnosis; in 32 patients with de novo ALL with mean age 9.8 years and 19 children patients with relapsed ALL with mean age 9.9 years. 10 healthy children with matched age (11.9 years) and sex were used as controls.
Results: Lower serum adiponectin and higher serum resistin levels are associated with de novo and relapsed ALL (compared to healthy controls).The mean values ± S.D of serum adiponectin level in control group, de novo ALL and relapsed ALL which were 11.91 ± 2.234, 10.031 ± 1.934 and 7.731 ±1.083 (ng/mL) respectively. The mean values ± S.D of serum resistin in control group, de novo ALL and relapsed ALL group were 4.92± 1.55, 7.353 ±1.582 and 9.784 ± 1.656 (ng/ml) respectively. A significant decrease of adiponectin levels was found in relapsed ALL compared to de novo ALL, P (<0.05). In contrast resistin was significantly increased in relapsed ALL compared to de novo patients P (<0.05). Adiponectin in ALL subjects inversely correlated with resistin level (r = -0.51, P <0.001).
In conclusion: Decreased serum adiponectin and elevated serum resistin levels are associated with childhood de novo and relapsed ALL, suggesting that adiponectin and resistin may represent not only a potential clinically significant diagnostic marker but also may be implicated in ALL pathogenesis.

M196

HYPERGLYCEMIA-INDUCED S100A8 AND S100A9 EXPRESSION TARGET AKT, MTOR AND NF-KB SIGNALLING IN PANCREATIC CANCER CELLS

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Background: S100A8/S100A9 inflammatory proteins are suggested to be involved in pancreatic cancer (PaCa) progression and in cancer-associated diabetes mellitus (DM). We analyzed S100A8/S100A9 expression levels in PBMC of patients with PaCa, chronic pancreatitis (ChrPa) or pancreatobiliary tract tumors (PBT) and ascertain whether they differently affect Akt, mTOR and NF-kB signalling in PaCa cells with different aggressiveness.
Methods: S100A8 and S100A9 mRNA was quantified by RT-PCR in 55 PaCa, 12 ChrPa, 15 PBT. S100A8, S100A9 and S100A8/A9 effects on Akt (Ser473,Thr308), mTOR (Ser2448) and NF-kB (p-IkB-a) were WB analyzed using BxPC3, Capan1 and MiaPaCa2.
Results: S100A8 and S100A9 expression levels correlated with each other (r=0.660, P <0.0001), but no differences were found between the three groups for variations in these proteins ($\chi 2$ =0.913, p = 0.663 and $\chi 2$ =3.925, P=0.140) or, in PDAC, between localized and advanced tumors (z= -1.292, P=0.196 and z=-0.951, P= 0.341). S100A8, mainly S100A9, were directly correlated with fasting plasma glucose, HbA1c and insulin levels, but not with the tumor marker CA 19-9. In BxPC3 Akt was Thr308 phosphorylated by S100A8/A9, while in Capan1 and MiaPaCa2 S100A8/A9, S100A8 and S100A9 phosphorylated both Akt sites. In BxPC3 mTOR was phosphorylated (Ser2448) by S100A8. In Capan1 and MiaPaCa2 S100A8, S100A9 and S100A8/A9 caused significant Ser2448 phosphorylation. S6RP, downstream effector of mTORC1, was phosphorylated (Ser235/236) only in S100A8 treated MiaPaCa2. A strong NF-kB activation was induced by S100A8 in BxPC3, by S100A9 and S100A8/A9 in Capan1; NF-kB was inhibited by both molecules in MiaPaCa2.
Conclusions: In PaCa-associated DM high expression levels of these proteins might favour cancer cell growth by inducing Akt, mTOR and NF-kB. In the less invasive BxPC3 cells S100A8 activates NF-kB. In more aggressive Capan1 and MiaPaCa2 cells S100A8, S100A9 and S100A8/A9 activate mainly Akt and mTORC1, not NF-kB pathways.

M197
PREVALENCE AND CLINICAL SIGNIFICANCE OF ENORMOUSLY INCREASED CA 19.9 CONCENTRATIONS IN HOSPITALISED PATIENTS

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Background: Markedly elevated CA 19.9 concentrations in serum are regarded as specific enough to reliably identify pancreatic cancer, even if a consistent body of literature shows CA 19.9 concentrations >1000 kU/L in a variety of benign conditions. Scarce data are, however, available on the prevalence and clinical significance of CA 19.9 values >10,000 kU/L. Here we present a case series of consecutive patients admitted to our hospital with CA 19.9 concentrations >10,000 kU/L, with the aim to assess the association of such concentrations with the presence of pancreatic cancer and, more in general, with tumours of the gastrointestinal system. We also tried to define whether the exact measurement of CA 19.9 concentrations in this range, which needs serial sample dilutions, is cost-effective.
Methods: CA 19.9 measurements, including a 1:10 sample dilution in accordance to manufacturer's instructions allowing the determination of concentrations up to 10,000 kU/L, were performed on Roche Modular EVO system. Samples with higher CA 19.9 values were diluted according to a defined laboratory protocol to obtain estimates up to 100,000 kU/L.
Results: During 14 months, 18 patients showing an enormous elevation of CA 19.9 concentrations (11,568 to >100,000 kU/L) were identified (55% males; median age 73.5 years, range: 58-85). Accordingly, the yearly prevalence of hospitalized patients tested for CA 19.9 and with marker concentrations >10,000 kU/L was 2.9%. All recruited patients were diagnosed as malignancies: 15 had primary or secondary pancreatic cancer, two had gastric cancer and one a cholangiocarcinoma. CA 19.9 concentrations ranged between >10,000-30,000 kU/L in 9 cases, >30,000-60,000 kU/L in two, >60,000-100,000 kU/L in three and >100,000 kU/L in four cases, respectively. A surgical resection of the tumour was performed in 5 patients, independently of CA 19.9 concentrations. The median patient's survival was <6 months.
Conclusions: CA 19.9 concentrations >10,000 kU/L unequivocally identify a gastrointestinal malignancy, more frequently (~83%) a primary or secondary pancreatic cancer. Exactly measuring CA 19.9 concentrations >10,000 kU/L after multiple sample dilutions does not add relevant information for patients' prognosis and treatment.

M198
SERUM LEVELS OF MATRIX METALLOPROTEINASE-9 (MMP-9) IN PANCREATIC CANCER PATIENTS (PC)

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Background. Pancreatic cancer is characterized by aggressive behavior and poor prognosis. Matrix metalloproteinases (MMPs) play a crucial role in tumor progression, including tumor growth, migration and invasion of malignant cells, metastasis, and angiogenesis within the tumor. The aim of the study was to compare clinical significance of serum MMP-9 with classic tumor markers, including carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) in the diagnosis of patients with pancreatic cancer.
Methods. The study included 30 patients with pancreatic cancer, 37 patients with chronic pancreatitis and 30 healthy subjects. The ELISA method was used for the assessment of MMP-9 levels, while MEIA method for the concentrations of classic tumor markers. The diagnostic criteria, such as diagnostic sensitivity and specificity, predictive value for positive (PV+ve) and negative (PV-ve) results as well as accuracy of all proteins tested were assessed.
Results. The serum levels of MMP-9 and classic tumor markers were statistically higher in patients with pancreatic cancer when compared to healthy subjects. The MMP-9 concentrations increased with more advanced stage of PC, similarly as classic tumor markers. Moreover, the levels of MMP-9 were higher in patients with lymph node and distant metastases in comparison to patients without nodal involvement and distant metastasis. The percentage of elevated results for MMP-9 as well as predictive value for negative results and accuracy were higher than for CEA. In addition, diagnostic sensitivity increased for combined used of MMP-9 with classic tumor markers.
Conclusions. Our present findings suggest the potential role of serum MMP-9 as tumor marker in pancreatic cancer, especially in combination with classic tumor markers.

M199

PEPTIDES FROM ADIPOSE TISSUE IN CHILDREN TREATED WITH STEM CELL TRANSPLANTATION

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Background: Hematopoietic stem cell transplantation (HSCT) is an efficient method of treatment of many neoplastic and non-neoplastic diseases in children. The complications of HSCT treatment are early like pancytopenia, mucositis, or malnutrition and late like developmental disorder, hormonal complications, and obesity. The metabolic late complications can be connected with disturbances in release of the regulatory peptides from adipose tissue - adipocytokines. The aim of our study was to evaluate the concentration of adiponectin, apelin, leptin, resistin or visfatin in patients treated with HSCT.
Methods: Four groups of patients were involved to the study. Group A- patients treated with HSCT (n=27), before graft (mean BMI 18,6 kg/m^2; mean age 10,5 y),. Group B – the same patients (n=27), at least 6 months after HSCT. Group C age matched children with obesity (n= 41; mean BMI 31,7 kg/m^2; mean age 13,5 y), and group D – healthy, lean children (n= 21; mean BMI 20,5 kg/m^2; mean age 12 y). Fasting plasma concentration of visfatin, apelin, resistin and adiponectin were measured using Phoenix Pharm. kits, leptin - R&D Systems kits.
Results: Similar concentrations of apelin (Ap) as well as visfatin (Vi) in group A and C were noted (group A – Ap median 1,25 ng/mL; Vi median 7.30 ng/mL; group C – Ap median 3,99 ng/mL; Vi median 6.76 ng/mL). After HSCT levels of both peptides increased to the concentrations observed in group D (Ap 4,18 ng/mL P >0,05 v. A & C group; Vi 17.0 ng/mL; P >0,05 v. A & C group). The only significant correlations were observed between apelin and visfatin in group A (R=0,74; P >0,05) and B (R=0,82; P >0,05). There were no significant differences in concentration of leptin, resistin and adiponectin between group A and B. Levels of Leptin (Le) and resistin (Re) were significantly higher than in the remaining groups in group C (Le median 37.5 ng/mL; P >0,05 v. A,B & D group) and group D (Re median 3.92 ng/mL; P >0,05 v. A,B & C group).
Conclusions: The recovery after HSCT is accompanied with increasing of apelin and visfatin concentrations into ranges observed in healthy lean children. Among the studied peptides secreted from the adipose tissue, only resistin could be involved in the development of metabolic complications observed after HSCT.

M200

MULTIPLEX MUTATIONAL PROFILING OF KRAS, BRAF AND PIK3CA GENES IN FORMALIN FIXED PARAFFIN EMBEDDED (FFPE) COLORECTAL CANCER TISSUE ON A BIOCHIP ARRAY PLATFORM

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Background: Individualised treatment based on mutational profiling of tumours is now a reality for metastatic colorectal cancer (mCRC) patients since the discovery of mutant KRAS status and resistance to anti-epidermal growth factor receptor (EGFR) antibodies. However, only a subset of patients respond to anti-EGFR treatment. Mutations in other downstream effectors of the EFGR signalling pathway, i.e. BRAF and PIK3CA, may have a negative impact on patient response to anti-EGFR therapy. This study reports the analytical performance of a biochip array platform for the rapid simultaneous detection of 20 mutations within KRAS (codons 12, 13, 61, 146), BRAF and PIK3CA genes in CRC FFPE tissue specimens.
Methods: Anonymised FFPE tissue-derived DNA samples were analysed using the KRAS/BRAF/PIK3CA Array. The assay is based on a combination of multiplex PCR and biochip array hybridisation, which enables high multiplexing detection. Innovative PCR priming technology permits high discrimination between multiple wildtype and mutant DNA regions. Providing there are enough copies of DNA present, approximately 1% of mutant can be detected in a background of wildtype genomic DNA. Sample analysis can be completed in less than 3 hours. Mutational status of samples was previously confirmed using routine, validated real-time PCR methods for KRAS codons 12/13 and BRAF codon 600. Further confirmation of results obtained was achieved using Sanger sequencing.
Results: Mutations were detected in 86%(24/28) of samples assessed. 100% concordance was achieved between methodologies for KRAS codons 12/13 and BRAF codon 600. Five samples harboured both a KRAS and PIK3CA mutation. Three samples in total resulted in detection of a mutation beyond KRAS codons 12/13 and BRAF codon 600. Additional mutations detected were therefore confirmed using Sanger sequencing.
Conclusions: These findings demonstrate that the KRAS/BRAF/PIK3CA Array can rapidly and simultaneously detect mutations within these genes in CRC FFPE tissue. The detection of additional markers besides KRAS, and extending the profile of this gene to include other codons beyond 12 and 13, may aid in the selection of candidate patients to receive anti-EGFR therapy thereby maximising drug efficacy and minimising adverse patient effects

M201

THE DIAGNOSTIC AND PREDICTIVE VALUE OF SERUM HUMAN EPIDIDYMIS PROTEIN 4 (HE4) AS A TUMOR MARKER IN LUNG CANCERS

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Background: Lung cancer is the most common malignancy in males worldwide. Currently available tumor markers show variable sensitivity and specificity, thus, novel biomarkers are desirable to increase our laboratory skills in this disease. Human epididymis protein 4 (HE4) is now an available tumor marker for ovarian cancer. In this study, our aim was to evaluate whether HE4 is also a reliable biomarker in lung tumors.
Methods: Serum HE4 levels were measured at diagnosis in 98 male patients with non-small cell (NSCLC; 84.7%) and small cell lung cancer (SCLC; 15.3%) at different stages, and these results were compared to an age-matched healthy male cohort (n=98). Chemiluminescent microparticle immunoassay (Architect®, ABBOTT) was used for analyzing concentrations of HE4, and electrochemiluminescent immunoassay (Modular E170®, Roche Diagnostics) was to determine the levels of CEA, Cyfra 21-1, CA 125, and NSE. TPA level and TK activity were measured by chemiluminescent immunoassay (Liason®, DiaSorin). The efficacy of HE4 was compared to that of these classic tumor markers.
Results: Patients with lung neoplasm showed significantly higher HE4 levels (P <0.001) compared to controls (118.2 [80.6-150.1] pmol/L vs. 62.2 [47.2-76.1] pmol/L), while no difference was found between NSCLC and SCLC. Age and smoking habits did not affect HE4 values in patients, but modulated these results in normal subjects. Significantly elevated HE4 concentrations were already measured in stage I (70.6 [60.5-114.4] pmol/L), and further elevation was detected to stage IV (136 [98-194] pmol/L). HE4 values showed a significant correlation (r=0.227, P=0.030) with the size of the tumor based on the evaluation of CT-scan and histology. In addition, higher area under the ROC curve (AUC) value with HE4 was found in SCLC (0.939 [0.866-1.000]) than in NSCLC (0.827 [0.760-0.895]), which was larger than that of the majority of other tumor markers. However, we did not find a significant correlation between HE4 levels and the overall survival rate.
Conclusions: Serum HE4 is a promising novel tumor marker for lung cancers since increased HE4 levels were significantly correlated with the disease progression, and showed a good discriminative power but without prognostic information.

M202

TUMOR MARKERS IN THERAPEUTIC MONITORING OF MALIGNANCIES. A CASE REPORT

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The best indication for use of tumor markers-TuM is a therapeutic monitoring-ThM after chemiotherapy - CheTh,radiotherapy-RaTh,surgical therapy-STh.The ideal TuM isn't yet found, so that is the best TuM use in monitoring of therapy-Th in still diagnosticated tumor,ph-verificated.The aim of this paper is that in a case report indicate on significance of determination TuM in ThM of malignancies. Male patient SM born 1935.from Arandjelovac first was in your Health Center. Because from suspection on GI malignancy was sended on examination in Clinical Center "Kragujevac". It was indicated next dg.: Ca recti meta in hepate.St.post laparatomiam explorativa et anus bipolaris et biopsiam hepatis.St.post ChTh cum 5-FU-LV NoIII. Progressio morbi (meta in hepate). St.post ChTh cum Folfox IV NoIV. Partial response (meta in hepate).Progressio morbi (meta in hepate). ChTh cum Folfiri in coursu. PH:AdenoCa mucosae int.crassi hg.II, ng.II infiltrativum str.superf. musc. Par.int.crassi. Also,patient was medicated with ChTh by Protocols 5-fluorouracil with leucovorin (5-FU-LV) in 3 cycles, next by Protocol Folfox IV in 8 cycles and finally was included a 3rd Protocol Folfiri – in this moment just 1 cycle and counsel to new determinations of TuM. From TuM was determined CEA and CA 19-9. After, started very increse values of CEA: 166.78, 410.27, 824.51 and 447.72 ng/mL, values start to decrease on 79.88, 53.86 and 53.58 ng/mL, but still are increased (rv <5 ng/mL). On the other hand,CA 19-9 was after STh 28.66 U/mL (rv <37 U/mL) that be after, 1st small increase on 37.16 U/mL and bigger on 73.12 and 91.97 U/mL, and after V cycle of Protocol Folfox IV were returned values of CA 19-9 on 20.34, 16.04 and 17.23 U/mL in interval of rv. During the Th, US- and with abdominaly CT-scanning,was diagnosticated progression of focal signs in the liver. Included is in 3rd Protocol named Folfiri in this time just 1 cycle and sended to home with the counsel to come on next cycles and monitoring of TuM (CEA, CA19-9, maybe AFP) for the QC of Th t.e. ThM. In this case report the authors discuss obtained results and indicate to signifficance of determinations of TuM in a ThM of malignancies.

M203
THE SIGNIFICANCE OF GM1 GANGLIOSIDES IN CUTANEOUS MELANOMA

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Background: GM1 gangliosides influence cell growth and differentiation, cell proliferation and adhesion, immune response, modulate signal transduction. The authors evaluated the umoral immune response anti GM1 gangliosides and a possible link between IgG/IgM antibodies and progression of melanoma.
Material and Method: The study included 176 patients with melanocytic lesions (128-cutaneous melanoma, 48 – dysplastic nevi) and control group (48 cases). We monitored the participants in the study for: - Serum level of IgG/IgM type antibodies against GM1 gangliosides (immunodot method) - Lactate dehydrogenase (LDH) activity (spectrophotometry)
Results: We did not determine detectable levels of antibodies against GM1 in any patient from control group. Positive values of antibodies against GM1 were identified in a small number of patients with dysplastic nevi (IgG in 4,68%cases, IgM in 6,25% cases). IgG antibodies were found positive in 4,68% melanoma patients, while IgM antibodies were found positive in 15,63% patients. Variation of IgM antibodies level was statistically significant when compared melanoma group with control group (P=0,001, CI=95%).
We determined a statistically significant correlation between IgM antibodies and tumors site (P=0,022), histological type (P=0,001), Breslow index (P=0,004), Clark level (P=0,001) and presence/absence of ulceration (P=0,0001). Also, a statistically significant correlation was obtained between antiGM1 IgM type and LDH (P=0,01, CI=95%).
Conclusions: The endogenous response against GM1 of IgM type is an immunological event associated with advanced phases of melanoma. It is necessary to test a larger number of patients in a longer period of time to show the clinical importance of autoantibodies against GM1.

M204
GD1A GANGLIOSIDES ASSOCIATED WITH ANGIOGENESIS IN MALIGNANT MELANOMA

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Background: The authors aimed to analyze the mechanism through which GD1a could influence melanoma progression, by: - determining the content of GD1a in melanoma tumors; - determining the level of antiGD1a antibodies in melanoma patients; - determining VEGF-A and sVEGFR1 (factors involved in angiogenesis).
Material and method: The study included 128 adult patients with primary melanoma evaluated before the surgical removal of the tumor and 48 healthy volunteers.
Extraction and quantifying of tissue gangliosides were made by Folch method and chromatographic techniques. The serum level of VEGF-A (ELISA method), sVEGFR1 (ELISA method) and antiGD1a antibodies IgM and IgG class (IMMUNODOT method) were determined in samples from patients before the surgical removal.
Results: GD1a gangliosides represent 6% from total gangliosidic sialic acid determined in tumor. Detectable values of GD1a gangliosides were registered in maximum 32% from analyzed specimens. AntiGD1a antibodies were present in 15% of melanoma cases, most of IgM class. Serum levels of VEGF-A were significantly increased in melanoma patients versus control (352.38±153.20 pg/mL versus 149.23±81.6 pg/mL, P <0.05). Furthermore, sVEGFR1 had similar variations (0.09±0.11 ng/mL versus 0.06 ng/mL). sVEGFR1: VEGF-A ratio was decreased in melanoma patients compared to control group(0.24±0.17 versus 0.4±0.11). A statistical significant correlation was determined between concentration of GD1a in tumor and serum sVEGFR1: VEGF-A ratio but no statistical significance was observed between concentration of GD1a in tumor and serum levels of VEGF-A, respectively, sVEGFR1.
Conclusions: GD1a gangliosides had a dual effect on melanoma evolution. GD1a could exert both a positive effect, by stimulating an efficiently antitumor immune response, and, a negative effect, by stimulating proangiogenic activity of VEGF-A and sVEGF-R1.

M205
ISCHEMIA-MODIFIED ALBUMIN AS A MARKER OF OXIDATIVE STRESS IN PROSTATE CANCER

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Background: Prostate cancer (PCa) is the most frequent cancer among men in developed countries. It is known that cancer cells are characterized by enhanced oxidative stress and reactive oxygen species (ROS) overproduction. The generation of ROS can transiently modify the N-terminal region of albumin and produce an increase of ischemia-modified albumin (IMA). Since PCa is associated with an increase in ROS production, the aim of this study was to evaluate the IMA levels, a new biomarker of oxidative stress indicative of protein oxidation, in PCa patients.
Methods: Total prostate-specific antigen (PSA), free PSA, albumin and IMA levels were assessed in 25 patients with PCa (67.6 ± 12.3 years) and 30 healthy controls (62.2 ± 8.6 years). IMA values were expressed with and without adjustment for albumin concentration. The following formula was applied to correct IMA values for serum albumin: [(individual serum albumin concentration/median albumin concentration of the population) x IMA value].
Results: Total PSA and free PSA were significantly higher in subjects with PCa, and serum albumin levels were significant reduced in these patients (control: 45.7 ± 2.6 g/L and PCa: 42.5 ± 3.6 g/L, P <0.05). The serum levels of IMA were 0.255 ± 0.056 ABSU for healthy subjects and 0.308 ± 0.090 ABSU for patients with PCa (P <0.05). However, no significant differences were observed when IMA values were adjusted for serum albumin (0.259 ± 0.055 ABSU versus 0.290 ± 0.090 ABSU, P=0.122), despite the IMA values were slightly higher in the PCa group.
Conclusions: These results suggest that IMA values were slightly higher in patients with PCa, which may be associated with increased oxidative stress and changes in the structure of albumin occurred during prostate cancer. However, the difference between groups was no longer statistically significant when the results of IMA were adjusted by serum albumin.

M206
EFFECT OF MOBILE PHONE ON SOME PLASMA ENZYMES IN RATS

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Background: Mobile phones are essential in our day to day activities however concerns have been raised on the effect of the radiation that emanates from it. We investigated the effect of radiation from global system of mobile communication handset on some plasma enzymes.
Materials and methods: Twelve rats were employed for the study. Two test groups of rats comprising three males and females were exposed to electromagnetic frequency from GSM handset for eight hours daily for 7 days after which the rats were sacrificed. Blood was obtained from their hearts into heparin bottles and the plasma separated from whole blood immediately. Total acid phosphatase (ACP), prostatic acid phosphatase (PAP), gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) levels were determined by colorimetric method. ACP, PAP, GGT, and ALP activities were determined in males while GGT and ALP were determined in females.
Results: The mean ACP and PAP levels in male test group (43.01 iu/L and 14.05 respectively) was significantly higher in tests than in the controls (26.69 iu/L and 12.69 respectively) with P <0.05 however there was no statistical significant difference in the levels of GGT and ALP between the test and control male rats. There was no significant difference in the activities of ALP and GGT between the irradiated females and males on one hand and the irradiated females and non-irradiated females on the other hand.
Conclusion: The elevated activity of prostatic acid phosphatase observed in irradiated male rats may be indicative of increased risk for prostate cancer. It is recommended that measures be taken to reduce human exposure to electromagnetic rays of mobile phones.

M207
HUMAN EPIDIDYMAL PROTEIN 4 (HE4) IN MONITORING RECURRENT OF OVARIAN CARCINOMA

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Background: Human Epididymis Protein 4 (HE4) is a 25 KD single peptide chain poorlyexpressed in epithelia of respiratory and reproductive tissues, but highly expressed in ovarian cancer tissue. It was recently approved by the Food and Drug Administration to monitor recurrence or progressive disease in epithelial ovarian cancer in conjunction with CA125. As a single tumor marker, HE-4 had the highest sensitivity for detecting ovarian cancer, especially in stage I disease, and it correlates with clinical response to therapy.
Methods: Serum samples were collected from 21 recidival ovarian cancer patients (mean age: 63 years [range: 38-81]). Out of these patients, 16 were affected by serous ovarian cancer, and 5 by undifferentiated histological type. Chemiluminescent immunoassay Cobas 6000 (Roche - USA) and Centaur XP (Siemens - Germany) were used to measure HE-4 and Ca125 concentrations respectively. Multiparametric analysis and Mann-Whitney test were used for statistical analysis.
Results: The median concentrations of HE4 in undifferentiated and serous histological type were 165.60 [range 87.96- 937.40] pmol/L and 104.52 [range: 46.30- 273.90] pmol/L respectively (P= NS). Reference interval for HE4 ranges between 0 to 74. The median concentrations of CA125 in undifferentiated and serous histological type were 18.10 [range 8.10- 385.60] U/mL and 107.75 [range 18.70- 780.40] U/mL respectively (P= 0.06 - NS). Reference interval for Ca125 ranges between 0 to 35. The linear multiparametric regression analysis (stepwise method) showed a significant correlation of the HE4 to the histological type even when it was adjusted with CA125 and age.
Conclusions: The HE4 assay seems to play an important role in the follow up of recurrent undifferentiated ovarian cancer by integrating the information provided by the Ca125 measurement.

M208
THE ACTIVITY OF ALCOHOL DEHYDROGENASE (ADH) ISOENZYMES AND ALDEHYDE DEHYDROGENASE (ALDH) IN OVARIAN CANCER AND OVARIAN CYSTS

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Background. The metabolism of cancerous tissue is in many ways different than in healthy cells. In ovarian cancer, cells exhibit activity of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), which participate in metabolism of many biological substances. The aim of this study was to compare the metabolism of ovarian cancer cells with ovarian cysts and normal ovarian cells by the measurement of ADH isoenzymes and ALDH activities.
Methods. Biopsy specimens were taken from 36 women with ovarian cancer and from 40 women with ovarian cysts. The control group contains histologically unchanged ovarian tissues obtained from 34 women with BRCA1 mutations. Class I and II ADH isoenzyme activity and ALDH were measured by fluorometric method with class-specific fluorogenic substrates. For measurment of class III, IV and total ADH activity we emloyed photometric method.
Results. The total activity of ADH was significantly higher in ovarian cancer (0.735+/-0.175 nmol/min/mg of protein) than in ovarian cysts (0.722 +/- 0.199 nmol/min/mg of protein) and healthy subjects (0.721+/- 0.194 nmol/min/mg of protein). The activity of the class I ADH isoenzyme was also significantly higher in ovarian cancer (0,156+/-0.063 nmol/min/mg of protein) as compared to ovarian cysts (0,128+/-0.048 nmol/min/mg of protein) and to the control group (0.122 +/- 0.047 nmol/min/mg of protein). The other classes of ADH tested, did not show significant differences between activity of cancerous cells, ovarian cysts and healthy ovary. The analysis of ALDH activity did not indicate significant differences between all tested groups.
Conclusion. The activity of ADH in cancer cells is disproportionately higher compared to the activity of ALDH, what can lead to the induction of acetaldehyde production and supports carcinogenesis in ovary. The activity of class I exhibited a statistically significant difference between cancerous and healthy or benign changed tissues, what could be a factor for the disturbances in the metabolism of many important biological substances, including retinoic acid.

M209
THE DIAGNOSTIC VALUE OF ALCOHOL DEHYDROGENASE ISOENZYMES AND ALDEHYDE DEHYDROGENASE MEASURMENT IN THE SERA OF ENDOMETRIAL CANCER PATIENTS

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Background. In previous experiments, we have found an increased level of class I ADH and total ADH activity in endometrial cancer tissues. Changes in this enzyme in cancer cells may be reflected by ADH activity in the serum and could thus be helpful for diagnostics of endometrial cancer. The aim of this study was to investigate a potential role of ADH and ALDH as tumor markers for endometrial cancer. We defined diagnostic sensitivity, specificity, predictive value for positive and negative results, and receiver-operating characteristics (ROC) curve for tested enzymes.
Methods. Serum samples were taken from 43 women with endometrial cancer, 56 with myoma uteri and 56 as a control group. Class I and II ADH isoenzyme activity and ALDH were measured by fluorometric method with class-specific fluorogenic substrates. For measurment of class III, IV and total ADH activity we employed photometric method.
Results. The total activity of ADH was significantly higher in the serum of patients with endometrial cancer (0.816 +/- 0.486 U/l) than in patients with myoma uteri (0.574 +/- 0.464 U/l) and healthy subjects (0.546 +/- 0.306 U/l). The activity of the class I ADH isoenzyme was also significantly higher in endometrial cancer (1.743 +/- 0.963 mU/l) as compared to myoma (1.249 +/- 0.649 mU/l) and the control group (1.214 +/- 0.755 mU/l). In the cancer patients, the diagnostic sensitivity for ADH I was 69%, specificity 77%, positive and negative predictive values were 75 and 71% respectively. Area under ROC curve for ADH I was 0,682.
Conclusion. The activity of class I ADH isoenzymes and the total activity of ADH were elevated in the sera of patients with endometrial cancer as compared to the healthy control and to the group of patients with myoma uteri. This is the first study showing all the diagnostic criteria for ADH and ALDH in endometrial cancer patients. These results suggest a potential role for ADH (especially ADH I) as a non-specific marker of gynecological cancers, which may also allow for differentiation of benign lesions to the cancer.

M210
INTERACTIONS OF ANTIOXIDANTS WITH CHEMOTHERAPEUTIC DRUGS

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Background: Numerous in vitro studies have demonstrated that a wide range of anticancer agents generate Reactive Oxygen Species (ROS) in malignant cells. Damage to the mitochondrial membrane via ROS may activate the apoptotic pathway. There are conflicting views on the concurrent use of antioxidants with conventional cancer treatments. This argument is based on the fact that some chemotherapy drugs generate ROS and antioxidants may inhibit ROS and prevent cancer cells to be killed by ROS induced apoptosis.
Methods: In order to clarify the roles of antioxidants in chemotherapy, we investigated Quercetin, N-acetylcysteine (NAC), and Curcumin in different cells treated with different anticancer drugs. We studied the effects of Quercetin on the cytotoxic activity of Topotecan in human breast cancer cell lines, MCF-7 and MDA-MB-231. We investigated the effect of NAC on doxorubicin and vincristine cytotoxicity in MRP1 transfected (293MRP) cells. We studied the effects of Curcumin and NAC on Bleomycin induced apoptosis in NTera-2 and NCCIT human testicular cancer cells.
Results: Our data indicated increased oxidative status in MCF-7 and MDA-MB-231 cells exposed to Topotecan. Quercetin didn't inhibit ROS generation and enhanced cytotoxicity of Topotecan in both cells. In contrast, NAC enhanced resistance against doxorubicine and vincristine in MRP1 overexpressing cells. Our data showed that NAC inhibits oxidative stress generated by Bleomycin and diminishes apoptosis in both testicular cancer cells. Curcumin has synergistic effect with Bleomycin in NTera-2 cells, but inhibits Bleomycin induced apoptosis in NCCIT cells.
Conclusion: We conclude that Quercetin, NAC and Curcumin have diverse effects in the cytotoxicity of chemotherapeutic drugs depending on the cancer cell type. These studies provide a better understanding in the development of new therapies involving induction of apoptosis to kill cancer cells selectively.

M211

SERUM LEVELS OF LDH AND GAMMA GT IN LIBYAN BREASE CANCER PATIENTS

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Background: Carcinoma breast is one of the major surgical problems in Libya particularly among the younger subjects. It is important to know the etiology of the breast cancer in Libya since it has become a major health problem. The alteration of enzyme levels in breast cancer is a best guide for its prognosis and treatment outcome. Earlier studies had shown a positive correlation with serum LDH (Lactate dehydrogenase) and GGT (Gamma glutamyl transpeptidase) in carcinoma breast. The present study is undertaken to observe any alterations in the levels of LDH and GGT in patients suffering from carcinoma breast.
Methods: 40 patients in various stages of breast cancer have been selected from 16 to 65 years of age with mean age of 37 years retrieved from the department of surgery, 7th October hospital, Benghazi, Libya during the years 2009 and 2010. There were 38 healthy controls with age group of 20 to 55 years with mean age of 35 years. Out of 40 cases of carcinoma of breast, 25 cases are premenopausal and 15 were post menopausal. This demarcation has been done by separating both groups selecting the group less than 47 years as premenopausal and 47 and more as postmenopausal patients. Serum LDH and GGT estimation had been done in the patients as well as in the healthy controls after an overnight fast.
Results: The serum LDH had been elevated significantly in carcinoma of breast when compared with the control group among both premenopausal and post menopausal (P=0.0001). Between postmenopausal and premenopausal women, postmenopausal women had significantly higher levels of LDH than premenopausal (P=0.0234). Serum GGT was significantly higher in cases when compared to the controls (P=0.006) and in Premenopausal women (P=0.0059) when compared with those of the postmenopausal women.
Conclusion: In this study, LDH levels were higher in cases of carcinoma breast, more so in postmenopausal women. Serum GGT levels are also increased in cases of carcinoma breast but with more preponderance among Premenopausal women.

M212

CORRELATION OF CIRCULATING TUMOR CELLS (CTC) AND CA27.29 BEFORE, DIRECTLY AFTER AND TWO YEARS AFTER CHEMOTHERAPY IN FEMALE PATIENTS WITH PRIMARY BREAST CANCER IN THE SUCCESS A TRIAL

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Background: In the SUCCESS a trial levels of CA27.29 and circulating tumor cells (CTC) have been measured before, directly after and 2 years after adjuvant chemotherapy. Evaluation of circulating tumor cells (CTC) and CA27.29 five years after chemotherapy are currently taking place. Aim of this study was to analyze the prevalence and correlation of the two prognostic factors during the course of the disease.
Methods: The SUCCESS A trial is a phase III trial which includes primary breast cancer patients with either positive lymph node involvement or high risk nodal negative disease. It compares the treatment of FEC-Docetaxel (Doc) vs. FEC-Doc-Gemcitabine (Doc-G), followed by treatment with zolendronat for either two or five years. CTC were analysed with the CellSearchSystem (Veridex, USA). The cells were first immunomagnetically enriched with an anti-Epcam-antibody, followed by labeling with anti-cytokeratin (8, 18, 19) and anti-CD45 antibodies to distinguish between epithelial cells and leukocytes. The ST AIA-PACK CA27.29 reagent using MUC-1 for AIA-600II (Tosoh Bioscience, Belgium) was applied to measure CA27.29. The cutoff level for positivity of CA27.29 was >31 U/mL.
Results: In total, CA27.29 levels and CTC were collected of 2011 patients before chemotherapy, 1525 patients directly after and of 1000 patients 2 years after completion of chemotherapy. Before chemotherapy, 7.9% of the patients were positive for Ca27.29 and in 9.4 % of the patients CTCs could be detected. A positivity for CA27.29 together with CTC detection could be shown for 1,29 % (P=0.0015) of the patients. Directly after chemotherapy, 8,6% of the patients were CTC positive and 20.9% were CA27.29 positive. Both markers were expressed by 1,2% of the patients (P=0.0346). 2 years after chemotherapy, 7.4 % of the patients were positive for CA27.29 and CTCs could be detected in 2.6% of the patients. 0.4% of the patients were positive for CA27.29 and CTC (P=0.1115).
Conclusion: There is a weak correlation between CA27.29 levels and CTCs before chemotherapy. This correlation decreases during the course of treatment and no correlation between CA27.29 and CTC can be shown 2 years after the completion of chemotherapy. CTC and CA27.29 are independent markers after chemotherapy.

M213

INSULIN LIKE GROWTH FACTOR RECEPTOR I (IGF-IR) AND VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR 2 (VEGFR-2) ARE EXPRESSED ON THE CIRCULATING EPITHELIAL TUMOR CELLS OF COLORECTAL AND LUNG CANCER PATIENTS

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Background: Circulating epithelial tumor cell analysis is a promising diagnostic field for monitoring the risk for metastatic relapse and progression in patients with malignant disease. CETCs detection is possible in peripheral blood using a non-dissipative approach comparable to other cell counting methods from blood as a liquid biopsy for prognostic and predictive purposes in colorectal, lung and other cancers. IGF-IR and VEGFR-2 play an important role in the growth of tumor and progression of cancer disease. Therefore the purpose of the current study was to investigate their expression on the CETCs.
Methods: CETCs were determined from blood of 60 patients suffering from colorectal and lung cancer. The number of vital CETCs and the expression of IGF-IR and VEGFR-2 were investigated using the maintrac® method.
Results: IGF-IR expression on the surface of CETCs was detected in 91,4% of patients whereas expression of VEGFR-2 was observed in 97,1% of patients with colorectal cancer. 76% of patients with lung cancer showed IGF-IR and VEGFR-2 expression on the CETCs. The number of living CETCs was higher in colorectal cancer patients, in comparison to lung cancer patients. A statistically high correlation was found between IGF-IR and VEGFR-2 on the CETCs in both, colorectal ($r = 0,659$; $P < 0,001$) and lung ($r=0,772$; $P < 0,001$) cancer patients. No statistically significant correlation was observed between the number of CETCs and IGF-IR or VEGFR-2 expression in colorectal and lung cancer patients. The co-expression of both receptors was confirmed and ranged between 70% and 100%.
Conclusion: Our results demonstrate for the first time the expression of IGF-IR and VEGFR-2 on CETCs in patients with colorectal and lung cancer and thus constitute the basis for anti-IGF-IR and anti-angiogenic therapy for their elimination.

M214

CYFRA 21-1, SELECTED ACUTE PHASE PROTEINS AND SYSTEMIC INFLAMMATION BASED SCORES IN MUSCLE-INVASIVE BLADDER CANCER PATIENTS

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Background: The aim of the study was the evaluation of potential diagnostic utility of CYFRA 21-1 and measure of the systemic inflammatory response factors in muscle-invasive bladder cancer patients treated conservatively.
Methods: The assessment of CYFRA 21-1, C-reactive protein (CRP), alpha-1 acid glycoprotein (AAG), prealbumin (PRE), Neutrophil Lymphocyte Ratio (NLR) and the Platelet Lymphocyte Ratio (PLR) were performed, before treatment, in the group of 86 patients with bladder carcinoma and in the reference group consisting of 37 healthy persons.
Results: Significantly higher levels of CYFRA 21-1, CRP, AAG, NLR, PLR and significantly lower concentration of PRE were found in patients with bladder carcinoma, in comparison to the reference group. A significant reciprocal correlation was found between NLR and PLR, and negative correlation between CRP and PRE and poor positive correlation between CRP and NLR. When analyzing concentrations of the studied factors in respect to tumour stage (T2 vs. T3-4), significantly higher CYFRA 21-1, CRP, AAG, and NLR levels were observed in more advanced patients. The percentage of pathological results of CYFRA 21-1, CRP, AAG, PRE, NLR and PLR in T2 and T3-4 were 36 v. 63%; 21 v. 49%; 15 v. 51%; 5 v. 30%; 26 v. 51%; 32 v. 55%, respectively. In the studied group, no differences were found between concentration of the determined factors in respect to histological grade. In the group selected according to CRP level, significantly higher concentration of CYFRA 21-1, AAG, NLR and PLR were observed and significantly lower PRE level in the group with higher CRP level (>5 mg/L) in comparison to the group with lower level. Patients treated radically with complete response (CR) had pretreatment levels of CYFRA 21-1, CRP, AAG and NLR significantly lower than others. When the group of patients was divided in respect to 12-months survivals, patients deceased before 12 month in comparison with those surviving longer, had significantly higher pretreatment CYFRA 21-1, CRP, AAG and NLR levels.
Conclusions: The preliminary study demonstrated that elevated pretreatment markers of systemic inflammatory response levels may be associated with poorer response to therapy and are likely to affect adversely on survival.

M215

ROCHE C6000 ANALYTICAL VALIDATION OF NEW ASSAYS FOR DOSE ADJUSTMENT OF PACLITAXEL AND DOCETAXEL

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Background: Rational dose adjustment may benefit patients with oncology drugs which are highly toxic, demonstrate a high degree of inter- and intra-patient pharmacokinetic (PK) variability, and a relationship between a PK parameter and pharmacodynamics (PD) such as toxicity. For paclitaxel (Taxol®) and docetaxel (Taxotere®) used to treat a variety of solid tumors, PK parameters have been identified for targeted dose adjustment. This study was undertaken to demonstrate analytical performance on the Roche c6000 for a routine immunoassay method which is less expensive and more easily accessible than physical methods.
Methods: The MyDocetaxel™ (DTX) and MyPaclitaxel™ (PTX) immunoassay reagents (Saladax Biomedical, Inc.) have been validated on other instrument platforms. For this study, analyzer specific performance characteristics were validated according to CLSI guidelines and established protocols for linearity, precision on 3 control concentrations, and two-instrument method comparison using the c501 clinical chemistry module of the Roche c6000. Cross-reactivity of related and unrelated compounds, and interferences were evaluated on the Beckman AU400.
Results: Both assays were linear through the reportable range: DTX (29-1000 ng/mL) slope =1.012, intercept=-4.2, observed error =5.9%; PTX (19-320 ng/mL) slope=0.997, intercept =8.0 ng/mL, observed error 3.9%. The assays were precise: DTX (control level/repeatability CV% within-laboratory CV%) low/2.8/3.8, med/2.3/3.1, hi/1.2/1.4; PTX: low/6.7/8.6, med/4.3/4.4, hi/4.0/5.5. Method comparison to an Olympus 400 (validated with comparison to LC-MS/MS), gave clinically equivalent results: DTX Coeff(R) =0.9982, slope = 1.008, intercept=3.7 ng/mL; PTX Correlation Coeff(R) =0.9924, slope =1.057, intercept=0.9 ng/mL. Relevant metabolites would have no clinical impact because of the metabolite concentrations and cross-reactivities established in the assays: DTX≤13%; PTX ≤3.4%. Effect of unrelated drugs or co-administered drugs was insignificant at <1%.
Conclusion: The MyDocetaxel and MyPaclitaxel assays have demonstrated performance on the c501 analyzer providing a tool for appropriate PK-guided dose management of docetaxel and paclitaxel to control systemic exposure and the toxicity often associated with treatment.

M216

CIRCULATING TUMOR CELL DETECTION USING A FILTRATION-BASED METHOD IN ADRENOCORTICAL CARCINOMA

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Background: Adrenocortical carcinoma (ACC) is an uncommon and heterogeneous malignancy which gains access to the systemic circulation early during disease progression. Circulating tumor cells (CTC) have never been researched in blood from ACC patients.
Methods: We applied a method for CTC detection based on filtration of peripheral blood through polycarbonate membranes with 8 μm pores. The method enables the easy, rapid and sensitive isolation of CTC (up to one cell for ml of blood), and their subsequent morphological and immunohistochemical characterization. CTC analysis was performed in 10 ACC patients and 5 subjects undergoing surgery for adrenocortical adenoma (ACA). Weiss score was between 6 and 8, with presence of venous or sinus invasion in all cases. CTC analysis was performed before and after surgery and a longitudinal study was performed during the follow-up period.
Results: CTC were isolated in all 10 (100%) patients. Hematoxylin and eosin stain of isolated CTC preserved cell morphology. Isolated cells were characterized by cell size >16 μm, nucleo-cytoplasmic ratio >50%, irregular nuclear shape and a hyperchromatic nucleus. Immunoistochemistry performed in parallel on CTC and the primary tissue showed the expression of the same markers on both specimens, thus confirming the ACC origin of the isolated cells. As expected , no CTC were identified in the ACA patients, considered as the control group.
Conclusions: We demonstrated for the first time the presence of CTC in ACC patients These results build the basis for future larger prospective studies to ascertain whether CTC analysis may be a promising diagnostic tool, providing prognostic information to guide monitoring and treatment in ACC patients.

M217

MEASUREMENT OF BRAFV600E ALLELE IN PLASMA CELL-FREE DNA FOR THE DIAGNOSIS AND FOLLOW UP OF DIFFERENTIATED THYROID CANCER

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Background: Accurate diagnostic tools for thyroid cancer are needed in order to avoid unnecessary surgery in patients affected by benign thyroid nodules. Although overall accuracy of fine needle aspiration (FNA) in identifying thyroid cancer is considered excellent, 20-30% of aspirates do not allow definitive diagnosis of malignancy. The presence of proto-oncogene somatic mutations such as BRAFV600E in FNA strongly suggest the presence of malignancy. Only few studies reported the use of circulating BRAFV600E-mutated alleles in plasma as an useful marker for non invasive diagnosis and follow up of this disease.
Methods: We propose an allele specific Taqman-based real-time PCR assay to measure plasma-circulating BRAFV600E concentration in patients affected by thyroid nodules (n= 100), non-nodular thyroid diseases (n=21) and healthy subjects (n=33).
Results: A significant difference in plasma-circulating BRAFV600E concentration (ng/mL) was found between the control group (mean±SE: 0.3±0.1) and cytological groups of undetermined (Thyr3; n=47, mean±SE: 2.2±0.9, P=0.04) and suspected plus certain carcinomas (Thyr4+Thyr5; n=29 mean±SE: 1.7±0.5, P=0.01). Plasma-circulating BRAFV600E levels in control subjects were not significantly different when compared to Thyr2 group (n= 27 mean±SE: 0.8±0.4, P=0.6). In 18 subjects affected by differentiated thyroid carcinoma the level of circulating BRAFV600E decreases significantly after surgery (from 3.1±0.8 to 0.8±0.3 ng/mL, P=0.02). ROC curve analysis indicated that BRAFV600E absolute concentration has the maximal diagnostic relevance with 76% sensitivity and 82% specificity. At present all the patients with Thyr3 cytology are submitted to surgery. In this group, the comparison of the BRAFV600E status with the histological examination demonstrate a negative predictive value of 73%.
Conclusions: The present results suggest that circulating BRAFV600E might be used in the diagnosis of differentiated thyroid cancer. It may be also a helpful tool in the follow up of these type of cancer, especially in the cases in which tireoglobulin (Tg) is not informative i.e when positive antiTg antibodies are present, preoperatively Tg is undetectable and patients were not subjected to radioablation.

M218

DIAGNOSTIC USEFULNESS OF D-DIMER, FIBRINOGEN AND APTT MALIGN AND BENIGN DISEASE

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Background: The elevated D-dimer levels have been detected in patients with thromboembolic events, disseminated intravascular coagulation, myocardial infarction, pneumonia and in vaso-occlusive crisis in sickle cell disease. D-dimer levels also are elevated in patients with solid tumors including lung cancer. We have make determination of D-dimer, fibrinogen and APTT at patents with lung cancer and patients with infection disease.
Material and methods: The concentrations of D-dimer, fibrinogen and APTT in 150 samples were determined using BCS autoanalyser, Simens Healthcare Diagnostic. All of 100 patients were hospitalized at Department of Oncology and Department for Infection disease at the University Clinics Center of Sarajevo and 50 healthy subjects. The normal serum range of D-dimer 0-0.55 mg/L FEU, fibrinogen 1.8-3.8 g/L and APTT 27.7-37.0 s. Collected data were statistically analyzed using programs SPSS version 11.0 and Microsoft Office Excel 2003.
Results: We had 50 patients with lung cancer a mean value of D-dimer 4.78±0.34 mg/L FEU, fibrinogen 4.02 ± 2.0 g/L and APTT 36.9±2.39 s. The mean values of the control group were in reference range. The group of patients with infection disease (50) have mean value D-dimer 16.77±0.34 mg/L FEU, fibrinogen 5.8±2.6 g/L and APTT 35.7±2.34 s. In group of patients with infection disease we have patients with sepsis and meningitis. Our study have show the low correlation between D-dimer and APTT with correlation coefficient r = 0.147. The correlation between D-dimer and fibrinogen was very low r = 0.014.
Conclusions: The patients with malign and infection disease have higher concentration of D-dimer and fibrinogen then control group. The high plasma D-dimer concentration was found to be a strong predictor of poor outcome, independent of age and tumor stage in patients with lung cancer. Patients with cancer have a higher risk of developing venous thromboembolism. We concluded that measuring plasma D-dimer may be helpful for predicting prognosis and risk of venous thrombotic disease in newly diagnosed lung cancer patients. Therefore the concentration of D-dimer rice in patients with infection and it could be a possible reactant of acute phase too.

M219
ESTROGEN AND PROGESTERONE STATUS OF CARCINOMA BREAST IN WESTERN MALAYSIA

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Background: Carcinoma breast is one of the most common malignancies in Malaysia. The present study involves role of estrogen and progesterone receptors in carcinoma of breast. In Malaysia there are 3 major ethnic groups are living out of which 43.3% of the cases were Malays, 40.0% were Chinese, 15.0% of the cases were Indians and 1.7% from other races and there are three major religions out of which 45.0% of breast cancer cases involved Muslims, 40.0% Buddha, 13.3% Hindu and 1.7% for other religious denominations.
Materials and Methods: 200 patients were selected for this study from Ipoh General Hospital, Ipoh, Malaysia during the year 2010-2012. All these patients were underwent surgical treatment and tumor biopsy was done for histopathological examination. All the specimens were examined for Estrogen and Progesterone receptor status which was determined by the immunohistochemical methods.
Results: 60% of cases involved cases who were pre-menopausal (12-55 years old) women and 40% post menopausal (>55 years old) women. 48.3% of the tumors demonstrated an ER positive status while 51.7% tumors were ER negative. 48.0% tumor biopsies demonstrated PR positive while 51.7% were PR negative.
Conclusion: The Estrogen and Progesterone status of these patients believed to be proper tool for assessment of the prognosis though they are counting less than 50%. Complete details of the patient's demographics, methods and follow up will be discussed.

M220
SOMATIC MUTATIONS IN BRAF AND NRAS GENES IN PRIMARY CUTANEOUS THICK MELANOMA

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Background: Identification of mutations that drive malignant trans¬formation in melanoma cells has opened new perspectives to patient-tailored targeted therapies. The aim of the study was to evaluate the occurrence and type of BRAF/NRAS mutations in the subset of thick cutaneous melanomas and to analyze the molecular status in relation to clinical-pathological features and outcome.
Methods: Ninety-eight patients with a diagnosis of primary cutaneous melanoma with Breslow thickness >4 mm were retrieved from the archives of Registro Tumori Toscano. Histopathological slides were re-evaluated for histotype, thickness, level, ulceration, mitotic index, TILs, microsatellites, vascular/perineural invasion, regression, solar elastosis and pigmentation. Median follow-up time was 39 months. Molecular characterization of the primitive tumor in terms of BRAF -exon-15 and NRAS genes, analyzed by sequencing of respective coding sequences, was performed. The association of each variable with mutational status was assessed using the Student test for continue and Chi square test for categorical variables. The survival rates were calculated using the Kaplan Meier method and differences in survival were tested using the log rank test.
Results: BRAF mutations were identified in 35 (36.5%) cases, including 32 V600E mutations and 3 V600K mutations. NRAS mutations were detected in 31 (31.6%) cases, specifically p.Q61R (24 cases), p.Q61K (3 cases), p.Q61L (2 cases) and p.Q61H (2 cases). Among BRAF/NRAS mutated cases, 37 (58.7%) were males and 26 (41.3%) were females, median age was 72 years. BRAF/NRAS mutational status significantly correlated with anatomical site of the primary tumor (P=0.002) and sun damage (P <0.01). Mutational status did not show correlation with other clinico-pathological variables and did not have prognostic effect on overall survival.
Conclusions: We found a high frequency of NRAS mutations in the subset of thick cutaneous melanomas and our analysis confirms the significant association of BRAF/NRAS mutations with anatomical site and degree of sun exposure. In this subgroup of advanced melanoma patients BRAF/NRAS genotype was not a prognostic factor.

M221

PERFORMANCE OF EARLY PROSTATE CANCER ANTIGEN-2 (EPCA2), PROPSA, FREE PSA (FPSA) AND PROSTATE HEALTH INDEX (PHI) IN DETECTION OF PROSTATIC CARCINOMA

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Background: Prostatic specific antigen (PSA) is an established tumor marker for screening, diagnosis and follow up of carcinoma of the prostate. PSA is organ specific, but not disease specific and benign diseases of the prostate influence PSA value and hence affect its clinical performance. The aim of this study was to compare novel tumor markers EPCA2, proPSA, fPSA and PHI to the PSA.
Methods: 46 men undergoing the transrectal biopsy of the prostate were included in the study. Blood sample for measurement of tumor markers was obtained prior to the procedure. Patients were classified into 5 groups according to the patohistological report: benign prostatic hiperplasia (BPH), prostatitis, high grade prostatic intraepithelial neoplasia (HGPIN), suspected prostatic carcinoma (spCa) and prostatic carcinoma (pCa). Values of tumor markers were compared among patohistologic gropus using Mann-Whitney U test (benign vs malign) and Kruskal-Wallis test (comparing each of the 5 groups). ROC curves were derived for each tumor marker.
Results: Only PSA (P=0.006) and fPSA/PSA (P=0.002) reached statistically significant difference in median tumor marker concentrations between benign (BPH, prostatitis, HGPIN) and malign (spCa, pCa) groups. The same was calculated by comparing each of the patohistological groups via Kruskal-Wallis test (PSA: p=0.015 and fPSA/PSA=0.021). Areas under the ROC curve were: 0.740 (PSA), 0.578 (fPSA), 0.578 (proPSA), 0.531 (EPCA2), 0.765 (fPSA/PSA), 0.633 (proPSA/PSA) and 0.619 (PHI).
Conclusion: On this small populatiom of prostate patients only the PSA and fPSA/PSA have shown a moderate performance in differentiating between pCa and benign prostatic disease.

M222

THE ROLE OF CALIBRATORS OF SERUM PROSTATE-SPECIFIC ANTIGEN IN THE PROSTATE CANCER DIAGNOSIS

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Background: Despite its low specificity and low positive value, the serum prostate-specific antigen (PSA) is the most widely used serum biomarker for the detection, management, monitoring and screening of prostate cancer (PCa). However, the PSA [total (t) and free (f)] levels measured with the different assays are very discordant. The goal of this study was to investigate the differences between the results of the Beckman Coulter Access PSA Immunoassay (with the Hybritech calibrator) and the Roche Elecsys 2010 PSA assay [with the WHO 96/670 (90% tPSA and 10% fPSA) calibrator]. Methods. From May 2010 to October 2012, the tPSA concentrations of 179 serum samples of patients with PCa (ages 53-89 years), and 538 samples of healthy subjects (ages 40-92 years) were measured with the Beckman Coulter Access PSA Immunoassay (Beckman Coulter, Inc., USA) and the Roche Elecsys 2010 PSA assay (Roche Diagnostic Corp., Germany). Prostate biopsies were performed if the tPSA concentrations were >4.0 ng/mL. Statistical calculations were performad with SPSS 13.0 for Windows.
Results: The tPSA range was 1.38-147.5 ng/mL (mean: 11.49+8.98 ng/mL) in the PCa group and 0.16-17.8 ng/mL (mean: 3.10+2.05 ng/mL) in the control group with the Access assay, and 1.06-119.8 ng/mL (mean: 8.92+6.27 ng/mL) and 0.12-15.27 ng/mL (mean: 2.53+1.78 ng/mL), respectively, with the Elecsys assay. The results with the Access assay were higher by 23% vs the Elecsys assay.
Conclusion: The reference range of the Access tPSA assay with Hybritech calibrator is significantly higher (23%) than that of the Elecsys assay with WHO calibrator. It is very important that, due to the variability of the assays modifications of the PSA cut-off values are needed which can cause changes in the guidelines in connection with the indications of prostate biopsy. The discrepancies between results with the two assays (the two calibrations) can lead to significant misinterpretations in the detection of PCa.

M223

PLASMA LEVELS AND DIAGNOSTIC UTILITY OF SELECTED CYTOKINES (M-CSF AND VEGF) IN BREAST CANCER PATIENTS

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Background: M-CSF and VEGF may play a role in the pathogenesis of cancer disease. We investigated plasma levels of selected cytokines (M-CSF and VEGF) in comparison with the tumor marker (CA15-3) in breast cancer patients and in relation to the control groups: benign breast tumor patients and healthy subjects.
Methods: The group tested included 50 breast cancer patients (adenocarcinoma ductale). The control groups consisted of 40 benign breast tumor patients and 40 healthy volunteers. Plasma levels of the cytokines tested were determined using immunoenzyme assay (ELISA), CA 15-3 concentrations with the use of chemiluminescent microparticle immunoassay (CMIA).
Results: Plasma levels of M-CSF, VEGF and CA 15-3 were significantly higher in breast cancer patients as compared to the healthy controls. Statistically significant differences in the level of M-CSF were also observed between the cancer group and benign breast tumor patients. The diagnostic specificities of the cytokines tested and CA 15-3 received high equal values (95%). The diagnostic sensitivity, positive and negative predictive values were higher for M-CSF than for VEGF and CA 15-3. The combined use of the parameters tested resulted in an increase in the sensitivity range, but the highest values were obtained analyzing all tested parameters (94%). The areas under the ROC curve (AUC) for M-CSF (0,8864) and VEGF (0,7944) were the largest and slightly lower than the AUC of CA 15-3 (0,9024).
Conclusions: These results suggest a potential usefulness of the cytokines tested in the diagnosis of breast cancer, particularly in combination with CA 15-3, but only M-CSF - in discriminating between cancer and non-carcinoma (benign) lesions.

M224

COX-1 AND COX-2 EXPRESSION IN TISSUE AND SERUM ARACHIDONIC ACID LEVEL IN COLORECTAL CANCER PATIENTS IN RELATION TO DISEASE STAGE

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Background: Cyclooxigenase (COX), the enzyme necessary for the first step in the biosynthesis of prostaglandins (PGs), catalyzes the conversion of arachidonic acid (AA) to prostaglandins and related eicosanoids. COX exists in two isoforms: COX-1, constitutively expressed in many cells and tissues and COX-2, which is not expressed under normal condition but is highly inducible in response to proinflammatory cytokines, hormones and tumour promoters. The aim of the study was to investigate the relation between serum AA level and tissue COX-1 and COX-2 expression in colorectal cancer patients at different stage of disease.
Methods: The study included 28 patients with colorectal cancer (mean age 63,1±10,2 years, M/F 15/13). Nineteen patients were at 0, I or II stage of disease (group I) and the remaining nine patients were at III or IV disease stage (group II). Serum AA level of phospholipid fraction was measured by gas chromatography. RNA was isolated from homogenized tissue samples using the Rneasy Mini Kit (Qiagen) than RT-PCR (with COX-1 and COX-2 gene probes, TaqMan, Applied Biosystem) was performed. The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for transcript normalization.
Results: The mean value of serum phospholipid AA level was higher for group I as compared to group II ($P < 0,05$). No difference in the COX-1 expression between both groups were observed. COX-2 expression was four times higher for group II than for group I ($P < 0,007$).
Conclusions. Low level of arachidonic acid in more advanced stages of colorectal cancer parallels over expression of COX-2.

M225

FATTY ACIDS (FA) OF MUCOSA TISSUE PHOSPHOLIPIDS IN COLORECTAL CANCER PATIENTS DOES NOT CORRELATE WITH TUMOR STAGE

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Background: Increasing evidence show that essential fatty acids play a role in etiology of colorectal cancer. Tissue phospholipids are the most sensitive indicator of FA fluctuations. Limited data on the FA content in cancer tissue and stage of the disease exist. Also, most information are related to the cancer tissue FA content of only essential fatty acids and no other laboratory calculation were taken into account. The aim of the study was to compare the FA of phospholipids in colon cancer tissue in colorectal cancer patient in relation to disease stage.
Methods: The study included 19 patients with colorectal cancer (Duke 0,I, II, mean age 63.6±10.5 years, M/F 10/9, group I) and 11 patients with colorectal cancer (Duke III, IV, mean age 61.1±10.7 years, M/F 5/6, group II). Tissue level of cancerous (CA) and non-cancerous (NCA) tissues saturated FA (C12-lauric, C14-myristic, C16-palmitic, C18-stearic) and unsaturated FA (C16:1-palmitooleic,C18:1-oleic,C18:2-linoleic,C10:4-arachidonic) were measured by gas chromatography. The results were expressed in mmol/L , than calculated mmol/g protein, as well as the percentage of total tissue FA. Total concentration of all FA, saturated FA (SSFA), monounsaturated FA (MUFA) and the ratio of C18 to C18:1 were calculated.
Results: The mean values of each fatty acid and the percentage of C16:1 and MUFA were significantly higher in cancer tissue as compared to non-cancerous tissue, regardless the disease stage (group I, II: P <0,003; group I: P <0,02, group II: P <0,04; respectively). The stearic to oleic acid ratio and SFA were higher in NCA as compared to CA when patient from both groups were taken into account. The percentage of C18:1 was higher in CA as compared to NCA regardless the disease stage.
Conclusions: In colorectal cancer patients, tissue fatty acids from phospholipids fraction does not correlate with disease stage. Higher amount of FA in cancer mucosa reflect their increased metabolism in cancer tissue.

M226

HUMAN EPIDIDYMIS PROTEIN 4 (HE4) REFERENCE INTERVALS IN A MULTIETHNIC ASIAN WOMEN POPULATION

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Background: Ovarian cancer is ranked as the fifth most common cause of cancer death in women. CA125 has been the tumor marker of choice in ovarian cancer with a poor diagnostic specificity of 50% in the early stages. Hence, there is a critical need to identify an alternative tumor marker that is capable of detecting detect ovarian cancer at an early stage. HE4 is a new tumor marker proposed for the early diagnosis of ovarian cancer and disease recurrence. Currently, none of the normal ranges of HE4 quoted in the literature were conducted in a multiethnic Asian population. Therefore, the aim of this study is to determine the reference intervals of HE4 in Asian population presenting in University Malaya Medical Centre, a tertiary reference hospital.
Methods: 300 healthy women were recruited comprising 150 premenopausal and 150 postmenopausal women, aged from 20 to 76 years. All women were subjected to a pelvic ultrasonography screening and were confirmed to be free from ovarian pathology on recruitment. Serum HE4 levels were determined by chemiluminescent microparticle immunoassay (CMIA) Abbott Architect. The reference intervals were determined following CLSI guidelines (C28-A2) using a non parametric method.
Results: The upper limit of the 95th percentile reference interval (90% CI) for all the women collectively was 64.6 pmol/L and 58.4 pmol/L (premenopausal) and 69.0 pmol/L (postmenopausal) respectively. The concentration of HE4 was noted to be increasing with age especially in women who were more than 50 years. We also noted that our proposed reference limit was lower compared to the level given by manufacturer Abbott Architect HE4 kit insert, (58.4 vs 70 pmol/L for premenopausal group and 69.0 vs 140 pmol/L in the postmenopausal group). The study also showed a significant difference in HE4 concentrations between the ethnicities (Malay and Indians). The levels of HE4 in Indian appeared higher than Malays (P <0.05) and no significant differences were noted between the Malays and Chinese ethnic groups.
Conclusions: More data is needed to establish a reference interval that will better represent the multiethnic Malaysian population. Probably a larger sampling size of equal representation of the Malay, Chinese, Indians as well as the other native ethnic communities will give us a greater confidence on whether genetics plays a role in the reference interval determination.

M227

ANALYTICAL PERFORMANCE CHARACTERISTICS OF HM-JACKARC SYSTEM FOR FECAL OCCULT BLOOD TESTING (FOBT)

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Introduction. Faecal occult blood testing is widely used in colorectal cancer screening and new devices are now available in European market. In this report analytical performance of HM-JACKarc (Kyowa Medex-Japan) was investigated and compared to OC-Sensor Diana (Eiken-Japan), currently used at ISPO.
Methods. Calibration stability (3 calibrations) and method imprecision were investigated using 2 levels of control materials and 3 biological samples obtained by addition of Hb to Hb-free faeces. Linearity was investigated using serial dilutions of 2 biological samples. Inter calibration bias and intra/inter/total method imprecision were assessed on control and biological samples. Methods comparison was performed using 35 samples to investigate the analytical phase, Hb measurements was measured running ARC-tubes and Diana-tubes on both instruments. Comparison including sampling was run on 153, system-specific samples, positives were obtained by addition of Hb to Hb-free faeces. Positivity or negativity of samples was assessed using cut-off values reported by manufacturers.
Results. Inter calibrations bias results <5% on control materials. Total imprecision (CV%) results <6.7 in control materials and <12.1 in biological samples. Pearson's correlation coefficients relative to linearity analysis result >0.98 in both investigations. Linear regression curve relative to systems comparison performed on 35 ARC tubes results x=0,84y+0,13 and x=0,84y+1.12 on 35 Diana tubes. Pearson's coefficients analysis results R2=0,92 and R2=0,93 respectively. Bland-Altman analysis showed a bias of 9% (ARC–Diana on ARC tubes) and 11% (ARC–Diana on Diana tubes). Methods comparison performed on 153 system-specific samples showed 72 positives and 81 negatives on both systems and no correlation among analytical values in ng/mL or ug/g faeces.
Discussion. Methods characteristics (linearity and imprecision) results in according to manufacturer reported characteristic, whereas the stability of calibration seems to be longer than reported. Our investigation also confirms the importance of pre-analytical phase in FOBT. A full investigation of method in terms of sensibility and specificity in a screening program was mandatory to full understand method's performances and the useful cut-off.

M228

CALPROTECTIN (S100A8/A9) IS NOT PREDICTIVE OF BACTEREMIA AND SEPSIS IN FEBRILE NEUTROPENIA PEDIATRIC ONCOLOGY PATIENTS

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Background and aims: Early diagnosis of infection complications, which are usually related to chemotherapy still remains an open challenge due to lack of reliable markers especially at the beginning of infection. Calprotectin (S100A8/A9) is a heterodimer of the two calcium-binding proteins S100A8 / S100A9 and up-regulation of these proteins were observed in various types of tumors. Of note, calprotectin is a well-known marker of an inflammatory conditions associated with several autoimmune diseases. The aim of the study was to evaluate the potentiality of calprotectin for the prediction of an infectious process at the beginning of febrile episode in childhood oncology patients.
Methods: A total of 66 febrile neutropenic episodes in 40 children suffering from hematological and solid tumors were evaluated. Serum samples were collected after confirmation of febrile neutropenia on day 1. Microbiological evaluation and calprotectin determination in serum were performed. According to microbiological and clinical findings, patients were divided into two groups: (1) septic and (2) fever of unknown origin. A receiver operating characteristic (ROC) curve was used to determine a cut-off level for the calprotectin; the result was estimated as statistically significant when P value <0.05.
Results: Area under the curve (AUC) for calprotectin was 0,49 (P=0,93). These results show that calprotectin is not a significant predictor of infection at the beginning of febrile episode.
Conclusions: Calprotectin serum levels were not able to discriminate between microbiologically or clinically documented infections or fever of unknown origin due to lack of sensitivity and specificity in childhood oncology patients. New and reliable laboratory markers are still required for an infection determination in febrile neutropenia pediatric oncology patients.

M229
UNUSUAL CA19-9 ELEVATION IN A 37-YEAR-OLD MOTHER AND 16-YEAR-OLD DAUGHTER

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Background: Serum carbohydrate associated antigen (CA19-9) is widely used tumor marker in clinical practice. It shows small increases in a number of benign diseases, with highest levels in pancreatic, hepatocellular, gastric, colorectal and cholangiocellular cancers. Here we report two healthy cases from the same family, 37-year-old mother and 16-year-old daughter with slightly elevated CA19-9 levels.
Method: Case report 1: The mother with mildly elevated CA19-9 level (89.92 U/mL; upper limit of normal range (ULNR) <37U/mL) was referred to our department for evaluation. Extensive laboratory data, imaging procedures and clinical evaluations were within the normal ranges except elevated CA19-9 level. A similar increase was determined in her daughter incidentally.
Case report 2: Daughter's serum CA19-9 level was 123.92 U/mL. Afterwards, similar medical procedures were also performed for the girl and any pathology compatible with the elevated CA 19-9 level could not be determined. In order to avoid laboratory errors and interferences, we measured the samples twice at four different immunassay platforms (UniCel® DxI 800, Elecsys E170, Architect i2000SR, Advia Centaur® XP) with same ULNR (<37 U/mL).
Results: Mother's CA19-9 levels were 28.80 U/mL, 35.80 U/mL, 59.70 U/mL and 64.90 U/mL, respectively. Daughter's CA19-9 levels were 64.40 U/mL, 42.99U/mL, 87.84 U/mL and 76.85 U/mL, respectively. Also, the recheck levels were similar to the first ones.
Discussion: In these cases, we tried to rule out the usual causes of CA19-9 elevation. Although most of the results were over the ULNR, there were discrepancies between assaying methods. These differences may result from the application of different types of antibodies and epitopes by different assay methods and suppliers. As manufacturers specified in their test manuals, there are healthy people in excess of the upper limit of the normal range. Moreover, the upper 97.5% reference limit for all methods can differ from the manufacturers' upper limit of expected values for healthy subjects. Also the reason for the CA19-9 elevation might be familial etiology. Therefore, clinicians need to be aware of these rare situations without missing the malignant disorders.

M230
EVALUATION OF SERUM LEVELS OF ADHESION MOLECULES BY BIOCHIP ARRAY TECHNOLOGY IN ACUTE MYELOID LEUKEMIA PATIENTS

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Background: Cytokines and adhesion molecules have been studied as markers of immune system activation in various diseases including hematological malignancies. The objective of our study was to evaluate serum levels of adhesion molecules by biochip array technology in patients treated for acute myeloid leukemia (AML).
Methods: A total of 15 AML patients (mean age 48.7± 12.1 years, median 51, 8 males and 7 females) treated with cyclic chemotherapy (3+7, 2+5, HiDAC) alone or in combination with high-dose chemotherapy (preparative regimen Bu/Cy2 or Cy/TBI) followed by autologous hematopoietic stem cell transplantation were studied. We evaluated serum levels of the following adhesion molecules: E-Selectin, L-Selectin, P-Selectin, Intercellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1). All biomarkers were measured by biochip array technology on Evidence Investigator analyzer (Randox) at the diagnosis of AML (active leukemia) and at 6 months after completion of chemotherapy (durable complete remission /CR/ in all patients).
Results: Comparing serum adhesion molecule levels in active leukemia and in durable CR, we found significant decrease in E-Selectin (30.19±20.46 mcg/L vs. 12.99 ±8.00 mcg/L; P <0.01), L-Selectin (2179.35±1169.39 mcg/L vs. 1533.35 ± 540.69 mcg/L; P <0.05), ICAM-1 (659.61± 259.50 mcg/L vs. 492.81±236.96 mcg/L; P <0.05), VCAM-1 (716.22±364.38 mcg/L vs. 514.52 ± 115.66 mcg/L; P <0.05). Levels of P-Selectin were without significant difference (89.56±67.60 mcg/L vs. 106.43±52.74 mcg/L; ns).
Conclusions: Our results indicate that serum levels of some adhesion molecules (E-Selectin, L-Selectin, ICAM-1, VCAM-1) are altered in patients treated for AML, showing activity of the disease. Whether these alterations could serve as a prognostic marker for AML is not known. Further studies in a larger number of patients and comparing adhesion molecule levels with established prognostic markers (cytogenetics, molecular genetics) will be needed to define the potential role of these and additional markers in the risk stratification of AML patients.

M231
MANAGMENT OF URINALYSIS FOR ATYPICAL CELLS DETECTION IN BLADDER UROTHELIAL NEOPLASIA

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Background: The prevalence of bladder tumour is 6,6% in male and 2,4% in females.The careful observation of urinalysis allows the identification of atypical cells (AC) in the urinary sediment. With the collaboration of the Department of Surgical Pathology, we have created a protocol for AC confirmation. Our purpose is the prompt detection of urothelial bladder tumour.
Methods: 89 samples were processed and classified in 3 groups: 1) Suspected presence of AC in urinary sediment (48 cases); 2) Patients with normal reagent strip but without observation of AC as a positive control (20 cases); 3) Patients with normal urianalysis as a negative control (21 cases). We standardized the sediment analysis. We confirm the presence of AC using the Papanicolau staining by the pathologists and we define it as the Gold Standard. The reliability test was performed in 32 samples with 3 observers, (α Cronbach=0,712). The data were introduced in statistical software SPSS 15.0
Results: In group 1, 27% of samples with suspected presence of AC in urinary sediment were confirmed by the pathologists (P=0,023, Sensibility 85%, Specifity 51%). The 68,8% were males (media age 54 years). The 83,3% have not urologic previous diagnosis (P=0,482). The 31% were followed by biopsy and subsequent surgery. In group 2, 10% of samples (without suspected of AC) were anomalous and were confirmed by the pathologist (P=0,023). The 40% were males (media age 57years). It weren´t followed by biopsy. The found tumours types were: 40% in situ (TIS), that is of high degree, aggressive and treatment to the infiltrant type. The 20% presented high degree tumours (G2-G3) or infiltrant (that are the ones that more desquamates) with different characteristics from normal urothelium, with high metastatic ability and also high mortality. Finally the 40% presented 2 tumours:high degree (pT1) and carcinoma in situ (TIS).
Conclusions: The median age of our group patients (54 years) with AC that were diagnosed of bladder tumour is less than the one found in the literature (72 years). It was detected, in the 27% of the samples with suspected presence of AC in the urinary sediment, aggressive tumours and of high grade in asymptomatic patients. For this, prognosis was better because of the early detection. In all cases, was a laboratory finding. We consider this study as the first one in medical literature in which the simple observation of routinary sediment allows the detection of AC and therefore the save of time and money.

M232
A UK NATIONAL AUDIT OF TUMOUR MARKER SERVICE PROVISION

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Introduction: As tumour markers form an essential part of the tests offered by laboratories an effective service should be provided.
Aim of the audit: Assessing the tumour marker service provided by UK laboratories.
Method: A questionnaire asked what markers were offered and their availability, availability of guidelines for their appropriate use, whether marker requests were routinely reviewed, reasons for rejecting requests, measurement of markers in fluids other than serum or plasma, reporting and telephoning of results, guidance for when to measure PSA in relation to a number of procedures and whether an increase in CA125 requests from primary care had occurred since the 2011 NICE guideline for screening for ovarian cancer. Questions relating to reference ranges and how the markers were used were not asked.
Results: 80 responses were received. More routine markers such as AFP, HCG, PSA, CEA and CA125 were generally assayed locally, more specialised markers being referred. 30 accepted requests for free PSA .Guidance as to when to measure total PSA in relation to procedures such as digital rectal examination was only offered by 16. 61 routinely reviewed requests for tumour markers, mostly for those sent to another laboratory. Main reasons for rejecting requests were PSA in females.CA125 in males, a panel of requests or lack of or inappropriate clinical information. 33 offered markers in fluids other than serum or plasma. 64 only offered a weekday service but all accepted urgent requests outside these hours after discussion with the requesting clinician. Whereas all 80 reported the upper limit of the reference range and 41 always offered some form of interpretative comment on their reports, only 15 mentioned the method used. 51 regularly telephoned results if there was an unexpected result, high level in a new patient or sudden or rapid change. With the exception of 16, CA125 requests from primary care had increased since the NICE guideline. Only 23 had guidelines for the appropriate requesting of tumour markers.
Conclusions: The audit showed a generally good service offered nationally but highlighted the need for production of evidence based guidelines to ensure an appropriate, cost effective tumour marker service.

M233

INFLAMMATION AND THE LEVEL OF SELECTED HAEMATOLOGICAL INDICES, TUMOR MARKERS, AND IL-6 IN PATIENTS UNDERGOING SURGERY FOR COLORECTAL CANCER

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Background: CEA is considered a marker of choice for colorectal cancer, it is recommended to include consideration of his signs, in addition to clinical assessment of tumor characteristics, surgical treatment planning and assessment of prognosis of patients. Reviews for utility in this regard CA 19-9 determinations are controversial. Relevant information for the assessment of prognosis of patients assigned to the intensity of the process of angiogenesis, which is one of the markers of the level of vascular endothelial growth factor (VEGF). The aim of the study was to assess in patients with colorectal cancer correlation between serum CEA, CA 19-9, and VEGF taking into account the effect of selected markers of inflammation.
Material and methods: In the group of 62 patients with colorectal cancer scheduled for surgery were measuring the level of CEA, CA 19-9, VEGF, and interleukin (IL-6), as well as hemoglobin (Hgb) and platelets counts (PLT). Cutt of valves of the study indicators were as follow: for CEA-5 ng / mL, CA 19-9 -37 U/mL, VEGF - 405 ng/mL, IL-6-6 pg/mL, PLT - 400x10 9 /L, and Hgb <12 g/dL for women and <14 g/dL for men.
Results: In patients with colorectal cancer before treatment were observed: increased levels of CEA in - 32.2% of patients, CA 19-9 -11.3%, 30.6%-VEGF, IL-6 - 53.2%, PLT - 16.1%, and decreased hemoglobin at 45.2% patients. Significant correlations were found between tumor stage and the level as well as the frequency of elevated results CEA and platelet count. In the study group before surgery also confirmed significant relationships between the level of IL-6 and VEGF levels (r=0.343, P=0.006) and CEA (r=0.311, P=0.014). It also showed the impact of inflammation on the level of haematological indices. While between IL-6 and PLT positive correlation was observed (r = 0.340, P=0.007) between the IL-6 and hemoglobin - negative correlation (r = - .404, P=.001).
Conclusion: In patients with colorectal cancer before surgery, from evaluated markers, only the level of CEA is helpful in planning surgery. As has been shown, inflammation has a significant impact on the intensity of angiogenesis, thrombocytosis and anemia.

M234

BIOCHEMICAL ASSESSMENT OF THE RADIOLOGICAL RESPONSE TO TREATMENT OF SMALL CELL LUNG CANCER PATIENTS

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Background: The aim of this study was an attempt to determine the degree to which changes in tumor markers and CRP levels may be useful for assessment of response to radio- and chemotherapy in patients with small cell lung cancer.
Material and methods: The study was carried out in 73 patients with limited disease of small cell lung cancer, treated according to scheme: 5 cycles of chemotherapy, chest radiation dose of 60Gy in 33 fractions, and prophylactic cranial irradiation (PCI) at a dose of 30 Gy in 15 fractions between the 3rd and the 4th cycle. In all patients, NSE, ProGRP and C-reactive protein levels were determined before each cycle of chemotherapy.
Results: In previously untreated small cell lung cancer patients with limited disease, NSE level higher than 20 ug/L was observed in 57.5%, higher than 50 pg /mL concentration of ProGRP in 79.5%, and greater than 10 mg /L of CRP in 34.2% of patients. During treatment, markers showed a tendency to decrease, but a significant decrease in the concentration of ProGRP and NSE compared to the previous study was observed before the 2nd and the 3rd cycle of chemotherapy, and of CRP just before the 2nd cycle. The proportion of patients with elevated levels of studied indicators before the 2nd, the 3rd, the 4th and the 5th cycle of chemotherapy were as follows: for NSE: 6.8%, 4.1%, 4.15 and 2.7%, for ProGRP: 57.5%, 31.5%, 19.2% and 19.2%, for CRP: 17.8% 17.8%, 26.0% and 24.6%, respectively. In the group of 65 out of 73 patients with complete remission, PCI was applied after the 4th cycle of chemotherapy. Among disqualified from PCI, compared to those who qualified, significantly higher levels of CRP, both pre-treatment and before the 3rd, the 4th, and the 5th cycle of chemotherapy, and a trend to higher levels of NSE and ProGRP were observed.
Conclusions: During combined treatment of small cell lung cancer patients with limited disease, a slower decrease of ProGRP than NSE concentrations was observed. Higher levels of CRP before the 4th and the 5th cycle of chemotherapy in patients disqualified, compared to qualified for PCI, indicate a poorer response of patients to treatment.

M235
CHANGES IN TOTAL PROTEIN DURING A LYMPHOPROLIFERATIVE SYNDROME

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Background: Non-Hodgkin lymphoma (NHL) is one of lymphoproliferative disorders that affect B cells, T or NK. Its location is mainly nodal, however all other organs that contain lymphoid tissue is a possible starting point for developing lymphoma. The incidence of this disease is very high in both sexes; in fact it increased in the last twenty years. Moreover, the change rate of total protein has been associated with several pathological conditions and cancer.
Methods: The aim of this study was to determine circulating levels of total protein (albumin, alpha 1, alpha 2, beta and gamma globulins) by zone electrophoresis: densitometric method (HELENA) in twenty patients with non-Hodgkin lymphoma admitted to the University Hospital of Tlemcen (Algeria) and 20 healthy control subjects. Some parameters: age, sex and inbreeding have been considered and collected using a questionnaire.
Results: We find that sex is comparable between the two groups ($P > 0.05$), the results show a population composed of 10 men and 10 women. In contrast, age and frequency of consanguinity family are significantly higher in patients ($P < 0.01 = 0.004$ and 0.007) compared to controls. The rate of gamma globulin in patients is significantly lower than the rate found in controls ($P < 0.01 = 0.000$), in contrast to albumin and alpha 1, which are present at higher levels in patients with NHL compared to healthy subjects ($P < 0.05 = 0.030$ and 0.034). No significant difference was observed between the two groups regarding the total protein, alpha 2 and bêtaglobuline.
Conclusions: The NHL is associated with pathological changes in total protein, with increased reached in the elderly and inbred patients.

M236
THE CLINICAL VALUE OF HE4 IN DIFFERENTIAL DIAGNOSIS OF OVARIAN CANCER AND GYNECOLOGICAL PELVIC BENIGN DISEASES

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Background: Human epididymis protein 4 (HE4), a recently found serum biomarker with improved sensitivity and specificity over cancer antigen 125 (CA125) in detecting ovarian cancer, is postulated to play a discriminating role in differential diagnosis between benign pelvic diseases and malignant ovarian tumors in women with pelvic mass. Our study is to explore the value of HE4 in the early diagnosis of ovarian cancer as well as the clinical value of the differential diagnosis in ovarian cancer and benign pelvic disease.
Methods: In this study, we measured serum concentration of HE4 and CA125 in 31 patients with ovarian cancer, 133 patients with gynecological pelvic benign disease (including 44 patients with adenomyosis, 32 patients with ovarian cysts, 30 patients with endometriosis) and 65 healthy volunteers. Serum CA125 was analyzed by Chemiluminesent Microparticle ImmunoAssay (Abbott Inc, Architect system i2000SR, USA) and HE4 concentrations were analyzed in serum samples by ELISA analysis (Fujirebio Diagnostics Inc, USA).
Results: The concentration of HE4 in ovarian cancer were markedly higher than those in gynecological benign groups and healthy controls (312.4 ± 513.8) ($P < 0.01$). In addition, the concentration of HE4 in gynecological pelvic benign diseases with high CA125 have no significant difference compared with the healthy controls ($P > 0.05$).
Conclusion: HE4 is a novel promising tumor marker. It can differentiate malignant ovarian tumors from benign disease. As the further study, HE4 may eventually be proven as an important marker in the diagnosis of ovarian cancer, and is better than CA125 in distinguishing patients with malignant ovarian disease from those with benign ovarian disease at high specificity.

M237

CYP2C19 GENOTYPE AND VORICONAZOLE LEVEL IN IMMUNOCOMPROMISED PATIENTS

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Background: Infections in immunocompromised patients pose a management challenge due to narrow therapeutic index, drug-drug interaction and significant inter-individual variability of antifungals such as voriconazole. We present case studies of invasive fungal infection on voriconazole therapy. Their CYP2C19 gene variant analysis, pre-dose voriconazole levels and co-medication history was assessed and correlated with clinical outcome.
Methods: A patient each with lung transplant, CNS infection & liver transplant receiving recommended voriconazole dose was assessed for pre-dose level by HPLC (therapeutic range 2-6mg/l), CYP2C19 genotyping by PCR – RFLP method for poor metabolizers *2, *3 and ultra rapid metabolizer *17 alleles. Clinical & drug history and follow up drug analysis were done to assess pharmacogenetic efficacy.
Results: Case 1 – A 41 yr/female lung transplant patient with homozygous poor metabolizer variant *2 had voriconazole level of 3.9 mg/L. Her tacrolimus level was in toxic range (>30 ng/mL). Voriconazole limits tacrolimus metabolism & increases its level; also seen in our patient. Dose adjustment of both drugs resulted in reduction of Tacrolimus. The patient being a poor metabolizer could attain a therapeutic level of 2.8 mg/L even after dose reduction. Case 2 - A 31 year/female with acquired fungal infection post-surgery was compound heterozygous for *2 & *17 variants suggesting to be a normal metabolizer. However her drug level was sub therapeutic i.e 1.2mg/L. Co-administered Rifmapin is known to decrease voriconazole level. Discontinuation of Rifampin increased voriconazole level to 2mg/L. Case 3 – A cadaver liver transplant patient (8 year/male) was compound heterozygous for *2 & *17 however his voriconazole level was significantly low i.e 0.76 mg/L. The patient was on no other interacting drugs. Voriconazole is metabolized in liver and the donor genotype may influence the drug level. However the donor genotype in our patient was not known to correlate the findings.
Conclusions: There is a remarkable genotype-phenotype correlation and drug-drug interaction affecting voriconazole level; hence genotyping and clinical and drug history may help in management of fungal infections.

M238

METABOLIC ENZYME AND DRUG TRANSPORTER GENE VARIANTS AND FLUVASTATIN ADVERSE DRUG REACTIONS IN RENAL TRANSPLANT RECIPIENTS

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Background: Premature cardiovascular disease is the most important contributor to the reduced life expectancy of renal transplant recipients (RTRs). Studies have shown that fluvastatin reduces the risk of cardiovascular events in RTRs. Statin use in transplant recipients had been hindered by concern about adverse drug reactions (ADRs), especially myotoxicity. Data from available pharmacokinetic studies indicate that polymorphisms in genes encoding metabolic enzymes and drug transporters could be valuable predictors of fluvastatin ADRs. Fluvastatin is substrate of metabolic enzyme CYP2C9 and drug transporters ABCB1 and ABCG2.
Methods: Fifty-two renal transplant recipients that experienced fluvastatin induced myotoxicity or hepatotoxicity and 52 control patients, matched for age, gender, dose and cyclosporine use, were enrolled in the study. Blood samples of all participants were genotyped for CYP2C9*2,*3, ABCG2 421C>A, ABCB1 2677C>T/A, 3435C>T and 1236C>T by means of Real-Time PCR methods (TaqMan® Drug Metabolism Genotyping Assays, Applied Biosystems).
Results: We found that variants of ABCG2 421C>A (p=0.005), CYP2C9*3 (p=0.012) and ABCB1 1236C>T (p=0.05), were associated with fluvastatin ADRs. Genotypes ABCG2 421CA, CYP2C9 *1/*3 and ABCB1 1236CC were statistically significantly more prevalent in the group of patients with adverse effects than in control group. Our results showed that polymorphism of ABCG2 gene (OR=3.81; 95% CI=1.27-11.45) is of more importance than of CYP2C9 (OR=2.44; 95% CI=1.05-5.71) and ABCB1 1236C>T (OR=2.38; 95% CI=1.04-5.47) in a subgroup of RTRs, being different from results of pharmacokinetic studies on healthy volunteers. Carriers of CYP2C9 mutant alleles (*2 , *3), who had inhibitor in their therapy, had more than six times greater odds of having adverse effects than those without inhibitor in their therapy (OR=6.59; 95% CI=1.24-35.08).
Conclusions: ABCG2, CYP2C9 and ABCB1 gene variants could be valuable predictors of fluvastatin ADRs. Presented results also pointed that in real clinical settings and depending on other risk factors like a large number of inhibitors in RTRs, pharmacogenetic predisposition at the level of individual gene could be of even more importance than in healthy volunteers.

M239
CATECHOL-O-METHYLTRANSFERASE – COMT VAL158MET POLYMORPHISM IN CROATIAN POPULATION

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Background: Catechol-o-methyltransferase (COMT) is an enzyme included in dopamine metabolism and therefore is a regulator of it's function. COMT Val158Met polymorphism is associated with reduction in activity of COMT enzyme. Biological hypothesis of schizophrenia is based on increased dopamine function and that is what makes COMT a gene candidate for schizophrenia. Some studies suggest association of COMT Val158Met polymorphism and certain symptoms of schizophrenia while others failed to support this thesis. Pharmacogenetic role of COMT has also been proposed. The aim of this study was to assess genotype frequency in Croatian population.
Methods: 235 healthy individuals were included in this study. They all underwent physical exam and their mental status was assessed by MINI psychiatric interview for mental disorders exclusion. DNA was isolated from whole blood by commercially available kit (Roche diagnostics). Genotyping was performed by PCR-RFLP reaction. PCR products were digested with NlaIII restriction endonuclease followed by electrophoresis on Elchrom Scientific Spreadex gel.
Results: Our results were in an accordance with Hardy-Weinberg equilibrium (P=0.163). Allele frequencies were 48% for Val allele and 52% for Met allele. Genotypes were distributed as follows: 20,9% (Val/Val); 54,5% (Val/Met) and 24,7% (Met/Met).
Conclusion: This results suggest there is no presence of population stratification, selection bias or genotyping error. In previous studies, reported genotype frequencies were similar to our findings.

M240
DAT1 AND COMT POLYMORPHISMS ARE NOT ASSOCIATED WITH POSITIVE AND NEGATIVE SYNDROME SCALE IN SCHIZOPHRENIA

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Background: Dopamine transporter has important role in regulation of dopamine neurotransmission. Its polymorphism is consisted of 40bp variable number tandem repeats (VNTR) which can range from 3 to 11 copies. The most frequent is allele with 10 repeats followed by 9 repeats allele. Previous studies showed that 10 repeats allele is associated with higher expression in contrast to 9 repeats allele, but some other studies failed to achieve the same result. This polymorphism has been associated with attention deficit hyperactivity disorder. Catechol-O-methyltransferase is enzyme included in metabolism of dopamine and its SNP polymorphism COMT Val158Met is associated with lower enzyme activity. Because of its function it has been proposed as a gene candidate for schizophrenia. Schizophrenia is a complex psychiatric disorder whose diagnosis is based on Diagnostic and Statistical Manual of Mental Disorders (DSM IV) criteria. Positive and negative syndrome scale (PANSS) is used for typological and dimensional assessment of schizophrenia. With PANSS it is possible to score positive, negative and general symptoms. The aim of this study was to assess association between DAT1 and COMT polymorphisms and positive, negative and general symptoms in patients with schizophrenia.
Methods: 118 patients with schizophrenia were included into this study. Positive, negative and general syndrome scores were assessed by PANSS. DAT1 genotyping was performed by simple PCR reaction followed by electrophoresis on 3% agarose gel. COMT genotyping was performed by PCR-RFLP with NlaIII restriction endonuclease. Digested PCR products were separated on Elchrom Scientific Spreadex gel.
Results: Kruskal Wallis test was used to assess association between positive, negative and general PANSS score among COMT (P=0.964; P=0.935; P=0.859) and DAT (P=0.315; P=0.374; P=0.301) genotypes, respectively. Genotype frequencies for COMT Val158Met polymorphism were Val/Val: 26.3%, Val/Met: 49.2%, Met/Met: 24.6%. Allele frequencies for this polymorphism were Val: 51% and Met: 49%. Results were in a Hardy- Weinberg equilibrium, P=0.856.
Conclusion: There is no significant difference in positive, negative or general PANSS score between COMT and DAT genotype.

M241

EXTREMELY LOW ACENOCOUMAROL DOSE REQUIREMENTS IN A PATIENT WITH VKORC1 A-1639A, VKORC1 T1173T, CYP2C9*1/*2 AND CYP2C9*1/*3 GENOTYPES

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Oral anticoagulation with acenocoumarol is the most common therapy for the treatment and prevention of thromboembolic events in several European countries. However, this medication has a narrow therapeutic range and there are large inter-individual variations in drug response. The allelic variants of genes like CYP2C9 and VKORC1 are closely associated with the maintenance dose of acenocoumarol. We describe the case of a patient, 47 year old female, who required extremely low dose of acenocoumarol (0.25 mg/day) to reach the target INR of 2.5 – 3.5. A 47 years woman with heart failure underwent surgery for mitral valve replacement (MVR) in year 2001. After surgery an anticoagulant therapy with acenocoumarol was added to the main treatment. At the beggining she started with the usual recommended dose of acenocoumarol: 4 mg/day for the first two days. After haematuria acenocoumarol dose was gradually reduced after every INR laboratory monitoring and finally a dose of 0.25 mg/day was required for maintain stable anticoagulation. Genome DNA was extracted from venous blood sample and genotyped for CYP2C9*2, CYP2C9*3, VKORC1 1173C>T and VKORC1 -1639G>A variant alleles. To evaluate the genotypes we used real time PCR followed by High Resolution Melting Analysis (HRMA). The patient was homozygous for VKORC1 -1639G>A and VKORC1 1173C>T and heterozygous for CYP2C9*2 and CYP2C9*3 alleles. This combination of genotypes points to extreme sensivity to anticoagulant acenocoumarol leading to the extremely low dose (0.25 mg/day) needed to reach the target INR of 2.5 -3. This is the first report in the literature of an extremely low requirement for acenocoumarol in a patient after MVR. Our case, presented in this report, supports the need for prospective genotyping of polymorphic variants in CYP2C9 and VKORC1 prior to initiation of acenocoumarol therapy. The effects of the CYP2C9 and VKORC1 genotypes suggest that the safety and efficacy of coumarins therapy could be improved by using knowledge of the genotype in dose algorithms. In addition, there is a growing interest in the use of High Resolution Melting Analysis (HRMA) in clinical diagnosis. We show that this technique is highly reliable for routine diagnostics in acenocoumarol monitoring.

M242

THE INFLUENCE OF SNPS IN CYP3A4 AND MDR1 ON PHARMACOKINETICS OF CYCLOSPORINE AND TACROLIMUS: THE CASE OF LUCANIA'S KIDNEY TRANSPLANT RECIPIENTS

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Background: Genes involved in the pharmacokinetics of immunosuppressive drugs are CYP (encoding cytocrome P450 enzyme) and ABCB1 (encoding Multi Drug Resistance 1 enzyme). Single Nucleotide Polymorphisms (SNPs) of these genes is one of the most important factor affecting the pharmacokinetics and availability of immunosuppressive drugs, Cyclosporine and Tacrolimus (CNI). The aim of the study is to determine the presence of SNPs, their effect on the kinetics of CNI and on long term graft survival in renal transplant recipients.
Methods: 139 patients who received renal transplantation between 1995 and 2012, treated with TAC or CsA immunosuppressant therapy, were selected. Genomic DNA was extracted from whole blood and fragment was amplified by PCR using specific primers. All recipients were genotyped for the SNPs CYP3A4*1B, CYP3A5*3 for CYP3A gene, and for C1236T, G2677T/A, C3435T SNPs of ABCB1 gene, by direct sequencing. SNPs was correlated with blood levels of immunosuppressor, proteinuria value, delayed graft function.
Results: 29 patients were wild-type for the 3 SNPs of MDR1 (WT), 71 carried at least one allelic variant in heterozygosis (WT/MT), 39 at least one variant in homozygosis (MT). The three groups were comparable for renal function, proteinuria, delayed graft function, number of acute rejection and number of patients who returned to dialysis after transplantation. Only blood pressure was highest in MT group. 132 patients were homozygous for CYP3A5*3 allelic variant and only 3 patients carried a mutation in heterozygosis in CYP3A4. In patients treated with CsA, the WT group show a lower dose of drug required to reach the target level of immunosuppression. Kaplan-Meier analysis showed no differences of long term graft survival between groups and Cox analysis confirmed SNPs in MDR1 don't increase the risk to returned to dialysis after transplantation.
Conclusions: Exists a correlation between SNPs and amount of blood pressure and dose of drug to reach therapeutic target, but there are not correlation with long term survival graft in patients with renal transplantation.

M243

THE RELEVANCE OF PLATELET GLYCOPROTEIN GP IIB/IIIA POLYMORPHISM TO ANTI-PLATELETS RESPONSE IN ACUTE CORONARY SYNDROME [ACS]

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Background: Pharmacogenomics is intervening in cardiovascular therapeutic armamentarium to tailor therapy to individual's genetic makeup. Thus, this case-control study probes in the potential implication of PlA gene variants of GPIIIa subunit of platelet's GP IIb/IIIa, in provocation of ACS and/or modulation of its anti-platelet's therapeutic outcome.
Methods: 22 controls and 44 ACS patients (NSTEMI vs STEMI) were enrolled and sampled for genotyping and for estimation of platelet aggregation (Turbidometric/aggregation by ADP), malondialdehyde, and other routine diagnostic tests. Patients were further risk stratified (TIMI score), then subdivided according to add-on anti-platelet therapy into: clopidogrel or tirofiban subgroups. After 48 hours, the therapeutic outcome was assessed clinically [pain relief or complication prevalence (symptomatic, electrocardiographic or hemorrhagic)] and re-assessed investigationally. Intra-procedural evaluation of chest pain, ECG tracing and angiographic findings (thrombus extent, TIMI flow, myocardial blush) were reported in patients who underwent per cutaneous intervention[PCI].
Results: Frequency of PlA2 vs PlA1 allele was higher in ACS patients (significant in <60 years /doubled in STEMI vs NSTEMI). TIMI score, stratification permitted considering PlA2 variant as independent risk factor in UA/NSTEMI subsets. This was fostered by intra-procedural finding of more stenotic and thrombotic lesions in PlA2 carriers. A lack of significant association between PlA variants and changes in platelet aggregation or oxidative indices, debate their causal relation to PlA2 variant being an ACS risk factor. A positive correlation was observed between PlA variants and the therapeutic response outcome to both clopidogrel and tirofiban regarding platelet aggregation and relief of chest pain while their antioxidative potentiality was negatively correlated only to PlA1 carriers.
Conclusions: PlA2 variant could be considered a genetic risk factor contributor rather than an anti-platelet therapeutic response modulator when speaking of ACS. This awaits larger scale pharmacogenomic studies before a final statement is declared so as to individualize anti-platelet therapy in ACS, to the best of its therapeutic outcome.

M244

INFLUENCE OF CYP2D6 AND ABCB1 GENOTYPES ON THE SERUM STEADY-STATE CONCENTRATIONS OF RISPERIDONE AND 9-OH RISPERIDONE IN PATIENTS USING LONG-ACTING INJECTABLE RISPERIDONE

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Background: Long-acting injectable (LAI) risperidone should have improved pharmacokinetics and less side effects. Risperidone is metabolized to its active metabolite, 9-OH risperidone, mainly by CYP2D6. Its antipsychotic effect is assumed to be related to the active moiety. Both risperidone and 9-OH risperidone are substrates of P-glycoprotein (ABCB1). The data on the influence of CYP2D6 and ABCB1 on LAI risperidone bioavailability are limited
Methods: Thirty-five male patients with schizophrenia receiving 25, 37.5 and 50 mg LAI risperidone were genotyped. Serum steady-state concentrations of risperidone and 9-OH risperidone were measured on 5th and 13th day following risperidone injection. TaqMan real-time PCR analysis was used for genotyping CYP2D6*3, *4, *6, ABCB1 C1236T, G2677T/A and C3435T variants. CYP2D6*5 and duplications were genotyped by long-distance PCR. Serum concentrations of risperidone and 9-OH risperidone were measured by high-performance liquid chromatography with diode array detection (HPLC-DAD). All patients were monitored for concomitant therapy and extrapyramidal side effects (EPS).
Results: The median active moiety concentrations were on 5th day 78.8 nmol/L (95%CI=58.9-102.1), and on 13th day 39.7 nmol/L (95%CI=28.8-48.8). On 13th day concentrations and concentration/dose (C/D) ratios of risperidone, 9-OH risperidone and active moiety were significantly lower (P=0.0015, P <0.0001 and P <0.0001 respectively). The active moiety concentrations on 13th day were significantly different according to CYP2D6 genotype (P=0.0451). The median active moiety concentration on 13th day for extensive CYP2D6 metabolizers was 31.2 nmol/L (95%CI=17.0-48.7), and for intermediate and poor CYP2D6 metabolizers was 47.1 nmol/L (95%CI=29.0-78.0). No significant difference was found in concentrations and C/D ratios of risperidone, 9-OH risperidone and active moiety according to ABCB1 genotypes. EPS were not related to variations in concentrations or genotypes.
Conclusions: The CYP2D6 genotypes had a strong influence on the steady-state serum levels of risperidone, 9-OH risperidone and active moiety, while for the ABCB1 genotypes we did not confirm such effects. The active moiety concentrations on 13th day were bellow recommended therapeutic range.

M245
INFLUENCE OF CYP2C9 POLYMORPHISM ON SERUM LEVELS OF PHENORBABITAL METABOLITES

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Background: Genotype-phenotype relationship in epilepsy is fairly complex but studies have shown involvement of genetic factors affecting pharmacocinetics of the AEDs (antiepileptic drugs). One of the most widely used antiepileptic drugs is phenobarbital (PHB), the majority of PHB is metabolized by CYP2C9 in the liver to form an inactive metabolites, p-hydroxy-phenobarbital. Genetics polymorphisms of CYP2C9 may affect the inter-individual differences in drug metabolism. Subjects with the CYP2C9*2 allele are low-metabolizers and therefore could require a lower dose of the drug. The aim of the study is to evaluate the association between CYP2C9 polymorphism and phenobarbital metabolism in subjects with epilepsy.
Method: We studied 36 epileptic patients (29 males; age 40±11.7 years) under maintenance PHB therapy and the most common AEDs (72% Carbamazepine, 28% Valproate, 11% Primidone, 6% Phenytoin, 33% Levetiracetam, 11% Lamotrigine). The mean dosage of PHB was 2.13 ± 0.80 mg/kg. Total genomic DNA was extracted from whole blood by commercial kit (Roche). CYP2C9*2 430C>T allelic discrimination was performed by PCR-RFLP. The dosage of PHB was performed in a fasting state and after 4h from the administration of the drug to evaluate the conversion of PHB into its metabolite. Serum dosage of PHB was performed by HPLC with isocratic elution on a C18 column (Sigma) and UV detection at 220 nm.
Results: Among the study group, 89% were CYP2C9 430CC, 11% were heterozygous 430CT and no homozygous were identified. The fasting mean value of PHB in non carriers and carriers of the variant allele was 24.18 ± 7.67 and 20.42 ± 4.49 mcg/mL respectively; after 4h the mean value were 25.31 ± 8.68 and 20.20 ± 6 mcg/mL, in the two groups. When we compare the variation of PHB after 4h among the non carriers group and the carriers one, we didn't find statistical significant differences (P >0.05).
Conclusions: From the results obtained, we assume that the serum values of PHB, instead of CYP2C9*2 variant, could be influenced by AEDs as Lamotrigine and Valproate that are inhibitor of CYP2C9. The major limits of the present study is the low number of patients; moreover clinical and pharmacological variables could interfere with PHB pharmacocinetics.

M246
META-ANALYSIS OF THE EFFECT OF THE POLYMORPHISM OF DONORS AND RECIPIENTS CYP3A5 GENE ON TACROLIMUS PHARMACOKINETICS IN LIVER TRANSPLANTATION

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Background: Tacrolimus is one of the most commonly immunosuppressants used in organ transplant. Its pharmacokinetic is highly variable so there is risk of administering doses inappropriate and suffer secondary consequences as graft rejection or toxicity. One factor that contributes to this is the polymorphism of its metabolizing enzyme CYP3A5. Studies evaluating the association between variants non-expresser (CYP3A5 * 3) and tacrolimus blood levels are discordant on stage liver transplantation, which has led to a lack of consensus on its usefulness. We aim is making a meta-analysis of published studies about the effect of polymorphism of the donors and recipients CYP3A5 6986A>G gene on tacrolimus pharmacokinetics in liver transplantation.
Methods: Selection criteria: cohort studies that evaluated the relationship between polymorphism of CYP3A5 of donors and recipients of liver transplantation and tacrolimus blood concentration weighted by the daily dose per kilogram body weight (C/D) until one year after transplantation. There was no restriction by age, language or publication status. Search strategy and selection: A literature search was performed up to March 2012 by using the Cochrane Library, MEDLINE, EMBASE and grey literature. Data analysis. Data were pooled (random effects model) and the results expressed as mean difference (MD) of the C/D and corresponding 95% confidence interval.
Results: Seven studies involving donors (316 patients) and five recipients (469 patients). The meta-analysis demonstrated that, in donors, the C/D ratio was significantly higher in patients with non-expresser polymorphism in all periods, but the quality of the evidence was adequate only for the first month. In recipients, the type of polymorphism did not influence on C/D ratio, but the quality of evidence was low.
Conclusion: In donors, the polymorphisms of CYP3A5 6986A>G affects the pharmacokinetics of tacrolimus. In recipients it has no effect, but the quality of the evidence is not conclusive.

M247

ALLELIC VARIANTS IN VKORC1 AND CYP2C9 INFLUENCE ACENOCOUMAROL DOSE REQUIREMENTS IN BULGARIAN PATIENTS

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Oral anticoagulant therapy with acenocoumarol is used worldwide for the treatment and prevention of thrombotic diseases. The clinical use of oral anticoagulant therapy is complicated by the narrow therapeutic range and the large interindividual variation that exists in response to the same dosage. Dosing is influenced by a variety of factors such as sex, age, smoking status, medications, diet, weight and genetic factors. The aim of the present study was to determine the prevalence of the polymorphic variants CYP2C9*2, CYP2C9*3, VKORC1 1173C>T and VKORC1 -1639G>A in the Bulgarian population and investigate whether these polymorphisms, along with the patient demographics (age, sex, weight) could explain the interindividual variability of acenocoumarol dose requirements for efficient anticoagulation in Bulgarian patients. A total of 59 outpatients with stable control of anticoagulation recruited were included in the study. DNA was isolated from peripheral blood samples. Polymorphic variants CYP2C9*2, CYP2C9*3, VKORC1 1173C>T and VKORC1 –1639G>A were genotyped by High Resolution Melting Analysis. Allelic frequencies of CYP2C9*2, CYP2C9*3, VKORC1 1173T and VKORC1-1639A were found to be 0.229, 0.025, 0.330 and 0.364, respectively. Carriership of at least one CYP2C9*2 allele led to the most pronounced reduction in the required mean dose (P <0.001) In contrast, the CYP2C9*3 allele played a minor role (P=0.24). Patients carrying the VKORC1 -1639A and VKORC1 1173T alleles needed approximately half of the dose required by wild-type patients to achieve the target INR (for VKORC1-1639A and VKORC1 1173T:p = 0.001). Age was the only demographic factor significantly affecting acenocoumarol dose (p = 0.006). In a multivariable analysis of variance CYP2C9 and VKORC1 genotypes, and age explained 51% of acenocoumarol dosing variability. This is the first study about the influence of VKORC1-1639G>A and VKORC1 1173C>T polymorphic variants on acenocoumarol dose requirements in the Bulgarian population. These polymorphisms in VKORC1 together with CYP2C9*2 and CYP2C9*3 were found to predispose to acenocoumarol sensitivity in Bulgarians. Other hereditary and nongenetic parameters can be incorporated in an individualized dosing algorithm to achieve a safer anticoagulant effect.

M248

ASSOCIATION BETWEEN THE NEW CYP3A4*22 ALLELEL AND THE PHARMACOKINETICS OF THE CYP3A4 PHENOTYPING PROBES MIDAZOLAM AND ERYTHROMYCIN

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Background: The enzymatic activity of CYP3A4 varies widely in the general population, affecting the metabolism of 50% of all drugs. Although a genetic cause for this variability has been implied quite some time ago (Odzimir et al, 2000), no clinically relevant CYP3A4 polymorphisms have been described so far due to either low alleic frequency (<0.1%) or absence of clinical effect. In 2011, a new CYP3A4 SNP (CYP3A4*22; intron 6 C>T) was described, with an allele frequency of 5%. This variant was associated with reduced CYP3A4 activity (Wang et al 2011). The main question now is if this effect on simvastatin reflects a general reduced CYP3A4 activity or is only relevant for simvastatin. In our study, we therefore investigated the impact of CYP3A4*22 on the pharmacokinetics of the gold standard CYP3A phenotyping probes Midazolam (MDZ) and Erythromycin.
Methods: A total of 108 cancer patients were given MDZ and 45 underwent the erythromycin breath test (EBT) for phenotyping of CYP3A activity. Genomic DNA was analyzed for CYP3A4*22 (rs35599367 C>T) and CYP3A5*3 by TaqMan analysis.
Results: Midazolam dose-adjusted concentrations ([MDZ]Dadj) were 32% higher (P <0.001) whereas [1OH-MDZ] was 20% lower (P=0.011) for CYP3A4*22 carriers compared to CYP3A4*1/*1 patients. Combining CYP3A4*22 and CYP3A5*3 genotypes showed a 50% increase in [MDZ]Dadj for poor (CYP3A4*22 carriers, CYP3A5*3/*3) and 17% increase for intermediate (CYP3A4*1/*1, CYP3A5*3/*3) compared to extensive (CYP3A4*1/*1, CYP3A5*1 carriers) CYP3A metabolizers. CYP3A4 erythromycin N-demethylation activity proved 40% lower in CYP3A4*22 carriers compared to CYP3A4*1/*1 patients (P=0.032).
Conclusion: Our results confirm a strong impact of the CYP3A4*22 allele on CYP3A phenotype, as determined using the established CYP3A4 phenotyping probes MDZ and erythromycin. This is the first study indicating a potential role for CYP3A4 genotyping for predicting CYP3A4 activity. This finding may have identified a potential factor predicting CYP3A4 variability which may affect 50% of all currently prescribed drugs.

M249
EARLY IDENTIFICATION OF WARFARIN MAINTENANCE DOSAGE: A PHARMACOGENETIC ALGORITHM READY FOR CLINICAL TRANSLATION

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Background: Warfarin has a very narrow therapeutic window and wide and unpredictable inter-individual variability in dose-response. We have previously developed a pharmacogenetic algorithm (PG) for warfarin maintenance dosing prediction based on VKORC1, CYP2C9 and CYP4F2 gene polymorphisms, Body Surface Area and age (Zambon CF, et al. Pharmacogenomics. 2011;12(1):15-25). We verified in a prospective randomized study (ClinicalTrials.gov Identifier: NCT01178034) whether our algorithm has any clinical advantage over standard warfarin dosing.
Methods: 180 patients were enrolled (atrial fibrillation; age >18yrs; target INR 2.5) and randomized to receive initial warfarin doses either established according to the standard care (STD arm; n=92) or determined by the pharmacogenetic algorithm (PG arm; n=88). VKORC1 (-1639G>A SNP), CYP2C9 (*1,*2,*3 alleles) and CYP4F2 (*1,*3 alleles) (Taqman chemistry) polymorphisms were analysed within 24 h from enrolment. INR was monitored on days 0,5,7,9,12,15 and 19. Twenty-two patients were considered drop-out.
Results: subjects in the PG arm spent a significantly reduced time at INR >4 (0.72% vs 1.83%) (chi2=8.33; P <0.01) (RR=0.39; 95% C.I. 0.21-0.74, P <0.005). This observation was confirmed in patients expected to require low warfarin doses (< 26.25 mg/week) (RR=0.04; 95% C.I. 0.01-0.16, P <0.0001) being lower in PG than in STD arm both the number of patients with INR >4 (1/23 vs 6/24) (Fisher's exact=0.097) and the time spent at INR>4 (0.23% vs 5.47%)(chi2=21.90, P <0.0001). By contrast, among patients expected to require high warfarin posology (>43.75 mg/week) those with INR <1.5 were significantly lower in the PG arm, (4/17 vs 12/14) (chi2=11.89, P <0.001) being lower also the time spent at INR <1.5 (15.17% vs 29.51%) (chi2=17.49, P <0.0001; RR=0.52; 95% C.I. 0.38-0.70, P <0.0001). No significant difference was recorded considering time to stable anticoagulation in patients overall or subdivided on the basis of expected warfarin dose.
Conclusions: The pharmacogenetic prediction of warfarin dose significantly reduce the risk of over-anticoagulation and under-anticoagulation in patients requiring low and high warfarin doses respectively.

M250
COMPARISON BETWEEN IDMS-TRACEABLE JAFFE AND ENZYMATIC CREATININE ASSAYS FOR ESTIMATION OF GLOMERULAR FILTRATION RATE BY THE CKD-EPI EQUATION IN HEALTHY AND DIABETIC INDIVIDUALS

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Background: Accurate measurement of serum creatinine for estimation of the glomerular filtration rate (eGFR) is the cornerstone of diagnosis of chronic kidney disease. The aim of this paper was to compare the agreement between creatinine measured by Jaffe and enzymatic methods and their putative influence on eGFR as calculated by the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation in healthy and diabetic individuals with normal renal function.
Methods: Cross-sectional study conducted in 123 adult Southern Brazilians with normal renal function (53 with type 2 diabetes, 70 healthy volunteers). Mean age was 49±16 years (range 19-86). Most were female (67, 55%) and white (102, 83%). Creatinine was measured by a traceable Jaffe method (Modular P, Roche Diagnostic) and by an enzymatic method (CREA plus, Roche/Hitachi 917). GFR was measured by the 51Cr-EDTA single-injection method.
Results: Serum creatinine measured by the Jaffe and enzymatic methods was similar in healthy individuals (0.79±0.15 vs. 0.79±0.15 mg/dL, respectively, P=0.76), and diabetic patients (0.96±0.22 vs. 0.92±0.29 mg/dL, P=0.17). However, the correlation between the two methods was higher in the healthy group (r=0.90 vs. 0.76, P <0.001). The difference between Jaffe creatinine and enzymatic creatinine was <10% in 63% of cases in the healthy group and 40% of cases in the diabetes group (P=0,018). In the subset of patients with diabetes, eGFR based on enzymatic assay results showed better agreement with measured GFR than did eGFR based on Jaffe results.
Conclusion: Jaffe and enzymatic creatinine methods show adequate agreement in healthy subjects, but in the presence of diabetes, the enzymatic method performed slightly better.

M251
β-TRACE PROTEIN AS MARKER FOR GLOMERULAR FILTRATION RATE (GFR) IN RENAL TRANSPLANT RECIPIENTS

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Background: After renal transplantation monitoring and detection of slight-to-moderate changes in GFR is a prerequisite for an optimal patient management. Due to the limitations of serum creatinine and lack of validation of creatinine based GFR estimation equations in transplantation setting, β-Trace protein (BTP) has been proposed as an alternative marker for GFR. Aim: The aim of this study was to evaluate the relationship between serum levels of beta-trace protein and glomerular filtration rate (GFR) in renal transplant recipients (RTRs).
Methods: We measured true GFR by99mtechnetium-diethylenetriaminepentaacetic acid (99mTc-DTPA) and BTP and for comparison cystatin C and creatinine in 60 RTRs . We also conducted a study of the GFR estimates of the Cockcroft and Gault (C&G), and the abbreviated modification of diet in renal disease ((aMDRD).
Results: Serum levels of BTP progressively increased with the reduction of GFR. A good correlation was found between GFR and serum levels of BTP (r=0.938), Creat (r=0.823), Cys (r=0.907)..BTP has the highest sensitivity of 96% and specificity of 91% at a cutoff of 2.01 mg/L with area under the curve of 0.965. The BTP correctly classified 89% of patients compared to to only 80% with cystatin-c ,75% with aMDRD) equation , 69% with the Cockcroft–Gault equation.
Conclusions: On the basis of the above results, we believe that BTP may be a useful and reliable analyte to estimate GFR in RTRs.

M252
THE ASSESSMENT OF ALBUMINURIA AND PROTEINURIA IN DIABETIC PATIENTS

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Background and scope: Assessment of albumin and/or protein excretion in the urine is a key step in the early detection of diabetic nephropathy. Our main goal is to present the results of albumin and protein excretion in 41 newly diagnosed type 2 diabetic patients , who were treated in our department of diabetology.
Patients and methods: 41 newly diagnosed type 2 diabetic patients with mean age 62.8 ± 9.8 years were enrolled. They were presented with median values of glucose and HbA1c (7.55 (4,3 – 15.4) mmol/L and 7.9 (5.7-11.6)%, respectively). Accordingly with the recommended screening tests, in all participants, the following parameters were further determined: U-albumin/U-creatinine (UACR), U-protein/U-creatinine (UPCR), calculated estimation of daily proteinuria (e DP) and estimated glomerular filtration rate (e GFR). The values of UACR>3 g/mol, UPCR >20 g/mol and e DP >0.150 g/day are defined as pathological. A threshold of e GFR <60 mL/min/1.72 m2 indicates chronic kidney disease.
Results: Clinically important albuminuria and proteinuria were detected in 15 and 12 out of 41 patients, respectively. Accordingly with determined e GFR value, 29 patients demonstrated stage 2 and 8 stage 3 chronic kidney disease. 7.3% patients who were screened as positive for UACR, were negative for UPCR and e DP. In comparison with other similar studies, in our study we found albuminuria to break up more frequently than proteinuria.
Conclusions: Screening for albuminuria and proteinuria in newly diagnosed type 2 diabetic patients is the most efficient method for early detection of diabetic nephropathy. Since the relationship between albuminuria and proteinuria is complex there is no reliable way for using one test only.

M253
SERUM CYSTATIN C COMPARED TO GLOMERULAR FILTRATION RATE IN CANCER PATIENTS RECEIVING PLATINUM-BASED CHEMOTHERAPY

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Background: Cystatin C (CysC) has been proposed as a marker of kidney function. Our aim was to evaluate the diagnostic accuracy of CysC in predicting a reduction in glomerular filtration rate (GFR), in cancer patients (pts) receiving platinum-based chemotherapy (CT).
Methods: Serum creatinine (enzymatic, on Cobas C6000 Roche), serum CysC (immunoenzymatic, on AIA 360 Tosoh) and GFR (Cockcroft-Gault equation) were determined simultaneously in all pts, before the first cycle of CT and before subsequent administrations. Pearson correlation coefficients (r) were calculated. To assess the diagnostic accuracy of serum CysC in predicting GFR<80 mL/min/1.73m^2 and GFR <60 mL/min/1.73m^2, ROC (receiver operating characteristics) plots were performed (pROC package for R), calculating the area under the curve (AUC) and 95% confidence interval (CI).
Results: Overall, 31 pts were studied (7 males, 24 females; 18 carboplatin-based, 13 cisplatin-based CT). Overall, 155 measurements were performed (range 1 – 12 per patient). Median baseline CysC value was 1.39 mg/L (range 0.75–1.94). Median baseline GFR was 87.8 mL/min/1.73m^2. Out of 31pts, 14 had a baseline GFR <80: all 14 pts had a baseline CysC > upper normal limit, whilst serum creatinine value was within normal range in 13 (93%). Of pts starting treatment with baseline normal GFR, 2 pts subsequently developed GFR <80, both with high baseline CysC and normal creatinine value. In the overall series, linear regression showed a significant relationship (P <0.001) between GFR and concomitant value of serum CysC (r=0.56). The relationship was statistically significant in both males and females, but r was higher in females (0.62 vs 0.43 in males). In the overall series, for GFR <80, the AUC of ROC curve for CysC was 79.5% (95%CI 72.6% - 86.5%). The best threshold was 1.285 mg/L. AUC was 81.2% (95%CI 73.6% - 88.8%) and 74.5% (95%CI 57.1% - 91.9%) in females and males, respectively. For GFR<60, the AUC for CysC was 88.0% (95%CI 81.0% - 94.9%). Best threshold was 1.62 mg/l. The AUC for CysC was 88.6% (95%CI 80.8% - 96.5%) and 88.5% (95%CI 73.9% - 100%) in females and males, respectively.
Conclusions: In patients treated with platinum-based CT, CysC is a more sensible marker than serum creatinine in early evaluation of renal damage.

M254
URINARY MARKERS OF KIDNEY DISEASE IN HIV-INFECTED PATIENTS

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Background: HIV infected patients require long term antiviral therapy to reduce mortality and progression to AIDS. Anti retroviral therapy (ART) is however associated with side effects which include acute and chronic kidney damage. Liver fatty acid binding protein (L-FABP) has been associated with tubular damage und kidney injury molecule 1 (KIM-1) is believed to be involved in tubular repair while albumin in urine reflects glomerular damage. The study intends to determine the role of urinary kidney markers in treatment of HIV infection.
Methods: The study was carried out in 300 HIV infected persons, 21 did not receive ART while all others received different combinations of antiretroviral therapy. 149 healthy subjects who were not HIV infected served as control. Urine samples were received from clinically stable patients and were tested for the presence of U-L FABP, KIM 1 and urinary albumin using commercially available tests, in addition the MDRD-GFR was calculated. Results were related to urinary creatinine.
Results: HIV infected individuals had increased urinary markers of tubular and glomerular damage when compared to non HIV infected controls. Tubular damage was highest in patients receiving a drug combination containing nucleotide reverse transcriptase inhibitors, however there was significant individual variation. GFR was slightly lower in patients not on ART, GFR did not differ significantly in patients on different ART schedules.
Conclusions: The study clearly shows that there is significantly increased glomerular and tubular damage in treated and untreated HIV patients if compared to healthy controls. Extent of kidney damage may require treatment adaptation in individual HIV infected patients in order to avoid acute or chronic kidney damage. Longitudinal assessment of kidney function (GFR) will elucidate which of these markers will be of importance for the development of chronic kidney disease.

M255

STUDY OF THE CORRELATION BETWEEN CYSTATIN C VALUES AND THE CONSERVATIVE TREATMENT OF CHRONIC RENAL FAILURE IN PEDIATRIC PATIENTS

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Background: The prevention of complications in pediatric chronic kidney disease (CKD) is one of the objectives of the pediatric nephrologist today. The need for a simple marker of glomerular filtration rate (GFR), accurate and minimally invasive, remains a limiting factor in clinical practice to assess renal function. Cystatin C (Cys) is an unglycosylated protein of low molecular weight that it´s synthesized in all nucleated cells in the body. After correlating the GFR by Cys and creatinine in a pediatric population since two years, we found that the GFR with Cys is better than GFR with creatinine to discriminate the early stages of CKD.
Methods: we studied 104 children diagnosed with pediatric chronic renal failure (stages I, II, III and pre-dialysis) whose stage is determined by comparing creatinine and cystatin C in the evaluation of GFR. Cystatin C in serum was determined by particle enhanced immunonephelometry with the BNII Nephelometer by Siemens.
Results: In the evaluation of the GFR using creatinine we found the following results in the 104 children: 54% in the stage I of chronic renal failure (CRF), 35% in stage II and 7% in stage III. In the evaluation of the GFR with cystatin C we found 70% of the children in stage I of CRF, 16% in stage II and 7% in stage III.If we relate the analytical parameters according to clinical severity, the patients that were classified into stages of lower gravity by cystatin C, corresponding to patients which requiring minor or no treatment.However, there are children who are classified in advanced stages of CRF using creatinine for GFR, which remain asymptomatic without treatment. Although there were children classified as stage 1 by creatinine were classified by cystatin C is stage 2 and correlating with the clinic, they actually needed more treatment.
Conclusions: Cystatin C is more effective for establish the stage of the disease in pediatric chronic renal patients than creatinine.So, there are a better clinical correlation between cystatin C and the need for treatment. The GFR with cystatin C in pediatric patients is better indicator in the early stages of CRF than GFR using Cr, leading to changes in the monitoring, treatment and prognosis.

M256

MONITORING OF KIDNEY FUNCTION DURING AMINOSIDE TREATMENT OF SEPTIC PATIENTS: COMPARISON OF NGAL, CYSTATINE C AND CLASSICAL MARKERS

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Background: Despite their nephrotoxicity, aminosides may be used in first attempt. Prior and during aminoside treatment (AT), kidney function has to be explored, mostly by plasma creatinine (CRE). But rise of plasma creatinine is not fast in case of acute kydney disease. In this study we compared the classical parameters of kydney function: cre and bun, with cystatine C (Cys C) and Neutrophil Gelatinase Activated Lipocalin (NGAL).
Methods: Six patients presenting confirmed sepsis were included in the study. They received intra-venous AT (2-5 days). Blood samples were collected during 3-6 days. CRE, bun and Cys C were measured onto COBAS 6000 Roche Analyzer with dedicated reagents, NGAL was measured onto Triage Meter ALERE analyzer. To estimate GFR, we used MDRD formula (GFRM) and cys C formula (GFRC).
Results: The mean±sd of measured parameters on the 28 samples studied are respectively: 75±28 μmol/L, for CRE,7.4±2.8 mmol/L for bun,1.7±0.48 mg/L for Cys C (normal value <1.09 mg/L),681±503 ng/mL for NGAL(normal value <150 ng/mL), 95±33 mL/min/1.73m^2 for GFRM and 75±24 mL/min/1.73 m^2 for GFRC. 5/6 patients presented high value (>95th percentile) of NGAL and Cys C before the first injection of AT, and among these five patients, two presented high CRE value (>110 μmol/L) four high bun value (>7.5 mmol/L) three presented a value of GFRM below 90 mL/min/1.73m^2 and four a value GFRC below 90mL/min/1.73m^2. All six patients presented a documented bacteriemia. We do not observe correlation between sepsis (NPN or CRP) and increase of NGAL, but in our study, patients with higher value of NGAL were also those with higher value of NPN. In all cases the elevation of NGAL correlates with CysC and precedes increase of CRE or decrease of GFR. There is a significant difference between GFRM and GFRC among the 28 samples (P <0.0001), this significant difference is also found for 3 patients. In case of normal value of CRE, if NGAL is increased, Cys C is also increased and GFRC is decreased.
Conclusion: Before starting or during AT the determination of plasma creatinine is not sufficient. To detect early kidney dysfunction, NGAL is a good parameter. In case of septic patients, the association of both elevated Cys C and NGAL may be useful to confirm the origin of kidney damage.

M257

SERUM B2-MICROGLOBULIN/CYSTATIN C RATIO AND SERUM CYSTATIN C AS A CELL-PROLIFERATION MARKERS IN RENAL TRANSPLANTED PATIENTS

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Background: β2-microglobulin (β2M) and Cystatin C (CysC) have been showed to be good endogenous markers of GFR but only β2M have shown to be affected by increased cellular population. The aim of this study is to evaluate whether serum β2M/CysC ratio could be used as a marker for the proliferation because the ratio should not be influenced by GFR.
Methods. The serum concentration of CysC and β2M were determined with DAKO immunoturbidimetric methods and serum creatinine with Cobas Integra 400. We analyze these laboratory parameters in 30 normal subjects and 42 patients with renal transplantation.
Results: Serum β2M was significantly higher in patients with renal transplantation and stable graft function (P <0.001) than normal subjects but, in both groups, serum β 2M and β2M/CysC ration showed poor correlation with serum creatinine (Spearman correlation <0.5; P >0.15). However, serum β2M shows significant correlation with β2M/CysC ratio (r=0.90, P <0.001) in transplanted patients with stable renal function including that the increased b2M is due to increased cellular population. The β2M/CysC ratio is significantly (P=0.02) higher in transplanted patients with elevated serum CysC that those with normal serum CysC and the correlation of serum β2M/CysC ration with CysC and β2M are 0.34 (P=0.03) and 0.78 (P <0.001) respectively.
Conclusion: The study showed that serum β2M/CysC ratio, a marker of cellular proliferation, is independed of the GFR in patients with normal functioning renal transplantation. In transplanted patients with stable renal function, increased serum β2M concentration is due to increased cellular population but as renal function declines, diminished GFR and catabolism contribute. The ratio could be used as a marker in immune activation and as an adjunct for the detection of transplant rejection.

M258

SERUM LEVELS OF THE TNF-ALPHA AND IL-6 VERSUS URINARY EXCRETION OF B-NAG, ALPHA/PI-GST AND ALPHA 1- MICROGLOBULIN IN PATIENTS WITH ACUTE RENAL FAILURE

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Background: Urinary enzymes and proteins also serum levels of inflammatory markers have been recommended for the detection of changes in the kidney tissue in cases with acute renal failure, acute tubular necrosis and acute rejection episode after renal transplantation.
Methods: We have analyzed 75 patients (46 males and 29 females, mean age 46.6 +/-19.6 years) with favorable outcome of acute renal failure (ARF): 35 dependent dialysis patients (DDP), and 40 dialyses independent patients (DIP) from different origin in relation with the urinary excretion of alpha1-Microglobulin , NAG, alpha/pi-GST, versus serum concentration of IL-6 (as a “far active” cytokine) and TNF-alpha - in the first ten days of hospitalization in Nephrology intensive care unit. The concentration of alpha 1-microglobulin were determined with DAKO tests, alpha/pi-GST with Biotrin tests (ELISA methods), NAG-urine-Roshe tests and IL-6/TNFalpha – with RD systems.
Results: The urinary alpha/pi GST (155.0+/-30.6; 26.8+/-7.2 mg/L) , NAG activity (3.8+/-2.0 U/mmol creatinine) and serum concentration of IL-6 (46.0+/-8.7 pg/L), presents a maximal values and very strong positive correlation between 2th and 5th days of polyuric phase of ARF (18 DIP). Inversely, the urinary excretion of alpha1-microglobulin and blood presence of TNFalpha have demonstrated a highest positive correlation and maximal values a little bit later, namely between 4th and 7th days (24 DDP; alpha1MG-137.4+/-12.4mg/L; TNFalpha - 108.8+/-17.5 pg/L). An obvious overlap is present in the days 4th and 5th of polyuric faze estimation the all investigated parameters (8 DOP and 10 DDP).
Conclusion: The urinary detection of NAG, alpha/piGST and alpha-1M may be useful and cheaper parameter in differentiation of dialysis requesting from dialysis non-requesting ARF in the recovery phase in the syndrome.

M259

PROTEOMIC PROFILE OF RETAINED PROTEINS FROM HFR CARTRIDGE

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Background: Hemodiafiltration with on-line endogenous reinfusion (HFR) is a dialytic method, which combines the processes of diffusion, convection and adsorption. The performance of this system is linked to the optimal combination of the membrane permeability and cartridge resin bed. Lupus nephritis (LN) remains one of the most severe manifestations of systemic lupus erythematous (LES), associated with considerable morbidity and mortality. In this preliminary study, ESI-QTOF Mass Spectrometer was used for identification of protein ultrafiltrate (UF) and for the protein captured by resin bed, obtained from one dialysed patient with LN.
Methods: Plasma and UF (pre and post cartdrige) samples of one patient with LN treated with HFR, were collected at 15 min and at 235 min of the dialytic session. The cartridge utilized during treatment, containing styrenic resin, was then opened and the proteins kept by the resin were eluted by incubation O/N with 60% ACN and 1%TFA. Samples were desalted and separated by SDS-page, interesting bands were picked and “in-gel” tryptic digested before ESI-QTOF mass spectrometer analysis.
Results: ESI-QTOF results of the retained proteins allowed to identifies several biomarker of kidney injury in LN, such as Retinol binding protein 4, Neutrophil gelatinase-associated lipocain, and Cystatin-C (and also TRFE, A1AG1, PTGDS, TTHY). Moreover we identify several fragments of Immunoglobulin, which are implicated in the etiopathogenesis of LES.
Conclusion: The results of this preliminary study demonstrate that styrenic resin retain several proteins implicated in the LN pathogenesis, in fact the corresponding bands in the UF pre-cartdrige at 240 min disappear confirming the removal of this proteins from the cartridge.

M260

ASSOCIATION OF THE CIRCULATING INACTIVE FORM OF MATRIX GLA PROTEIN (DP-UCMGP) LEVELS AND ABDOMINAL AORTIC CALCIFICATION SCORE (AACS) IN HAEMODIALYSIS PATIENTS.

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Introduction: Patients suffering from end stage renal disease (ESRD) with vascular calcifications (VC) have the highest cardiovascular morbidity and mortality. Matrix Gla Protein (MGP) is the first protein known to act as a calcification inhibitor in vitro and in vivo. The aim of this study was to evaluate the association between the inactive, dephosphorylated, uncarboxylated MGP (dp-ucMGP) levels and aortic abdominal calcification score (AACS) in a cohort of hemodialysis patients.
Methods: 121 patients (69 male, mean age 68.7±14.4 years) on thrice-weekly hemodialysis (median dialysis vintage: 21months, range: 3-396) entered the study. 51 patients (42.5%) had history of diabetes, 98 (81.7%) of hypertension and 78 (65.0%) of cardiovascular diseases. Plasma dp-ucMGP levels were determined with the Inactive MGP ELISA kit (IDS, Boldon, UK). Lateral lumbar radiography of the abdominal aorta was used to determine the overall AACS. Intergroup comparisons were performed using the Mann-Whitney test. Multivariate linear regression analyses were used to select factors that were independently associated with dp-ucMGP.
Results: dp-ucMGP levels were positively correlated with AACS (r=0.244, P=0.0073), age (r=0.203, P=0.0262), CRP (r=0.306, P=0.0007), homocystein (r=0.215, P=0.0185) and troponin (r=0.276, P=0.0023). Homocystein and troponin levels had no association with AACS. Both dp-ucMGP levels and AACS did not show significant difference between the groups with and without history of diabetes, hypertension and cardiovascular diseases.
Conclusions: The Inactive MGP ELISA kit from IDS is the first test kit to be commercialized for the determination of the inactive, dephosphorylated, uncarboxylated MGP levels. This is the first report documenting the positive and significant correlation between dp-ucMGP levels and AACS. The data suggest that the dp-ucMGP concentrations might be a suitable, low cost tool to assess the vascular calcification status in CKD patients. The Inactive MGP blood test could be included as part of routine monitoring biomarkers after further proven validity data.

M261
QUALITATIVE AND QUANTITATIVE COMPOSITION OF URINARY PROTEINS IN INDIVIDUALS WITH ORTHOSTATIC PROTEINURIA

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Background: The qualitative and quantitative composition of urinary proteins were monitored in 5 urinary samples excreted during stress tolerance test in 27 subjects with and 30 healthy subjects without functional proteinuria of the type orthostatic proteinuria, all aged 7-17 years.
Methods: Individuals with orthostatic proteinuria were selected from the general population, after the evaluation of the concentration of total urinary proteins in three urinary samples: first and second morning urine and urine excreted after physical exercise. All necessary investigations were made to exclude the presence of organic renal disease or systemic disease with renal affection. From each subject five urinary samples were collected during stress test (first, second morning urine, urine excreted after physical effort, urine excreted before sleeping and next day first urine). Total urinary protein was determined according the Meulemans method. The detection of the type of excreted urinary proteins was made by a horizontal SDS-PAG electrophoresis and Coomassie Blue R-250 staining technique.
Results: There was a significant difference in excretion of total proteins and in SDS-PAG electrophoretic profiles between individuals with and without orthostatic proteinuria. In subjects without orthostatic proteinuria there was no significant difference ($P > 0.05$) in the mean total protein concentration between the 5 urinary samples. On the electrophoretic profiles only albumin fraction was detected in all five urinary samples. In individuals with orthostatic proteinuria a statistical significant differences in mean protein concentration ($P < 0.001$) was detected between urinary samples. SDS-PAG electrophoretic profiles have shown the presence of only albumin fraction in urinary samples 1 and 5 (first morning urine), but in sample 2,3 and 4 beside albumin fraction (67 kDa) and transferrin fraction (78 kDa), several fractions with molecular mass above 100 kDa were detected as well as thin, sharp fractions with a molecular mass of 28 kDa.
Conclusion: The results have shown that evaluation of the dynamics of urinary proteins excretion and qualitative changes of urinary proteins by SDS-PAGE during the stress test, could be useful in the detection of subjects with orthostatic proteinuria.

M262
ROLE OF SERUM AND URINARY URIC ACID THE DIAGNOSIS OF SYNDROME OF INAPPROPRIATE ANTIDIURETIC HORMONE SECRETION (SIADH)

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Background: Hyponatremia is the commonest electrolyte abnormality. Syndrome of inappropriate antidiuretic hormone secretion (SIADH) is a common cause of hyponatremia. This syndrome is defined by the hyponatremia and hypo-osmolality that results from inappropriate, continued secretion and/or action of antidiuretic hormone (ADH) by the kidney despite normal or increased plasma volume, which results in impaired water excretion. Till date SIADH does not have consensous diagnostic criteria and remains more of a diagnosis of exclusion. But diagnosis is of great importance because the management is fluid restriction rather than intravenous saline therapy used in cases of depletional hyponatremia like dehydration. Interestingly it has been suggested that serum and urinary Uric Acid (UA) levels can be useful in the diagnosis of SIADH.
Methods: Considering this background, a study was done to assess the importance of serum uric acid and renal handling of urate through Fractional Excretion of Uric Acid(FE-UA).Cases(n= 42) included all clinically and biochemically suspected SIADH patients admitted to our hospital with hyponatremia,who met inclusion/exclusion criteria. Serum/urinary UA & Creatinine were assayed on an automated chemistry analyzer. As control population (n= 42) we included those hospitalized patients who had confirmed depletional hyponatremia as a result of dehydration, diuretics etc.
Results: Result analysis showed that the SIADH group of patients had a significantly lower serum UA as compared to the other control group. The mean(SD) of serum UA in SIADH patients were 1.55(SD 0.28) and 5.89 (SD 0.95) All patients with SIADH had serum UA levels of less than 3 mg/dL. FE- UA was significantly higher in SIADH patients(Mean 16%) than the control group (Mean 7.2%). Strong inverse correlation between serum UA and FE-UA was seen in SIADH.
Conclusions: Our study shows that serum UA and FE UA are simple, cheap and readily available investigations that can help in the diagnostic approach of hyponatremia leading to identification of SIADH. Clinical laboratorians and physicians might consider incorporation of Serum UA & FE-UA in their diagnostic protocol for evaluation of hyponatremia which can help in timely diagnosis and treatment in hyponatremic patients with SIADH.

M263

DIAGNOSTIC SIGNIFICANCE OF LIPOCALIN-TYPE PROSTAGLANDIN D SYNTHASE AS RENAL BIOMARKER IN DIFFERENT URINE SAMPLES

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Background: Urinary lipocalin-type prostaglandin D synthase (L-PGDS) has been proposed as an alternative biomarker for detection of kidney damage. It is almost completely excreted via the kidneys and it is stabile at the urine pH. In clinical practice it has not yet been clarified which urine sample is most appropriate for proteinuria detection. The protein-to-creatinine ratio on random samples is considered as a convenient and suitable alternative to the 24h collection. The aim of this study was to compare the clinical usefulness of L-PGDS levels in different urine samples (from same patient) for detection of renal dysfunction in patients with chronic kidney disease (CKD).
Methods: The study included 117 patients with a wide range of renal function that encompassed all five CKD stages. Urinary L-PGDS and creatinine were measured by immunonephelometric assay (Behring nephelometer) and a kinetic alkaline picrate method (ARCHITECT ci8200), respectively.
Results: The median (range) urinary concentrations of L-PGDS were 8.08 (0.28-63.5) mg/day in 24h samples and 0.67 (0.02-5.43) mg/mmol creatinine in second morning samples. L-PGDS values in 24h urine highly correlated (P <0.0001) with values in second morning urine (r=0.880). The mean difference (SD) value, from Bland-Altman analysis, between two urine samples was -18.4% (45.9%). ROC analyses showed that L-PGDS in both urine samples had high diagnostic accuracy for estimated GFRs of <15, <30, <60 and <90 mL/min/1.73 m^2. The areas under ROC (AUC) for 24h sample were from 0.795 to 0.903, and for second morning sample from 0.793 to 0.959. The AUCs between 24h and second morning samples, for mentioned decision limits, were not significantly different.
Conclusion: The results from this study show that L-PGDS has a high diagnostic significance for the detection of severity of renal dysfunction and the reduction in GFR, independently of the type of urine sample.

M264

CREATININE-OR CYSTATIN C-BASED EQUATIONS TO ESTIMATE GLOMERULAR FILTRATION: IMPACT ON THE EPIDEMIOLOGY OF CHRONIC KIDNEY DISEASE

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Background: Prevalence of chronic kidney disease (CKD) (defined as glomerular filtration rate (GFR) under 60 mL/min/1.73 m^2) is increasing according to recent epidemiological studies. Prevalence has been calculated using the creatinine-based equations like the Modified Diet in Renal Disease (MDRD) study and Chronic Kidney Disease Epidemiology Collaboration study (CKD-EPI) equations for estimating GFR. Recently, new equations based either on cystatin C (CKD-EPI Cys) or both cystatin and creatinine (CKD-EPI mix) have been proposed by the CKD-EPI consortium. The aim of the study was to measure the difference in prevalence of CKD in a population using these different equations.
Methods: CKD screening is organized in the Province of Liège, Belgium. On a voluntary basis, people aged over 50 years are invited to be screened. GFR was estimated by the four equations.
Results: The population screened consisted in 4189 people (47% of men). Mean serum creatinine and plasma cystatin C were 0.88±0.21 mg/dL and 0.85±0.17 mg/L, respectively. The prevalence of CKD in this population using the MDRD, the CKD-EPI, the CKD-EPI Cys and the CKD-EPI mix equations was 13%, 9.8%, 4.7% and 5%, respectively. The prevalence of stage 3 CKD is significantly higher with the creatinine-based (MDRD and the CKD-EPI) equations compared to the new cystatin C-based equations.
Conclusions: Prevalence of stage 3 CKD varies strongly following the method used for estimating GFR. Such discrepancies are of importance and must be confirmed and explained by additional studies, notably studies using GFR measured with a reference method.

M265

URINARY ALKALINE PHOSPHATASE IN RELATION WITH E-GLOMERULAR FILTRATION RATE

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Chronic kidney disease (CKD), defined as sustained kidney damage indicated by the presence of structural or functional abnormalities, and/or reduced estimated glomerular filtration rate (eGFR) to less than 60mL/min/1.73 m^2 for at least 3 months. Its symptoms are unspecific and common throughout every stage of the disease. Patients with normal or slightly elevated eGFR are at risk of developing CKD. Determination of albumin creatinine ratio (A/Cr) allows a precise stratification.The activity of urinary alkaline phosphatas (ALPur) reflects damage in the tubular structure with a cutoff >8UI/L. The aim is to relate the ALPur with A/Cr and eGFR, in patients with different arterial pressures and glycemia.
Materials and methods: Patients, n=136, ambulatory, divided into four groups according to arterial pressure and glycemia:1) normotensive-normoglycemic (NTNG, n=45); 2) normotensivehiperglycemic (NTHG, n=39); 3) hipertensive-normoglycemic (HTNG, n=28), y 4) hipertensive-hiperglycemic (HTHG, n=24), with no significant differences in age, body mass index (BMI) and sex. None of the patients were dialyzed or presented kidney transplant.Nor did physical activity. Age, gender, weight, height were registered.Arterial pressure was measured; Glycemia and creatinine were determined in serum,and ALPur, Creatinine (Cr) and albumin (A) in urine; eGFR calculated by MDRD-4 formula. BMI and A/Cr (mg/g) was calculated. Ethics Committee consent: Clinical Hospital UBA.
Methods: IFCC (ALPur, UI/L); Jaffe (Cr, g/L) and immunoturbidimetry (A, mg/L). All determinations were made in Cobas 6000 Roche Autoanalyzer. Statistic: Parametric tests, significance P <0.05 (InfoStat2011).
Results: Expressed as mean ± SD, we observed significant differences between groups NTNG and HTHG regarding: ALPur: (7,4±4,8 vs 4,3±3,4) UI/L; P=0.018-A/Cr: (6,8±12,3 vs 30,0±65,8) mg/g; P <0.0001-no significant differences for MDRD between all groups
Conclusions: Patients with normal arterial pressure and glycemia,and elevated ALPur associated with a low A/Cr and normal eGFR, may indicate tubular and inflammatory damage. These markers allow the identification of patients presenting structural kidney damage with conserved functionality. Early detection of CKD may prevent or delay dialysis. Thus in the biochemical evaluation we recommended include the measurement of these parameters

M266

CHRONIC KIDNEY DISEASES IN MIXED ANCESTRY SOUTH AFRICAN POPULATIONS: PREVALENCE, DETERMINANTS AND CONCORDANCE BETWEEN KIDNEY FUNCTION ESTIMATORS

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Background: Population-based data on the burden of chronic kidney disease (CKD) in sub-Saharan Africa is still very limited. We assessed the prevalence and determinants of CKD, and evaluated the concordance of commonly advocated estimators of glomerular filtration rate (eGFR) in a mixed ancestry population from South Africa.
Methods: Participants were a population-based sample of adults selected from the Bellville-South community in the metropolitan city of Cape Town. eGFR was based on the Cockroft-Gault (CG), Modification of Diet in Kidney Disease (MDRD) and CKD Epidemiology Collaboration (CKD-EPI) equations (with and without adjustment for ethnicity). Kidney function staging used the Kidney Disease Outcome Quality Initiative (KDOQI) classification. Logistic regressions and kappa statistic were used to investigate determinants of CKD and assess the agreement between different estimators.
Results: The crude prevalence of CKD stage 3-5 was 14.8% for Cockcroft-Gault, 7.6% and 23.9% respectively for the MDRD with and without ethnicity correction, and 7.4% and 17.3% for the CKD-EPI equations with and without ethnicity correction. The highest agreement between GFR estimators was between MDRD and CKD-EPI equations, both with ethnicity correction, Kappa 0.91 (95% CI: 0.86-0.95), correlation coefficient 0.95 (95% CI: 0.94-0.96). In multivariable logistic regression models, sex, age and known hypertension were consistently associated with CKD stage 3-5 across the 5 estimators.
Conclusion: The prevalence of CKD stages greater than 3 is the highest reported in Africa. Our study provides evidence against the local CKD guidelines that recommend the use of Cockcroft-Gault or MDRD equations for eGFR.

M267
URINE PROTEOME IN DOGS AFFECTED BY LEISHMANIASIS AND CUSHING'S SYNDROME

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Background: Urinary proteome is a topic not deeply investigated in dogs. Leishmaniasis (L) and Cushing's Syndrome (CS) are frequently associated with persistent proteinuria. Evaluation of urine proteome with sensitive methods is considered a non invasive procedure to monitor the therapy and select novel biomarkers.
Objectives: Comparison of urinary electrophoretic profiles between healthy (H) and diseased dogs (L, CS) at the time of diagnosis and during the treatment monitoring.
Methods: Urine samples were collected from 49 dogs (12 H, 26 L, 11 CS) and Urine Protein and Albumin to Creatinine ratios (UPC, UAC), HRE and SDS-PAGE were performed. Electrophoretic profiles were analyzed by ImageJ©; proteins were identified by ESI-Q-TOF.
Results: UPC and UAC (mean ± SD) were significantly higher ($P < 0.01$) in L (3.47±4.76 and 1.69±3.19) and CS (2.55±3.69 and 1.79±3.65) compared to H dogs (0.11±0.11 and 0.008±0.008). HRE was useful with urine protein concentration higher than 40 mg/dL, and revealed different electrophoretic patterns between healthy (mainly albumin band is present) and diseased dogs (also other bands can be present). Urinary albumin concentration determined by HRE correlated well with the data obtained by routine immunoturbidimetric method ($r=0.91$, $P < 0.01$). SDS-PAGE allowed to visualize an "healthy profile" with 20-25 different bands in urine of H dogs; the two most abundant ones have been identified as uromodulin and albumin. Urine samples from L and CS dogs presented a significantly higher number of protein bands (35-40), a decrease of uromodulin and an increase of albumin band intensities and the appearance of other bands, particularly in the range of 55-14 kDa; among them haptoglobin, superoxide dismutase1 and arginine esterase were identified by ESI-Q-TOF in urine of L dogs. Qualitative changes of protein profiles were evidenced during the treatment in CS and L dogs, indicating in some cases an improvement of renal damage.
Conclusion: HRE could represent a rapid screening test for diagnosis of renal damage. SDS-PAGE is a sensitive and promising technique to investigate qualitative proteinuria in healthy and diseased dogs. Urine proteome analysis could help clinicians to better characterize the proteinuria even in absence of renal histopathology.

M268
ESTIMATED GLOMERULAR FILTRATION RATE BY CKD-EPI FORMULA MAY HELP DETECT CHRONIC KIDNEY DISEASE IN ELDERLY PEOPLE WITH SERUM CREATININE STILL IN THE NORMAL RANGE

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Background: Chronic Kidney Disease (CKD) is a condition of great impact on both the quality and quantity of life, as well as on healthcare costs. It is an age-related disease, with important and common conditions as risk factors: cardiovascular disease, diabetes, hypertension, obesity. So, prevention and early diagnosis are crucial for healthcare. A simple and cheap lab test correlating closely with the glomerular filtration rate (GFR) would be the ideal approach. Serum creatinine alone is not a reliable marker of CKD, especially in the elderly population, because of the so-called creatinine-blind range. In fact, serum creatinine decreases with age despite the decline in GFR, resulting in an increased number of patients with CKD but a normal creatinine value.
Methods: The aim of this study was to test the effectiveness of estimated GFR (eGFR) to identify the presence of CKD in elderly patients with serum creatinine in the blind range, i.e. still normal. Seventy patients from INRCA hospital in Ancona, both outpatients and hospitalized (excluding nephrology and dialysis clinics), aged 75-95 years, with serum creatinine 0.6 to 1.2 mg/dL, were randomly selected. GFR was measured by means of the creatinine clearance test and was estimated using the CKD-EPI formula. Diagnostic cut-off was set at 60 mL/ min (CKD stage ≥3). Creatinine clearance was used as comparison test. The concordance between the two methods was investigated.
Results: Mean±SD age of the study population was 82±5 years. The prevalence of CKD in this population was 63 ± 11% (95% CI). The eGFR (CKD-EPI) showed the following performances: Sensitivity = 65% ± 14%; Specificity = 73% ± 17%; PPV = 80% ± 13%; NPV = 56% ± 17%.
Conclusion: The prevalence of CKD is high in the elderly population with normal serum creatinine. The eGFR (CKD-EPI) at the cut-off of 60 mL/min was able to identify two-thirds of these cases, with a false-positivity rate of 20%. It looks like a pretty good result for a free-of-charge information, obtained from a serum creatinine value which otherwise might be almost useless.

M269
ASSESSMENT OF ALBUMINURIA USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Background: Many studies have found higher values of urinary albumin reported using high performance liquid chromatography (HPLC) in comparison with immunochemical methods. The aim of our study was the implementation of HPLC method for albuminuria, testing the hypothesis about co-eluting proteins, monitoring of the storage temperature influence on urinary albumin stability, and comparison of albuminuria assessed using HPLC and immunoturbidimetric (IT) methods in patient samples.
Methods: We developed the HPLC method for detection of albuminuria. We analyzed two mixtures of urine. The first one was prepared from 30 patient urine samples with immunoturbidimetrically physiological albuminuria, to which we added albumin standard. The second mixture was prepared from 30 patient urine samples with mild albuminuria. We stored the mixtures at room temperature, 4 ˚C, -20 ˚C, and -70 ˚C. We compared albuminuria assessed using HPLC with the immunoturbidimetric method in a group of 1077 patients (519 diabetics and 558 nondiabetics, 589 males and 488 females, age 43 ± 26 years).
Results: Transferrin, α-1-acid glycoprotein, α-1-antitrypsin, α-1-antichymotrypsin and hemopexin do not interfere with albumin in HPLC method. The elution curve of prealbumin splits into several peaks, of which a few interfere with albumin. This interference has no clinical importance. In mixture 1 we did not find a significant difference between the albuminuria assessed using both methods (79 mg/L vs. 82 mg/L), while in mixture 2 we measured over 26% higher albuminuria using HPLC (79 mg/L vs. 99 mg/L). We found albumin instability after 3-month storage at room temperature and -20 ˚C. Urinary albumin was stable after 9-month storage at -70 ˚C, but also at 4 ˚C. We found a statistically significant difference between the methods in patient urine samples (167 ± 499 mg/L IT vs. 197 ± 562 mg/L HPLC, P <0.0001, Mann-Whitney test).
Conclusions: Our results prove that the HPLC method for albumin detection is more sensitive than immunoturbidimetry. We did not confirm nonspecificity of the HPLC method. We found lower urinary albumin stability at -20 ˚C compared to 4 ˚C.

M270
THE ROLE OF NGAL AND CYSTATIN C MEASUREMENT AFTER HEART TRANSPLANTATION

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Background: Renal function is deteriorated after heart transplantation (TxHT). Creatinine and glomerular filtration rate have been used as predictors of kidney function. However, these markers are unable to detect early stages of renal dysfunction. Neutrophil gelatinase-associated lipocalin (NGAL) predicts acute kidney injury, albuminuria and alpha-1-microglobulin (A1M) in urine predict glomerular and tubular dysfunction, respectively. Cystatin C is a predictor of cardiorenal pathology. The purpose of our study was to evaluate the best prediction model of sampling for biochemical examination of kidney function after heart transplantation.
Methods: 75 patients were evaluated on Day 0, 1, 7, 10, 30 after TxHT, 50 of them after 3 and 6 months. NGAL and albumin in urine and cystatin C in plasma were measured on Abbott Architect, alpha-1-microglobulin on Beckman Immage.
Results: Extremely increased concentrations of NGAL in urine were found on Day 0 and 1 after TxHT without any relation to kidney function. However, NGAL measured on Day 3 correlated with cystatin C evaluated 6 months after TxHT (P <0,01) and correlated also with the necessity to use renal replacement therapy soon after TxHT. Concentrations of A1M in urine on Day 10 correlated with kidney function 6 months after TxHT. Cystatin C on Day 7 and 10 correlated with prognosis (Day 10: Cystatin C >2,0 mg/L is connected with shortened survival, Kaplan-Meier analysis, P <0,0001; similarly, cystatin C on Day 7 above 1,4 mg/L means worse prognosis, P <0,05). Albuminuria was increased in only 16% of patients at Month 6, but A1M was increased in 56% of patients at that time.
Conclusions: Markers of kidney dysfunction (NGAL, alpha-1-microglobulin and albumin in urine, cystatin C in plasma) measured 3 to 10 days after heart transplantation predict renal dysfunction and prognosis during 6 months of follow-up.

M271
BNP PLASMA CONCENTRATION IN PATIENTS WITH END STAGE RENAL DISEASE BEFORE AND AFTER HEMODIALYIS

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Background: End-stage renal disease is a clinical situation in which the cardiac natriuretic peptides are almost universally raised. Extracellular volume expansion, concomitant heart disease, and severely reduced renal clearance are the main factors responsible for the high plasma concentration of brain natriuretic peptide (BNP) in uremic patients who are on chronic dialysis. It has been suggested that the measurement of the plasma concentration of BNP may help to define better the "dry weight"), however cardiac function is a major confounder for the interpretation of prevailing BNP plasma concentration in chronic renal failure. We investigated variations in BNP concentration, before and after dialysis, in a cohort of patients without heart failure.
Methods: BNP plasma concentration was evaluated by using a commercial kit and an automated analyzer (AIA 2000) supplied by Tosoh. A total of 60 patients with end-stage renal disease (37 men and 23 women) who had been on regular hemodialysis treatment for at least 6 months, with LV ejection fraction (LVEF) >35% and without history of clinical evidence of circulatory congestion were considered.
Results: In pre-dialysis samples we observed a BNP mean concentration of 148+126 pg/mL, in post dialysis samples we observed a BNP mean concentration of 82+63 pg/mL, this difference was statistically significant (P <0.001). Pre-dialysis BNP values were higher than post-dialysis in 54 patients but in 6 subjects we observed higher post-dialysis BNP concentration.
Conclusions: BNP is eliminated during hemodialysis, it is cleared by both high- and low-flux membranes, with high-flux membranes giving higher clearance (mass balance) and reduction rates. Furthermore in the majority of considered subjects shown a correlation between BNP concentration ad body weight pre and post dialysis but BNP seem to be released into the circulation during the hemodialysis session as shown by increasing post dialysis plasma concentrations observed in some patients in spite of demonstrated clearance. Additional studies are needed to test the influence of dialysis treatment on plasma concentrations of BNP and to elucidate the interdependence of the production, release, and elimination of these peptides in dialysis treatment.

M272
EVALUATION OF SERUM CYSTATIN C AS A NEW INDICATOR OF RENAL DYSFUNCTION

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Background: Serum cystatin C (CysC) is examined as a marker of glomerular filtration rate (GFR), compared to determination of Creatinin (Cr), Creatinine Clearance (CrCl) measured with Cockcroft and Gault (C&G) formula, and albuminuria in Diabetes mellitus type II (DM) and hypertensive (HBP) patients.
Methods: We examined 57 patients (pts) with no obvious renal disease; 32 (56%) males, mean age 62.7±10.15, mean weight 74.7±14.25 kg, mean height 166.9±7.55 cm, mean BMI 26.93±4.4 kg/m^2 (range 19,03 - 36,80). 21 pts were with HBP - mean age 65 (48-84) yrs, 25 pts with DM - 59 (45-77) yrs and 11 pts with HBP and DM - 64,5 (53-76) yrs. CysC is measured with ELISA, CrCl with C&G formula. Statistical analysis is made with SPSS19.0. Significant p-value <0,05.
Results: Resulted pathologic values of CysC to 19 (33.3%) cases, Cr- 4 (7%), and microalbuminuria-26 (45.6%), macroalbuminuria-2 (3.5%). Mean calculated CrCl -75,04 mL/min/1,73 m^2, mean serum Cr- 1,045 mg/dL (0,7-2,0), mean serum CysC - 0.9995 mg/L (0,16-3,67), mean albuminuria was 609,5 mg/24h. We found significant correlation between Cr and CrCl (P=0.001), significant correlation between CysC and ClCr (P=0,004). GFR resulted in significant correlation with both Cr and CysC (P=0,001). In pts with ClCr interval values <75 ml/min/1.73 mean CysC was 1.362mg/L (P <0.001), mean Cr 1,2 mg/dL (P <0.001) and mean albuminuria 88.04 mg/24h (P=0.4). In pts with ClCr intervals >75-85 mL/min/1,73, mean CysC was 0.944 mg/L (P <0.002 and mean Cr 0,956 mg/dL (P <0.001) and mean albuminuria 30.23 mg/24h (P <0.001). In pts with ClCr intervals >85-100 mL/min/1,73 mean CysC was 0.754 mg/L (P <0.02)and mean Cr 0,862 mg/L (P <0.001) and mean albuminuria 51.38 mg/24h (p <0.033). In pts with CrCl >100 mL/min/1.73 m^2, mean CysC was 0.458 mg/L (P <0.001) and mean Cr 0,866 mg/dL (P <0.001) and mean albuminuria 27.5 mg/24h (P <0.001).
Conclusions: Cistatin C is a valuable marker of GFR, particularly in situations in which Creatinin fails to provide an accurate estimation of GFR. Cystatin C is more sensitive in early stages of reduced GFR than Creatininemia and microalbuminuria. Virtually unaffected by non-renal factors.

M273

LIQUID STABLE ENZYMATIC ASSAY FOR THE MEASUREMENT OF CREATININE ON THE RX DAYTONA PLUS ANALYSER

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Background: Creatinine is derived from creatine and creatine phosphate in muscle tissue and is defined as a nitrogenous waste product. Creatinine is not reutilised but is excreted from the body in the urine via the kidney. As a consequence of the way in which creatinine is excreted by the kidney, its measurement is used almost exclusively in the assessment of kidney function. Creatinine is regarded as the most useful endogenous marker in the diagnosis and treatment of kidney disease.This study reports the development of an enzymatic assay kit with enhanced precision and stability for the measurement of creatinine in human serum and urine applied to the fully automated RX daytona plus. This is of value in the diagnosis and treatment of renal diseases as well as in monitoring renal dialysis.
Methods: The assay is enzymatic and has 4 steps the last of which utilizes hydrogen peroxide, in the presence of peroxidase, to yield a blue pigment. The absorbance of the color is proportional to the creatinine concentration. On-board and calibration stabilities were tested by storing the reagents uncapped on the RX daytona plus analyser for a period of 28 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 2 replicates twice a day for 20 days. Correlation studies were conducted using a commercially available enzymatic creatinine assay.
Results: The liquid enzymatic reagent presents an on-board stability and calibration frequency of 28 days. The assay was found to be functionally sensitive to 12.27 μmol/L and be linear up to 2517 μmol/L. The within-run and total precision for three different concentration levels typically had %C.V.'s of ≤5.0%. In the correlation study 99 serum patient samples were tested and the following linear regression equation was achieved: $Y=0.989x + 4.96$; $r^2 = 0.999$.
Conclusion: This enzymatic assay kit exhibits high sensitivity and reproducibility with the added advantage of using liquid reagents with good stability. This represents an improvement for use in the accurate and reliable determination of creatinine.

M274

A NEW EQUATION TO ESTIMATE THE GLOMERULAR FILTRATION RATE IN CHILDREN AND ADOLESCENTS

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Background: A new estimating glomerular filtration rate (eGFR) equation, specifically designed for isotope dilution mass spectrometry (IDMS)-standardized serum creatinine, that can be used for children and adolescent boys and girls is presented in this study.
Methods: The new equation is based on the concept of normalized serum creatinine(Scr): $eGFR=107.3/(Scr/Q)$. Q is the normalization value and is considered as the serum creatinine concentration for the average healthy child or adolescent of a specific length (L) and can be modeled as a polynomial of the 4th degree: $Q=3.94 - 13.4 \times L + 17.6 \times L^2 - 9.84 \times L^3 + 2.04 \times L4$.
Results: The new equation can be seen as an extension of the well-known Schwartz equation ($eGFR=kL/Scr$) for children between 1 and 14 years for which the normalization value Q is linearly dependent on the length of the child: $Q=0.0035 \times L(cm)$. This corresponds with a value of k=0.375, which is very close to the previously determined k-values of 0.373 (by the research group in Lyon) or 0.413 (by Schwartz). The new eGFR equation has been validated in a dataset of n=1054 children and adolescents aged 10-25 years, against the true GFR (inulin method) and outperforms the selected (but most used) creatinine-based eGFR equations for children, mainly in the healthy GFR region.
Conclusions: The new Q(L)-eGFR equation serves as an excellent screening tool for kidney disease in 1 to 25 year old children and young adults.

M275
NGAL: NEW EARLY BIOMARKER OF KIDNEY INJURY

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Background: Traditional laboratory tests such as plasma creatinine are not able to identify acute kidney injury in its early stages. Recently, the neutrophil gelatinase associated lipocalin (NGAL) have been proposed as new early emerging marker of renal damage. Moreover the synthesis of NGAL increases in case of inflammation, bacterial infections of low urinary tract. Some studies have shown that plasma urine NGAL increases even before the creatinine levels if a dangerous condition for the kidney occurs. NGAL levels vary more during the different hours of the day in the same subject, rather than among days and for this reason, as suggested from other studies, in our investigation we want evaluate the standardised cut-off of NGAL to urine creatinine, as well as the absolute cut-off.
Methods: For the study, urine samples were collected from 50 healthy volunteers, HV, (24 M, 26 F) aged between 31 and 60 years (mean 44 years). Exclusion criteria were: diabetes, hypertension, proteinuria and acute infections. NGAL on ARCHITECT 1000 SR (Abbott, USA) by chemiluminescent microparticle immuno assay and urinary albumin and creatinine on Cobas C501 (Roche Diagnostics, Germany) by immunoturbidimetric and enzymatic method, respectively, were evaluated. The statistical analysis was performed by MINISTAT 2.1 program.
Results: Our results showed that NGAL data are not distributed in parametric way and that the cut-off at 95th percentile, identified for our population, was 134.1 ng/mL (Abbott, cut-off: <131.7 ng/mL). The standardised cut-off of NGAL to urinary creatinine found was:116 ng/mg creatinine.
Conclusions: Our study confirm the results reported in literature and Abbott cut-off. We suggest to evaluate standardised cut-off.

M276
PLASMA NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AS A BIOMARKER FOR ACUTE KIDNEY INJURY IN CRITICALLY ILL PATIENTS WITH SUSPECTED SEPSIS

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a biomarker for the early diagnosis of acute kidney injury (AKI), which is very common and the most serious problem in sepsis patients. We investigated the diagnostic utility of plasma NGAL as an early objective biomarker to predict AKI in critically ill patients with suspected sepsis.
Methods: The plasma NGAL in 231 samples obtained from clinically suspected sepsis patients was measured using the Triage NGAL Test (Alere Inc., San Diego, CA, USA). The NGAL results were compared with those of procalcitonin (PCT). Renal failure was assessed using the renal subscore of the Sequential Organ Failure Assessment (SOFA) score. AKI was defined according to the Acute Kidney Injury Network criteria.
Results: Plasma NGAL concentrations were significantly different according to the 5 PCT concentration groups (ANOVA, $P < 0.0001$) and the renal subscore of the SOFA score (ANOVA, $P < 0.0001$). Plasma NGAL was significantly increased in patients with AKI compared to those without AKI (416.5 vs. 181.0 ng/mL, $P = 0.0223$).
Conclusions: Plasma NGAL is a highly sensitive and objective predictor of AKI in suspected sepsis patients. Plasma NGAL seems to be added for the diagnosis and staging of renal failure in sepsis.

M277
EFFECTS OF PERITONEAL-AND HEMODIALYSIS ON LEVELS OF PLASMA PROTEIN AND LIPID OXIDATION MARKERS IN DIABETIC PATIENTS

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Background: . On renal dialysis, diabetes is an independent risk factor for CVD as higher levels of oxidative stress occurs - which is based on decreased antioxidant capacity due to loss of, low to middle molecular weight molecules including antioxidant substance via dialysis in addition to- continuous interact with the dialyze membrane.
Objectives: To evaluate the effects of dialysis procedures on oxidative stress in diabetic patients.
Methods: The study was performed on 15 nondiabetic hemodialysis (HD) patients, 30 nondiabetic perinoteal dialysis (PD) patients, 18 diabetic HD patients (DHD), 15 diabetic PD patients (DPD), and 20 healthy controls. Plasma thiobarbituric acid reactive substances (TBARS), protein carbonyl (PCO), and oxidized LDL (oxLDL) were determined as oxidative stress markers. Plasma thiol (P-SH), erythrocyte glutathione (GSH) levels, and serum paraoxonase (PON1) activities were measured as antioxidants. PCO, TBARS, PON , P-SH and GSH levels were determined using colorimetric methods, and oxLDL levels were determined by Enzyme-Linked Immun Assay.
Results: Compared to PD patient; HD patients have significantly - higher plasma oxLDL (P <0.05), TBARS (P <0.05), and PCO (P <0.05) and lower plasma P-SH levels (P <0.05) .In HD group, DP have significantly; higher PCO and PON1 activities (P <0.05 and P <0.05) and lower erythrocyte GSH levels (P <0.05) than non-diabetic patients. Plasma oxLDL levels were significantly higher (P <0.001) and plasma P-SH levels (P <0.01) were significantly lower in non -diabetic HD patients than in non-diabetic PD patients. DHD patients had significantly higher plasma PCO levels than DPD patients (P <0.05).
Conclusion: Oxidative stress is exacerbated by HD in diabetic patients. Treatment strategy with antioxidants in dialysis patients may be associated with a worsened survival.

M278
CREATININE, CYSTATIN C AND RENAL FUNCTION

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Background: Evaluation of renal function is based on the measurement of serum creatinine concentration. However, creatinine is not an early indicator of impairment of kidney function, because it rises significantly only in case of decrease of the renal function more than 50%. Cystatin C is a basic protein of 13 KDa not glycosylated, produced and released into the blood at a constant speed by all nucleated cells. It's filtered to Glomerular layer, reabsorbed and completely catabolized by the renal tubules. Gender, age, race, muscle mass, steroidal therapies, inflammation, liver disease (that modify significantly creatinine serum concentrations) do not affect the concentrations of Cystatin C. We wanted to test the feasibility of using Cystatin C as a marker for early kidney damage more quickly and accurately in place of Creatinine.
Methods: We studied 180 patients (of various age including diabetes and hypertensive followed at the Day Hospital Units) compared with a sample of normal subjects going to our Laboratory Unit for a blood control. Over the blood samples we assayed. Creatinine values (measured with standardized enzymatic method by Unicel DxC 600 Beckman Analyser) Cystatin C (measured with a FEIA-Fluorimetric Enzyme Immuno Assay - on AIA-1800 by Tosoh Bioscience Tokyo, Japan) Albuminuria (IMMAGE with Nephelometric method by Beckman).
Results: We found high correlation between Creatinine, Cystatin C and renal function. Both markers increase with age; however there was a greater increase of Cystatin C from 65 upwards, more linked to the renal decline age-dependent. In all groups we didn't found correlation between Creatinine and Albuminuria, whereas Cystatin C and Albuminuria showed a more evident relation in hypertensive patients.
Conclusions: Creatinine is the current gold standard Marker of Renal Function (easy and cheap). Our preliminary data suggests the usefulness of Cystatin C as a marker for early kidney damage (as mirror of endothelial lesion) with greater diagnostic accuracy especially in the elderly and in the atherosclerotic patients. The wide availability of automated analytical methods will enable an effective use for the evaluation of early impairment of renal function.

M279
GLOMERULAR FILTRATION BY MDRD AND CREATININE CLEARANCE BY COCKCROFT-GAULT IN PARAGUAYAN PATIENTS

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Background: In the practice of clinical medicine, the endogenous creatinine clearance (ECC) is used as a marker of renal function but its routine use is not always possible and has some limitations. Due to this, different equations have emerged based on the value of serum creatinine, age, weigh and sex. The most used equations are the Crockcroft-Gault (C-G) equation and 4-MDRD (four variables modification of diet in renal disease) equation recommended by the National Kidney disease Education Program. The objective of this study was to demonstrate that the equations used to measure renal function can be used in the Paraguayan adult population (mostly mestizo) instead of the ECC that uses 24-h urine collection comparing the glomerular filtrate formulas with the ECC.
Methods: This was a descriptive analytical study performed in 197 male and female patients older than 18 years old that attended the Central Laboratory of the Hospital de Clínicas to have the creatinine depuration test in 24-h urine. Glomerular filtration was simultaneously evaluated by the 4-MDRD and creatinine clearance by the equation C-G considering age, weight, height, body mass index (BMI), sex and plasma creatinine using the renal function calculator of the Spanish Society of Nephrology available in its website. The serum creatinine was measured by the kinetic colorimetric Jaffe method being the normal values for women up to 1.1 mg/dL and in men up to 1.4 mg/dL.
Results: Of the 197 patients, 72% was women, the mean age was 48±17 years and 65% had overweight and obesity similar to the finding of the national survey that found a 60.7%. The correlation found in the general population between ECC and C-G was r=0.95 and between ECC and 4-MDRD r=0.89. When correlations were made with the different degrees of BMI, significant differences were not found between normal BMI and those of overweight and obesity. The correlation between ECC and CG was r=0.94 in women and r=0.97 in men. The correlation between ECC and 4-MDRD was r= 0.84 in women and r=0.94 in men.
Conclusion : The data found in this study were similar to those of different scientific publications in other populations. The use of Crockcroft-Gault and 4-MDRD equations to measure renal function in the Paraguayan adult population represents a practical, economic and reliable procedure to inform about the clinical and diagnostic usefulness of the renal function status. Also, apparently they can be used in overweighed and obese patients though this still needs a larger number of patients evaluated.

M280
"HIDDEN" RENAL INSUFFICIENCY BY THE VALUATION OF RENAL FUNCTION BY 4-MDRD EQUATION

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Background: Most physicians of primary care and other specialists, who are not nephrologists, use the value of serum creatinine as marker of the renal function and only send patients to a nephrologist when they find a creatinine value of 2 mg/dL, which means that the glomerular filtration (GF) is already 50% less than normal value. In this status, it is not possible to avoid the progression towards a terminal renal insufficiency. Currently, treatment goal of patients with a renal disease in the prevention of the progression and that can only be achieved in the first stages of the chronic renal disease (CRD) when plasma creatinine is still in normal ranges or slightly increased. The objective of this study was to evaluate the GF in ambulatory patients that had analytical tests in the laboratory and to verify how many of them had renal insufficiency (GF ≤60 mL/min/1.73 m^2).
Methods: This was an analytical descriptive study performed in 197 male and female patients older than 18 years that attended the Central Laboratory of the Hospital de Clínicas to have some analytical tests. They were subjected to glomerular filtration by the 4-MDRD equation considering age, weight, height, sex and plasma creatinine. Only those patients with creatinine lower than 2.5 mg/dl were evaluated using the renal function calculator of the Spanish Society of Nephrology available in its website. Serum creatinine was measured by the kinetic colorimetric Jaffe method being the normal values in women up to 1.1 mg/dL and in men up to 1.4 mg/dL. Hidden renal insufficiency was defined when the GF was ≤60 ml/min/1.73m^2 and serum creatinine was within the limits of the normal range of the laboratory. Of the 197 patients, 72% (n=142) was women, mean age was 48±17 years and the mean plasma creatinine was 1.02±0.4 mg/dl. Data were analyzed considering two groups according to the creatinine values; Group 1: with normal values of creatinine (W: up to 1.1 mg/dL, M: up to 1.4 mg/dL). Group 2: with values higher than normal values up to 2.5 mg/dL.
Results: Group 1: Women: 46.32±17.45 years, serum creatinine: 0.78±0.13 mg/dL GF: 88.40±21.9 mL/min, Renal insufficiency: 5%. In 31.35%, GF was not normal between 60 and 89 mL/min. Men: 51.19±16.4 years, creatinine: 0.95±0.13 mg/dL, GF: 92.13±16.52 mL/min, renal insufficiency: 7.6%. In 46.2%, GF was not normal between 60 and 89 mL/min Group 2: Women: 54.04±14.13 years, creatinine: 1.52±0.39 mg/dL, GF: 40.87±10.96, renal insufficiency: 75%. Men: 50.05±19.16 years, creatinine 1.7±0.14 mg/dL, GF: 80.8±39 mL/min, renal insufficiency: 44.8%.
Conclusions: The use of the 4-MDRD equation allows the detection of an important group of patients with hidden renal insufficiency and normal levels of creatinine. It also allows the detection of a group of patients that with slight increases of creatinine already have advanced renal insufficiency. The laboratory could be a great help to detect these patients, performing routine determination of GF by the 4-MDRD equation in every patient that has plasma creatinine; it only needs age, weight and sex.

M281
MEASUREMENT OF URINARY CYSTATIN-C CAN DIAGNOSE ACUTE KIDNEY INJURY AS EARLY AS SIX HOURS AFTER SURGICAL ABDOMINAL AORTIC ANEURYSM REPAIR

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Background: Acute kidney injury (AKI) is a common and often serious complication following surgical abdominal aortic aneurysm repair (AAAr). Recent findings demonstrate that elevated levels of urinary Cystatin-C (uCys-C) may reflect tubular dysfunction. Our aim was to evaluate the utility of uCys-C as a diagnostic marker of AKI in patients undergoing AAAr surgery.
Methods: In a prospective study we included 123 patients undergoing elective AAAr surgery. All patients had serum and spot urine collections before surgery (baseline) and at 6, 24 and 48 hours post-surgery thereafter. A final collection was made on day-5. Exclusion criteria included neoplasia, end-stage renal and liver disease, and recent surgery. uCysC was quantified by latex particle-enhanced turbidimetric immunoassay using a commercial CysC kit (Sentinel, Milan, Italy) on Architect ci8000 analyzer (Abbott Laboratories, Il.) with intra- and inter-assay coefficient of variation <5.0%. Serum Creatinine (sCr) was determined with the Jaffe reaction using Abbott reagents on the same analyzer. AKI was defined by using the AKIN (Acute Kidney Injury Network) criterion: an absolute increase in sCr above baseline of at least 0.3 mg/dL or a percentage increase of at least 50%.
Results: The mean age (SD) of the patients was 69.2 (8.8) years. Twent-seven patients (21.43%) developed AKI. The baseline median (IR) values of uCysC was similar (t-test=NS) among patients who developed AKI and those who did not [0.08 (0.06) vs 0.06 (0.04) mg/L] Subsequent analysis showed that 6 hours after surgery those who developed AKI increased their uCysC levels significantly from baseline [0.58 (0.38) mg/L] as well as from those who did not develop AKI [0.05 (0.04) mg/L]. Among AKI patients median value of uCysC continued to increase at 24 hours [1.41 (1.72 mg/L)] and peaked at 48 hours [2.94(7.08) mg/L] while no increase was observed among those who did not develop AKI [0.06 (0.10) vs 0.08 (0.15)]. The diagnostic accuracy of uCysC at 6 hours post-surgery was excellent (AUC-ROC=0.966) and a cut-off value set at 0.30 mg/L could diagnose AKI with sensitivity 85.19% and specificity 98.95%
Conclusions: Our results indicate that uCysC can facilitate in early diagnosis of AKI following abdominal aortic aneurysm repair surgery.

M282
EFFECTS OF DIFFERENT IMMUNOSUPRESSIVE DRUGS ON LIPID LEVELS IN RENAL TRANSPLANT PATIENTS

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Background: The aim of this study is to investigate the effects of different immunosuppressive agents on lipid levels in renal transplant patients. A few mmunosuppressive agents including cyclosporine A (CyA), corticosteroids and tacrolimus have a significant pathogenetic role.
Methods: There were two groups of renal transplant recipients, one treated with cyclosporine A combined with steroids and the other with tacrolimus and steroids. Values of plasma lipids cholesterol (CHOL), low-density lipoprotein (LDL)-CHOL, high-density lipoprotein (HDL)-CHOL and triglycerides (TG) were compared one day before transplantation, 6 months and 12 months posttransplantation.
Results: In patients treated with cyclosporine was significant increase in mean cholesterol and mean LDL-cholesterol values at the 6 months posttransplantation study compared with pretransplant levels (CHOL: 205.9 +/- 47.3 vs. 266.5 +/- 41.2 mg/dL, P=0.004; LDL: 117.3 +/- 48.8 vs. 197.6 +/- 41.1 mg/dL, P=0.002. At 12 months, LDL-cholesterol levels were significantly elevated compared with pretransplant levels (LDL: 119.3 +/- 49.7 vs. 147.8 +/- 47.8 mg/dL, P=0.034. In cyclosporine treated patients, plasma triglyceride levels were reduced at the 6- and 12-months follow-up (TG: 292.8 +/- 59.1 vs. 181.9 +/- 47.8 mg/dL, P=0.03; 292.7 +/- 58.1 vs. 177.8 +/- 73.1 mg/dL, +/- =0.023.Cholesterol levels at 12 months posttransplantation were significantly lower than the pretransplant measurements (CHOL: 181.7 +/- 43.2 vs. 163.1 +/- 38.1 mg/dL, P=0.024.
Conclusions: Tacrolimus and cyclosporine microemulsion have specific effects on cardiovaskular risk factors that affect the predicted rate of coronary artery disease. In patients with stable renal function cyclosporine therapy is associated with increased cholesterol and LDL-cholesterol levels. Hyperlipidemia is less pronounced in patients given tacrolimus.

M283

POLYMORPHISMS IN THE NON-MUSCLE MYOSIN HEAVY CHAIN GENE (MYH9) AND CHRONIC KIDNEY DISEASES IN MIXED ANCESTRY DIABETIC SUBJECTS FROM SOUTH AFRICA

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Background: Single nucleotide polymorphisms (SNPs) in the non-muscle myosin gene (MYH9) which explain most the excess risk of nondiabetic chronic kidney disease (CKD) in African Americans, have been linked with diabetic nephropathy. We investigated the association of MYH9 SNP's with renal traits in a mixed-ancestry South African population prone to diabetes.
Methods: Three SNPs known to be associated with CKD (rs4821480, rs5756152 and rs12107), were genotyped using Taqman assay in 716 adults (198 with diabetes) from Bellville-South community, Cape Town. Glomerular filtration rate was estimated (eGFR) and urinary albumin albumin/creatinine ratio (ACR) assessed. Multivariable regressions were used to relate the SNPs with renal traits.
Results: Mean age was 53.6 years, with the expected differences in characteristics by diabetes status. Significant associations were found between rs575152 and serum creatinine, and eGFR in the total population, and in participants with diabetes (all P <0.003), but not in non-diabetics (all P >0.16), with significant interactions by diabetes status (interaction P <0.009). The association with ACR was borderline in people with diabetes (P=0.05) and non-significant in non-diabetics (P=0.85), with significant interaction (P=0.02). rs12107 was associated with fasting-, 2-hour glucose and HbA1c in participants with diabetes only (interaction P <0.003), but not with renal traits.
Conclusion: MYH9 SNPs were associated with renal traits only in diabetic participants in this population. Our findings and other studies suggest that MYH9 may have a broader genetic risk effect on different types of kidney diseases.

M284

NT-proBNP AND SDMA ARE ASSOCIATED WITH EGFR IN CHRONIC KIDNEY DISEASE PATIENTS AND RENAL TRANSPLANT RECIPIENTS

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Background: The aim of study was to find the ralationship between serum levels of symmetric dimethylarginine (SDMA) and N-terminal natriuretic propeptide B-type (NT-proBNP) and the degree of estimated glomerular filtration rate (eGFR).
Methods: This study involved 98 chronic kidney disease (CKD) patients and 44 renal transplant (RT) recipients. Measurement of SDMA was determined using ELISA (DLD Diagnostica GMBH) and NT-proBNP was determined with ELFA (bioMerieux). GFR was calculated according to the Modified Diet in Renal Disease (MDRD) equation.
Results: To evaluate the changes of examined parameters according to renal function we stratified CKD and RT patients to GFR categories. All patients have moderate (GFR 30–59 ml/min/1.73 m^2) to severe (GFR <30 mL/min/1.73 m^2) impaired renal function. Both NT-proBNP and SDMA were significantly higher in CKD and RT patients with severe than moderate impaired renal function. In CKD group, NT-proBNP values rose from 88,09 ng/L in patients with moderate eGFR to 988,78 pg/L in patients with severe eGFR (P <0.001) and from 197,05 ng/L to 996,87 ng/L in RT group (P=0.003). Obtained values for SDMA between moderate and severe eGFR were 1,09 μmol/L vs 1,62 μmol/L in CKD group and 0,58 μmol/L vs 1,18 μmol/L in RT group (P <0.001).
Conclusion: NT-proBNP and SDMA are usefull for detection of severity of renal disfunction in CKD patients and renal transplant recipients.

M285
RESISTIN CONCENTRATION IN PATIENTS WITH CHRONIC RENAL FAILURE TREATED BY HEMODIALYSIS

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Background: Resistin is a small (12.5-kDa) cysteine-rich protein, secreted mainly by adipocytes and apparently inhibiting insulin action in vitro. Recent studies have shown that human resistin is expressed also on monocytes and macrophages, and a relationship to inflammation. High levels of some adipocytokines have been reported in patients with chronic renal failure, but little information is available on resistin concentrations evolution after hemodialysis. The aim of our study was to quantify the resistin levels in patients with chronic renal failure (CRF), before and after undergoing hemodialysis.
Methods: Our research was performed on 48 patients with chronic renal failure (CRF) treated by HD (hemodialysis). A group of 30 healthy individuals served as the control. Serum resistin level was determined using the DRG Leptin ELISA Kit - a solid phase enzyme-linked immunosorbent assay based on the sandwich principle on automated ELISA Adaltis instrument. Routine biochemical parameters on Advia 1800 instrument (Siemens) with Diasys reagents were also obtained. The efficiency of hemodialysis was estimate by dialyzer clearance of urea (Kt/V).
Results: Pre-treatment resistin serum levels were significantly increased in hemodialysis patients compared to healthy controls (20.59±6.25 ng/mL vs. 5.15±2.31 ng/mL; P <0.001). After hemodialysis the resistin concentrations were more increased (34.70±5.57 ng/mL, P <0.001). No significant correlation was observed between Kt/V and after-dialysis serum resistin levels (r = 0.166, p=0.259). A signficant correlation was found betweeen the resistin levels before and after treatment (r = 0.632, P <0.001).
Conclusions: In patients with chronic renal failure treated by hemodialysis resistin serum levels are significantly elevated. The hemodialysis procedure increases the resistin levels.

M286
MICRO PROTEINS SCREENING TESTS FOR BALKAN ENDEMIC NEPHROPATHY IN AN ENDEMIC VILLAGE POPULATION

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Background: Balkan endemic nephropathy (BEN) is a familial chronic tubulointerstitial disease with insidious onset, slow progression and also an environmentally induced disease in genetically predisposed patients. So far, there is no specific and sensitive laboratory marker of early BEN detection. The aim of this investigation was to evaluate different urinary micro proteins such as micro albumin (MA), and to compare with low molecular weight Beta2 micro globulin (B2M), Cistatin C (CysC), as a markers of tubular kidney disfunction.
Methods: In the first morning urinary portion, MA was examined in 224 patients in an endemic village Batkovići near Bijeljina (Republic of Srpska - Bosnia and Hercegovina), with semi quantitative test strips (Micral - Roche Diagnostics). Urines with positive MA results were stored and further analyzed for the concentration of B2M and CysC by immunonephelometric (SIEMENS DADE Behring BN II) method. In patients with high MA values, total proteins were analyzed by turbidimetric (SIEMENS DADE Behring X-pand) method, as well as, urine electrophoresis pattern detection on (sebia Hydrasys) analyzer.
Results: Positive values for MA were detected in 41/224 (18.3%) of patients. Mean average values for MA with test strips were 64.87±27.58 mg/L in spite of 117.84±265.05 mg/L, obtained by quantitative nephelometric method and that differences was statistically significant (P <0.05). Average values for Total urinary proteins was also elevated 271.24±351.91 mg/L in these patients. All 43 patients have CysC concentrations inside reference interval (0.04±0.05 mg/L) and only 6 patients with confirmed glomerular proteinuria have elevated B2M (0.22-2.88 mg/L).
Conclusion: Obtained result showed that urinary MA has an advantage in comparison to CysC and B2M as a markers of tubular disfunction. Frequency of positive test for MA (18%) in examined endemic village BEN patients is similar to previous few decades ago investigation. Values for MA about 100 mg/L obtained by the test strips, always has to be remeasured by exact quantitative method.

M287

SIMPLE HPLC METHOD FOR ROUTINE SINGLE PLASMA IOHEHEXOL DETERMINATION

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Background: The single plasma sample methods is widely used for later determing the gromelural filtration rate.The aim of the present study was to evaluate the potentaal simple HPLC method for the routine determination of iohexol for latter clearance deternination. Iohexol was determined in plasma samples withdravewen from patients, whose were admistrated iohexol, under controlled conditions in Department of Nefrology, Clinical Centre of Serbia. Method: Iohexol was determined in EDTA-plasma samples. 100 μL was deprotenized with 400 μL of 5% perchloric acid. 50 μL aliquots was injected automaticlly in isocratic HPLC line, consisted of: HPLC pump "BIO-Rad 1350T", autosampler "AS-100 BIO-RAD", UV detector "Lamda max 481 Waters". All chromatografic data were evaluated by "Chrom – Line 4.20. Bio-rad" program. Isocratic HPLC separation has been done on "Lichrospher 60 RP Merck", 125 X 4.6 mm with mobile phase cosisting of sodium dihidrogen phosphate solution, 60 mM, methanol 8% v/v, tetrahydrofuran 2 % v/v. Standard solutions (30- 1200 mg/L), has been made from iohexol supstance, delivered from "Sigma-Aldrich". Results: The basiline separation of exo and endo isomers was better than 2.0. Quantitative analysis has been performrd on assessment of peak heights, as a sum of heights of both iohexol isomers. The method was validated as linear in concentration range between 5.0 and 500.0 mg/L.Within run precision for "low" sample concentration, as 50 mg/L was 4.8%, and for "high" sample concentration, as 400 mg/L, was 3.5%. Between run precision, for same concentration were 6.0% and 5.1%, respectively. Analitical and absolute recovery was 97% and 105%, respectively.
Conclusion: An accurate determination of iohexol concentration in plasma samples can be obtained, using this simple extraction and separation technique in biochemical laboratories which performed HPLC technique for different analitical purposes. This modification can be easily performed as, fast, reliable and cheap manner for iohexol determination and latter glomerular filtration ratio measurement.

M288

SUPERHIGHFLUX THERAPIES FOR HEMODIALYSIS: ULTRAFILTRATE PROTEOMIC PROFILE AND PROTEIN IDENTIFICATION BY ON-CHIP ELUTION

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Background: End-stage renal disease is often related with the uremic syndrome that leads to an increase in the morbidity and mortality rate. Uremia pathophysiological process is not completely understood but the retention of a high number of toxic solute compounds, normally eliminated by healthy kidneys, seems to play an important role. Uremic toxins are heterogeneous group of substances, including organic compounds and peptides. Hemodiafiltration with on-line endogenous reinfusion (HFR) dialytic method needs an optimal combination of the membrane permeability and cartridge resin bed. In this study, SELDI-TOF and ESI-QTOF Mass Spectrometries were used for protein profiles and identification of pre cartridge ultrafiltrate (UF) obtained from dialysed patients using three different membranes.
Methods: 25 dialysed patients were treated with three membranes with different albumin sieving coefficients: polyphenylene High Flux (pHF), polyphenylene Super High-Flux (pSHF) and Synclear 0.2 (Sync0.2). UF samples, taken at 30, 60, and 240 min during the dialysis, were loaded on ProteinChip and analysed by SELDI-TOF. Differences in protein profiles, in terms of molecular weight and peaks intensities, were detected by statistical analysis. Bounded proteins were eluted by a suitable buffer, and digested before ESI-QTOF analysis.
Results: Proteomic profiles of UF at 30 min of dialysis showed higher cluster peaks intensities (range 1.5-30 kDa) for the pHF respect to the pSHF and Sync 0.2 membranes. On the contrary, at 60 and 240 min, the cluster peaks intensities decreased for pHF and increased for pSHF and Sync0.2. Cluster peaks in the range of 30-60 kD and 60-100 kDa had the lower number of detected peaks and intensities, for the pHF, while the Sync0.2 showed the higher permeability, especially for the species >60 kDa, during all treatment. Several inflammatory proteins were identified by ESI-QTof.
Conclusions: The results of this study demonstrate that, compared to pHF and pSHF, the Sync0.2 membrane offers the higher permeability and efficiency, showing its potential use for the clearance of high molecular toxins. The on-chip elution is a method that allows obtaining protein identification that is crucial to better understand uremic disease pathophysiological processes.

M289
SERUM CYSTATIN C IN TRANSPLANTED PATIENTS TREATED WITH GLUCOCORTISOUID IMMUNOSUPPRESSION

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Background: The present study aimed to elucidate the influence of glucorticoid immunosuppression on Cystatin C concentration in serum in renal transplanted patients.
Methods: Clinically stable patients (38) on immunosuppressant therapy with low-dose glucocorticoids were matched with 30 clinically stable patients receiving cyclosporine A alone and 18 clinically stable patients receiving cyclosporin A together with azathioprine Serum Cystatin C was measured by a particle-enhanced turbidimetric immunoassay (PETIA; Dako) on a Cobas Mira (Roche) . Serum creatinine was measured with a modified kinetic Jaffe method. Creatinine clearance was estimated by the formula of Cockroft and Gault. The Cystatin C GFR was measured by the formula of Grubb (CysCGFR=84.69.CysC-1.680).
Results: Patients receiving long-term, low-dose glucocorticoid therapy showed higher cystatin C concentrations than controls (2,25;1.9-2.9, P <0.05). High-dose methylprednisolone given intravenously led to significant differences in Cystatin C values at different time points (before administration, after three doses, and 8 days after discontinuation; P <0.001). After three daily doses of 500 mg, Cystatin C concentrations increased from 2.13 mg/L (1.72–2.80) to 2.69 mg/L (2.34–3.5; P <0.05). Eight days after discontinuation, Cystatin C concentrations significantly decreased to 1.96 mg/L (1.63–2.4; P <0.05. At these time points, neither the CrCl estimate (54 ± 13 mL • min-1 • 1.73m-2, 51 ± 15 mL • min-1 • 1.73m-2, and 56 ± 14 mL • min-1 • 1.73m-2; P =0.05), nor the serum creatinine concentrations (165 μmol/L,158 μmol/L, and 162 μmol/L, P = 0.19) underwent significant changes.
Conclusion: Our data illustrate the need for specific reference intervals in patients on glucocorticoid therapy. In clinical routine settings, as well as in future clinical studies, it is important to take glucocorticoid medication into account when interpreting serum cystatin C concentrations in renal transplant patients presumably, in other patient groups.

M290
LEVEL OF CYSTATIN C IN PATIENTS WITH TYPE 2 DIABETES

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Background: Kidney failure can occur due to a number of causes. The laboratory marker that has long served as the mainstay for detecting impaired kidney function is serum creatinine. Serum creatinine is an insensitive marker of kidney injury, since it depends on sex, age and race. Furthermore, it does not reflect mildly diminshed renal function. Some of these shortcomings can be overcome by the creatinine clearance. However, creatinine clearance may be inaccurate because of tubular creatinine secretion and errors in specimen collection. Cystatin C seems to be a promising candidate to assess GFR, but also other functional chnages in the kidney and their progression. It is produced by all nucleated cells and is small enough to be freely filtered at the glomerulus and completely removed from blood. It is then fully catabolized in the tubules, meaning that its serum concentration depends mainly on GFR, because we aimed to determine is serum cystatin C a reliable marker of renal function in nephropathy caused by type 2 diabetes.
Methods: We investigated 31 patients with type 2 diabetes (15 men and 16 women), mean age 57.29 years. Duration of the illness was >5year. All individuals gave their informed consent for participation the study, accordnig to the Declaration of Helsinki. Laboratory investigations were performed in the Center for Medical Biochemistry at the Clinical Center of Serbia. All blood and urine samples were taken in the morning. Cystatin C serum concentration was determined by the PENIA method (Particle-Enhancesd Nephelometric Immuno-Assay), using the SIEMENS (Marburg, Germany) tests, on a laser nephelometer BN II. Results were statistically analyzed using the ANOVA.
Results: Mean serum creatinine, creatinine clearance and serum cystatin C values were 273,6 ±234,0 μmol/L, 58±54 mL/min, and 2,35±1,19 mg/L, respectively. Cystatin C was significantly reversely correlated with creatinine clearance (P <0,0001) and directly correlated with serum creatinine (P <0,0001) in diabetic patients with creatinine clearance under 60 mL/min. No correlation between these parameters was found in diabteic patients with creatinine clearance over 60 ml/min.
Conclusion: Cystatin C is a reliable marker of renal function in nephropathy caused by type 2 diabetes.

M291
CKD-EPI AND MDRD FORMULAS COMPARED

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Background: GFR is widely accepted as the best measure of kidney function. More recently, calculating estimated GFR (eGFR) using an empirical algorithm has been encouraged through the supply of a Creatinine Clearance (CLCR) test measured on a timed urine collection. From the 46 proposed equations, there are two validated ways to calculate eGFR expressed in ml/min/1.73 m2. The first one is the well known MDRD formula which estimates GFR adjusted for body-surface area. The second one is CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation that was developed in order to create a more precise formula, especially when actual GFR is > 60 mL/min. In this retrospective study we compared the formulas with CRCL.
Method: Consecutive laboratory data (CLCR, serum creatinine and eGFR-MDRD) from 9359 patients >18 years of age seen at Careggi Hospital between January 2008 and June 2010 were collected. On these data eGFR (CKD-EPI) calculations were performed. All creatinine measurements were performed with an assay calibrated to the isotope dilution mass spectrometry traceable method on the ADVIA 2400 platform using an enzymatic method (Siemens, Germany). Statistical analysis was performed using a Bland and Altman plot.
Results: The studied subjects (4807 M age 56,4±15,8, 4552 F age 55,7±16,5) were divided in two groups: subjects aged 18-65 years and over 65 years old. In the 18-65 age range subjects, the median and 5th and 95th percentiles of the distribution of the difference between CLCR and MDRD or CKD-EPI were respectively 8.95, -113, 164.8 and 9.4, -103.1, 164.4 mL/min (P=0.045), and -0.001, -105, 104.7 and -3.8, -84.8, 111.5 mL/min (P <0.005) in the over 65 age group. Considering only those patients with CRCL >90 in the 18-65 age ranged subjects, the median and 5th and 95th percentiles of the distribution of the difference between CLCR and MDRD or CKD-EPI were respectively 26.7, -110.8, 225.8, and 28.8, -84.8, 223.2 mL/min (P <0.005), and in the over 65 years group were 17.6, -91.6, 169.8 and 29.3, -42.8, 166.4 mL/min (P <0.005).
Conclusions: This study confirms the improved usefulness of GFR calculated with the CKD-EPI equation in respect to the MDRD formula, especially when actual GFR is > 60 mL/min and in over 65 aged patients.

M292
HYPERLIPIDEMIA MANAGEMENT IN KIDNEY TRANSPLANT PATIENTS

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Background: Post-transplant hyperlipidemia increases cardiovascular morbidity and mortality rate in kidney transplant patients. It also leads to graft loss due to atherosclerosis and glomerular damage. It is essential to control hyperlipidemia in kidney transplant patients to prevent these events.
Method: In our study, we determined lipid profiles in 59 kidney transplant patients. 20 of the patients had hyperlipidemia; 9 patients had type IV, and 11 patients had type II hyperlipoproteinemia. 14 patients were treated with American Phase 3 diet for one month and 6 of the patients received their regular diet as a control group.
Results: Lipid profile was normalized in 9 patients on diet. The lipid profile of 5 patients on diet did not change. These 5 diet resistant patients were given gemfibrozil (600 mg twice a day) for two months. At the end of therapy period, their cholesterol and triglyceride levels decreased significantly. No change was observed in LDL-cholesterol and HDL-cholesterol levels.
Conclusions: We conclude that American phase 3 diet and/or gemfibrozil are effective in controlling post-transplant hyperlipidemia in kidney transplant patients.

M293
HDL AND LDL SUBFRACTIONS IN CHRONIC RENAL FAILURE PATIENTS

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Aim: Although the close association between atherosclerosis and kidney dysfunction is indisputable, mechanisms augmenting formation of plaque in chronic renal failure (CRF) patients are still unclear. CRF is in connection with disturbances in lipoprotein metabolism. Relations of LDL and HDL cholesterol concentrations with coronary artery disease (CAD) are rather complex. Many patients with CAD have normal plasma LDL and HDL concentrations. These findings led interest to subfractions rather than serum levels of these lipoproteins. Changes in lipoprotein subclass distribution are linked to differences in atherosclerotic outcome. In the present study we investigated LDL and HDL subfractions in CRF patient.
Methods: A total of 21 CRF patients (8 female, 13 male, age: 50.4 ± 14.6 years) and 19 healthy control subjects (7 female, 12 male, age: 48.6 ± 13.4 years) were included. Identification of LDL and HDL subfractions were performed by an electrophoresis method on polyacrylamide gel, using the Lipoprint® system. The different bands corresponding to ten subfractions of HDL (large HDL 1-3, intermediate HDL 4-7, and small HDL 8-10) and ten subfractions of LDL (Mid-C, Mid–B, Mid-A and LDL-1 through 7) were excised and separated. Serum levels of total cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol were measured with enzymatic method.
Results: Serum levels of total cholesterol (182.70±10.42 vs 188.40±8.32 mg/dL, P >0.05), triglyceride (218.00±32.83 mg/dL vs 150.10±20.14, P >0.05) and LDL cholesterol (96.67±8.17 mg/dL vs 105.9±7.46, P >0.05) were similar, but CRF patients had significantly lower serum levels of HDL cholesterol (41.52± 2.91 mg/dL vs 52.84±2.35, p=0.005) than control subjects. In CRF group, HDL 10 (5.06±3.85 % vs 3.24±1.96, P=0.006), small HDL (14.67±8.25% vs 12.34±4.85, P=0.028) and LDL 3 (2.85±3.34% vs 1.25±1.53, P=0.0027) subfractions were significantly increased compared to control. Concentrations of other HDL and LDL subfractions did not differ significantly between the groups.
Conclusion: We demonstrated an increment in atherogenic LDL and HDL subfractions in CRF patients which suggested that the increment in these atherogenic groups may be important in development and progression of atherosclerosis in patients with CRF.

M294
THE RELATION OF OSTEOPROTEGERIN LEVELS WITH SOME CARDIAC PARAMETERS IN PATIENTS WITH CHRONIC RENAL FAILURE

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Aim: The mortality in chronic renal failure (CRF) patients is higher than that in normal population. Cardiovascular events are the major contributors in increased mortality rate in CRF patients since more than 50% of deaths are due to cardiovascular events. Osteoprotegerin (OPG) has recently been implicated in the regulation of vascular calcification since it inhibits RANKL–mediated osteoclastic bone resorption. Arterial calcification is a prominent feature of atherosclerosis and is associated with an increased risk of cardiovascular events. CRF patients are among high risk patients for the development of atherosclerosis with vascular calcification. In the present study we investigated the level of OPG and it's relation with some cardiac parameters in CRF patients.
Methods: A total of 51 CRF patients (21 female, 30 male, mean age: 50.9±16.5 years) and 41 healthy control subjects (22 female, 19 male, mean age: 45.7 ± 11.6 years) were included in the study. Serum levels of OPG (ELISA method), NT-ProBNP and hsTnT (ECLIA method), CRP (turbidimetric method) and albumin (spectrophotometric method) were measured in all study subjects. Correlations of OPG levels with ejection fraction, CRP and albumin levels and it's relation with left ventricular hypertrophy (LVH) and hypertension (HT) were also investigated.
Results: CRF patients had significantly higher serum levels of OPG (105.98 ± 33.76 vs 28.20 ± 3.78 pg/mL, P= 0.045), NT-ProBNP (5382.00 ± 1115.00 vs 77.00±9.99 pg/mL, P<0.00001), hsTnT (0.070 ± 0.010 vs 0.004 ± 0.001 pg/mL, P <0.00001), CRP (1.13±0.21 vs 0.15±0.08 mg/dL, P <0.00001) and significantly lower serum levels of albumin (3.93±0.08 vs 4.48 ± 0.03 g/dL, P=0.0015) than control subjects. In CRF patients serum OPG levels correlated significantly with CRP (r= 0.506, P <0.0001), albumin (r= -0.332, P=0.016) and ejection fraction (r=-0.657, P <0.0001) values. CRF patients with LVH or HT had significantly (P < 0.05 for both) higher levels of OPG compared to patients without LVH or HT.
Conclusion: The findings of higher OPG levels and it's significant relations with some clinical parameters of cardiovascular function suggested that serum OPG level might be a promising parameter in evaluation and follow up of cardiovascular function in CRF patients.

M295

ORAL PARICALCITOL IN PATIENTS WITH CHRONIC KIDNEY DISEASE STAGE 3-4 AND SECONDARY HYPERPARATHYROIDISM: A PRELIMINARY STUDY OF CLINICAL EXPERIENCE

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Background: Active vitamin D is an effective treatment for secondary hyperparathyroidism (SHPT) in chronic kidney disease (CKD) patients often complicated by hypercalcemia and hyperphosphatemia. Treatment with paricalcitol, a selective vitamin D receptor activator, has shown benefits by adequately reducing parathyroid hormone (PTH) levels with minimal changes in serum calcium (Ca) and phosphorus (P). The purpose of this study is to present data on the use of oral paricalcitol in real-life clinical practice in patients with CKD 3-4 and SHPT.
Methods: We studied 43 patients, M/F: 25/18, median age: 74 years (47-87), CKD stage 3/4: 16/27, with SHPT, who were prescribed oral paricalcitol at recommended doses for 6 months. Monthly measurements of serum intact PTH (iPTH) using immunochemiluminencence intact hormone assay (Modular E170, Roche Diagnostics Corporation, Indianapolis, IN, USA), Ca, P, alkaline phosphatase (ALP), hemoglobin, albumin (ALB), lipid profile using standard laboratory methods (Thermo Scientific Konelab Prime 60i Clinical Chemistry Analyzer), as well as proteinuria (nephelometric by the sulfosalicylic acid-sodium sulfate method) and 24-h urine creatinine clearance were performed 3 months before and 6 after treatment initiation.
Results: Paricalcitol induced a significant, early and sustained decrease in iPTH (282.6±134.4 at baseline vs 225.0±114.1 at 1st treatment month and 156.4±77.5 pg/mL at the study end, P <0.001). ALP levels showed a significant decrease from 4th treatment month (257.6±88.0 vs 234.3±79.8 U/L) until the study end (220.8±73.9 U/L). Serum ALB showed a significant increase by the end of the study period (3.66±0.45 vs 3.8±0.39 g/dL, P <0.001). No significant increase in Ca and P levels as well as in Ca×P product was observed during the study period. No significant changes were found in protein excretion, kidney function and the other measured parameters between baseline and last evaluation. Paricalcitol final median dose was 5 μg/week (range: 3-7).
Conclusions: In the context of real-life clinical practice, oral paricalcitol for 6 months is an effective, well-tolerated treatment of SHPT in CKD 3-4 with minimal effects on calcium and phosphorus metabolism

M296

NEUTROPHIL GELATINASE ASSOCIATED LIPOCALCIN (NGAL) – AN EARLY MARKER OF CONTRAST-INDUCED NEPHROPATHY IN PATIENTS FOLLOWING DIAGNOSTIC AND INTERVENTIONAL CARDIOLOGICAL PROCEDURES WITH CONTRAST MEDIA ADMINISTRATION

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Background: Contrast-induced nephropathy (CIN) is defined as acute kidney injury within 48 h of intravenous or intraarterial contrast media (CM) administration. CIN is identified by an absolute increase of ≥ 44 μmol/L from baseline creatinine value. Neutrophil Gelatinase Associated Lipocalcin (NGAL) is released by the kidney tubular cells in response to acute kidney injury. It is supposed that NGAL could be an early marker for CIN. The aim of this study is to incorporate a NGAL test in the everyday clinical practice for early detection of renal damage.
Methods: We used turbidimetry to measure NGAL in EDTA plasma of patients. The following reagents were used: NGAL Test Reagent, NGAL Test Calibrator and NGAL Test Control. All reagents were from BioPorto Diagnsotics, Denmark. The investigation was performed on Olympus 400 analyzer. The NGAL Test Reagent is a ready to use immunoparticle suspension containing polystyrene particles coated with anti-NGAL mouse monoclonal antibodies. Our study enrolled 12 high risk patients with arterial hypertension, diabetes and ischemic heart disease. All of them underwent invasive cardiological procedure with low-osmolar CM - Iohexol (100 to 350 mL) at National Heart Hospital, Sofia. Blood samples were collected before the angiographic procedure and at the 4-th and 24-th hours afterwards.
Results: The turbidimetric assay of NGAL demonstrated very good stability and low day-to-day reproducibility - VC = 7,2%. The baseline levels of NGAL were 108±48 ng/mL. In patients who had glomerular filtration (GF) rate under 60 mL/min an increase in the value of NGAL at the 24-th hour after administration of CM was observed. This event was accompanied by an additional decrease in glomerular filtration with 10%. CIN was observed in one patient – there was an early 20 ng/mL (baseline value 89.9 ng/mL) increase in NGAL value at the 4-th hour, about three times (268 ng/mL) increase of NGAL at the 24-th hour, a decrease in GF with 56%. Meanwhile, the increase in creatinine value was just 9.4 μmol/l at the 4th hour. The ≥44 μmol/L increase in creatinine value was observed at the 24-th hour.
Conclusions: CIN is associated with adverse long-term outcomes. NGAL testing allows for early identification of CIN.

M297

URINARY NGAL LEVELS IN HEALTHY SUBJECTS: HOW TO REPORT THE RESULTS? ABSOLUTE VALUE OR RATIO TO CREATININE?

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Background: NGAL is considered an emerging and sensitive biomarker of acute kidney injury (AKI) with diagnostic and prognostic significance. It can be reliably used in different clinical settings and in the screening of patients, prior to procedures putting them at higher risk for AKI, as the use of intravascular contrast media. It's still under debate, however, NGAL diagnostic ability in detecting situations of subclinical tubular damage, even if the assay in the urine seems to have better analytical and diagnostic performance than that in the plasma. The biological variability, the low specificity of the current kits for kidney-NGAL, make difficult to define accurate reference ranges and reliable diagnostic cut-off. Moreover for the lack of standardization and guidelines, the NGAL values are reported in literature in different measurement units. Then in this study we measured in a healthy population the urinary NGAL to evaluate the values distribution, expressed as "absolute" (NGAL) and as ratio to creatinine (NGAL/Cr).
Methods: 180 apparently healthy subjects (81W, age 31-78y), with normal renal function were studied. NGAL was measured on the second morning urine, by CMIA kit Abbott-Architect 2000i. Creatinine was measured by enzymatic method on Roche Cobas.
Results: The distribution of NGAL values was highly asymmetric and similar both expressed as "absolute" value (median 19.8; 95th perc 98.2 ng/mL) and as NGAL/Cr (median22.4; 95th perc 168.2 ng/mgCr). No significant difference was found between women and men, even if in women the creatinine was lower ($P < 0.01$). In 10 subjects, NGAL values reported in ng/ml were included within the 95th percentile, instead expressed in NGAL/Cr significantly overcame it.
Conclusions: NGAL values seem to have lower strength than those expressed as NGAL/Cr. NGAL in fact, is used on random urine sample and the ratio to creatinine reduces the intra-individual variation due to different osmolality. Up to date, the poor specificity of the kits together with the large variability in the population limit the predictive role of the single NGAL measure and confirm its usefulness only in longitudinal follow-up. Finally, we think that is more correct to report NGAL levels as NGAL/Cr.

M298

PROTEOMICS OF URINARY EXOSOMES: APPROACH TO BIOMARKERS DISCOVERY IN HEREDITARY TUBULOPATHIES

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Background: Urinary exosomes (UE) are nanovescicles released by every epithelial cell facing the urinary space and considered a promising source of molecular markers for renal dysfunction and injury. Proteomics has emerged as a powerful tool to understand the molecular composition of exosomes and to accelerate biomarker discovery. We applied this strategy to the study of hereditary renal tubulopathies (HRT), the Bartter and Gitelman syndromes, characterised by hypokalaemia, metabolic alkalosis and normotension. A specific gene is responsible for Gitelman disease, encoding the thiazide-sensitive Na-Cl cotransporter (NCCT) of the distal convoluted tubule, while many different genes have been shown involved in Bartter syndrome. Many of these disorders can be diagnosed by a combination of specific disturbances in plasma and urine parameters and by mutation identification. However, this field may also benefit from the developments in urinary exosomal proteomics. Therefore, the aim of this work is the isolation of urinary exosomes and the study of their protein composition in order to provide urinary biomarkers for HRT.
Methods: 2nd morning urines were collected from HRT patient, characterized by gene mutational analysis, and from age- and sex-matched control subjects. Urinary exosomes were isolated by ultracentrifugation, and characterized by immunoblotting (IB) with known markers. Proteomic analysis were performed by gel electrophoresis: classical SDS-PAGE and diagonal bidimensional electrophoresis to improve hydrophobic protein resolution; the disease-related transporters were analyzed by IB with specific antibodies.
Results: Results show that NCCT signal is absent in urinary exosomes from Gitelman patients, while present in control subjects' ones, and in other HRT patients', showing high sensitivity and specificity. We also investigated the Na-K-2Cl symporter, involved in Bartter I syndrome, and also its analysis by IB show a similar performance, allowing Bartter patients identification.
Conclusions. These findings suggest that urinary exosome isolation may provide an efficient first step in HRT biomarker discovery in urine. Grants from FIRB: Rete Nazionale per lo studio del proteoma umano (n. RBRN07BMCT).

M299
NEUTROPHIL GELATINASE-ASSOCIATED LIPOKALIN (NGAL) - AN EARLY MARKER OF ACUTE KIDNEY INJURY (AKI) IN PATIENTS UNDERGOING CARDIOVASCULAR SURGERY

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Background: One of the major complications after massive surgery using cardiopulmonary bypass long, is the development of acute kidney injury (AKI) are mostly composed of multiple organ failure (MOF). Along with the above in the etiology AKI important place syndrome, low emissions, the use of nephrotoxic drugs, reperfusion complications, bleeding, infection-related septic processes.
Delaying treatment AKI often due late diagnosis, since benchmarks are not specific enough and sensitive for early detection of kidney damage, which affects the prognosis of patients with AKI. In the widely discussed possibility of new laboratory markers of renal damage, one of which is NGAL (neutrophil gelatinase-associated lipokalin). Aim of this presentation is analyzed the results of the research level uNGAL patients after “open” heart surgery and blood vessels in long-term dynamics of the cardiopulmonary bypass (CPB) in the postoperative period.
Methods: A total of 48 adults and 9 children who underwent surgical treatment for hypothermia and long-CPB (120 min) Study uNGAL, creatinine and urea in the blood serum, the content of microalbumin in urine was carried out in three stages: 1 immediately after the operation, 2 - 2 h and 3 phase - 24 h after surgery.
Results: Our work clearly observed increase in u-NGAL 2 hours after the operation in the CPB, as well as significant (P <0.001) elevated levels of u-NGAL in this period in patients with a duration of more than 150 min CPB regarding patients whose duration CPB was 120-150 min. Later (after 4, 24 h) decreased concentration u-NGAL. In contrast, 24 h after cardiac surgery saw an increase in the levels of creatinine and urea in the blood and urine microalbumin in the examined patients.
Conclusions: The data suggest that the introduction of a new marker laboratory practice of renal damage-u-NGAL, allow earlier in the diagnosis and risk stratification of conduct AKI in patients after cardiac surgery with long-term CPB, more thoroughly assess the causes of complications, and to determine tactics of therapeutic measures and to monitor the effectiveness of the therapy.

M300
BIOLOGICAL VARIABILITY OF URINE NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) IN HEALTHY ADULTS

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) i s one of the emerging biomarkers for early detection of acute kidney injury. Rational clinical interpretation of any marker is impossible without thorough knowledge of biological variability and analytical precision. The aim of our study was to evaluate these characteristics for urine NGAL in 26 healthy middle-aged volunteers.
Methods: We enrolled 26 healthy volunteers (58 % of woman, median [IQR] age 36 [28.25 - 51] years). NGAL was measured with the automated immuno-assay from Abbott Laboratories (Abbott Park, Chicago, IL, USA) on the Architect I 2000 SR platform and expressed either as concentration in μg/L or as a ratio with urine creatinine (μg of NGAL/mmol of urine creatinine). Each participant served with 5 fresh urine samples collected within 1 day in following times: 6 AM, 10 AM, 14 PM, 18 PM and 22 PM. One of the volunteers was excluded from further analysis because of outlaying results (according to Reed´s criterion). Biological variabilities were calculated according to Fraser using nested ANOVA.
Results: Median [IQR] range of urine NGAL in all samples was 4.3 [1.8-8.4] μg/L (0.52 [0.52-1.29] μg/mmol of urine creatinine. Day-to-day analytical precision expressed as coefficient of variation was 5.5%. Intraindividual and interindividual variabilities of urine NGAL accounted for 90% and 4% resp. Ratio of urine NGAL to urine creatinine significantly decreased intra- and increased interindividual proportions of variabilities to 59.5% and 35%.
Conclusions: Intraindividual variability is the major source of total urine NGAL variability. However, ratio of urine NGAL to urine creatinine can decrease proportion of this variability.
Supported by the project Ministry of Health, Czech Republic for conceptual development of research organization 00669806 - Faculty Hospital in Pilsen, Czech Republic.

M301

FACTORS OTHER THAN THE GLOMERULAR FILTRATION RATE THAT DETERMINE THE SERUM BETA-2-MICROGLOBULIN LEVEL: IS THERE AN ASSOCIATION WITH NUTRITIONAL STATUS AND OTHER PARAMETERS?

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Background: Beta-2-microglobulin is increasingly investigated as a diagnostic marker of kidney function and a prognostic marker of adverse outcomes. To date, non-renal determinants of beta-2-microglobulin levels, including nutritional status, have not been well described.
Methods: This cross-sectional analysis was performed within the framework of the www.seniorlabor.ch study, which includes subjectively healthy individuals aged >60 years. Nutritional status (assessed with geriatric nutritional risk index, GNRI) and other factors known to have a non-renal influence on kidney function markers were investigated for a non-renal association with serum-beta-2-microglobulin. As a marker of kidney function, cystatin C based estimated glomerular filtration rate (eGFR) was employed.
Results: A total of 1250 participants (690 females and 590 males) were enrolled in the study. The use of a multivariate regression model revealed that beta-2-microglobulin levels, unlike creatinine levels, did not show a significant association with the GNRI. Nevertheless, beta-2-microglobulin levels were significantly associated with body weight, gender, TSH, CRP and systolic blood pressure independent of the eGFR.
Conclusions: Although not associated with nutritional status, serum-beta-2-microglobulin levels are related to several other non-renal factors, similar to those known to be related to the levels of cystatin C and creatinine. This result suggests that the beta-2-microglobulin level will not be able to serve as a valuable, alternative parameter to the levels of creatinine and cystatin C when estimating the GFR in routine clinical practice.

M302

NEUTROPHIL GELATINASE – ASSOCIATED LIPOCALIN (NGAL) AS A BIOMARKER FOR ACUTE KIDNEY INJURY, MORBIDITY AND MORTALITY AFTER PEDIATRIC CARDIAC SURGERY

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Background: AKI after cardiac surgery is one of the most serious postoperative complications. Although, in clinical practice, AKI is typically diagnosed by measuring serum creatinine, it is an unreliable indicator during acute changes in kidney function.
Methods: All selected individuals were subjected to the following: Full history , Complete clinical examination , renal function tests , plasma and salivery samples at baseline and at frequent intervals: (6, 24, 48 h) after initiation of CPB for measuring NGAL by ELISA using an established and validated assay.
Results: The present study was conducted on a total number of 20 pediatric patients who undergone elective CPB and 20 healthy individuals representing the study control group,and as regards to post-operative AKI development the studied patients were divided into two subgroups:AKI subgroup: 8 cases (40%) developed AKI, Non-AKI subgroup: 12 cases (60%) passed without AKI. The study showed no significant difference between AKI and Non-AKI subgroups as regards to serum creatinine levels 6 h after CPB ,While its levels after 24 (0.67±0.17 mg/dL) and 48 h (1.43±0.85 mg/dL) were significantly higher in AKI subgroup.The study showed no significant differences between AKI (325.13±50.61 ng/mL) and Non-AKI subgroups (278.95 ± 123.27 ng/mL) as regards to plasma NGAL levels after 6 hours of CPB. Whereas, our results showed that AKI subgroup had significantly higher plasma NGAL levels 24 hours after CPB than in Non-AKI subgroup ($p < 0.001$). In our study the mean saliva NGAL value after 6 h of CPB in Non-AKI subgroup was (864.45 ±21.39 ng/mL) and was (1413.93±414.17 ng/mL) in those developed AKI which was statistically significant difference. ($P < 0.001$)
Conclusions: Plasma NGAL is a promising diagnostic tool; the 24 hours plasma NGAL can diagnose AKI after CPB earlier one day than serum creatinine, which is of great significance for early intervention.Saliva NGAL is a highly promising diagnostic tool; it can diagnose post-operative AKI as very early as 6 hours after CPB which is more beneficial than even the 24 hours plasma NGAL.

M303

TUBULAR REABSORPTION OF PROTEINS ACCORDING TO A TUBULAR DAMAGE MARKER IN GLOMERULONEPHRITIS

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Background: Proteinuria is the hallmark of glomerulonephritis (GN) and a factor responsible of further renal damage as filtered proteins upregulate imflammatory molecules in proximal tubular cells (PTEC) with induction of interstitial fibrosis and nephron loss. Proteinuria is dependent on two mechanisms: 1) glomerular loss by damaged glomerular filtration barrier; 2) tubular reabsorption by PTEC. Aim of study: evaluate tubular reabsorption of proteins of different molecular weight (MW) [α2-macroglobulin/urinary creatinine ratio (α2m/C, 720 kDa), Fractional Excretion (FE) of IgG (150 kDa), Transferrin (Tf 78 kDa), Albumin (Alb 68 kDa), α1-microglobulin (α1m 31.8 kDa)] according to the tubular damage marker βNAG/C/eGFR [βNAG/urinary creatinine ratio divided by estimated glomerular filtration rate (eGFR) as βNAG excretion is dependent on surviving nephrons] in 188 GN patients with Focal Segmental Glomerulosclerosis (FSGS), Membranous Nephropathy (MN), Membrano-Prolipherative GN (MPGN). Crescenting IgA Nephropathy (CIgAN).
Methods: Proteins have been measured by immunonephelometry (BN II); βNAG by colorimetric method at 580 nm. The excretion values of all proteins have been compared between 4° and 1° quartile of βNAG/C/eGFR.
Results: In 4° vs 1° quartile α2m/C excretion is 63, 59, 58 and 31-fold higher in CIgAN, MPGN, MN, FSGS, respectively (mean 53); FE IgG is 76, 40, 27, 18-fold higher (mean 40); FE Tf is 47, 17, 12 and 11-fold higher (mean 22); FE Alb is 36, 13, 10, and 12-fold higher (mean 18); FE α1m is 25, 15, 7, and 5-fold higher (mean value 13). Reduction of reabsorption is about double for α2m/C and FE IgG vs FE Tf, FE Alb and FE α1m.
Conclusions: Different reabsorption may be dependent on different expression/efficiency of the tubular molecular machinery for reabsorption: α1m is normally freely filtered and completely reabsorbed by PTEC, indicating a very efficient reabsorption mechanism; albumin loss is normally very low and almost completely reabsorbed, indicating efficient mechanism for low excretion values; α2m and IgG are normally absent in urine; their reabsoption machinery may be less efficient. Reduced reabsorption of high MW proteins is an ominous factor as progression to renal failure is associated with high MW proteins excretion.

M304

ASSOCIATION OF SERUM HEPCIDIN WITH MARKERS OF INFLAMMATION AND ATHEROSCLEROSIS IN PERITONEAL DIALYSIS PATIENTS

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Background: Hepcidin is a small peptide produced by liver, which plays a significant role in iron homeostasis, by reducing the amount of circulating iron. It has been suggested that hepcidin may provide a link between inflammation and anemia in renal failure patients. In this study, hepcidin levels were assessed in serum of peritoneal dialysis (PD) patients in comparison to healthy individuals (NC) and their correlation with C-reactive protein (CRP) and lipid concentrations was evaluated.
Methods: Twenty-seven patients with chronic kidney disease on peritoneal dialysis (17M /15 F, 59 ±15 years) without clinical manifestations of inflammation and twenty-nine healthy controls (10M /19 F, 66±16 years) were recruited. Serum hepcidin levels were measured by competitive ELISA method and high-sensitivity CRP by nephelometry.
Results: In PD patients, hepcidin levels were significantly higher than in NC (313.7±32.7 vs. 131.4±55.9 ng/mL, mean ±SD, P <0.001). Hepcidin in patients was positively correlated to CRP (r=0.384, P=0.048) and triglycerides (r=0.398, P=0.040), while it was negatively correlated to HDL-C (r=-0.385, P=0.047) and apo-A (r=-0.383, P=0.048), as defined by simple Pearson's correlation analysis. Multiple regression analysis showed that hepcidin was independently related only to CRP and triglycerides levels.
Conclusions: Elevated hepcidin in serum of peritoneal dialysis patients may be associated to inflammation but not to the alterations of the classical lipid- associated markers of atherosclerosis.

M305

MEASUREMENT OF PLASMA AND ERYTHROCYTE MEMBRANE FATTY ACIDS IN END STAGE RENAL DISEASE PATIENTS UNDERGOING HEMODIALYSIS AND EVALUATION OF RELATIONSHIP WITH COMPLICATIONS

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Background: Patients with end-stage renal disease (ESRD) accompanied by type II Diabetes Mellitus (DM), including those undergoing hemodialysis (HD), have a high risk for death, particularly from cardiovascular causes. Omega-3 fatty acids (ω-3 FAs) exert anti-inflammatory actions by inhibiting pro-inflammatory signal transduction pathways whereas ω-6 FA (C20: 4 ω-6) facilitates inflammation by mediating inflammatory signals and serving as precursor of pro-inflammatory eicosanoids. In this study, it was aimed to evaluate the relationship between plasma and erythrocyte membrane (EM) FAs and complications observed in ESRD patients undergoing HD.
Methods: 32 ESRD patients undergoing HD treatment in Gulhane School of Medicine (15 with type II DM) and 29 control patients (17 with type II DM, 12 healthy controls with no chronic disease) have been included in the study. A modified extraction procedure was adopted to extract the lipids from plasma and EM, followed by transesterification to FA methyl esters using methanol and concentrated hydrochloric acid. Afterward, C14, C15, C16, C16:1, C18, C18:1 ω-9, C18:2 ω-6, C20, C22, C20:3 ω-6, C20:4 ω-6, C24, C20:5 ω-3, C24:1, C22:6 ω-3 FAs are determined by gas chromatography-flame ionization detector.
Results: Plasma and EM C18 and ω-6 (only C20:4 ω-6) FAs levels, besides membrane C16, C16:1, C24:1, C22:6 ω-3, C20:4 ω-6, C18:1 ω-9. FAs levels were significantly lower in ESRD patients than healthy controls (p 0.01). Additionally, plasma C14 and ω-6 (both of C20:4 ω-6 and C20:3 ω-6) FAs levels were significantly lower whereas C22, C24 FAs levels were significantly higher in all patient groups than healthy controls (P <0.01). When ω-3/ω-6 ratio was assessed, EM ω-3/ω-6 ratio in patients with ESRD+type II DM and only with type II DM were significantly lower than controls (P <0.01).
Conclusions: Our results show that there are FA abnormalities and especially a depletion in EM ω-3/ω-6 ratio in patients with ESRD+type II DM and only with type II DM as distinct from patients only with ESRD. And this is thought to be associated with the development of complications secondary to inflammation, particularly cardiovascular pathologies, in patients with ESRD accompanied by type II DM and treated with HD.

M306

LOW RATES OF AUTOMATIC-REPORTING OF ESTIMATED GLOMERULAR FILTRATION RATE IN SOUTHERN-BRAZILIAN LABORATORIES

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Background: Automatic reporting of estimated glomerular filtration rate (eGFR) is now formerly recommended for clinical laboratories in order to increase the detection of chronic kidney disease (CKD) and improve its clinical outcomes. Different countries inform highly variable rates of eGFR reporting, ranging from 10 to 90%. The aim of this study was to analyze the proportion of laboratories that routinely report eGFR in Southern-Brazil.
Methods: A cross-sectional survey was conducted in a representative sample of clinical laboratories of the state of Rio Grande do Sul (RS), the southernmost state in Brazil, from July 2010 to July 2012. A standardized questionnaire to obtain eGFR automatic reporting (Modification of Diet in Renal Disease [MDRD] and/or Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] creatinine-based equations) was given out by mail or email. Eight hundred and eighty (880) clinical pathology laboratories are registered in Regional Pharmacy Council of the state.
Results: Five hundred and fifty laboratories, equally distributed in the different state regions, completed the questionnaire. eGFR was automatically reported by 54 (9.8%) laboratories and, of these, 94% reported MDRD equation and 2%, CKD-EPI equation. Jaffe method was the most employed procedure to measure serum creatinine (94% of laboratories).
Conclusion: The automatic eGFR reporting rate is unacceptably low in our state, pointing out the crucial importance of educating patients, medical teams and laboratories about the magnitude of having these tools available to optimize renal disease detection and proper treatment.

M307

PRESENCE OF ANEMIA IN RENAL TRANSPLANT PATIENTS UNDER IMMUNOSUPPRESSIVE DRUG THERAPY

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Background: The aim of the study was to investigate the degree of anemia under tacrolimus treatment in comparison to cyclosporine (CsA) treatment in patients after renal transplantation.
Methods: We examened 25 kidney transplant recipients who were under immunosuppressive therapy. The renal function tests such as blood urea, serum creatinine and creatinine clearance and the hematological tests such as Hb,Htc and white blood cell count (WBC) were carried out at the Center for Medical biochemistry, Clinical center of Nis. Student t-tests were used to compare the difference between anemic and non-anemic patients and analysis of variance (ANOVA) was used for comparing indicators of anemia (i.e. Hb and Htc) in the groups of patients according to the immunosuppressive treatments which they received.
Results: Anemia was defined as Hb <12 g/dL for male patients and Hb <11 g/dL for female patients consistent with the gender-specific K/DOQI (Kidney Disease Outcomes Quality Initiative) guidelines. We identified anemia in 25% of patients. The anemic patients had a significantly higher mean blood urea and serum creatinine levels and lower mean creatinine clearance level than the non-anemic patients. Among the immunosuppressant drugs, patients on tacrolimus had significantly lower Hb and Htc compared with patients receiving cyclosporine. The presence of a hemoglobin <12 g/dL at 3 months after kidney transplantation is a major risk factor for persistent anemia at the end of the first posttransplant year. Anemia at 12 months after transplantation is independently associated with reduced patient survival.
Conclusions: We have shown that anemia is a common complication in kidney recipients who are under tacrolimus immunosupressive therapy. Immunosuppressive drugs contribute to the development of anemia through a variety of mechanisms. Most of the evidence suggests that impared erytropoetin production by the renal allograft is the most important pathogenic factor of post-transplant anemia.

M308

ESTABLISHMENT OF AGE AND GENDER DEPENDENT REFERENCE VALUES FOR NOVEL URINARY BIOMARKERS FOR RENAL DAMAGE IN THE HEALTHY POPULATION

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Background: Recent research has focused on the discovery of novel urinary biomarkers for kidney damage. Amongst others, urinary KIM-1 and NGAL are promising biomarkers in a wide variety of renal pathologies. However, little is known about the normal biomarker concentrations in urine of healthy subjects. Therefore, the goal of our study was to establish reference values for urinary KIM-1, NGAL, NAG and Cystatin C in a healthy population, taking into account possible effects of age and gender.
Methods: Urine samples were collected from 338 healthy, non-smoking men and women between 0 and 95 years old. KIM-1, NGAL and Cystatin C levels were determined by means of commercially available sandwich ELISA. Urinary NAG levels were determined colorimetrically. Creatinine levels and specific gravity of urine samples were determined, in order to correct for urinary dilution, via the kinetic Jaffe method (compensated rate-blanked) and by refractometry respectively.
Results: Absolute values as well as values corrected for specific gravity of KIM-1, NAG and NGAL were significantly associated with age ($P < 0.01$). After correction for creatinine a significant age effect was present for all 4 biomarkers ($P < 0.0001$). A significant effect of gender was observed for absolute values of KIM-1, NAG and Cys C ($P < 0.05$). After normalization for creatinine, sex only affected NGAL and NAG ($P < 0.01$). Gender and values normalized for specific gravity were significantly associated for KIM-1 and Cys C ($P < 0.01$).
Conclusions: Age and gender have distinct effects on KIM-1, NGAL, NAG and cystatin. Based on this knowledge, age and gender specific reference values for KIM-1, NGAL, NAG and Cystatin C were established.

M309

THE INFLUENCE OF LP(A) IN ATHEROSCLEROTIC RISK AND HYPERCOAGULABILITY IN HEMODIALYSIS PATIENTS WITH ACTIVATED ACUTE PHASE RESPONSE

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Background: Patients on chronic hemodialysis, have one of the highest risks for atherosclerosis, which has been characterized as an inflammatory disease. Lp(a) is important risk factor for cardiovascular disease in general population, as well as in dialysis patients. The impaired haemostasis which is common in this group of patients increase their cardiovascular risk. The aim of this study was to explore in patients on chronic hemodialysis, the correlation of Lp(a) elevated levels influenced by activated acute phase response (APR) with HDL, serum albumin, fibrinogen and D-dimer.
Methods: In 50 hemodialysis patients (27 male and 23 female) with CRP levels over than 10 mg/L, Lpa was determined in relation to HDL-C, serum albumin, D-dimer and fibrinogen. The same parameters were determined in 30 healthy people as a control group. The serum concentration of CRP was measured by the turbidimetric method based in combines of CRP with specific antibody. Diazyme's Lipoprotein (a) assay is based on a latex enhanced immunoturbidimetric method, whereas D-dimer by immuno turbidimetric assay with monoclonal antibodies F(ab)2 fragments to the D-dimer epitope. HDL-C was measured after precipitation with MgCl2. Fibrinogen was determinated based on Clausse's method by measuring of ratio of fibrin formation. Measurement of serum albumin was carried out by a timed endpoint method, using the bromocresol purple.
Results: Serum concentration of CRP, Lp(a), fibrinogen and D-dimer in hemodialysis patients were significantly higher than in controls (34,5 mg/L versus 9,5 mg/L, P <0.001; 29.34 mg/dL versus 18.22 mg/dL, P <0.001; 3.99 g/L versus 2.58 g/L, P <0.001; and 1.69 μg/mL versus 0.51 μg/mL, P <0.001). Concentration of HDL-C and serum albumin was significantly lower in hemodialysis patients than in controls (0.91 mmol/L versus 1.29, P <0.01 27 g/L versus 40 g/L; P <0.001). Lp(a) correlated positively with D-dimer (r= 0.37) in patients with elevated CRP and negatively with HDL-C (r= - 0,53) and serum albumin (r= -0.57) but not in patients with CRP in normal range and healthy controls.
Conclusions: In hemodialysis patients with APR, high levels of Lp(a) increase atherogenic risk profile namely elevated fibrinogen and D-dimer as well as decreased HDL-C and serum albumin.

M310

THE ROLE OF THE BIOCHEMISTRY LABORATORY IN IMPROVING PATIENT DETECTION OF PATIENTS WITH ACUTE KIDNEY INJURY STAGE 3

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Acute kidney injury (AKI) has now replaced the term acute renal failure and a universal definition and staging system has been proposed to allow early detection and management of AKI which affects up to 20% of hospital admissions. Retrospective studies have demonstrated a linear relationship between mortality and AKI grade. AKI stage 3 represents a powerful predictor of inpatient mortality and is a risk factor for chronic kidney disease. In 2009,the UK National Confidential Enquiry into Patient Outcome and Death report entitled "Adding Insult to Injury" showed serious deficiencies in AKI care in UK hospitals and as a result an AKI network was established in North Central London to try and rectify this situation. In early 2011 following a meeting between the Renal Consultants and Consultant Biochemist it was agreed that early each weekday morning a search would be made of those patients unknown to the renal team with a serum creatinine above 300 umol/l with their details e mailed to the Renal Consultant responsible for the inpatients and these would be copied to the renal registrars to enable follow up of these patients.Patients over the weekend would be alerted early on a monday morning. The management and outcome of those patients fulfilling criteria for AKI stage 3 were audited for each day during 2011. Of the 282 patients generated by the alert system,123 (43.6%) comprising 80 males and 43 females with ages ranging from 21 to 98 years with an average age of 70.2 years fulfilled the criteria. Although there was no improvement in the mortality data when compared to the year prior to introduction of the alert system perhaps as abnormal renal function may represent the end stage of an underlying disease process,the alert system identified more patients with AKI stage 3 at an earlier stage and improved the chances of full renal recovery. 30 patients (24.4%) made a partial recovery and 62 (50.4%) a full recovery. We therefore feel that this alert system has been beneficial as a result of the renal team being involved at an earlier stage of management of such patients.

M311

PLASMA NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AS A MARKER FOR EARLY DETECTION OF ACUTE KIDNEY INJURY AFTER CARDIOPULMONARY BYPASS IN KOREAN CARDIAC PATIENTS

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Background: Acute kidney injury (AKI), previously called acute renal failure, is a rapid loss of kidney function. AKI development after cardiopulmonary bypass (CPB) procedure is associated with increased postoperative morbidity and mortality in patients with cardiac surgery. Recently, plasma neutrophil gelatinase-associated lipocalin (NGAL) level appears to be of diagnostic value for prediction of AKI development. We investigated clinical usefulness of NGAL as a predictor of AKI development in patients with cardiac surgery.
Methods: Eighty-nine adult patients undergoing cardiac surgery with CPB were included. Pre- and postoperative blood samples were analyzed by a Biosite Triage plasma NGAL test (Alere Medical, San Diego, CA). Postoperative samples were collected at the time of intensive care unit (ICU) arrival after surgery completed. AKI development was defined as an absolute increase in serum creatinine of more than or equal to 0.3 mg/dL, or a percentage increase in serum creatinine of more than or equal to 50% after undergoing CPB.
Results: In patients with AKI developed (n=10), median value of postoperative NGAL levels on ICU arrival was significant higher than that of preoperative NGAL levels [223 (range, 79-576) vs. 108 (49-516) ng/mL, P=0.043, Wilcoxon rank sum test]. However, creatinine concentrations (median) showed no significant change between the ICU arrival and the baseline [1.01 (range, 0.7-1.7) vs. 0.99 (0.77-1.80) mg/dL, P=0.393]. ROC area of postoperative NGAL levels on ICU arrival (n=89) to predict AKI development was 0.824 (95% CI, 0.729-0.897) with a cut-off of 168 ng/mL (sensitivity, 70.0%; specificity, 88.6%). Postoperative NGAL levels on ICU arrival showed a significant independent predictor of AKI development after cardiac surgery in multivariate logistic regression analysis with various clinical factors (age, sex, creatinine concentration, CPB time and NGAL level) as covariates (P = 0.016; odds ratio, 1.006; 95% CI, 1.001-1.011).
Conclusions: This study showed that postoperative plasma NGAL levels on ICU arrival were significantly increased in AKI developed patients, with a good diagnostic performance. The plasma NGAL could be used as an early biomarker for detection of AKI development following CPB in Korean cardiac patients.

M312

TAURINE METABOLISM IN PATIENTS EARLY FOLLOWING KIDNEY TRANSPLANTATION

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Background: Ischemia/reperfusion injury (IRI) remains a major problem in renal transplantation. Recent experimental animal studies have shown that body taurine (Tau) is a major determinant of renal IRI. The goal of the present study was to examine plasma Tau and related compounds early following kidney transplantation, comparing alterations in kidney recipients (KR) with living kidney donors (LKD).
Methods: Arterial blood samples were taken from KR and LKD before, after anesthesia and surgery (0-time) and 1, 2, 3, 7 and 14 days after transplantation. KR got triple immunosuppressive therapy consisting of steroids, mycophenolate mofetil and tacrolimus, according to the protocol. Plasma tacrolimus level was measured by chemiluminescent microparticle immunoassay. In both groups of patients Tau, cysteine (Cys) and methionine (Met) levels were analyzed in plasma samples by ion-exchange chromatography (automatic amino acid analyzer model AAA-400, Ingos, Czech Republic). Molar Tau/Cys and Tau/Met ratio was used to evaluate Tau synthesis. The results are presented as mean ±SE Two-way analysis of variance and regression analyses were used for statistical evaluation.
Results: Before surgery plasma Tau level was higher ($P < 0.05$) in KR (68.1±3.6 μmol/L) than in LKD (56.0±5.5 μmol/L), while at 0-time its level increased in both groups reaching 99.7±5.6 and 115.9±11.5 μmol/L in LKD and KR, respectively. In the later time intervals gradual normalization of plasma Tau in LKD occurred. However, in KR reduction of plasma Tau to the level representing almost half of the baseline level in healthy subjects occurred. Increase of both Tau/Cys and Tau/Met ratios in KR suggest decrease of Tau synthesis early following transplantation. In addition, Tau level negatively correlated with administrated tacrolimus ($r = -0.263$, $P < 0.039$) and its plasma level ($r = -0.363$, $P < 0.004$).
Conclusions: Both nephrectomy and kidney transplantation produced transient increase in circulating Tau immediately after surgery. Within later time-intervals normalization of Tau level in LKD occurred, while kidney transplantation resulted in increased reduction of Tau level, mainly due to its decreased synthesis, probably caused by immunosuppressive therapy.

M313

ALTERATIONS OF GLOMERULAR FILTRATION RATE IN RECIPIENTS AND LIVING DONORS EARLY FOLLOWING KIDNEY TRANSPLANTATION

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Background: Estimation of glomerular filtration rate (GFR) is important for detecting and staging renal function. In this study we estimated GFR in kidney recipients (KR) and living kidney donors (LKD) before and in several time intervals during the early period after kidney transplantation.
Methods: Plasma urea, creatinine, and albumin levels were analyzed in blood samples taken from KR and LKD before, after anesthesia and surgery (0-time) and 1, 2, 3, 7 and 14 days after surgery. For GFR estimation the following equation: GFR=170 x (SCr)-0.999 x (age)-0.176 x (BUN)-0.170 x (Alb)0.318 x (0.762 for women) x (1.18 for black) was used. The results are presented as mean±SE. Two-way analysis of variance was used for statistical evaluation.
Results: Before surgeries in both groups of patients albumin was within referent interval, while plasma creatinine and urea levels were several times higher in KR than in LKD. At this baseline level estimated GFRs were 85.1±22.1 and 0.0 ±0.0 ml/min per 1.73m2 body surfaces in LKD and KR, respectively. After surgeries similar decrease in albumin levels in both groups occurred. On the other hand, kidney transplantation has produced gradual decrease and normalization of both plasma creatinine and urea while GFR increased up to 57.4 ml/min per 1.73m2 at the end of 2-weeks follow-up. Interestingly, during the examined period gradual increase of creatinine and concomitant decrease of GFR in LKD occurred.
Conclusions: Kidney transplantation has significantly improved GFR in KR, while nephrectomy has decreased it, suggesting moderate reduction in overall renal function in LKD. At the end of examined early period GFRs were similar in LKD and KR, but in the both groups of patients it was significantly bellow the level observed in healthy subjects, which has to be considered in their therapy.

M314

ELEVATED URINARY ARSENIC AND BLOOD LEAD LEVELS IN TURKISH MINE WORKERS

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Background: Exposure to heavy metals are known to have neurotoxic, nephrotoxic, immunotoxic and genotoxic effects on human populations, of varying degrees, depending on the dose. In some regions of Turkey, toxic heavy metal concentrations have been found in living organisms, but little information is available on heavy metal profiles of mine workers. In this study, our aim was to investigate the heavy metal profiles among mine workers, according to their work sections.
Methods: Blood and random urine samples were collected from 758 mine workers and 52 healthy control subjects. Blood lead, zinc, copper and urinary mercury, arsenic levels were analyzed by Inductively Coupled Plasma – Mass Spectrometer.
Results: Blood lead levels were significantly higher in workers of “crushing and screening”, “mineral characterization laboratory” sections compared to other departments ($P < 0.0001$) and urinary arsenic values were significantly higher in workers of “crushing and screening”, “drilling and blasting” sections compared to other departments ($P < 0.0001$).
Conclusions: In our best knowledge, this is the first study evaluating heavy metal profiles among mine workers in such a large occupational group in Turkey, and we plan to conduct longitudinal follow-up of the miners and report their progress periodically. It is imperative that occupational standards in mines should be improved in order to avoid further long-term exposure to heavy metals.

M315
THE FOLLOW-UP OF LEAD EXPOSURE IN TUNISIAN WORKPLACE

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Background: Lead poisoning is a preventable environmental disease. Long-term exposure to Pb can cause serious neurological and renal health problems. Pb intoxication is considered as the first professional disease in Tunisia. Therefore, the assessment of Pb poisoning of workers who have direct contact with Pb is crucial in term of occupational health monitoring. The aim of this study is the biological and toxicological follow up of 30 subjects working in a lead acid batteries industry.
Method: The population of this study consisted of 29 workmen and a woman. Five biological parameters was studied (blood Pb, urine Pb, 5 aminolaevulinic acid (ALA), ALA/Creat ratio). Blood and urinary Pb were analyzed by Electrothermal Atomic Absorption Spectrometry. 5 aminolaevulinic acid was determined by colorimetric method on an exchanging ions column.
Results: The standard profile of exposed workers was represented by male sex, average age (42 years old) and exposure duration (up to 40 years of exposure). The indicators of exposure were over the acceptable thresholds with 80% of the studied subjects having a blood-Pb concentration over 400 µg/L and a urine-Pb concentration over 100 µg/L. Regarding the clinical signs, 73.3% of the studied population suffered from neurological disorders and 70% presented abdominal syndromes among them 23,3% complained of Pb colics. Correlation between Pb exposure parameters has been studied. For more than half of the patients (66.7%), a good correlation was found between blood -Pb, urine-Pb and ALA/Creat. Results showed that 26.4% of workers with blood -Pb concentrations ranging between 400 and 600 µg/L had an ALA/Creat ratio lower than 5 mg/g of creatinin and 11.3% of workers with blood Pb exceeding 600 µg/L had also an ALA/Creat ratio less than 5 mg/g of creatinin. These results confirmed the non sensitivity of the ALA/Creat ratio.
Conclusion: The results of this investigation showed that the association of blood-Pb, urine-Pb and ALA/Creat ratio constitutes a reliable tool for the assessment of Pb exposure and suggested the necessity of introducing other parameters (urinary coproporphyrine, ALA deshydrogenase , heamoglobin and hematocrit) for a thorough interpretation of Pb exposure in occupational toxicology.

M316
CYANIDE POISONING AS A MODE OF SUICIDE

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Background: Cyanide is a powerful toxic agent used as suicidal but also as homicidal agent, particularly among health care and laboratory workers and they can potentially be used by the jewellers as polishing agents for gold. In this work, we report a voluntary fatal intoxication case by cyanide salt oral ingestion.
Methods: 25-year-young jeweler was found dead in his sale local few days after his disappearance. In autopsy, the external examination revealed an intense cyanosis of the face and conjunctiva hyperemia without evident trauma and external violence signs. Internal examination revealed congestion of inner organs, slight pulmonary edema and congestion of sub mucosal vessels of the upper respiratory tract. At the autopsy, sample of blood, urine, brown stomach content were obtained. Two flasks containing white and brown fluid were found nearly the victims were sent to the laboratory for examination and identification. Alcohol and abuse drugs were analyzed using enzymatic and gas chromatography/mass spectrometry technics. Cyanides were analyzed in the biological fluids and in fluid contained in the two flasks using potentiometric method based on ion-selective electrodes (ISE) (Jenway). Quantitative determination of cyanide was carried out in the linear concentration range between 6 and 1 ppm. A good inter-assay precision was obtained with a slope of the curve equal to 57.
Results: The measured blood alcohol concentration was 0.00 g/L. The cyanide concentration in urine, gastric contents and two liquids, white and brown were respectively 15.48, 146.2, 16.52 and 4.38 mg/L. These of cyanide Concentrations proved a massive oral ingestion of cyanide and were corralled with the clinical and the external and Internal autopsy examinations . In the present case a jeweler committed suicide by oral ingestion of potassium cyanide which was usually used by the victim for polishing the gold on sliver.
Conclusions: In this report case, the death was attributed to the toxicity of cyanide related probably with massive oral ingestion of cyanides salt. Fatal intoxication with cyanide salts is relatively rare and a treatment is largely described. The prognostic depend to the quantities and the moment of the hospitalization in intensive care.

M317

HARMONIZATION OF CARBOHYDRATE-DEFICIENT TRANSFERRIN MEASUREMENT: A MULTICENTER STUDY

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Background: Lack of standardization of Carbohydrate-Deficient Transferrin (CDT) represents a critical issue, particularly when CDT positivity could have legal consequences. In this multicenter study, CDT harmonization and repeatability were evaluated using a commercial kit and two different HPLC instrumentations.
Methods: 100 samples, calibrators and controls were measured by "%CDT by HPLC" commercial kit (Bio-Rad Laboratories), using Agilent (Laboratories 1-5) or Variant HPLC systems (Laboratories 6-10) in 10 runs. CDT was expressed as % of disialotransferrin (%CDT) (cut-off 1.7%). Raw and calibrated %CDT were compared to those obtained by the HPLC candidate reference method previously reported by Helander. Concordance was evaluated by Passing-Bablok regression, Bland-Altman analysis and Cohen's k. Within-laboratory repeatability was evaluated by Variance component analysis (2 levels, 2 replicates, 10 runs).
Results: Agilent systems (n=5): neither proportional (slope from 1.02 to 1.12) nor constant (intercept from -0.12 to 0.02) errors were evident for raw %CDT values, with mean bias from -0.16 to -0.01. However, after calibration, a statistically significant constant error (intercept from 0.08 to 0.29) was present, with an increase of the mean bias (from -0.21 to -0.38). Agreement was similar for raw (92-95%, k=0.83-0.89) and calibrated data (92-95%, k=0.83-0.90). Repeatability, at %CDT close to 0.9% and 3.0%, was respectively 3-15% and 1.5-11%. Variant systems (n=5): results were similar to data yielded by the Agilent systems (presence of a constant systematic error (intercept from -0.07 to 0.29) with increase of mean bias (up to values ranging from -0.17 to -0.45) after calibration). Agreement was similar before and after calibration (93-96%, k=0.85-0.92 vs 90-95%, k=0.80-0.90). Repeatability was respectively 6-15% (at 0.9%) and 4-13% (at 3.0%).
Conclusion: Good repeatability and high agreement between all laboratories and the candidate reference method were observed in this multicenter study independently by the HPLC instrumentation. According to our results the use of calibrators obtained by spiked serum does not improve the harmonization of %CDT; however, their use is strongly recommended when %CDT is measured by other analytical techniques.

M318

EVALUATION OF THE TRANSFERRIN GENETIC VARIANT FREQUENCIES IN THE POPULATION OF MODENA IN THE YEARS 2007-2011

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Background: The detection of Carbohydrate-deficient Transferrin (CDT), a marker for chronic alcohol abuse, is performed using chromatographic, electrophoretic and immunoassay methods. In these assays the presence of genetic variants (GV) may bring to misleading interpretations with false positive or negative results. There are at least 38 GV that differ by one or more amino acids. There are three types of transferrin with a prevalence greater than1%: Transferrin C is the most common one in the Caucasian population, and it has at least 16 known subtypes (C1-C16), of which C1 is the most prevelent (95%), Transferrin B presents a more anodic migration and Transferrin D has a more cathodic migration. It needs also to consider the possibile heterozygote associations of the various C, B and D forms. Our aim was to assess characterizing transferrin GV in consecutive serum patient samples for CDT analysis between 2007 and 2011 using Isoelectric focusing (IEF), the gold standard for the identification and classification of GV.
Methods: In our study we analyzed 20868 serum samples collected between 2007 and 2011. All our samples were tested using capillary electrophoresis (CZE) P/ACE MDQ Beckman Coulter using CEofix CTD Kit (Analis, Belgium) for routine analysis. In case of uncertain results (41) and in case of samples suggestive of GV (61), these samples were further analyzed using IEF/Immunoblot, by a specific anti-transferrin antibody (Dako) after neuraminidase (Clostridium perfringens, Sigma) digestion for 24 hours at 37°C, to eliminate the sialic acid residues from the protein chain.
Results: In our samples, we found 12 (0,7%) GV in 2007, 29 (0.74%) in 2008, 23 (0,49%) in 2009, 27 (0.52%) in 2010 and 29 (0.54%) in 2011, adding up to a total of 102 (0,49%).
Conclusions: Nevertheless the GV frequency was slightly lower than previously reports, the assessment of GV is mandatory when the test has medico-legal implications. The use of the described method, even not easy and time consuming for confirmation, allows us more precise and defendable results.

M319

EARLY RECURRENT ISCHEMIC LESION ON DIFFUSION-WEIGHTED MRI BASED ON THE CYTOCHROME P450 2C19 GENOTYPE IN STROKE PATIENTS TREATED WITH CLOPIDOGREL

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Background: The early recurrent ischemic lesions on diffusion-weighted magnetic resonance imaging (DW-MRI) scan of brain are commonly observed in patients with stroke. The recurrent lesions on DW-MRI have been used as a possible surrogate marker for clinical recurrence of stroke. Clopidogrel is an inhibitor of adenosine diphosphate-induced platelet aggregation and it is widely used for secondary prevention of stroke. The metabolism of clopidogrel is affected by cytochrome P450 2C19 (CYP2C19) genotypes. We aim to analyze the incidence of DW-MRI recurrence based on the CYP2C19 genotype in stroke patients treated with clopidogrel.
Methods: We enrolled 24 stroke patients treated with clopidogrel. The DW-MRI scan of the brain was carried out the onset of stroke and at subsequent times within the first week after stroke. The CYP2C19 genotyping was performed using the Verigene system (Nanosphere, USA). Depending on the CYP2C19 genotype, the CYP2C19 enzyme activity was classified into three groups including extensive metabolizer (*1/*1 or wild-type), intermediate metabolizer (*1/*2 and *1/*3) and poor metabolizer (*2/*2, *3/*3 and *2/*3). And we compared the incidence of DW-MRI recurrence based on the CYP2C19 alleles in patients with stroke.
Results: The overall DW-MRI recurrence rate was 33.3% (8 patients). Twenty four patients classified based on the CYP2C19 genotype into extensive metabolizer (n=88, 33.3%), intermediate metabolizer (n=10, 41.7%) and poor metabolizer (n=6, 25.0%), respectively. The highest incidence of DW-MRI recurrence was observed in poor metabolizer (66.7%), followed in descending order by intermediate metabolizer (40.0%), and extensive metabolizer (12.5%). The poor metabolizer revealed a significantly high incidence of DW-MRI recurrence than extensive metabolizer (P=0.09). In addition, the DW-MRI recurrence rates have largely been attributed to an increase a number of variant alleles of CYP2C19 (P=0.04).
Conclusions: In our small series of stroke patients treated with clopidogrel, the rate of early ischemic recurrent lesion on DW-MRI was higher in poor and intermediate metabolizer than extensive metabolizer. We believe that the consideration should be given to routine CYP2C19 genotype testing in stroke patients treated with clopidogrel.

M320

MICROHETEROGENEITY OF SERUM β-HEXOSAMINIDASE IN CHRONIC ALCOHOL ABUSERS

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Background: Serum β-hexosaminidase (β-HEX) has been put forward as a marker for chronic alcohol abuse. It was already suggested that increased serum activity of β-HEX B (isoforms B, I and P) may be a more sensitive marker. Although high specificities have been reported for alcohol abuse, increased levels of serum β-HEX activity were found in patients with cholestasis and liver diseases. In the latter, the rise in total β-HEX activity is mainly due to increases in P isoenzyme which exhibited a pI around pH 6.0. Preparative iso-electric focusing (IEF) enables us to quantify β-HEX activity in different fractions, each corresponding to a bracketed pH gradient. In this study we describe a novel method to quantify different β-HEX isoforms in serum of social drinkers and heavy alcohol abusers and investigate the correlation with carbohydrate-deficient transferrin (CDT).
Methods: CDT was assayed by CZE, measured on the Capillarys 2™ system. In 68 subjects, serum samples were separated in to 10 fractions, using preparative IEF, over a total pH gradient of 5.0 to 8.0. Afterwards total β-HEX activity was measured in each fraction using 4-methylumbelliferyl-2-acetamido-2-deoxy-b-D-glucopyranoside as a substrate. The total β-HEX activity in each fraction was compared between social drinkers (CDT and liver enzyme activities within normal range) and heavy alcohol abusers. Mean β-HEX activity between pH 6.8 and 7.7 and total β-HEX activity were compared to gamma-glutamyltransferase (GGT) and %CDT.
Results: Median β-HEX activity between pH 6.8 and 7.7, designated as HEX-7, of heavy alcohol abusers is significantly higher when comparing to social drinkers (P <0.0001). There is a highly significant correlation between HEX-7 and %CDT (r=0.75), which is significantly better when compared to β-HEX B activity (r=0.39) and GGT (r=0.39).
Conclusions: The data suggests that there is a cathodal shift of β-hexosaminidase in the serum of chronic alcohol abusers when compared to moderate and abstinent drinkers. It extends previous findings by of increased β-HEX activity in heavy drinkers. HEX-7 could be a more specific marker for chronic alcohol abuse, by exclusion of the β-HEX P isoform. The measurement of HEX-7 could proof to be of value for detecting chronic alcohol abuse.

M321

GENETIC BASIS OF ALCOHOL DEPENDENCE: TRI-ALLELIC POLYMORPHISM OF THE SEROTONIN TRANSPORTER GENE IN A POPULATION OF ALCOHOL DEPENDENT SUBJECTS FROM ITALY

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Background: The involvement of genetic variants in the neurobiology of addiction and in the pathways of reward circuits is well established. Serotonin transporter (5-HTT) regulate serotonergic neurotransmission and has been associated also with alcohol dependence (AD). Aim of this study is to assess a possible link between a tri-allelic polymorphism of the 5'-UTR region of 5-HTT gene (SLC6A4) and AD. This tri-allelic polymorphism is made up of the long (L) allele of 5-HTTLPR polymorphism together with the A (La) or G (Lg) allele of the rs25531 single nucleotide polymorphism (SNP) and the short allele (S). Lg and S are the low-functioning alleles.
Methods: Genotyping was performed by PCR amplification of the 5-HTTLPR region; this produces long (L) or short (S) amplicons depending on the variable number of repeated elements, that results, in most cases, in a 43 base pair insertion/deletion. The rs25531 genotype was obtained by an L specific PCR amplification followed by a mini sequencing reaction. We investigated 439 Italian alcoholic outpatients (male 350, female 89) at Alcohol Unit of Umberto I Hospital - Sapienza University of Rome and 423 healthy controls (male 285, female 138).
Results: From allelic point of view, no statistically significant difference could be evidenced between alcoholics and controls, considering either overall population or males separated from females. However, AD women showed higher frequency of both the L (56.2%) and La (51.7%) allele than control women (respectively 50.4% and 47.5%), although not statistically significant probably because of the smallness of the sample analysed. An higher frequency of the L allele statistically significant ($P < 0.05$), can be only observed in women with an early first contact with alcohol (≤16 yo, 63.5%) compared to control (50.4%).
Conclusions. According to our results, the 5HTTLPR and rs25531 polymorphisms of the serotonin transporter gene (SLC6A4) seems not to be generally involved in AD. However, considering males and females together can lead to underestimate important differences in allelic distribution. In fact, at least some categories of female patients show an higher percentage of "fully working" alleles (L and La). Genotypic analysis and studies of larger populations are mandatory.

M322

LABORATORY MEDICAL TECHNIQUES IN THE IMMUNOTOXICOLOGICAL CHARACTERIZATION OF CARBON-NANOTUBES

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Background: The carbon-nanotubes (CNT) are producing in an excessively amount and widely used in the different field of chemistry, biology and medical sciences. They have several advantageous chemical properties but their impact on the living cells is very controversial yet. Our purpose was to investigate the effect of nanotubes on the function of different types of blood cells. Beside these we studied the level of activation of complement- and hemostatic system.
Methods and materials: we investigated single walled CNT's functionalized in different ways. (CNT-OH, CNT-COOH) The CNT solutions (100 mg/mL final concentration) were applied on the human blood samples. We measured the rate of the cell activation (basophilic, dendritic cells, NK, monocytes, etc.) - after a short incubation - according to a flow cytometric protocol; and the generation of complement activation fragments (e.g. sC5b-C9) by ELISA method. Furthermore we studied the involvement of JAK/STAT signaling pathway in the process of "nanomaterial-derived" activation of human NK cells and monocytes.
Results: The expression rate of surface CD11c and CD123 (dendritic cell markers) was a slightly elevated in presence of CNT, independently the type of functionalization. The coagulation and complement activation parameters did not show any differences none of the examined nanoparticles compared with control samples. The CNT-OH had significantly greater basophilic activating capacity than the CNT-COOH according to the CD63 antigen expression. We didn't find any sign of activation of JAK/STAT signaling pathway in NK cells and monocytes in presence of CNT's.
Discussion: The CNT has a number of beneficial properties but unfortunately in most cases it has serious problems in biological applications. Our work pointed out, that the surface modification of nanomaterials (CNT also) determines the basophil activation. It could lead to a severe allergic reaction in the human body. In spite this we didn't observed dramatically changing in the antigen presenting DC cells and monocytes nor the rate of complement activation.
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M323
SYNERGISTIC ANTIBACTERIAL ACTIVITY OF ACACIA HORRIDA LEAF EXTRACTS IN COMBINATION WITH ANTIBIOTICS

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Aim of the study: The evaluation of the antibacterial activity of total oligomer flavonoids (TOF) and methanolic extracts from leaf of Acacia horrida against: Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus clinical strains, paired with antibiotic.
Methods and Results: Using MTT assay method, fractional inhibitory concentration (FIC) values were calculated to characterize interactions between antimicrobials and antibiotics. Substantial susceptibility of these bacteria toward the natural antimicrobials and a considerable reduction in the minimum inhibitory concentrations (MIC's) of the tested antibiotics, were noted when paired combinations of antimicrobials and antibiotics were used in the interaction study. Conclusions: We have showed that TOF and MeOH extracts affect the potent growth of some bacteria strains. This means that bacteria were sensitive to the studied plant derived natural antimicrobials. Also the extracts can have a synergistic effect in combination with some antibiotics. Significance and Impact of Study: In this paper we have examined the synergistic antibacterial activity of antibiotics in combination with Acacia horrida extracts, which could improve potentially the performance of antibiotics.

M324
SCREENING OF SEVERAL DRUGS OF ABUSE IN WORKPLACE DRUG TESTING: THE ROLE OF PUBLIC HEALTH LABORATORY ASL BRESCIA

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According to the Provision of the Government-Regions Conference,2008 some categories of workers entrusted with duties possibly costituting a threat to security, have to be screened to exclude the use/abuse of several drugs. This Provision requires that all laboratories testing urine specimens have to be certified and that they put into operation a chaine of custody, security and quality control procedures. It also requires that certified laboratories use immunoassay for initial testing and Gas-Chromatography/Mass Spectrometry (GC/MS) for confirmation. Cut-off values (the values serving as threshhold for evaluating a test result as positive) were manly dictated by this Provision. During the medical examination, the subject is questioned on drug-taking and is informed that he will be screened for drugs by a urine test. Within 24 h from the notification, as the law requires, workers provide a urine sample to be screened for opiate, methadone, buprenorphine, cocaine, amphetamines, ecstasy (MDMA), and cannabinoids. We examined and elaborated data collected on a large group of workers (8593 samples) involved in public/private transportation during the years from January 2010 to August 2012; these data generally included pre-employment, reasonable suspicion and post-accident testing. Specimen collection (urines) takes place in the laboratory or in various medical centres of the province. As a result of this study 103 out of 5120 workers (2,0%) screened positive; only 80 of them (1,6%) were confirmed with the GC/MS. Confirmed result included: cannabinoids 48 (0,9%), cocaine 17 (0,3%),methadone 5 (0,09%), buprenorphine 4 (0,08%). Only one out of 24 samples screened positive for amphetamines and ecstasy was confirmed MDMA with GC/MS. Drug testing is only one of a comprehensive workplace anti-drug program, which should also include a written and communicated policy, training for supervisors, education for employees and an employee-assistance resource.

M325

SERUM CONCENTRATION OF LIVER FUNCTION INDICATORS IN HIV PATIENTS ON DIFFERENT CLASSES OF HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

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Abstract: Backgroud of study: Soon after the introduction of HAART , drug related toxicities became recognized and well characterized, thus liver toxicity has become a growing problem among HIV patients on HAART hence this study.
Method: Liver function was assessed in 290 HIV patients {80 on Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)-based HAART, 80 on Nucleoside Reverse Transcriptase Inhibitors (NRTIs)-based HAART, 80 on Protease Inhibitors (PIs)-based HAART and 50 newly diagnosed and not on any antiretroviral medication serving as test control} using plasma concentration of alanine transaminase (ALT) and aspartate transaminase (AST) as test indicators. Each of the three groups on HAART were further subdivided into four subgroups of 3 months, 6 months, 12 months and >12 months based on the duration on HAART with each subgroup consisting of twenty patients. The ALT and AST concentration were determined using standard methods.
Result: When the patients on different classes of HAARTS were compared with the control group, the differences in enzyme (ALT and AST) activities were significantly higher in 3 and 6 months duration on HAART for all the three classes of HAART used. The NNRTIs and PIs-based groups also showed a significant difference only in ALT activity in the 12 months duration on HAART. When the ALT and AST activities of patients on NRTIs- based HAART group were compared with the NNRTIs and PIs- based HAART groups, statistically significant differences were observed only in the 3 and 6 months duration on HAART.
Conclusion: Conclusively HAART has a duration and drug dependent effects on the liver cells integrity and functions. This effect is lesser with NRTIs as compared with NNRTIs and PIs.

M326

STATUS OF LIVER FUNCTION TEST OF GASOLINE ATTENDANTS IN EDO CENTRAL ZONE OF EDO STATE, NIGERIA

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Backgroud: Extensive exposure to petrol fumes has been shown to be a significant population and health hazard. This present study investigated Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total bilirubin (T-BIL), Conjugated bilirubin (C-BIL) and Albumin (ALB) as liver function tests of gasoline attendants.
Method: A total number of ninety (90) subjects, comprising of sixty (60) gasoline attendants (35 males and 25 females) and thirty (30) apparently healthy non gasoline attendants volunteers as control(18 males and 12 females) were recruited for this study. ALT, AST, ALP, T-BIL, C-BIL and ALB concentration were determined using standard spectrophotometric methods.
Results: The mean values obtained for gasoline attendants were 12.16 + 0.83IU/L (ALT), 11.26 + 2.60IU/L (AST), 24.93 + 8.82IU/L (ALP), 1.16 + 0.37 mg/dL (T-BIL), 0.26 + 0.31 mg/dL (C-BIL) and 37.7 + 4.22 g/L (ALB), while the corresponding values of control subjects were 11.52 + 0.71 IU/L (ALT), 11.21 + 1.93 IU/L (AST), 23.97 + 7.68 IU/L (ALP), 1.18 + 0.39 mg/dL (T-BIL), 0.24 + 0.21 mg/dL (C-BIL) and 38.77 + 5.29 g/L (ALB). This study showed that there was no significant difference between the mean values of ALT, AST, ALP, T-BIL, C-BIL and ALB of gasoline attendants when compared with the controls ($p > 0.05$).
Conclusion: Conclusively, the parameters of liver functions investigated were within normal range in gasoline attendants in Edo central zone of Edo State, Nigeria.

M327
FLUORIDE EXCESS IN A PATIENT TRANSPLANTED BONE MARROW RECEIVING LONG-TERM VORICONAZOLE

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Background: Invasive fungal infections are an important cause of morbidity and mortality among patients with hematologic malignancies undergoing intensive chemotherapy with or without autologous or allogeneic hematopoietic stem cell transplantation. In most patients, the diagnosis of invasive aspergillosis triggers prolonged antifungal treatment voriconazole, a fluorinated triazole compound. A long-term voriconazole, as a risk factor for the development of fluoride excess and subsequent painful periostitis and exostoses in post transplant patients. In this work, we report a case for acute toxicity of voriconazole in a patient transplanted bone marrow who was receiving long-term therapy with this medication.
Methods: A patient (21 years) transplanted bone marrow following a medullary aplasia who complains periostitis and exostoses treated with voriconazole therapy for a pulmonary Aspergillus infection. Bone pain and radiographic evidence of periostitis were exclusively observed .There is no history of rheumatologic disease. To determine whether voriconazole is a cause of fluoride excess, we measured urinary fluoride levels. Fluorides were analyzed in the biological fluids using potentiometric method based on ion-selective electrodes (ISE) (Jenway).Quantitative determination of fluoride was carried out in the linear concentration range between 6 and 1ppm. The detection limit of this sensor was observed at 4, this sensitivity is sufficient for concentrations which are toxic for humans.
Results: The patient has an elevated alkaline phosphatase of 400UI/l and elevated urine fluoride level (20 ppm). Discontinuation of voriconazole therapy in the patient resulted in an improvement in pain and a reduction in alkaline phosphatase and fluoride.
Conclusion: Voriconazole is associated with painful periostitis, exostoses, and fluoride excess in post-transplant patient with long-term voriconazole use for prophylaxis and treatment of Aspergillus infection.

M328
A NEW POSSIBLE MECHANISM OF FLAVONOID-DRUG INTERACTION: FLAVONOIDS ARE ABLE TO DISPLACE WARFARIN FROM HUMAN SERUM ALBUMIN

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Background: Warfarin is a widespread anticoagulant binding to serum albumin with high efficiency (99%) therefore only 1% displacement of warfarin can lead to doubling its free concentration. Because the binding property is coupled to a narrow therapeutic window displacement can result in serious biological consequences. The flavonoid molecular group also shows very strong albumin binding characteristics at the same binding site as known for warfarin. It seems to be plausible to hypothesize that dietary flavonoid aglycones maybe able to displace warfarin from human serum albumin (HSA) which in turn may lead to unexpected bleeding complications.
Methods: In our study the competing activities of different flavone, flavonol and flavanone aglycones (0-3 μM) vs. 3 μM racemic warfarin binding to albumin were investigated using fluorescence spectroscopic and fluorescence polarization techniques. Beside the examination of displacing capacities binding constants were also quantified based on the fluorescence emission data of warfarin-HSA-flavonoid systems. OriginPro8 software was used for background fluorescence correction while binding constants (logK) were determined with the Hyperquad2006 software.
Results: Our data suggest that flavone and flavonol aglycones show similar or higher binding affinity towards albumin than warfarin does. The logK value for chrysin-HSA was 6.17±0.15, while that of warfarin-HSA complex was calculated to be 5.45±0.27. Our results represent that flavones and flavonols are able to displace warfarin from the surface of HSA already at a few hundreds of nanomolar concentrations occurring physiologically in human plasma; on the other hand flavanones show much lower competitive effect. In our in vitro experimental model system as low as 300 nM flavone and flavonol aglycone concentrations caused visible interaction and 800 nM resulted in more than 7% displacement of bound warfarin.
Conclusions: Our investigations strongly suggest that flavonoid-warfarin interaction should be considered in anticoagulated patients. In order to explore the degree of the impact of natural flavonoids on warfarin therapy further in vivo investigations are needed.

M329

SIGNIFICANT DECREASE OF PLUMBEMIA IN LEAD-EXPOSED WORKERS DUE TO EFFECTIVE PREVENTIVE MEASURES

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Background: Lead can have an adverse effect on human organism as it affects the synthesis of heme, has neurotoxic and nephrotoxic effects and can possibly reduce fertility. Lead is accumulated in bones and some of the organs and is released from these pools during stress of organism. Monitoring of lead blood concentration (plumbemia), which reflect the total lead level in the organism, is therefore very important, especially for persons with higher lead exposition. The maximal permitted value of plumbemia in lead-exposed workers is 400 μg/L. Plumbemia of all such workers is periodically monitored. Preventive measures are taken to keep plumbemia within ranges defined by law. We studied the effect of preventive action on plumbemia of lead-exposed workers from battery industry.
Methods: The study was performed on 236 workers of a battery industry factory (Johnson Controls) in period 2005 – 2012. Workers were divided into 4 groups based on their initial plumbemia in 2005 (<200 μg/L, 200 – 299 μg/L, 300 – 399 μg/L, ≥400 μg/L), plumbemia of all of the workers was controlled at least once every year. Plumbemia was measured using graphite furnace atomic absorption spectrometry using Varian 220 FS with GTA 110 device. Analysis was performed from whole blood samples collected in special containers for trace metal analysis. Used method has CV 8.44% and expanded uncertainty 18.6%. Paired t-test was used for statistic interpretation.
Results: The highest significant decrease in plumbemia between years 2005 – 2012 was in the group of workers with initial plumbemia over 400 μg/L (average decrease to 37% by the year 2012, $t(63) = 29.105$; $P < 0.001$), significant decreases were measured also in other groups of workers with initial plumbemia 300 – 399 μg/L (average decrease to 41%, $t(70) = 32.704$; $P < 0.001$), 200 – 299 μg/L (average decrease to 42%, $t(67) = 34.208$; $P < 0.001$) and under 200 μg/L (average decrease to 51%, $t(32) = 11.663$; $P < 0.001$).
Conclusions: In all groups of lead-exposed workers we find significant decrease in plumbemia during years 2005 - 2012. Our data show that applied preventive measures (high sanitation standards, personal protective equipment) are highly effective in decreasing plumbemia. Support: Research project RVO-VFN64165/2012-

M330

APPLICATION OF BENZODIAZEPINE IMMUNOASSAY IN ACUTE POISONING

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Background: Benzodiazepines are often prescribed drugs and most common cause of acute poisoning. Immunoassays are widely used tests for fast estimation of intoxications. These assays are qualitative or semiquantitative. When used semiquantitatively, the assay yields approximate, cumulative concentrations of the drug and metabolites. The aim of our study was to compare concentrations obtained by immunoassay with cumulative concentrations of benzodiazepines measured by HPLC method.
Methods: We have analysed 26 samples of patients who were suspected for intoxications and had positive serum benzodiazepines measured by EMIT method (VIVA E, SIEMENS). All samples were confirmed for presence of benzodiazepines and metabolite using GC-MS analysis. Same samples were analysed by HPLC method in order to determine concentrations of each drug from this group using reagent kit for benzodiazepines (CHROMSYSTEM). Sample comparison was performed using Passing - Bablock regression.
Results: Although some major outliers were noted, regression equation with 95% CI obtained from Passing - Bablock analysis showed no significant deviation from linearity ($y = -0.01+1.09$). Recovery of immunochemical method calculated against HPLC as a reference is 142,08% with CV of 126,56%. More significant deviation was observed in area of higher concentrations.
Conclusion: Although clinically important discrepancies could be observed, application of concentrations obtained by immunoassay could give useful information about risk or level of intoxication, when it is known which benzodiazepines (with known therapeutic/toxic ranges) are cause of intoxication.

M331
URINARY LEVELS OF HIPPURIC AND METHYLHIPPURIC ACIDS AS A BIOMARKERS OF EXPOSURE TO TOLUENE AND XYLENE

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Background:The exposure to solvents such as benzene and derivatives is considered dangerous for our health even in low concentrations.These solvents are frequently used by the workers of some clinical laboratories.To determine the degree of exposure we use hippuric and methylhippuric acids levels in urine samples.These acids are considered biomarkers of exposure to toluene and xylene respectively.The aim of our study is to assess the degree of exposure of workers at the department of Pathology Anatomy in our hospital
Methods:It was made a cross-sectional descriptive study about the quantification of the levels of hippuric and methylhippuric acids in urine samples of workers at the Pathology Anatomy Service of the San Cecilio Hospital in Granada.The personnel exposed samples were collected at the end of the working day.The control group was formed by workers from the same Hospital, which did not belong to this service.The hippuric and methylhippuric acids were analyzed by high performance liquid chromatography (HPLC).We determined creatinine in urine of each sample to correct the values.It was used a statistic descriptive analysis.
Results:The hippuric and methylhippuric acid levels in urine showed an average for the exposed group of 1.2 g/g (g acid/g creatinine) and 0.17 g/g respectively. Both values were below the Biological Limit Value (BLV) for hippuric acid (<1,6 g/g) and methylhippuric acid (<1.5 g/g) adopted by the Spanish Institute of Health and Safety at work (INHST). In the control group,the average value in urine for hippuric acid was 1.1 g/g. Methylhippuric acid levels were not detected.These values were also below the BLV.The t student test was applied for two independent samples, in order to contrast the concentration obtained.There was no statistically significant differences in hippuric acid levels (P >0.05) between the two groups
Conclusions:The results found in this study are within the range of reference values to healthy people, which suggests that, they were working in suitable conditions regarding the exposure to xylene and toluene. Methylhippuric acid levels were detected only in exposed group which means it could be useful to biomonitoring occupational exposure to xylene.

M332
INTEREST OF EXPERIMENTAL DESIGN IN THE DEVELOPMENT OF AN ASSAY FOR THE DETERMINATION OF AMIODARONE AND DESETHYLAMIODARONE BY HPLC-UV

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Background: Our objective was to study the interest of Experimental Design in the development of an HPLC-UV method for the determination of amiodarone and desethylamiodarone in plasma in the presence of an internal standard (promethazine).
Methods: We used plasma samples spiked with amiodarone and desethylamiodarone at different concentrations. Sample treatment consisted on acetonitrile deproteinization. We applied a simple cubic plan with Hadamard matrix and studied the influence of acetonitrile percentage, pH of the buffer and the flow rate of the mobile phase. Three types of responses were considered: the retention times of amiodarone and desethylamiodarone as well as the resolution between promethazine and desethylamiodarone peaks. Mathematical modeling was performed using the software Nemrodw®.
Results: We used as stationary phase a bonded silica C18 and a wavelength of measurement equal to 245 nm. After six experiments, we obtained the optimal method using a mobile phase consisting of acetonitrile/phosphate buffer pH= 3.7 (55/45 v/v) and a flow rate of 1.2 mL/min. The retention times were 6.00, 7.46 and 9.04 min respectively for desethylamiodarone, promethazine and amiodarone. The total duration of the analysis did not exceed 10 min. Our method was successfully applied in patients treated with amiodarone.
Conclusion: Experimental design allowed us to rationally and economically develop a simple, fast and reliable method. Our assay can be applied in therapeutic monitoring of patients treated with amiodarone as well as in pharmacokinetic studies.

M333

VALIDATION OF AN ANALYTICAL METHOD FOR THE DETERMINATION OF LEAD IN HAIR BY ELECTROTHERMAL ATOMIC ABSORPTION SPECTROMETRY (ETAAS)

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Background: Heavy metals exposure is the main source of poisoning in work places. In Tunisia, the most vulnerable are those who work in the metal industry including lead acid batteries and tanning. Hair samples can be of great interest in occupational health surveys because metallic cations form bonds with the sulphur of the keratin matrix of the hair. In this regard, it can serve as an alternative matrix to monitor past or recent exposure. The aim of this work is to develop and validate a method for the quantification of Pb in hair samples by electrothermal atomic absorption spectrometry.
Method: Hair samples were washed by ultrasonic cleaning (10 min) with ultrapure water, with a 1% Triton X-100 solution and with acetone then dried at 80 °C overnight before being ground to a fine powder with a bill mill. Approximately 50 mg of hair samples were digested during 2H on a hot plate at 70 °C after addition of HNO3/H2O2 (2:1 V/V) mixture. The graphite tubes were pyrolytically coated with Na2WO4.2H2O solution following a specific heating program. Aliquots of 10 μL of digested hair were introduced directly into the graphite furnace with an equal volume of matrix modifier.The analysis of results and the building of experimental designs was carried out with the NEMRODW software.
Results: Pyrolysis and Atomization temperature as well as pyrolysis ramp and step time were selected as factors being able to influence the analytical signal. The optimization process was carried out by using Doehlert matrix. The graphite coating process revealed a great thermal stability with a pyrolysis temperature up to 900 °C. A maximum of absorbance was obtained at a pyrolysis temperature and atomisation temperature of 680 °C and 1650°C respectively. Optimal pyrolysis ramp and step times were 30s. The proposed method allowed Pb determination with a detection limit of 0.8 µg/L. Recoveries of known amounts of Lead added to the samples varied from 98.6 to 103.2%. Lead concentration in hair of 15 workers in Pb acid batteries factory varied between 8.8±0.5 μg/g and 59.4±2.2 μg/g.
Conclusion: The analytical features achieved demonstrated the feasibility of the proposed method which was successfully applied for Pb determination in hair samples of exposed workers in Pb acid–batteries industry.

M334

THE CONTRIBUTION OF CADMIUM TO OESTRADIOL MODULATION AND SEMEN QUALITY IN NIGERIAN MEN

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Background: Declining male fertility is of global concern and has been linked to the effects of endocrine disruptors like Cadmium(Cd) on the modulation of oestradiol(E2). This study aimed at identifying the possible contribution of Cd to sperm defects in Nigerian men.
Methods: One hundred and twenty males (20-54 years), 77 dyspermics and 43 normospermics were recruited from Urology Clinics of 2 teaching hospitals in Nigeria after informed consent. Demographic and anthropometric indices were obtained using a structured questionnaire and standard methods respectively. Semen samples were collected from subjects by masturbation after 3-5 days abstinence. Spermiogram were done using WHO guidelines and Tygerberg strict criteria. 10ml of blood was obtained from each participant. Prolactin, luteinizing hormone and follicle stimulating hormone were estimated in serum while testosterone and E2 were estimated in serum and seminal plasma by enzyme-immunoassay. Cd, Se and Zn were assayed in serum and seminal plasma by atomic absorption spectrophotometry.
Results: Forty-eight (62.3%) dyspermics had reduced sperm motility and abnormal morphology while 17 (22%) and 12 (15.6%) had oligospermia and azoospermia respectively. Seminal plasma E2 was significantly lower in normospermics (0.7±0.04 nmol/L) than dyspermics (1.1±0.07 nmol/L) while testosterone/E2 ratio was significantly higher in normospermics (10.7±0.60) than dyspermics (7.3±0.70). Serum and seminal plasma Cd were significantly higher in dyspermics (0.3±0.02 µg/L; 2.0±0.07 μg/L) than normospermics (0.1±0.01 μg/L; 1.2±0.07 μg/L) respectively.Multiple regression models predicted increased seminal plasma Cd associated with increased seminal plasma testosterone (β=2.04) but decreased serum LH (β=-0.75), E2 (β=-1.84), Zn levels (β=-0.55) and seminal plasma E2 (β=-2.18), testosterone/E2 ratio (β=-2.24) in normospermics. Increased tail defects (β=1.21), cytoplasmic droplets (β=0.90) and seminal plasma Zn (β=0.29), but decreased % sperm motility (β=-0.50) and serum E2 (β=-1.33) in dyspermics.
Conclusion: Cadmium may cause dyspermia through direct toxicity, endocrine disruption, and depletion of Zn and Se. These events in both normospermics and dyspermics suggest that other may also be involved.

M335

STUDY OF INTOXICATIONS FOR DIGOXIN AT THE URGENCIES AREA OF SAN CECILIO HOSPITAL (GRANADA, SPAIN) IN THE PERIOD 2007-2012

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Background: Nowadays intoxications represent a major health problem causing critical situations in which patients should be treated as soon as possible. Therefore, in suspected toxicity, plasma levels above the therapeutic range reinforce the diagnosis. The monitoring of this drug is of a great importance because of the narrow therapeutic index that it presents. The objectives of our study are to evaluate the requests of digoxin, and the frequency of poisoning in the emergency department during the years 2007-2012.
Methods: A retrospective study was made of all patients admitted to urgency area of San Cecilio Hospital in which there was clinical suspicion of digoxin toxicity between 2007 and 2012. The digoxin was measured in a Dimension® Vista® (Siemens) by chemiluminescent immunoassay. The data base was obtained from the informatic system of laboratory. Data were grouped according age ranges in <20; [20-45]; [46-65] and >65.
Results: The total amount of requests was 3699. Of the total, the main sex was the female (64%) and the major number of requests was to patients above 65 years old (87.3%). The number of requests of digoxin in our urgency service increases since 2007 (361 requests) until 2011 (846). Otherwise, in 2012 there is a significant decrease (680). The 34.4% of the samples showed levels above the reference range (0.8 - 2.0 ng/mL), where the rate of intoxications (>2.0 ng/mL) represents a 44% in 2007, 34.2% in 2008, 31.6% in 2009, 31.7% in 2010, 25.9% in 2011 and 21.5% in 2012. The population above 65 years represents a 93.1% of the total of intoxications.
Conclusion: The implementation of an electronic petition system in 2012 slows the upward trend in the request of this test justified by a lower accessibility to the test. The percentage of intoxications has declined over the study period with the addition of protocols awareness in our region. The group of over 65 years is the most affected by these intoxications.

M336

DRUG CONSUMPTION AMONG TEENAGERS. DILEMMA ABOUT DIAGNOSTIC APPROACH

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Background: Actually, there is an early consumption of drugs of abuse between teenagers that could become in an important public health problem. Medical staff find themselves with this problem more and more often. Adolescence is one of the hardest stages, not only for the teenager, but also for parents. Many parents are demanding toxicological tests for drugs, taking into account the behavioral change of their sons or daughters in this stage of adolescence. Due to the increase in the request of this test, the objective of the study is to analyze all the drug tests that were carried out to teenagers in our laboratory, and the result of these tests.
Methods: A retrospective study was made of the drug tests realized at the Legal medicine and Toxicology department at the San Cecilio Hospital during a period of 3 years (2010-2012). The range of age of the teenagers to study was since 12 until 16 years old. It was used a screening qualitative test made by a panel drugs of abuse. This screening test uses a fluorescence immunoassay for the qualitative determination of the presence of drugs (and/or the major metabolites) of up to 8 distinct classes, including amphetamines, benzodiazepines, cocaine, methadone, opiates, cannabis, imipramine and salicylates. The results were studied looking at the amount of positive tests and the kind of drug.
Results: 156 tests of drugs of abuse were analized in the period of study. The 83% of the total had a negative result. All the positive tests were due to cannabis.
Conclusions: It confirms the prevalence of consume of cannabis compared to other drugs by adolescents; the start drug. We believe that there is an abuse of parental insistence on the request of drugs of abuse for fear of the consumption by their children. This type of requests should be restricted to suspected poisoning.

M337

PREPARATION OF ACETAMINOPHEN-GLUTATHIONE CONJUGATES AND ESTIMATION OF THEIR TOXICITY IN VITRO

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Background: Acetaminophen (APAP) is an analgetic and antipyretic drug. Its toxicity occurs after overdose where APAP is oxidized in cytochrome P450 to N-acetyl-p-benzoquinone imine. It can react with proteins or with glutathione producing 3 - (glutathione-S-yl) acetaminophen conjugate (APAP-SG). The conjugation has been considered as a detoxification pathway generally. However, based on the literature and our preliminary results, we postulated, that the conjugate could also possess a toxic effect. Therefore, the main aim of our study was to synthesize, purify and characterize APAP-SG conjugate and to assess its toxicity in biological material.
Methods: We prepared APAP-SG conjugate using organic synthesis. The conjugate was purified by preparative liquid chromatography (Labiosphere; C18; mobile phase – water/methanol/acetic acid – 87%/12%/1%, v/v/v) and after evaporation the grey powder was obtained. The structure was confirmed by mass spectrometry (LC/MS). Consequently, the possible toxic effect of the conjugate was tested in vitro in glutathione reductase and in isolated murine mitochondria and cells.
Results: We prepared APAP-SG conjugate using preparative liquid chromatography. The structure and purity was confirmed using LC/MS. The final purity was >97%. In addition to APAP-SG, we found two other conjugates in the synthetic sample that were identified as 2-(glutathione-S-yl)acetaminophen and (APAP)2-SG. The effect of 3-(glutathione-S-yl)acetaminophen conjugate on respiration was tested in isolated mitochondria. We found that APAP-SG presence decreased cellular dehydrogenase activity in relation to the dose; e.g. in 2 mM APAP-SG the respiration decreased by 30%. Recently, we found that APAP-SG is able to inhibit the activity of glutathione reductase (GR), therefore we estimated an inhibitory effect also in 2-(glutathione-S-yl)acetaminophen. We proved that also this compound is able to inhibit the activity of the enzyme; in 2 mM, GR activity decreased by 20%.
Conclusion: We proved our hypothesis that acetaminophen-glutathione conjugates possess a toxic activity. We found toxic effects in different biological material, thus we conclude that the acetaminophen-glutathione conjugates could countribute to the entire mechanism of acetaminophen toxicity.

M338

LIPID PEROXIDATION IN ACUTLY DRUG POISONED PATIENTS

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Beckground: The lipid peroxidation is an oxidative damage witch can hold cell membranes, lipoproteins and other molecules that contain lipids, under conditions of oxidative stress. Lipid hydroperoxides, different gassy products (pentane, ethane, ethene), as well as products that contain aldehyde group (malondialdehyde, hydroxytransnonenal), take rise in the lipid peroxidation process. Acute medicament poisoning presents appearance of symptoms and signs of illness, witch begin shortly after intake of individual or repeated toxic doses of medicaments in 24 h. The aim of this study was to show that poisoning with chlorpromazine, carbamazepine and diazepam initiates the process of lipid peroxidation.
Methods: First group was made from 32 patients who where acutely poisoned with chlorpromazine, carbamazepine and diazepam, which is confirmed by determinating of mentioned medicament in serum with HPLC method; based on this, the patients where divided on heavily and slightly poisoned. Second group was 39 psychiatric patients, which chronically recieved therapeutic doses of chlorpromazine, carbamazepine and diazepam (not less than 60 days). Controls where 30 volunteer blood donors, non-smokers, which did not use mentioned medicaments, and have no acute or chronic disease. The level of malondialdehide (MDA) was determined spectrophotometrically, in method with thiobarbituric acid for every patient after admission, and 24 h after the first sampling. Data are expressed as means ±SD. Comparision between groups were examined using analysis of variance. For testing correlation of MDA and the concentrations of medicaments, regression correlation anlysis is used.
Results: We found increased values of MDA in all severely poisoned patients, compared with controls and psychiatric patients ($P < 0.001$). Slightly poisoned and psychiatric patients show no differences compared with controls. Furthermore, levels of MDA in plasma of severely poisoned patients show very good correlation with concentration of carbamazepine ($r=0.96$) and diazepam in their sera ($r=0.85$).
Conclusion: Determination of MDA might serve in estimation of severity poisoning and lipid peroxidation, due to direct dependence of MDA level on concentration of medicament found in serum.

M339
EFFECT OF CURCUMIN ON ANTIOXIDANT ENZYMES ACTIVITY IN KIDNEY RAT EXPOSED TO ACETAMINOPHEN

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Background: Acetaminophen is one of the most popular analgesic and antipyretic drugs and its overdose, which can cause severe damage to liver and kidneys in human and animals. Although renal dysfunction and acute renal injury can occur without damage to the liver. Unlike kidney damage, mechanisms of liver damage is poorly understood. it is one of the most common reasons of emergency admissions. The aim of present study was to investigate the protective effect of curcumin, derived from plant Curcuma longa, on acetaminophen-induced kidney damage.
Methods: In this study rats were divided into five groups of five each: Group I as control. Group II treated with curcumin (200 mg/kg b.w & i.p). Group III received dimethyl solfoxide (DMSO) as vehicle control. Group IV were treated with a single dose of i.p injection of acetaminophen (1000 mg/kg b.w). Group V received acetaminophen+Curcumin. After 24 hours, all the rats were sacrificed with mild anesthesia. Urea and creatinine levels were measured in the plasma, and the levels of lipid peroxidation (TBARS) and superoxide dismutase (SOD) and catalase (CAT) activity in the kidney were determined. Data analysis was run using SPSS and the differences between the groups were analyzed applying T-test and Mann Whitney tests at the significance level of 0.05.
Results: Acetaminophen administration caused elevated level of urea and creatinine in plasma and TBARS in kidney. While the activities of SOD and CAT were decreased in kidney tissue. The presence of curcumin with acetaminophen significantly decreased the Urea, creatinine and TBARS but significantly increased the activity of SOD, CAT.
conclusion: Our results indicate that curcumin can be potent protective agent against acetaminophen induced biochemical alterations and oxidative damage in kidney rats but further studies are necessary to identify this subject especially mechanism of biochemical reaction before clinical application becomes conceivable.

M340
PHARMACOKINETICS AND SAFETY OF ESZOPICLONE IN HEALTHY CHINESE VOLUNTEERS

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Objective: The main objective of this study was to investigate the pharmacokinetic characters of eszopiclone (CAS: 138729-47-2) after oral single-dose and multiple-dose administration in Chinese healthy adult volunteers.
Methods: In single-dose study, twelve subjects were given oral administrations of 1.5, 3 and 6 mg eszopiclone in an open-label, randomized, crossover fashion. In multiple-dose study, eight subjects were given 3 mg eszopiclone once-daily consecutively for seven days. Blood samples were collected over 24 h and plasma eszopiclone were determined using a validated liquid chromatography/mass spectrometry (LC/MS/MS) assay. The safety and tolerability of eszopiclone was evaluated by adverse events recording, physical examination, laboratory testing, vital signs, and 12-lead ECG findings.
Results: The main pharmacokinetic parameters of eszopiclone after single-dose administration were as follows: at 1.5, 3 and 6 mg dose respectively, Cmax was 18.08±4.65, 38.29±15.41 and 76.38±23.34 ng/mL; Tmax was 0.94±0.39, 1.04±0.63 and 1.08±0.51 h; AUC0-24 was 110.90±23.06, 227.36±62.41 and 504.10±140.13 ng*h/mL; elimination half-life was 5.84±1.03, 5.53±1.91 and 6.17±1.23 h. After multiple-dose administration, the steady-state levels of eszopiclone were achieved by the 4th day, and the main pharmacokinetic parameters were as follows: Css_max was 33.43±5.63 ng/mL, AUCss (0-24) was 263.30±51.21 ng*h/mL. The most common adverse event was bitter or abnormal taste. All the adverse events were judged as mild to moderate and resolved without any medication.
Conclusion: The pharmacokinetic character of eszopiclone is linear and dose-proportional over the range of 1.5 to 6 mg. The systemic exposure does not accumulate with once-daily administrations. Eszopiclone has good safety and is well tolerated in Chinese populations.

M341

IMPACT OF CONCOMITANT THERAPY ON EVEROLIMUS WHOLE BLOOD CONCENTRATIONS IN ORGAN-TRANSPLANT IMMUNOSUPPRESSIVE TREATMENT

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Background: Therapeutic drug monitoring (TDM) of immunosuppressive drugs in organ-transplant patients is vitally important to prevent intoxication or rejection due to incorrect dosage. Everolimus (EVL), a macrolide immunosuppressant, is used in prophylaxis of acute rejection episodes of allograft after kidney and heart transplantation. EVL is a selective mammalian target of rapamycin (mTOR) inhibitor. mTOR is a key serine-threonine kinase, the activity of which is known to be upregulated in a number of human cancers. EVL is a substrate of cytochrome P450 CYP3A4,and also a substrate and moderate inhibitor of multidrug efflux pump P-glycoprotein (PgP). EVL is a competitive inhibitor of CYP3A4 and mixed inhibitor of CYP2D6. This is the reason why coadministration of other CYP3A4 and PgP inhibitors significantly increases EVL exposure (ketokonazole–strong, rythromycin–moderate, verapamil–moderate). EVL may predispose patients to bacterial, fungal, viral or protozoal infections. Itraconazole is a synthetic triazole antifungal agent used in treatment of fungal infections (blastomycosis, histoplasmosis, criptococcal meningitis, and aspergillosis) in immunocompromised and non-immunocompromised patients. Itraconazole decreases the elimination of EVL and causes toxic effects.
Methods: We monitored EVL concentrations by the QMS EVL Immunoassay on Modular biochemistry analyzer during the treatment of a 58 years old patient one year after heart transplantation. Her medical background comprised cardiac allograft vasculopathy, critical illness myopathy, state after anterior-wall STEMI, and respiratory insufficiency.
Results: We observed elevated EVL whole blood concentrations(between 10 and 20 mg/L) although a small therapeutic dosage (0.50 mg/day) was administrated. After itraconazole was discontinued, and therapeutic dosage of EVL readjusted, the concentration dropped to the levels between 3 and 8 mg/L.
Conclusions: Concomitant administration of itraconazole and EVL in organ-transplant patients should be avoided. A dose reduction of EVL may be warranted in order to decrease the risk of adverse effects. Closely monitoring of immunosuppressant concentration in whole blood is crucial, as the inhibitory effects of itraconazole may not be recognized during concomitant therapy.

M342

THE ANTIOXIDANT EFFECT OF CINNAMON AND CARDAMOM POWDERS ON RAT LIVER CELLS MEMBRANES

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Background: Destructive and pathogenesis effects of reactive oxygen species (ROS) on global macromolecules such as DNA, proteins and lipids are well established.For investigation of antioxidant effects of Cinnamon and cardamom powders on Rat liver cells membranes, the present study was done.
Methods and material: The rat hepatocytes separated by Percoll density-gradient centrifugation and then treated by oxidant agent “tertio butyl hydroperoxide” (1.5 mL). The levels of lipids peroxidation product, Malondialdehyde (MDA), measured in the presence and absence of cinnamon and cardamom powders. Results were evaluated by t-test and p-value ($P < 0.05$) obtained.
Result: Results showed Cinnamon and cardamom powders (1g/dL concentration) can reduce the level of MDA to 42.7 % and 39.8% respectively. The inhibitory effects of these agents were dose-dependent in the present study.
Conclusion: This study showed the Cinnamon and cardamom can be used as antioxidants agents and have inhibitory effects on oxidative damages on liver cells.

M343

VITAMIN D DEFICIENCY IN PATIENTS BEFORE BARIATRIC SURGERY (HIGH BMI) AND SCHEDULED SURGERY PROCEDURES (NORMAL BMI)

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Background: In Central Europe, where sunlight exposure is limited, vitamin D insufficiency might affect majority of the population. There is a well documented inverse association between BMI and serum levels of 25OHD and 1,25(OH)2D. Both factors are of great importance for patients undergoing bariatric surgery, because already existing vitamin D insufficiency might be exacerbated by postoperative nutritional deficiency.
Aim: To verify whether bariatric patients, residents of Central Poland, are vitamin D-deficient, to define the level of insufficiency/deficiency and to compare it to vitamin D levels in slim patients.
Methods: The studied group (O) consisted of 61 patients with high BMI 39-68kg/m^2 awaiting bariatric surgery, while control group (N) consisted of 30 patients with normal BMI 20-26kg/m^2, undergoing a scheduled surgery procedures (cholecystectomy, abdominal hernioplasty or stripping of vena saphena). All study subjects were free of acute inflammation, chronic inflammatory and autoimmune diseases, and were not treated with anti-inflammatory drugs or immunosuppressants. Concentrations of 25OHD and 1,25(OH)2D were measured in serum using the Roche Diagnostics vitamin D total assay and IDS manual immunoassay.
Results: The mean serum 25OHD and 1,25(OH)2D concentrations in the O group were 14.3±8.6 ng/mL and 130.4±47.1 pg/ml, respectively and were lower than in the N group, (17.0±10.5 ng/mL and 137.2±51.3 pg/mL, respectively). The optimal levels of 25OHD (30-80 ng/mL) were found only in 4.9% of the O and in 13.3% of the N groups. Mild insufficiency (20-30 ng/mL) was observed in 11.5% of the O and in 26.7% of the N groups. Vitamin D deficiency (<20 ng/mL) was present in 83.6% of the O and in 60% of the N groups. This difference was statistically significant (c2 test, P=0.013).
Conclusions: We show here that not only obese patients undergoing bariatric surgery, but also patients with normal BMI admitted for scheduled surgery procedures should be preoperatively screened for hypovitaminosis D and supplementation of this vitamin should be considered.

M344

A COMPARISON OF THE NUTRITIONAL STATUS OF HOSPITALIZED PATIENTS: NURSING HOME PATIENTS VERSUS HOME CARE PATIENTS

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Background: Malnutrition represents an important hospital problem among patients admitted to acute hospitals and even more among discharged patients. Depending on where patients come from, care facilities or home, nutritional risks can be different.
Methods: Study of the relations of age, gender and patient's location, as well as the Nutritional Alert Index (NAI) in 2404 hospital admissions. NAI is a complex indicator which collects three analytical data and classifies the patient in four categories; a. without nutritional risk; b. at mild nutritional risk; c. at moderate nutritional risk d. at severe nutritional risk. This indicator has been validated for the hospitalized Spanish population.
Results: A 65.9% of the analyzed admissions showed some risk of malnutrition. NAI was moderate or severe in 26.4% of cases. Distribution of the four NAI categories was similar in both genders (P=0.091) but different in each age group: as age increasing, higher proportion of abnormal NAI and, specially, severe NAI was exhibited (P <0.001). A 11.2% of admissions came from nursing homes. Despite the fact that some patients from care facilities were misclassified, differences between care facilities and the rest of locations were overwhelming; normal NAI: 17.0% vs. 36.2%; severe NAI: 18.5% vs. 5.8% (P <0.0001). However, the different age distribution of both groups (P <0.001) did not completely explain those percentages obtained.
Conclusions: Malnutrition risk assessed by NAI among patients admitted to our hospital was detected in two thirds of the evaluated admissions, a quarter of the total in a moderate or severe level.Admissions from care facilities were important. Their malnutrition risk measured by NAI was much higher than in admissions from other locations, although some patients from care facilities were classified in the other location group. Malnutrition risk assessed by NAI was significantly more common in admissions from care facilities than in the rest of admissions. This diversity was even bigger in proportions of severe NAI. The higher aging of the group from care facilities did not explain these differences but increased them.

M345
ASSOCIATION BETWEEN 25-HYDROXYVITAMIN D AND CARDIOMETABOLIC RISK FACTORS IN CLINICALLY HEALTHY NON-DIABETIC INDIVIDUALS

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Background: 25-hydroxyvitamin D (25(OH)D) concentration might be a predictor of cardiovascular disease, diabetes and metabolic syndrome. The aim of study was to present the association of serum 25(OH)D with anthropometric and biochemical indicators in clinically healthy non-diabetic individuals.
Methods: Study included 145 healthy normoglycemic, non-obese subjects, aged 25-40 (73 women and 72 men). Serum 25(OH)D, adiponectin, lipid profile, insulin, C-reactive protein (CRP), total bilirubin, apolipoproteins B100 and AI (apoB, apoAI), plasma fasting glucose and glycated hemoglobin (HbA1c) measurements were performed. LDL cholesterol (LDL-C), non-HDL cholesterol (non-HDL-C), body mass index (BMI), waist-hip ratio (WHR) and atherogenic indexes (TC:HDL-C, LDL-C:HDL-C, apoB:apoAI) were calculated. Subjects were divided according to 25(OH)D concentration: <15 ng/mL, 15-30 ng/mL, ≥30 ng/mL.
Results: 25(OH)D concentration was higher in women than men (20,5 vs. 15,3 ng/mL; P <0,001) whereas all anthropometric and biochemical indicators, except HDL-C, apoAI and adiponectin, were lower in women. In the whole group 25(OH)D was inversely correlated with waist circumference and WHR (R=-0,20; R=-0,21; P=0,02), systolic and diastolic blood pressure (R=-0,21 and -0,19; P=0,03), glucose and HbA1c (R=-0,21; R=-0,23 P=0,01), CRP (R=-0,18; P=0,03), apoB:apoAI (R=-0,21; P=0,02) and positvely related to HDL-C, apoAI (R=0,31; R=0,43; P=0,001) and adiponectin (R=0,23; P=0,01). Subjects with 25(OH)D <15 ng/mL had significantly higher HbA1c and CRP concentration and lower apoAI and adiponectin than other groups. Serum 25(OH)D <30 ng/mL was associated with glycemia >95 mg/dL (OR=3,66; CI 1,04-13,1; P=0,04) and more strongly with HbA1c ≥5,4% (OR=8,5; CI 1,11-16,2; P<0,04).
Conclusion: Association of insufficient serum 25(OH)D with indicators of obesity, proatherogenic profile, higher glycemia and HbA1c seem to be important public health concern in young non-diabetic subjects that may have substantial adverse health consequences in future.

M346
VITAMIN D STATUS IN THE SOUTHERN POLISH POPULATION

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Background: VitaminD is stored mainly as a 25OHD form which most fully reflects status of vitamin D in the body. 25OH D serum concentration is1000 times higher than the concentration of biologically active 1,25-OH D. The half-life of 25OHD is 2-3 weeks, compared with a half-life1,25D-OH, which is 4 h. It is recommended to measure 25OHD2 and 25OH inmost clinical situations. The aim of study was to evaluate the concentration of vitamin D in patients' samples(southern polish population) over the January to October 2011.
Methods: Concentration 25 OHD was measured on DiaSorin Liaison using ELISA method
Results: Results were analyzed in ninth ranges: I 0-10 ng/mL, II 10-20 ng/mL, III 20-30 ng/mL, IV 30-40 ng/mL, V 40-50 ng/mL, VI 50-60 ng/mL, VII 60-70 ng/mL, VIII 70-80 ng/mL and IX >80 ng/mL. Most of the results obtained in the ranges II and IV for all analyzed months. Strong focus on lower limit of reference concentration range was observed for most of samples (all analyzed months). 20% of all results in August were in range II and it was significantly lower compared to October (40,6%). Lower percentage of results per August in range I (4,6%) compared to the October (9,7%) was also observed. 26,9% of all results in August were in range IV and it was significantly higher compared to October (12,3%).
Conclusions: Changes in concentrations of vitamin D may be related to seasonal variation. Annual observation of vitamin D concentration allows to determine an individual's medical decision point of substitution vitamin D3 for each patient. Shortening determination of vitamin D to 24 hours enables quick clinical decisions.

M347

COMPARISON OF THE TOTAL ERROR OBTAINED WITH SIX IMMUNO-ASSAYS FOR 25-HYDROXY VITAMIN D WITH THE MINIMAL, DESIRABLE AND OPTIMAL TOTAL ALLOWABLE ERRORS CALCULATED ACCORDING TO THE BIOLOGICAL VARIATION

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Background: The number of requests for vitamin D determination has increased these last years. New assays have been developed and 25(OH)D testing has become part of the routine. We established the total error of six immunoassays to verify if their analytical performance were consistent with the goals based on biological variability of 25(OH)D.
Methods: We calculated the analytical CVA of 5 automated vitamin D assays (Roche-Elecsys, DiaSorin- LiaisonXL, IDS-iSYS, Abbott-Architect, Siemens-Centaur) and one radio-immunoassay (DiaSorin) by running 6 samples presenting values from 15 to 75 ng/mL in triplicate for 5 days. We evaluated the bias according to PerkinElmer LC-MS/MS, used arbitrarily as a reference, and calculated total error, measurement uncertainty and sigma metrics.
Results: Elecsys and Liaison-XL on the sample with LCMS value of 14.7 ng/mL and Centaur on the samples at 14.7, 25.3 and 38.5 ng/mL failed to meet the desirable CVA of 6%. Centaur was the only method that produced a total error greater than the total minimal allowable error of 30.6% (at 14.7 ng/mL). Measurement uncertainty was greater with Centaur. At 14.7 ng/mL, all methods failed to reach the 3.0 sigma level, except DiaSorin-RIA and IDS-iSYS. Liaison-XL also missed the target at 25.3 ng/mL whereas RIA and iSYS failed to reach 3.0 sigma in the upper range.
Conclusions: If all the CVA were well below 10% (except for Centaur with samples 14.7 and 25.3 ng/mL), the immunoassays (except the IDS iSYS) produced relative bias greater than 5%. IDS iSYS was the immunoassay that presented the best sigma performance throughout the measuring range. Improving the bias by recalibrating the assays against a reference method would certainly increase the sigma performance, but this would need an update of the cut-offs classically used for 25(OH)D.

M348

HYPOVITAMINOSIS D AND ASSOCIATED FACTORS IN PRESCHOOL CHILDREN IN A SUNNY COUNTRY

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Background: To estimate the prevalence and factors associated with sub optimal levels of vitamin D in a group of urban preschool children in Sri Lanka, where there is no routine vitamin D supplementation.
Methods: In a cross sectional study 340 children (172 girls and 168 boys) aged 2-5 years were selected by random sampling. Height, body weight, body mass index (BMI) and mid arm circumference were recorded. Diets were analyzed using a 7-day dietary recall questionnaire. Fasting levels of serum 25-hydroxyvitamin D [25(OH)D] and parathyroid hormone (PTH) were determined by chemiluminescent assay. Vitamin D status was categorized as deficient (<25 nmol/L), insufficient (25-50 nmol/L), sufficient (50-75 nmol/L) and optimal (≥75 nmol/L).
Results: Serum 25(OH)D ranged from 6.3 to 64.0 ng/mL (mean 23.5 ± 8.9 ng/mL); with 5.6 % deficient, 32.4 % insufficient, 42.8 % sufficient and 19.2 % optimal. Children with 25(OH)D levels <50 nmol/l had higher body weight, BMI and mid arm measurement than the children with 25(OH)D levels >50 nmol/l. No significant differences were found by age and gender. 25(OH)D levels were significantly associated with serum PTH levels (r=-0.112) , milk and dairy food intake (r=0.129), body weight (r=-0.123) and BMI (r=-0.181). Linear regression analysis of 25(OH)D adjusted for age and gender suggested that body weight and BMI z-score independently influenced 25(OH)D status.
Conclusion: Even in a sunny country, hypovitaminosis D is common among preschool school children. Body weight and BMI influence sub optimal levels of serum 25(OH)D in children. Vitamin D supplementation should be considered as part of child health policy in Sri Lanka.

M349
PREVALENCE OF VITAMIN B12 AND FOLIC ACID DEFICIENCY IN EAGEAN AREA CHILD

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Background: The aim of this study is to investigate the frequency of B12 and folic acid defiency and their effect on Hb levels in childhood.
Methods: Vitamin B12 (B12) and Folic Acid levels were measured in ambulatory children aged over 1 year, without known chronic disease and attended our hospital between 2006-2011 for routine screening. B12 <200 pg/mL and folic acid <5 ng/mL were defined as deficiency.
Results: A total of 1464 subjects (mean age 13,3+5,3; max: 18-min: 1 median age: 13,1 856 female/ 608 male) were included in the study Anemia was identified in 323 (%22,1) subjects. Mean B12 and folic acid levels were 337±195.4 (range:31-1500,median; 278) ,8.3+4.6 (range 2-24; median 7) respectively. B12 deficiency was identified in 324 subjects and folic acid deficiency was identified in 243 subjects. B12 deficiency was found in 81 (% 25.1) of 323 patients with anemia (P=0.149) and folic acid deficiency was found in 77 (%23.8) of 323 patients with anemia (P <0.001). Significant correlations were found between B12 (P <0.001: r= -0.242), folic acid (P <0.001: r= -0.507) and age. Mean serum levels of B12 were not significantly different between male (mean serum B12 = 340.6±204.4) and female (mean serum B12 = 332.1±188.2) subjects however a significant difference was found between male (mean folic acid = 8.7±4.8) and female (mean folic acid = 8.04 ±4.4) subjects for folic acid (P=0.005).
Conclusions: B12 and Folic acid deficiency may remain obscure without lowering Hb levels.

M350
VITAMIN D STATUS IN PATIENTS WITH KIDNEY TRANSPLANTATION

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Background: The role of Vitamin D (VD) in maintaining bone health has been known for decades. Recently, series of studies have demonstrated the importance of VD in preventing cancers, blood pressure, diabetes, cardiovascular diseases, autoimmune diseases, infections, risk of falls in the elderly, and even total mortality. Immunomodulatory and renoprotective properties of VD reveal potential for preventing allograft rejection after transplantation and optimization of immunosuppressive therapy. Plasma 25 hydroxyvitamin D (25D) is the functional indicator of VD status.
Methods: Determination of total 25-Hydroxyvitamin D (25D) (sum of 25D3 and 25D2) was performed by a LC-MS/MS validated according to FDA guidance with insignificant and matrix effect, accuracy and precision within 7.5%, extraction recoveries 57-73%, linearity 3.0-300.0 nmol/L, R2>0.99, and documented freeze-thaw, post-preparative, short-term, stock solution, and long term stability. Clinical study for the assessment of VD status in patients with kidney transplantation without supplementation of VD encompassed 159 subjects, who consented to participate.
Results: Subjects were included in this study with their first VD status analysis performed at least six months after operation, if they had stable graft function. Results presented as mean ± standard deviation (min-max) were as follows: 68 females aged 42±13 (18-69) years had total 25D of 42.6±22. 9 (14.0-96.7) nmol/L; 91 males aged 42±12 (20-70) years had total 25D of 51.7 ±18.4 (13.9-105.0) nmol/L; deficiency (<25.0 nmol/L) was found in 14.3% of the population studied; 39.4% of results were in the range 25.0-50.0 nmol/L, assessed as heavy insufficiency; 38.9.0% of values were in the range 50.0 – 80.0 nmol/L, judged as mild insufficiency, and only 7.4% of total levels were over 80.0 nmol/L, which is accepted as the lower limit of sufficiency range.
Conclusions: Our preliminary study demonstrates significant deficiency and insufficiency of VD status among Bulgarian kidney transplant recipients, confirming the need for development and implementation of supplementation strategy, in the effort to improve care for this patient population.

M351
BLUEBERRY TREATMENT ATTENUATES D-GALACTOSE-INDUCED OXIDATIVE STRESS AND TISSUE DAMAGE IN RAT LIVER

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Background: One of the mechanisms underlying the aging process is proposed to be oxidative damage caused by reactive oxygen species (ROS). The excess formation of ROS can damage cellular lipids, proteins, or DNA, and thus they inhibit their normal functions, disturb homeostasis and result in cell damage. Therefore, preventing of oxidative-stress may be a potential therapeutic strategy for the treatment of aging and age-related diseases. D-galactose (GAL) overload was reported to induce changes that resemble the normal aging process in rodents. Blueberries (BB; Vaccinium corymbosum L.) contain anthocyanins, polyphenols and flavonoids and appear to have highest antioxidant capacity among fruits and vegetables.
Methods: We investigated the effect of whole fresh BB on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities and hepatic malondialdehyde (MDA), protein carbonyl (PC) and glutathione (GSH) levels, and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione transferase (GST) activities together with histopathologic changes in GAL-treated rats. Rats received GAL (300 mg/kg; s.c.; 5 days per week) alone or together with 5% (BB1) and 10% (BB2) whole fresh BB containing chow for two months.
Results: Serum ALT, AST, MDA and PC levels were observed to increase together with histopathological structural damage and increased inflammatory reactions in the liver in GAL-treated rats. GAL treatment decreased GSH levels, and SOD and GSH-Px, but not GST, activities in the liver. BB1 and BB2 caused significant decreases in serum ALT and AST activities together with the amelioration in histopathological findings in GAL-treated rats. Both BB1 and BB2 were detected to reduce MDA and PC levels and to elevate GSH levels and SOD and GSH-Px activities.
Conclusion: Our results indicate that treatment with BB restored liver prooxidant status together with histopathological amelioration in accelerated aging model due to GAL.

M352
A CLOSER LOOK AT 25-HYDROXYVITAMIN D ASSAY VARIATIONS: VITAMIN D STATUS IMPLICATIONS

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Background: the last decade has seen a substantial increase in clinical interest in vitamin D (VitD) deficiency and laboratory testing for VitD status. Laboratories professionals are often confronted with challenged related to VitD testing, including controversy over optimal and target VitD concentrations, assay co-specificity, variable sample results across marketed assays and laboratories and lack of standardization of VitD assays. In this assessment, we focus on the implication of different VitD status produced by automated immunoassays.
Methods: remnant serum samples, targeting low concentration levels, from 74 subjects (48.2±18.7 years, 50 females and 24 males) were measured in eight 25(OH) VitD methods: Abbott Architect (Arch), Roche Elecsys (Elec), IDS-iSYS (iSYS), Siemens ADVIA Centaur (Cent), DiaSorin RIA (DSR), Liaison (LSN), LiaisonXL (XL) and Perkin Elmer LC-MS/MS (LCMS). The Centaur method also included adjusted standardization (CentA). Similarly, the DiaSorin's improved formulation LSNI and XLI were also evaluated. The VitD status were classified according to two commonly used scenario: a) deficient: <20 ng/mL; b) insufficient: 21-29 ng/mL and c) sufficient: >32 ng/mL. The agreement between the immunoassay and reference method was assessed by the inter-rater agreement Kappa.
Results: under the status classification, Arch, Cent, CentA, iSYS , LCMS, LSNI, XL and XLI are moderately agreed with DSR reference method, except the Elecsys and LSN are fairly agreed. With LCMS as reference, only the iSYS is in good agreement; the CentA, Elecsys and XLI are in moderate agreement while the remained methods are fair. Using the sufficient criteria and DSR as reference method, the CentA, Elec, iSYS, LCMS and LSN are in fair agreement, the Arch, LSNI, XL and XLI are in moderate while the Cent is in poor. With LCMS as reference, the iSYS is good; moderate agreement includes CentA, Elec, DSR and XLI; fair agreement comprises Arch, LSN, LSNI and XL; the Cent is in poor agreement.
Conclusions: while laboratories tend to focus on the correlation slope and although manufacturer indicated how the method is referenced (most LCMS reported to NIST SRM 272 or calibrated against DSR), this work further demonstrate that we should look beyond: to VitD status classification.

M353
VITAMIN D STATUS IN MEN OF ST. PETERSBURG

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Background: A total of 132 male residents of St. Petersburg, ranging in age from 21 to 52 were studied. Determination of 25-OH vitamin D (25(OH)D) level was conducted in winter (November-March) and summer (April-July) periods.
Methods. The ELISA was used for measurement of the 25-OH-Vitamin D in serum.
Results: In summer the average level of 25-OH vitamin D was 20 ng/ml. While analyzing the distribution of patients by the concentration level in summer period, in 40% cases it was optimal - in the range between 20-30 ng/mL. However, even in summer the reduced (below 20 ng/mL) level of vitamin D was detected. In winter the average level of 25-OH vitamin D was 12 ng/mL. 23% of patients had 25(OH)D serum level higher than 20 ng/mL. The dependence of 25(OH)D level on bone mineral density changes (r=0,45) was showed. In 34,4% of cases (31 patients) we observed the decrease bone mineral density by Z-score. Two patients had osteoporosis, localized in vertebrae L2-L4. Considering the importance of vitamin D in the pathogenesis of bone tissue an attention to the hypovitaminosis D should be paid. In winter period the serum levels below 20 ng/mL were observed in 76% of male patients. 40% patients had critical (<10 ng/mL) level of vitamin D in serum.
Conclusions. The summary of obtained data indicates the necessity of monitoring and correction of the vitamin D status, especially in winter, it can be used for prediction of pathology risks and selection a complex of remedial measures for each patient.

M354
DIFFERENCES IN PREVALENCE OF DEFICIENT VITAMIN D LEVEL DEPENDING ON THE USED ASSAY

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Background: The best indicator of vitamin D status is measurement of 25-hydroxyvitamin D [25(OH)D] since is its major circulating metabolite.With the recent launch of automated assays has exponentially increased his determination and recent studies shown a high frequency of insufficient serum vitamin D levels in the general population.Moreover, some authors have highlighted the lack of precision, reproducibility and standardization between assays.The aim of this study is to compare the prevalence of deficient levels of 25(OH)D measured by Elecsys Vitamin D total (Roche) and ADVIA Centaur Vitamin D Total (Siemens).
Methods: 166 routine serum samples were randomly selected and frozen at -20 ºC until analysis.25(OH)D levels were analyzed in a freshly thawed aliquot by both methods.Two groups of patients were considered: "supplemented group" (92 patients, 58.7% males and 41.3% females) formed by patients who received Vitamin D analogues and "untreated group" (74 patients, 44.6% males and 55.4% females) formed by the rest of the patients.Concentrations of 25(OH)D were expressed in ng/mL. The deficiency was defined as 25(OH)D values less than 20 ng/mL. Data were analyzed using Medcalc 12.3.0.
Results: 25(OH)D mean values measured by Roche and Siemens for the supplemented group were 40.6±14.5 and 25.4±13.1 ng/mL, and for the untreated group were 24.9±13.2 and 12.8±6.6 ng/mL respectively.The differences between these means were statistically significant (p <0,0001). The prevalence of Vitamin D deficiency in the case of samples analyzed by Siemens was 40.2% and 85.1% for supplemented and untreated group respectively. For serum samples evaluated by Roche prevalence of deficiency was 7.6% and 43.2% for supplemented and untreated group respectively.
Conclusions: The prevalence of vitamin D deficiency is higher when Siemens's assay is used. This means that some patients may receive treatment without needing it, if the determination is made with the Siemens test, or not to be treated if the determination is performed with the Roche assay. In brief, these tests do not seems to be interchangeable and laboratory specialists should inform clinicians if any change in 25(OH)D assays is perform since these differences between assays will have a significant impact on patients management.

M355
VITAMIN D DEFICIENCY IN THE UNITED ARAB EMIRATES AND ITS GLOBAL STATUS

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Background: Vitamin D deficiency is an epidemic of such magnitude and seriousness that it is not only alarmingly widespread, but also a root cause of many serious diseases such as rickets, osteoporosis, MS, cancer, CVD, tuberculosis and diabetes. Vitamin D is actually a steroidal hormone like estrogen or testosterone. It stands alone as the only 'vitamin' the body can produce on its own. Vitamin D is called the 'Sunshine Vitamin' because the body naturally produces it through exposure of your skin to the sun.Vitamin D receptors (VDR) have been found in almost every type of human cell, from brain to our bones. Vitamin D receptor controls (directly or indirectly) more than 3000 genes that regulate calcium, phosphorus and bone metabolism, modulate innate & adaptive immunity, control cell growth and maturation, regulate the production of insulin and renin, induce apoptosis and inhibit angiogenesis. Many clinical laboratories in the world have seen requests for vitamin D testing increased by 100% or more in the last 5 years. The most common laboratory test to assess vitamin D nutritional status is total 25(OH)D serum concentrations. All age-groups require optimal levels of vitamin D to support physiologic functions that are dependent on circulating 25 (OH) D.
Methods: A total of 278 participants (female, 208; male, 70) were randomly selected from a computer-generated sequence from Zayed University who were willing to participate in the study. 25(OH)D. Blood samples were taken from all subjects to analyze serum 25(OH)D as an indicator of vitamin D status. Status of 25(OH)D was measured using a modified high-performance liquid chromatography (HPLC) method. A written consent was signed by all the participants and the research projects was approved by the IRB and ethics committee.
Results: More than 90% of UAE students at Abu Dhabi were found D-deficient and only 1% students were found >75 nmol/L of 25(OH)D. This study documents the prevalence of vitamin D deficiency due to sun avoidance, dress code, and life style. The optimal range for 25(OH) D values lies above 30 to 32 ng/mL (75-80 nmol/L) for most populations.
Conclusion: This study offers evidence that vitamin D deficiency could be a major public health burden among young Emirati adults, mostly because of sun avoidance. To further evaluate the predictors of vitamin D status in this population, the study examined diet, obesity and sun exposure. In summer, the mean serum 25(OH)D concentration for females was 20.9±14.9 nmol/L, whereas that for males was 27.3±15.7 nmol/L. Females scored significantly higher than males on the sun avoidance inventory (SAI), indicating that females avoid sun exposure to a greater extent than males, possibly explaining the lower vitamin D status. Public education and awareness should be provided about the usefulness of vitamin D supplementation like Minisun vitamin D3 tablets (1) and drops and the value of sensible sunlight exposure.

M356
EFFECT OF CHLORPROMAZINE AND HALOPERIDOL COMBINATION ON LIPID PROFILE IN NIGERIA SCHIZOPHRENIC PATIENTS

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Backgroud: It is a known fact that dyslipidemia subsist in psychiatric patients on both typical and atypical antipsychotics mono-therapy. Could these regimens in combination be favorable against dyslipidemia? This study therefore investigated the effect of chlorpromazine and haloperidol combination on the lipid profile of schizophrenic patients.
Method: The test comprised of 26 schizophrenic patients recruited from psychiatric hospital, Uselu, Benin city, Edo state, Nigeria. Chlorpromazine 200mg nocte in combination with haloperidol starting at 15 – 20mg daily in divided doses were administered. Blood samples were collected prior to experiment (basal value) and six weeks (final value) later for lipid profile estimation and values compared to those of control (n=30), who are apparently healthy volunteers with no family history of any psychiatric illness. Lipid profile was determined using standard methods.
Results: Compared to control, the schizophrenic patients had significantly higher BMI, blood pressure and lipids levels prior (basal values) to the experiment. Final values showed significant increase ($P < 0.05$) in the levels of TG, TC, LDL and VLDL and significantly decreased ($P < 0.05$) levels of HDL compared to basal values and control. Atherogenic indices (TC/HDL and LDL/HDL) of patients were significantly higher ($P < 0.05$) than control and this effect was potentiated with six weeks of chlorpromazine/haloperidol combination therapy.
Conclusion: Conclusively, the observed changes suggest lipid disorders in schizophrenic patients which was exacerbated with chlorpromazine/haloperidol combination therapy.

M357

COMPARISON OF FOUR AUTOMATED SERUM VITAMIN B12 METHODS: BECKMAN ACCESS DXI 800 UNICEL, SIEMENS ADVIA CENTAUR XP, ROCHE COBAS E601, ABBOTT ARCHITECT I2000SR

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Background: Vitamin B12 deficiency manifests itself with signs and symptoms of neurological and hematological system. Although there is no gold standard diagnostic test yet for vitamin B12 deficiency, serum vitamin B12 assay is generally used for the diagnosis and follow up of treatment. In our study we aimed to compare four immunoassay methods for the serum vitamin B12 assay and investigated the correlation of immunoassay methods.
Methods: The study included sera of 69 patients for whom vitamin B12 were requested for routine clinical testing. Serum vitamin B12 levels were measured on all samples by four immunoassay analyzers and measurements were duplicated. Serum vitamin B12 concentrations were determined by four immunoassay methods based on the competetive protein binding assay: Access DxI 800 Unicel (Beckman Coulter, USA), ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY),Roche Cobas E601 (Roche Diagnostics,Germany), Architect i2000sr (Abbott Laboratories, Abbott Park, Illinois, U.S.A).
Results: Vitamin B12 results were significantly correlated between four immunoassay methods with slopes ranging from 0.798 to 1.140 and correlation coefficients (r) of 0.898 to 0.987, P <0.001. While four analyzers were compared between each other, we observed that the results of Unicel DXI 800 were to be lower compared to the other three methods.
Conclusion: Although observed values were significantly correlated among the four methods, the results of Unicel DXI 800 were to be lower compared to the other three methods. For the reason that there is no available reference method for vitamin B12 assay, the disagreement between serum cobalamin assays continues.Therefore it is not possible to determine a common reference value especially because of Unicel DXI 800. This study showed that more standardization studies are needed and clinicians should be aware of this disagreement while making clinical decisions especially.

M358

COMPARISON OF SERUM VITAMIN B12 LEVELS WITH SERUM METHYLMALONIC ACID AND HOMOCYSTEINE

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Background: There is no gold standard diagnostic test for vitamin B12 deficiency. Diagnosis of vitamin B12 deficiency usually made by measuring serum vitamin B12 levels. However, serum vitamin B12 levels alone is not enough. Therefore the measurement of serum methylmalonic acid and homocysteine combined with serum vitamin B12 is recommended to evaluate the diagnosis of vitamin B12 deficiency.
Methods: The study included sera of 69 patients with vitamin B12 levels (ranged from 85 pg/mL to 1669 pg/mL with normal serum folat levels >5.8 ng/mL) requested for routine clinical testing. From each sample, serum vitamin B12, serum methylmalonic acid and serum total homocysteine levels were determined. Serum vitamin B12 levels were measured by ADVIA Centaur XP (Siemens Diagnostics, Tarrytown, NY) immunoassay analyzer and measurements were duplicated. Serum methylmalonic acid concentrations were determined by liquid chromatography–mass spectrometry. Serum homocysteine levels were measured by high pressure liquid chromatography assay kit.
Results: In our study, serum vitamin B12 levels were significantly negatively correlated with methylmalonic acid and homocysteine levels (correlation coefficients (r) of -0.337, P <0.05 and -0.448, P <0.05 respectively). Methylmalonic acid levels were significantly correlated with homocysteine levels (r=0.639, P <0.05). Especially, elevated methylmalonic acid and homocysteine levels were found in 63.6% and 72.7% of serum samples with low vitamin B12 levels, respectively. However, methylmalonic acid and homocysteine levels were higher at 13% and 19% of cases with normal serum vitamin B12 levels, respectively.
Conclusion: Our study shows that MMA and homocystein levels correlates with serum vitamin B12 levels and evaluation of patients with low serum vitamin B12 levels or the patients clinically suspected of vitamin B12 deficiency even with normal serum vitamin B12 levels, should be made according to the MMA and homocystein levels. However, any of the test alone are not sufficient for the diagnosis of cobalamin deficiency.

M359

ESTIMATION OF BLOOD SERUM VITAMIN D LEVEL IN PATIENTS WITH CHRONIC NONINFECTIOUS DISEASE AND HEALTHY INDIVIDUALS

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Background: Importance of vitamin D (25(OH)D) insufficiency has been shown for the genesis of different diseases: cardiovascular, cancer, auto-immune pathologies, type I and II diabetes and other. Therefore laboratory assessment of serum 25(OH)D level is important as an indicator of vitamin D storage, as well as in the estimation of reference values of 25(OH)D in different population groups.
Methods: We investigated 140 individuals: 64 healthy volunteers and blood donors, 60 patients with cardiovascular disease, 16 – with type II diabetes and hyperparathyrosis. 25(OH)D concentration was analysed on ARCHITECT ci8200 system (Abbott Laboratories, USA) using a chemiluminescent microparticle immunoassay (CMIA) technology.
Results: 25(OH)D concentration in volunteers (n=34) was 60.07±23.004 nmol/L and 33.34±8.121 nmol/L in blood donors (n=30) respectively. Insufficiency of 25(OH)D was identified in 90.9% of patients with hyperparathyrosis (45.55±15.403 nmol/L) and in 100% of patients with type II diabetes mellitus (37.04±9.068 nmol/L). In patients with hyperparathyrosis increased concentration of parathyroid hormone influenced the decrease in blood 25(OH)D concentration (r=–0.613, P=0.045). 25(OH)D insufficiency was found in most of patients (98.3%) suffering from coronary heart disease (44.13±14.017 nmol/L). A decreased concentration of serum 25(OH)D (38.62±12.381 nmol/L) was found (r=–0.330, P=0.010) in the group of patients with cardiovascular disease and with higher troponin I values (mean concentration 15.75±11.101 mkg/L), whereas when mean concentration of troponin I was 0.047±0.017 mkg/L, the mean concentration of 25(OH)D was found 49.65±13.736 nmol/L.
Conclusions: Blood 25(OH)D concentration in patients, with hyperparathyrosis, type II diabetes mellitus and coronary heart disease, was lower than the recommended optimum level (75–100 nmol/L) (P <0.05). Concentration of 25(OH)D was found to be not sufficient in healthy individuals blood serum as well.

M360

A HIGH SENSITIVITY ANALYTICAL METHOD FOR THE DETERMINATION OF DERIVATIZED 1,25-DIHYDROXYVITAMIN D3 AND 1,25-DIHYDROXYVITAMIN D2 BY LC-MS/MS

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Background: For Research Use Only. Not For Use In Diagnostic Procedures. Current methods for the determination of 1,25-dihydroxyvitamin D3 (DHVD3) are hampered by low sensitivity (in the case of the underivatized species) or low selectivity (in the case of immunoassays). The accurate and sensitive determination of various dihydroxyvitamin D species continues to be an important research field in clinical chemistry. We report here the results of a recently developed workflow using a novel dieneophile Cookson type reagent to significantly improve the sensitivity of MS/MS based assays.
Methods: Serum samples (200 μL) were diluted with deionized water and cleaned up using a combination of Celite and silica gel SPE type cartridges. The target materials retained on the Celite cartridge were eluted on to the silica gel cartridge using di-isopropyl ether. Successive elution from the silica gel cartridge using mixtures of isopropanol and hexane allowed for the selective removal of most interferences including 24, 25-dihydroxy vitamin D. Isolation, followed by solvent evaporation afforded a sample that could be immediately derivatized using Ampliflex™ Diene reagent. After 1 h, the derivatized sample was diluted with deionized water and analyzed by LC/MS/MS on an AB SCIEX QTRAP® 6500 system. Baseline resolution of the derivatized DHVD3 and DHVD2 could be obtained in less than 7 min using a small particle size C-18 column employing a simple Water:Acetonitrile gradient.
Results: Using double charcoal stripped serum as the matrix, we were able to routinely obtain LODs as low as 5 pg/mL. Linear calibration curves were generated from the same matrix from 5 to 250 pg/mL. Precision values, based on multilevel calibrators and controls, were typically less than 10%, with accuracies ranging between 94-106%.
Conclusions: The use of this novel Cookson type reagent affords significant gains in sensitivity compared to previously used reagents. This improvement in sensitivity allows for small sample volumes and a simplified extraction derivatization workflow.

M361

COST EFFECTIVE, HIGH-THROUGHPUT ANALYSIS OF VITAMIN D USING MICRO-FLOW LC-MS/MS

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Background:Micro-flow liquid chromatography has become a compelling alternative to conventional HPLC for many analyses given its cost-reduction and time-saving potential. Lower solvent consumption resulting from the use of micro-flow rates (5-60 μl/min), as compared to a typical flow rate of 500 μl/min using conventional HPLC, significantly reduces solvent and waste disposal costs. In addition, high on-column linear velocities and low mixing and delay volumes allow for fast chromatography and thus higher sample throughput. These benefits are realized while sensitivity is maintained or enhanced as compared to conventional HPLC, making micro-flow LC an excellent fit for the analysis of Vitamin D given the sensitivity and throughput requirements of this assay.
Methods: Here we present the details of the analysis of 25-monohydroxy Vitamin D2 and 25-monohydroxy Vitamin D3 using the Eksigent ekspert™ microLC 200 system in combination with the AB SCIEX QTRAP® 4500 LC/MS/MS system. Chromatographic separation of the two compounds is achieved using a Halo C18 column (0.5x50 mm, 2.7 μm) and a water/methanol/formic acid gradient. Total runtime for the analysis is 3 minutes, which is achieved at a flow rate of 20 μl/min. To avoid introducing early-eluting matrix to the system, the first part of the gradient is diverted to waste thus improving robustness and column lifetime.
Results: The Limit of Quantitation (LOQ) for the analysis was determined using 'blank' stripped serum and spiked serum and found to be below 4 ng/mL, and 5 ng/mL for 25-monohydroxy Vitamin D3 and 25-monohydroxy Vitamin D2, respectively. Accuracies were within 92-109% across the calibration range (1–100 ng/mL), with CVs below 15% based on n=5 for each calibration concentration. Serum samples from donors were also analyzed using the method described above, and the results indicate that method performance is comparable to that of a conventional HPLC method.
Conclusions: An LC-MS/MS method has been developed for the analysis of 25-hydroxyvitamin D2 and D3 in human serum using micro-flow LC, resulting in a reduction of solvent consumption and faster analytical run-times.

M362

EFFECTS OF CURCUMIN SUPPLEMENTATION ON LIVER METABOLISM IN RATS FED A HIGH-FAT DIET: A PROTEOMIC APPROACH

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Background: In the modern world, many individuals routinely consume diets rich in animal fat, leading to a high incidence of obesity. Curcumin (diferuloylmethane, turmeric) extracted from the dried root of the rhizome Curcuma longa, is a popular Asian dietary spice used in curry. Curcumin is the most active component of turmeric, comprising 2-8% by weight. Proteomics is an important tool for elucidating the cellular response to various treatments including dietary factors.The aim of the study was to investigate the effect of curcumin treatment on hepatic proteome in rats fed high-fat diet (HFD).
Methods: Male Sprague-Dawley rats were divided into six groups. Group 1 was fed control diet (10% of total calories from fat). Groups 2 and 3 were given curcumin (100 and 400 mg/kg bw/day, respectively) by gavage for 8 weeks and were fed control diet. Group 4 was fed HFD (60% of total calories from fat). Groups 5 and 6 received HFD together with the two doses of curcumin, respectively. In order to understand the molecular changes in liver cells, we performed a nano LC-MS/MS analyses.
Results: Our results identified 539 proteins and of these, differential expression of 122 were calculated to be statistically significant (P <0,05). Proteomic analysis revealed 11 proteins which were associated with lipid metabolism. Curcumin treatment caused changes in the expressions of three hepatic proteins such as carnitine O palmitoyltransferase 1, ATP citrate synthase and acyl CoA binding protein in HFD fed rats. The results were confirmed by western blot.
Conclusions: The most outstanding effects of the curcumin treatment in HFD may be considered as the inhibition of hepatic lipid synthesis and the enhancement of fatty acid oxidation. Curcumin treatment is assumed to affect liver protein expressions. To the best of our knowledge, this is the first study reflecting the beneficial effects of curcumin treatment in fatty liver metabolism by performing a proteomic analysis.

M363

ZINC DIETARY SUPPLEMENTATION ALTERS HEPATIC LIPID PROFILING RECORDED BY 1H NMR SPECTROSCOPY IN HIGH FAT DIET EXPOSED ADULT MICE

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Background: High fat diets (HFD) induce metabolic alterations leading to atherosclerosis, fatty liver disease. Zinc, is essential trace element and has proved to protect tissues from metabolic distress, mainly, via antioxidative procedure. In the present study, we investigated the impact of dietary supplementation of Zinc on high-fat diet-induced changes in hepatic lipid profiling recorded by 1H NMR spectroscopy.
Methods: Male C57B6/J mice (n=40), age matched, were divided in the following groups: 6 control fed chow diet group (CD, AIN-93G, 30 mg Zn/kg) and 13 HFD3, 9 HFD30 and 12 HFD300 groups (Zn supplemented diet 3, 30 and 300 mgZn/kg, respectively, 55% calories from fat, Mucedola Co). All groups started their diets from 4 weeks of age. Body weight was registered once per week. Lipid content of liver was extracted and pattern recognition analysis was applied on the 1H NMR hepatic lipidomic data recorded on a Bruker DRX-500 Spectrometer.
Results: All HFD mice subgroups presented statistically significant higher levels of serum total cholesterol, HDL-cholesterol and non-HDL-cholesterol compared to control group. HFD300 mice group presented lower levels of serum triglycerides than those seen in the control group. The 1H NMR-based lipid analysis showed that HFD mice subgroups presented different hepatic lipid profiling compared to that recorded in the control group. The main hepatic lipid characteristics that differentiate HFD3 mice from the control group were the lower levels of saturated fatty acids, phosphatidylcholine and degree of unsaturation, and the higher levels of unsaturated fatty acids and cholesterol. HFD30 group presented lower levels of saturated fatty acids and higher levels of unsaturated fatty acids, phosphatidylcholine, cholesterol and degree of unsaturation compared to control. Finally, HFD300 group is characterized by a hepatic lipid profiling rich in atheroprotective constituents, including higher levels of unsaturated and diallylic fatty acids and degree of unsaturation, and lower levels of saturated fatty acids.
Conclusions: 1H NMR-based lipid analysis of liver samples support the protective role of Zinc in mice exposed to oxidative alterations induced by high fat diets.

M364

IS VITAMIN D DEFICIENCY ASSOCIATED WITH INFLAMMATION IN FREE LIVING OLDER ADULTS?

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Background: Inadequate vitamin D status may be implicated in the aetiology of autoimmune disease and immune dysfunction. In-vitro and in-vivo studies have reported vitamin D to be a powerful immune-modulator although the evidence is not entirely consistent. Few studies have investigated the modulatory effect of vitamin D in humans and particularly, in the older adult population who are more vulnerable to age-related immune system dysfunction. Therefore, the aim of this study was to investigate the association between vitamin D status, immune markers of inflammation and the ratio of TH-1:TH-2 cytokines in large sample of older adults.
Methods: This observational study was conducted as part of a larger investigation of Irish older adults (aged >60 yrs), namely the Trinity Ulster Department of Agriculture (TUDA) cohort study. Participants (n 998) provided blood samples and plasma concentrations of interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP) and interleukin-10 (IL-10) were measured along with serum concentrations of vitamin D (25(OH)D).
Results: A significant correlation was observed between vitamin D and IL-6, CRP, IL-6:IL-10 ratio, TNF-α:IL-10 ratio ($P < 0.001$) and with TNF-α and the CRP:IL-10 ratio ($P < 0.05$) after adjustment for age, sex, BMI, smoking and presence of inflammatory conditions. The concentrations of TNF-α, IL-6, CRP and the ratio of IL6:IL-10 were significantly lower in individuals with a sufficient vitamin D status (>75 nmol/L) compared with a deficient status (<25 nmol/L) following adjustment for age and BMI ($P < 0.05$). Vitamin D was a significant predictor of the IL-6:IL-10 cytokine ratio with vitamin D deficient (<25 nmol/L) participants significantly more likely to have an IL-6:IL-10 ratio >2:1 than those with sufficient status.
Conclusion: This is the first study to demonstrate an association between vitamin D status, markers of inflammation and the IL-6:IL-10 ratio within a free-living, older adult population. These findings demonstrate the in-vivo capability of vitamin D to significantly modulate inflammatory markers toward an anti-inflammatory profile and highlight the need for sufficient vitamin D status particularly within the older adult population, for optimal immune function.

M365
ASSOCIATION BETWEEN VITAMIN D AND TYPE 2 DIABETES?

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Background: It has grown exponentially the number of publications that relate the D vitamin complex with the development of diabetes mellitus in adults. Until the results are based on the evidence, we have studied our population. To evaluate the relationship between vitamin D and Diabetes Mellitus defined by glycosylated hemoglobin (HbA1c) and fasting plasma glucose and also as noted in other studies related to check for kidney and liver functions, which we defined by plasma creatinine and liver enzymes: aspartate aminotransferase (AST) and alanine aminotransferase (ALT).
Material and methods: We have selected 526 patients diagnosed with type 2 diabetes from outpatient hospital, specialty and health center, who had requested 25-hydroxyvitamin D, HbA1c, glucose, creatinine, AST and ALT, during the autumn- winter (January, February, March, October, November and December 2011). The determination of 25-hydroxyvitamin D was performed in the IDS-ISYS by enzyme immunoassay, that of HbA1c by HPLC (Menarini), and glucose, creatinine, AST and ALT in ARCHITEC c16000 (Abbott) by colorimetry. It has made the Kolmogorov-Sminorv for the variable under study and has been found so nonparametric was performed the statistical method Rho Spearman (RS) using SPSS version 19.0
Results: We studied 526 patients, of whom 291 (55.3%) were women and 235 (44.7%) were men. The mean of study population was 59.7 ± 14.5 years, range: 20.2–90.0 years, the mean HbA1c 6.34±1.25 %, range: 4.40–13.00% and the mean vitamin D 21.49± 12.68 ng/mL, range: 3.20 – 87.80 ng/mL. We observed a weak correlation between 25-hydroxyvitamin D and Age (RS: 0.088), Hb1Ac (RS: -0.07), Glucose (RS: -0.097), Creatinine (RS: 0.047), AST (RS: 0.077) and ALT (RS: 0.022).
Conclusions: Our results with biochemical parameters chosen, do not confirm the association between type 2 diabetes and 25-hydroxyvitamin D, neither the association with renal and hepatic function.

M366
DEVELOPMENT OF THE VIDAS 25 OH VITAMIN D TOTAL ASSAY

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Introduction: An assay for total 25-hydroxy vitamin D [25(OH)D] that measures both 25(OH)D2 and 25(OH)D3 is being developed by bioMérieux. Vitamin D is a fat-soluble steroid pro-hormone which deficiency can be associated with rickets, osteoporosis, secondary hyper-parathyroidism, as well as increasing risk of diabetes, cardiovascular or autoimmune diseases or various forms of cancer. Vitamin D is found mainly in two forms: vitamin D3 (cholecalciferol) synthesized by action of solar ultraviolet radiation on the skin and vitamin D2 (ergocalciferol) from exogenous origin only. The main storage form of Vitamin D in the body is 25(OH)D (calcidiol), found in high concentrations in serum or plasma, which makes 25(OH)D the preferred analyte for the determination of vitamin D nutritional status.
Material and Methods: Precision of the VIDAS 25-OH Vitamin D Total Assay determined across the dynamic range using assay controls and samples pools in a 5 day protocol according to CLSI EP15-A2 protocol. Method comparison was achieved using 130 specimens spanning the VIDAS calibrated range and 30 DEQAS samples. Serums were tested on multiple batches with each method. Recovery of 25(OH)D2 on the VIDAS assay was determined using serum with endogenous 25(OH)D2 (without spiking).
Results: Data obtained with the VIDAS 25-OH Vitamin D Total Assay demonstrated a limit of detection <7 ng/mL and a functional sensitivity (20% dose total CV) <10 ng/mL. Linearity was achieved from 7 ng/mL to an upper limit of 130 ng/mL. Total assay CVs (between run/day/lot) were 6.9% (at 20 ng/mL), 4.5% (35 ng/mL), 3.0% (71 ng/mL), and 1.7% (117 ng/mL). Detection of endogenous 25(OH)D2 was determined to be >75% cross-reactivity. A method comparison study proved the VIDAS 25-OH Vitamin D Total Assay to be well correlated to a FDA/CE-approved commercial immunoassay and to LC-MS/MS.
Conclusions: The VIDAS 25-OH Vitamin D Total Assay exhibits excellent analytical data and correlation to reference methods. The assay is a valuable tool in clinical laboratories for the accurate measurement of vitamin D deficiency in human sera.

M367

ROLE OF VITAMIN D AND FOLIC ACID IN CANCER PREVENTION

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Background: Experimental and epidemiological evidences have shown that some components of Western diets are important factors in the development of colorectal cancer (CRC). In this way, vitamin D (VitD) and folate have emerged as promising chemopreventive agents. The purpose of this study was to evaluate the prevalence of VitD and folate deficiency in patients with initial diagnosis of CRC and compare the results with patients with other gastrointestinal diseases.
Methods: Between November 2010 and September 2012, individuals diagnosed of CRC and patients with colorectal adenomas, both diagnosed by colonoscopy and histologically confirmed, and controls that received negative colonoscopy results were enrolled in the study. Subjects consuming supplements of vitamins were excluded. 75 patients with CRC (median 69, range from 45 to 90 years), 45 individuals with adenomas (median 71, range from 53 to 89 years) and 52 controls (median 73, range from 31 to 92 years) were collected. Serum VitD and folate levels were measured by a Roche Diagnostics automatic immunoassay for the Cobas e601 platform.
Results: Data analysis showed that 90% of the study subjects were VitD insufficient whereas incidence of folate deficiency was low since more than 85% of patients had concentrations higher than 3 ng/mL. Serum VitD levels in patients with CRC did not differ significantly from those of adenoma patients and controls (median (IQR), 11.0 (11.7) vs 13.4 (14.0) vs 10.6 (12.6) ng/mL). Serum folate levels were lower in patients with CRC compared to those of patients with adenomas and controls (median (IQR), 4.5 (2.4) vs 5.5 (4.9) vs 6.0 (4.6) ng/mL, respectively; $P < 0.05$).
Conclusions: VitD deficiency was highly prevalent in patients undergoing diagnostic colonoscopy but no differences in serum levels were observed among groups of patients. On the contrary, serum folate concentrations were significantly lower in patients with cancer but more studies are needed to relate these results with the chemopreventive effect of the folic acid in this disease.

M368

EFFECTS OF CURCUMIN SUPPLEMENTATION ON SERUM FETUIN-A LEVELS IN RATS FED A HIGH-FAT DIET: IS CURCUMIN USEFUL FOR TREATMENT OF FATTY LIVER DISEASE?

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Background: Fetuin-A (α2 –Heremans-Schmid glycoprotein) is mainly synthesized in the liver and secreted into the bloodstream. The clinical studies suggest the involvement of fetuin-A in metabolic disorders such as visceral obesity, insulin resistance, diabetes and fatty liver. Curcumin (diferuloylmethane) is extracted from the rhizome Curcuma longa, and has been shown to possess potent antioxidant, anticarcinogenic, anti-inflammatory and hypoglycemic properties. In this study, we investigated the effect of curcumin treatment on serum fetuin-A levels as well as hepatic lipids and prooxidant-antioxidant status in rats fed high-fat diet (HFD) .
Methods: Male Sprague-Dawley rats were divided into six groups. Group 1 was fed control diet (10% of total calories from fat). Group 2 and 3 were given curcumin (100 and 400 mg/kg/b.w/day) by gavage for 8 weeks, respectively. Group 4 was fed HFD (60% of total calories from fat). Group 5 and 6 received HFD together with two doses of curcumin. Biochemical parameters and lipid profile were measured using commercial ELISA kits. Prooxidant-antioxidant status parameters were determined in liver homogenates by spectrophotometrical analysis. Protein levels were determined using bicinchoninic acid.
Results: It was found that HFD caused triglycerides and cholesterol to accumulate in the liver but these increases were not associated with significant changes in prooxidant-antioxidant status. Serum fetuin-A levels were increased in the HFD group as compared to control and to Cur400 groups. On the other hand, serum fetuin-A levels were found to be significantly reduced in both HFD+Cur100 and HFD+Cur400 groups, the reduction being 24.5% and 22.9% respectively. High fetuin-A levels in HFD rats did not induce any difference in insulin resistance and dyslipidemia.
Conclusions: All these findings suggest that, curcumin treatment may reduce liver fat accumulation, in correlation with fetuin-A levels. To the best of our knowledge, this is the first study demonstrating the effect of curcumin on serum fetuin-A levels in an experimental obesity model. The reduction of fetuin-A may be considered to play an important role in the beneficial effects of curcumin treatment in obesity.

M369
HYPERCHOLOSTEROLEMIA, HEPATIC ENZYMES AND VITAMIN E

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Background: Hypercholesterolemia increases the levels of oxygen radicals through various mechanisms. Oxidative stress is cytotoxic. It peroxidizes membrane phospholipids resulting in cell damage. Vitamin E is an antioxidant and hence it could be protective against hypercholesterolemia-induced toxicity.
Objectives: The objectives are to determine: 1) if long-term hypercholesterolemia has deleterious effects on liver enzymes. 2) if vitamin E can prevent the delirious effects of hypercholesterolemia on liver enzymes.
Method: The rabbits were divided into three groups each comprising of 6 rabbits: Group 1) Regular diet for two months. Group 2) 0.25% cholesterol diet for four months, and Group 3) 0.25% cholesterol diet for two months followed by 0.25% cholesterol diet with vitamin E (40 mg/kg body wt. daily orally) for an additional two months. Fasting blood samples were collected from marginal ear artery before and at monthly interval thereafter for measurement of serum cholesterol, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT).
Results: High cholesterol diet increased the serum levels of total cholesterol in both group 2 (1.57±0.27 vs. 38.9±3.72 mmol/L) and group 3 (1.39±0.17 vs. 32.97 ±6.44 mmol/L). Hypercholesterolemia did not affect serum levels of ALT (38.3±7.8 vs. 49.7±14.9 U/L). Vitamin E did not alter the levels of ALT (43.0±6.0 vs. 41.0±4.2 U/L) in hypercholesterolemia. Hypercholesterolemia increased the serum levels of AST (23.3±2.0 vs. 36.3±3.5 U/L) and vitamin E did not affect AST level (34.0±2.5 vs. 37.7±3.2 U/L) in hypercholesterolemia. Serum levels of ALP (134.3±9.6 vs. 83.8±6.0 U/L) were reduced during hypercholesterolemia and vitamin E did not alter the levels of ALP (172.0 ± 15.3 vs. 141.0 ± 50.1 U/L) during hypercholesterolemia. Serum levels of GGT were not affected by hypercholesterolemia (9.5 ± 0.5 vs. 10.0± 1.5U/L) and vitamin E did not alter the serum levels of GGT (8.7±0.7 vs. 7.5±0.5 U/L) during hypercholesterolemia.
Conclusion: The data suggest that 1) high cholesterol diet produced hypercholesterolemia.
2) Hypercholesterolemia had not effect on ALT and GGT but reduced the levels of ALP and increased the levels of AST and 3) vitamin E had no effect on the serum levels of AST, ALT, ALP and GGT during hypercholesterolemia.

M370
ARE RED BLOOD CELL INDICES GOOD INDICATORS OF COBALAMIN, FOLATE AND IRON STATUS IN THE ELDERLY?

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Background: Red blood cell (RBC) indices such as MCV, MCH, as well as the red cell distribution width (RDW) are commonly known to indicate nutritional deficiencies (i.e. vitamin B12, folate, and iron deficiency). Elderly patients show an increased prevalence of several circumstances influencing RBC indices, often with concomitant occurrence. This could affect the diagnostic characteristics of RBC indices in this patient group.
Aim: To investigate the diagnostic characteristics of RBC indices to indicate nutritional deficiencies in a cohort of elderly individuals.
Methods: This cross-sectional analysis was performed in subjectively healthy individuals aged >60 years. B12 status was investigated by holotranscobalamin (HoloTc) and total cobalamin (CBL), folate status was assessed with RBC folate and serum folate, whereas iron status was assessed with ferritin and reticulocyte hemoglobin (Ret-Hb) content. Diagnostic characteristics of RBC indices were evaluated by ROC-analysis.
Results: A total of 1230 Caucasian participants (697 females; 533 males; mean age 72 + 8 years) were included in the study. MCV had a an area under the curve (AUC) of 0.86, 95% confidence interval [0.84,0.88], for recognizing ferritin <15 ng/mL, 0.91 [0.89,0.93] for recognizing Ret-Hb <30 pg, 0.50 [0.48,0.53] for recognizing HoloTC<30 pmol/L, 0.53 [0.50,0.56] for recognizing CBL <150 pmol/L, 0.62 [0.59,0.64] for recognizing folate <7 nmol/L and 0.52 [0.49,0.55] for recognizing RBC folate <370 nmol/L. The AUC for MCH were 0.89 [0.87,0.90] for ferritin <15 ng/mL, 0.96 [0.95,0.97] for Ret-Hb <30 pg, 0.50 [0.48,0.53] for HoloTC <30 pmol/L, 0.54 [0.51,0.56] for CBL <150 pmol/L, 0.59 [0.56,0.61] for folate <7 nmol/L and 0.57 [0.55,0.60] for RBC folate <370 nmol/L. The AUC for RDW were 0.74 [0.72,0.77] for ferritin <15 ng/mL, 0.79 [0.76,0.81] for Ret-Hb <30 pg, 0.50 [0.47,0.53] for HoloTC <30 pmol/L, 0.53 [0.50,0.56] for CBL <150 pmol/L, 0.60 [0.58,0.63] for folate <7 nmol/L, and 0.79 [0.76,0.81] for RBC folate <370 nmol/L.
Conclusions: RBC indices exhibit satisfactory diagnostic characteristics in the recognition of iron deficiency in elderly Caucasians. However, they do not possess a discriminatory ability to recognize Vitamin B12 and folate deficiency.

M371
VITAMIN D LEVELS IN ELDERLY SUBJECTS CORRELATE TO HUMORAL IMMUNITY

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Background: Vitamin D plays a pivotal role in musculoskeletal health, immune cell differentiation, and in the inhibition of proliferation and angiogenesis in cancer. Elderly healthy people are known for their lower 25-OH Vitamin D serum levels nevertheless studies on healthy subjects and the relationship to humoral immunity remain to be elucidated.
Aim: To evaluate the relationship between vitamin D and analytes of humoral immunity in elderly subjects.
Methods: The www.seniorlabor.ch study is a cross-sectional study in subjectively healthy subjects aged >=60 years. We analysed data on fasting serum 25-hydroxyvitamine D3 levels [25(OH)D3] together with total IgM/A/E/G levels, IgG subclass levels and complement C3 and C4. A cutoff of 30 μg/L (75 nmol/L) was regarded as lower limit of 25(OH)D3 sufficiency. Vitamin deficient subjects with levels lower than 10 μg/L (<25 nmol/L) were regarded as deficient. Immunoglobulin and complement concentrations were compared according to 25(OH)D3 status.
Results: A total of 1440 elderly subjects were included. We found 29.7% of elderly subjects with normal levels (M: 24.0%, F: 33.0%) whereas insufficient levels, i.e. those diminishing from 30 to 10 ug/L, were seen in 63.1% (M: 67.8%, F: 59.1%) subjects. Severe deficiency, i.e.levels <10 ug/L, were seen in 8.0% (M: 8.2%, F: 7.9%) of the subjects. IgG subclass 1 and IgE levels were significantly higher (P=0.03 and P <0.01) and IgG subclass 2 levels were significantly lower in deficient subjects (P=0.04). Further, C3 was significantly higher (P=0.02) and C4 was significantly lower (P <0.01) in deficient subjects. An inverse correlation of Vitamin D levels was observed with IgE, IgG and C3 whereas C4 levels were higher when vitamin D levels were above the deficiency cutoff.
Conclusions: A majority (about 3/4) of the subjectively healthy elderly Swiss population presents with Vitamin D insufficiency. The observed associations point to a further potential effector function of 25(OH)D3, namely the production of IgG1, IgG2, and IgE immunoglobulins. This observation offers a possible explanation for the increased frequency of bacterial infections and allergic diseases in patients with Vitamin D deficiency.

M372
ARE MEAN PLATELET VOLUME (MPV) , PLATELET DISTRIBUTION WIDTH (PDW) AND PLATELET COUNTS ASSOCIATED WITH MARKERS OF VITAMIN B12, FOLATE, AND IRON STATUS?

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Background: Mean volume and distribution widths of red blood cells and leukocyte subpopulations have been shown to be associated with iron deficiency and/or vitamin B12 and folate deficiency. It would be interesting whether platelets as other cellular components of peripheral blood also display morphological characteristics linked to these nutritional deficiencies.
Aim: To investigate, whether platelet counts, mean platelet volume and platelet distribution width are associated with markers of nutritional deficiencies.
Methods: This cross-sectional analysis was performed within the framework of the ongoing www.seniorlabor.ch study, which includes subjectively healthy individuals aged >60 years. Vitamin B12 status was investigated by holotranscobalamin and total cobalamin, folate status was assessed with red cell folate and serum folate, whereas iron status was assessed with ferritin and reticulocyte hemoglobin content. In a multivariate linear regression model, the association of these markers with platelet markers was controlled for age and gender.
Results: A total of 1230 participants (697 females; 533 males; mean age 72 + 8 years) were included in the study. Platelet counts were inversely associated with markers of iron status (ferritin and reticulocyte hemoglobin; both P <0.001), folate status (serum folate P=0.02; RBC folate P <0.001) and B12 status (total B12 P=0.02; HoloTc P <0.01). Ferritin was associated with both mean platelet volume (MPV) and platelet distribution width (PDW) (P <0.01), whereas reticulocyte hemoglobin was only associated with PDW (P <0.01). Neither MPV nor PDW was associated with markers of vitamin B12 and folate status.
Conclusions: Whereas quantitative platelet counts are associated with markers of iron, vitamin B12 and folate status, qualitative platelet characteristics (MPV and PDW) are only associated with markers of iron status. Morphological platelet markers are unrelated to vitamin B12 and folate status.

M373

A RAPID, SIMPLE, AUTOMATABLE METHOD FOR DETERMINING METHYLMALONIC ACID LEVELS IN SERUM USING LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LCMSMS)

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Background: Vitamin B12 deficiency can result in anaemia and neurologic symptoms, early intervention can prevent many complications e.g weakness, memory loss and dementia. Measuring total B12 levels in serum is not an adequate marker of deficiency because only about 20% of B12 circulating in the body is bound to its transport protein transcobalamin which can be taken up by cells. Methylmalonic acid (MMA) levels are increased in vitamin B12 deficiency and increases in MMA levels leads to neurologic symptoms. MMA is a more sensitive and specific marker of vitamin B12 deficiency. We investigated, developed and validated a simple, easy, rapid automatable method for the measurement of MMA levels in serum using liquid chromatography and tandem mass spectrometry (LCMSMS).
Methods: Samples, calibrators, and quality controls were diluted with MMA-d3 internal standard and acidified with formic acid. This was transferred to a 10 kDa molecular weight cutoff filter plate and centrifuged. The filtrate was collected in 96 well microtitre plate and analysed on a Waters Xevo TQMS mass spectrometer. The mobile phase was methanol and water with added formic acid. The chromatography was carried out using a High Strength Silica (HSS) C18 column.
Results: Both within batch and between batch precision gave a coefficient of variation of less then 5% at high, medium and low levels. Linearity between 0 and 1.5 umol/L gave a correlation of 0.9999. The lower limit of detection was 0.021 ummol/L and the lower limit of quantitation was 0.041 umol/L. The method was further validated by comparison with a reference laboratory r^2=0.9945.
Conclusions: We have developed an easy, rapid and cost effective method for the measurement of MMA levels by mass spectrometry. This method is now in routine use and has been automated using the Hamilton Robotics Starlet liquid handling instrument. We routinely use this method to assess MMA levels in B12 deficient patients and the monitoring of their treatment. Data collected so far shows that a total B12 of 250ng/L or less should be automatically reflexed for MMA to detect functional deficiency of vitamin B12 and to establish a baseline for patients receiving therapy.

M374

25 HIDROXI- VITAMIN D (25(OH)D) MEASURED BY DIFFERENT METHODOLOGIES IN PATIENTS WITH AND WITHOUT SUPPLEMENTATION WITH ERGOCALCIFEROL, CHOLECALCIFEROL OR BOTH

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Background: Analysis of 25(OH)D has become increasingly important in clinical practice. At present there are several methodological options for its measurement. Lack of standardization between them can yield different results. Discrepancy could be increased in the case of patients supplemented with different forms of vitamin D. We compared 25(OH)D results of untreated subjects and patients receiving ergocalciferol (D2), cholecalciferol (D3), or both, assessed by three methodologies.
Methods: Groups: G1: untreated (n 19), G2: D2-treated (n 21), G3: D3-treated (n 21) and G4: D2 + D3 treated (n 20). Treatment for at least 2 months. Centrifuged samples were stored at - 20 ° C until processed. Samples were analyzed by QLIA [Abbott Diagnostics (Architect i1000)], EQLIA [Roche Diagnostics (Cobas 601)] and RIA [Diasorin] - the latter as comparison method. Data were compared by analysis of variance (ANOVA), correlation coefficient r by Passing Bablok and means versus differences by Bland and Altman.
Results: RIA and EQLIA sample means were similar with and without supplementation. G1, showed a no significant differences (F: 1.57 p: 0.2175), but QLIA showed a lower mean. The difference was higher in G2 (F: 9.08 p: 0.0003), G3 (F: 3.28 p: 0.0443) and somewhat less in G4 (F: 4.55 p <0.02). Concordance of QMIA and EQLIA with RIA was r >0.67 in G1 to G3, but fell in G4, r ~ 0.5. Bland and Altman shows that QLIA underestimates concentrations versus EQLIA in all cases (RIA mean difference: - 5.69 to - 14) especially in those treated with D2 (G2 and G4). QLIA: G1 71% of patients had values below the RIA average, this difference increased to 90.5% in G2, not shown comparing EQLIA and RIA (diff.: - 3 to 0.47).
Conclusions: RIA and EQLIA showed comparable measurements in patients untreated, treated with vitamin D2, D3 or both. While correlation of RIA with QLIA and EQLIA is similar, QLIA tends to underestimate levels of 25 (OH) D in subjects treated or not. The difference is higher in patients supplemented with D2 or D2 + D3.

M375

COMPARISON OF THREE METHODS FOR 25 HIDROXI-VITAMIN D (25 (OH) D) MEASUREMENT IN SERA

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Background: Vitamin D, (Vit D) has an essential role in calcium and phosphorus homeostasis. Low concentrations have been associated with cancer, cardiovascular and autoimmune diseases. There are manual and automated available tests, not standardized between them. We compared three methods for quantification of 25 (OH) D in patients´ sera in order to evaluate their performance.
Methods: 81 patients´ samples were analyzed by Abbott Diagnostics QLIA (Architect i1000), EQLIA Roche Diagnostics (Cobas 601) (both recently introduced in the argentine market) and Diasorin (RIA), considered as comparison method as it was used routinely in our environment. We included patients supplemented with VitD or not. All three methods use different vitamin D capture strategies, two use polyclonal antibodies and one vitD binding protein, with different specificity towards the metabolites. Samples, once extracted in gel tubes, were centrifuged within an hour and stored at -20 °C until processed.
Results: There was a significant difference between the (25 (OH) D) mean obtained by the three methods (ANOVA, F: 14.80, P <0.0001). By Bonferroni test, means and percentiles of RIA methods and EQLIA were not significantly different, but both differ from the measurement QMIA (P <0.05). The correlation between data obtained with QMIA and EQLIA regarding RIA indicates that although the correlation is similar (r: 0.692 and 0.704 respectively), slopes differ significantly (0.59 and 0.91 respectively). EQLIA would have greater similarity with RIA.
Conclusions: There are methodological differences in the design and specificity of immunoassays as the three methods do not recognize Vit D and its metabolites in the same percentage. By consensus, preferred reference range is 30-60 ng/mL; so, patients could be missclassified depending on the methodology used. It would be interesting to study the possible impact of these differences in patients (treated or not with vitamin D).

M376

DETERMINATION OF 25 HYDROXY VITAMIN D3 AND TOTAL VITAMIN D LEVELS IN HUMAN BLOOD BY DIFFERENT IMMUNOASSAYS IN COMPARED WITH LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC/MS/MS)

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Background: Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol) are the most abundant forms of Vitamin D in the body. Vitamin D3 is synthesized in the skin from 7-dehydrocholesterol in response to sunlight which then metabolised in the liver to 25 hydroxy vitamin D3 abbreviated as 25 (OH) D3. The measurements of both 25 (OH) D3 and the total 25 (OH) D (the sum of vitamin D2 and vitamin D3), in the blood are available in many immunoassays. In this study we measured 25 (OH) D3 and the total 25 (OH) D levels by four immunoassays and compared with LC/MSMS method.
Methods: Blood samples were collected from 176 apparently healthy subjects. The total 25 (OH) D level were analyzed simultaneously in our lab for three immunoassay analyzers Liaison from DiaSorin (Vercelli) - Italy); Architect i2000 from Abbott (IL, USA); and Cobas 8000 from Roach (Basel, SWZ). In addition, Cobas 8000 immunoassay method was used to determine 25 (OH) D3 fractions. Determination of both 25 (OH) D3 fraction and the total 25 (OH) D levels were assayed by LC/MSMS method from Water (Austria). The regression and statistical analysis were performed using the Microsoft excel sheet version 2003. The significant of P value were set at <0.05 using 2-tailed t-test.
Results: The values of Pearson correlation coefficient (r) between LC/MSMS and the three immunoassay methods Cobas 8000; Architect i2000; and Liaison for total 25 (OH) D were found to be 0.903; 0.902 and 0.891 with P-values of 0.058; 0.063; and <0.0001 respectively. The mean and standard deviation of error (M±SDE) values were found to be 37.1±1.74; 33.8±4.07; and 25.6±1.79 respectively compared to that for LC/MSMS method (35.3±2.12). The (M±SDE) values for 25 (OH) D3 fraction by Cobas 8000 method compared with LC/MSMS method were found to be 21.5±1.05 and 34.72±2.13 respectively with r=0.816 and (P <0.0001). A positive bias was observed on the entire range between LC/MSMS and Cobas 8000 for the 25 (OH) D3 level, with average difference of 25.9%.
Conclusions: The total 25 (OH) D level by Architect i2000 and Cobas 8000 immunoassay methods were correlated very well with LC/MSMS method. On the other hand, Cobas 8000 immunoassay method underestimated the 25 (OH) D3 level.

M377
LC-MS/MS ANALYSIS OF PLASMA 25-HYDROXY-VITAMIN D: COMPARISON OF TWO EXTRACTION METHODS

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Background: 25-Hydroxy-Vitamin D [25(OH)D] testing is essential for investigating vitamin D deficiency. A level below 75 nmol/L may lead to bone softening diseases like rickets, osteomalacia and osteoporosis as well as chronic illnesses. The acknowledged reference method for measuring 25(OH)D levels is LC-MS/MS. This technology requires an extraction step prior to analysis where most of the matrix proteins are removed from human serum or plasma. The aim of this study was to compare the performance of 25(OH)D extraction by immobilized liquid on well plate with a validated method based on protein precipitation.
Methods: 25(OH)D status was determined in 88 routine patients serum samples by a LC-MS/MS method (Chromsystems Instruments & Chemicals GmbH, D-Munich) measured on a Thermo TSQ Quantum Ultra triple quadrupole mass spectrometer fitted with an APCI source (Thermo Fisher Scientific Inc., Waltham, USA-Massachusetts) after sample preparation by immobilized liquid (Tecan AG, Switzerland) and protein precipitation (Chromsystems P/N 62000 MassChrom®Kit). The Tecan extraction relies on the principle of absorption chemistry and is coated with a proprietary immobilized liquid. The analyte dissolves in the extraction phase, the disturbing proteins, majority of lipids and carbohydrates remain in the supernatant. After an additional wash step and applying an appropriate organic solvent the resulting eluate can directly be used for analysis. The sample preparation with the Chromsystems kit is minimized to simple and effective protein precipitation. A subsequent online trap column concentrates the analyte and separates interfering substances.
Results: 25(OH)D results of 88 patient samples (range: 11 to 151 nmol/L) obtained using the two extraction methods were compared. The correlation coefficient between extraction by immobilized liquid and protein precipitation was 0.9433. On Passing-Bablok regression the slope and the intercept were 1.10 and -0.24 nmol/L.
Conclusion: Both of the described extraction procedures are fast, simple, accurate, and automatable for separation and quantification of 25(OH)D and offer a cost-effective alternative to immunological methods in a diagnostic laboratory.

M378
IS AN AUTOMATED METHOD SUITABLE TO DIFFERENTIATE POLYNUCLEAR CELLS IN ASCITES?

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Background: Cell differentiation is essential in the differential diagnosis of elevated cell counts in ascites, especially the discrimination between mononucleated (MNC) and polynucleated cells (PNC). A definite cut-off of 0.25 G/L for the absolute PNC count in ascites is diagnostic for spontaneous bacterial peritonitis (SBP). For routine determination of absolute cell counts, a cyto-preparate is set and manually differentiated. We compared the routine method with an automated method on a routine hematologic 5-diff analyser.
Materials and Methods: In 26 ascites specimens we differentiated the cells into MNC and PNC with the routine method and with a HORIBA Medical Pentra 80 autoanalyser. In each cyto-preparate 100 cells were differentiated. Then the absolute cell counts of both subpopulations were calculated using the result of the total cell count of the Pentra 80. Regression analysis was performed using Excel.
Results: There was a good correlation of the PNC count between the manual and automated method with r = 0.997 (manual count = 1.038 x automated count – 0.043). Looking at the lower range up to 0.5 G/L the correlation was quite good with r = 0.908 (manual count = 0.760 x automated method + 0.016) with a higher mean value of the automated method. Looking at the SBP discriminator of 0.25 G/L there were two discrepant results between the two methods, each with a value above the cut-off with the automated method.
Conclusion: Automated cell differentiation of ascites into PNC and MNC gives similiar results compared to the manual method without loss of diagnostic significance. As the automated method counts and differentiates more cells in comparison to the manual method, thus providing more statistically accurate results, there could be an increase of diagnostic significance, especially in the diagnosis of SBP. Therefore, this study shows that the automated method seems to be a reliable and suitable method for routine purposes in differentiating an elevated cell concentration in ascites.

M379

EFFECTS OF METHACRYLATES PRESENT IN COMPOSITE RESINS ON REDOX STATUS OF HUMAN PULP CELLS

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Background: Composite resins, utilized in dentistry, are complex mixed materials composed also by methacrylic monomers like triethylenglycol-dimethacrylate (TEGDMA) and 2-hydroxyhethyl methacrylate (HEMA). Since the polymerization reaction of monomers is uncomplete the release of these compounds may implement adverse effects in the organism. To understand the causes of this toxic action, the effects of TEGDMA and HEMA on cellular redox status were investigated in this study. The analytical techniques utilized are usually applied in Clinical Biochemistry in patient' s serum for the evaluation o oxidative stress.
Methods: Pulpal Cells were used in this study. Sub-cytotoxic concentrations of monomers were identified by the MTT assay and the following parameters were analysed: 1) Production of reactive oxygen species (ROS), measured using the probe 2',7'-dichlorodihydrofluorescin diacetate by a Glomax Multi detection system fluorimeter (Promega, Milan, Italy) (490 nm excitation and 526 nm emission wavelengths). 2) Reduced Glutathione (GSH) and Total Glutathione (GSH+GSSG), determined by Ellman method. 3) GSH metabolism, investigated through the assay of glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH) activity. NADPH absorbance modifications at 340 nm were used to determine the activity of both enzymes. 4) Superoxide dismutase (SOD) and Catalase enzymatic activities, were measured (Packard Spectracount; Packard BioScience) using the appropriate SOD determination kit (19160 Fluka Analytical, Sigma-Aldrich, Milan - Italy) and Catalase Assay kit (Sigma-Aldrich, Milan - Italy) Statistical analysis Data are expressed as the mean ± statistical error of the mean (SEM) and compared by analysis of variance (ANOVA), $P < 0.05$ was considered significant.
Results: Monomers induced an increase (about 100%, $P < 0.01$) of ROS production with a consequent increase of SOD (about 10% $P < 0.05$) and catalase enzymatic activity (about 30% $P < 0.05$). Moreover, monomers provoked a depletion both of GSH and GSH+GSSG (40% $P < 0.01$), no changes in GR and G6PDH activity were observed.
Conclusions: These changes can then be considered as a mechanism able to trigger clinical and sub-clinical adverse effects, thus calling for further investigation on biocompatibility of dent

M380

DECREASED ANTIOXIDANT DEFENSE PARAMETERS ARE ASSOCIATED WITH DEVELOPMENT OF AGE-RELATED MACULAR DEGENERATION

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Background: Dysregulation of the production and removal of oxidative species and free radicals may be detrimental in the body. It has been documented in the past 20 years, that free radicals are potential causal agents of many diseases. The aim of this study was to test the parameters of antioxidant defense system in patients with age-related macular degeneration (AMD) in order to determine the impact of oxidative stress to the development of AMD.
Methods: A total of 110 patients with age-related macular degeneration and 87 age-matched healthy subjects were included in the study. Patients underwent complete ophtalmological examination including visual acuity, color fundus photography and fluorescein angiography. Antioxidant status was estimated by the activity of erythrocyte Cu,Zn-superoxide dismutase (SOD), Se-dependent glutathione reductase (GPx), glutathione reductase (GR) and total antioxidant status (TAS) using commercial assays manufactured by Randox Laboratories based on spectrophotometar determination methods. Data analysis was performed by SPSS v10.0 statistical package using Student's t-test, Mann-Whitney U test, Chi-Square and ANOVA test.
Results: Statistical processing data revealed significaltly lower values of GR activity ($P=0.007$) and TAS concentration ($P < 0.000$) in patients with AMD compared to healthy control subjects. We documented that GR <55 U/L (OR: 4.08; 95% CI 1.30–12.71, $P=0.01$) and TAS <1.25 mmol/L (OR: 193.2; 95% CI 27.7–1349.1, $P=0.000$) had a high association with AMD development.
Conclusion: Based on the obtained results, it may be concluded that reduced antioxidant defense in tested patients could be one of the major risk factors for development of AMD in elderly patients.

M381
THE FLOW CYTOMETRY ON BRONCHOALVEOLAR LAVAGE IN THE DIAGNOSIS OF SARCOIDOSIS

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Background: In the diagnostic pathway on the diffuse pulmonary diseases the laboratory tests on the bronchoalveolar lavage fluid (BAL) are fundamental elements along with clinical features, pulmonary function tests, imaging and biopsy results.
Methods: In the past five years we have examined in our immunology laboratory 740 bronchoalveolar lavage fluids. White blood cell counts, cytograms, lymphocyte immunophenotypings were carried out on all of the samples and furthermore a detailed morphological description was obtained from observation with the optical microscope. Interleukin 8 dosage was also tested to reveal the eventual presence of an inflammatory condition. We selected from the samples a group of 50 patients with a probable o confirmed diagnosis of "sarcoidosis". We examined the bronchoalveolar fluid samples of the selected patients to search for an increased lymphocyte presence and among the lymphocyte subpopulations, particularly a CD4+ subset increase (with the respective increase in CD4/CD8 ratio), evaluating in these cases also the CD103/CD4 ratio.
Results: In our 50 patients we have found in 24 cases an increased presence of lymphocytes and in 29 cases of the same population an increase of the ratio CD4/CD8. The most interesting results concerns the distribution of the CD103 that, in 46 cases on 50, was not much expressed on the CD4+ lymphocytes: consequently in these patients we have found the presence of a ratio CD103/CD4 lower than 0,31 (as expected according to bibliography on sarcoidosis). We have measured the interleukin 8 only in 38 of these patients and 20 have had a low value as expected (<70 pg/mL) but we cannot exclude, for the others 18 samples, inflammatory conditions contemporary.
Conclusions: These data suggest a significant validity in the use of the flow cytometry to study bronchoalveolar lavage fluids, for both the possibility to obtain an accurate white blood cell count and to research and evaluate more specific details on certain interstitial lung diseases like sarcoidosis even if the histological examination for the diagnosis of this pathology is at present still the "gold standard" .

M382
CHLOROGENIC ACID AS POTENTIAL ANTI-INFLAMMATORY ANALGESIC AGENT: AN INVESTIGATION OF THE POSSIBLE ROLE OF NITROGEN -BASED RADICALS IN RATS

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Background: Non-steroidal anti-inflammatory drugs represent one of the most widely prescribed drugs used for treatment of pain and inflammation. The prescription of current existing anti-inflammatory drugs is hampered by their adverse effects over time. In the recent years there is an upsurge in the areas related to newer developments in the prevention of disease especially the role of free radicals and antioxidants. Phenolic compounds are receiving increased attention as epidemiological studies have highlighted the association between the consumption of polyphenolic –rich food and beverages and the prevention of various human diseases.
Aim: The present study investigated the analgesic and anti-inflammatory effects of chlorogenic acid (CGA), a polyphenolic compound present in many foods and beverages using carrageenan (Carr)-induced paw edema in rats and formalin – induce analgesia in mice
Materials e methods: Swiss mice and Wistar rats , Formalin, Carrageenan, Indomethacin, CGA and Elisa Kits. To study the effect of CGA on Carr – induced paw edema, 0.1 ml of 1% suspension of Carr in 0.9%Nacl solution was injected.The antnociceptive effects of CGA were tested by the formalin – induced hindpaw licking procedurein the day light. Elisa Kits were used to study the effects of CGA on some indices of oxidative stress.
Results: Treatment of rats with CGA (50, 100, 150 mg/kg) significantly reduced the rats paw edema induced by Carr and the formalin- induced pain in mice (P <.0.05) as compared to control groups. A significant reduction in rat paw volume in nitric oxide induced edema was observed (P <0.05).CGA produced a significant reduction in malondialdehyde and significant increase in reduced glutathione in paw tissues (P <.05).
Conclusion: These results confirm that CGA has both analgesic and anti-iflammatory properties which may be related to the ability of this polyphenol to reduce the levels of superoxide and peroxynitrite anion radicals.CGA showed a promising potential drug of natural anti-inflammatory property to control oxidative stress.

M383

SEMEN QUALITY AMONG MALES CONSULTING FOR COUPLE INFERTILITY OVER A 2-YEAR PERIOD IN SPANISH POPULATION

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Abstracts: Considerable interest and controversy about a possible decline in semen quality during the 20th century raised concern that semen quality could have reached a critically low level where it might affect human reproduction. Infertility is a problem that affects 12-25% of couples of reproductive age.Recent reports have indicated a decrease in semen quality of men in some countries, and suggested regional differences. The aim of our study was to analyze the sperm quality of patients presenting to our andrology laboratory in the north of Spain Gijón) during 2 years (2010-2011).
Materials and methods: Semen samples of 698 men with 36 years (range: 18-42) were processed.Samples were collected after 3-6 days of abstinence. Measurements of seminal fluid volume, pH, sperm concentration, total sperm count, motility and detailed morphology of spermatozoa were performed according to WHO criteria 2010. The data extraction was performed using LMX computer system (Siemens).
Results: Volume per ejaculate: 11.17% of the samples had hypospermia (<1.5 mL) and 7.59% hyperspermia (>5.5 mL), being the total median 2.8 mL and the 5th percentile in 0.90 mL. Sperm concentration: 12.18% oligozoospermia (<15 million/ml), 4.87% were polizoospermia (>250 million/mL) and 3.58% were azoospermia (total median: 60.63 and 5th percentile: 2.29 millions). Only 8.6% had anormal Vitality (<58%), (total median: 77% and 5th percentile: 51.2%).
Sperm mobility: 29.94% asthenozoospermia (total motility <40% and progressive motility <32%). Sperm morphology: 7.59% patients had terazoospermia (<4% of sperm with normal morphology), being the medium of normal sperm morphology (13%) and an abnormal (87%).
Conclusions: According to data obtained by our study, the most sperm abnormality observed were the mobility defects, followed by low spermatic counts. Also seems to be a relationship between these two defects as the proportion of asthenozoospermia in oligozoospermic patients was significantly higher 73 of 85 (86%) than in non-oligozoospermic group 138 of 590 (23%) (P <0.001).

M384

THE RELATIONSHIP BETWEEN INTERLEUKIN 6 (IL-6), C-REACTIVE PROTEIN (CRP) FROM SERUM AND BRONCHOALVEOLAR LAVAGE FLUID (BALF), AND DIFFUSING CAPACITY OF THE LUNG FOR CARBON MONOXIDE (DLCO) IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD)

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Background: This study measured the concentrations of interleukin 6 (IL-6), C-reactive protein (CRP) from serum and bronchoalveolar lavage fluid (BALF) in patients with chronic obstructive pulmonary disease (COPD) during stable stage to determine their possible role as biomarkers of systemic and local inflammation. We also investigated their relationship with diffusing capacity of the lung for carbon monoxide (DLCO).
Methods: 51 patients (82% man) with COPD and 16 controls (43% man) were analyzed. The concentrations of IL-6 were measured by ELISA method (eBioscience); CRP were determined with turbidimetric method. DLCO was performed with single breath method on Master screen (Jaeger). BALF were performed with 4 portion of 50 ml sterile saline solution.
Results: Statistically significant differences were found between patients with COPD (P) and control group (C) concerning serum levels of CRP (10,94±30,13 mg/L) (P), (4,23±6,99 mg/L) (C) (P=0,02). No statistically significant differences were found between patients and control group concerning serum levels IL-6, BALF IL-6 as well as BALF CRP at P level <0,05. Statistically significant differences were found between patients and control group concerning DLCO (66,4±22,8%) (P), (94,4±23,0 %) (C) (P <0,001). Serum IL-6 and serum CRP showed a strong negative correlation to DLCO (r=-0,58, -0,33 respectively, P <0,05). The correlations between DLCO, BALF IL-6 and BALF CRP were not statistically significant (r=-0,14, -0,14 respectively, P <0,05).
CONCLUSIONS: Serum IL-6, unlike serum CRP, cannot be used as biomarker for systemic inflammation.
CRP and IL-6 from BALF cannot be used as biomarkers for local inflammation in COPD and they do not correlate with DLCO.

M385
TOTAL PROTEIN ASSAYS AND ELECTROPHORETIC DETECTION OF MONOCLONAL BANDS IN URINE

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Background: Routine quantitative assays for total protein concentration in urine display different sensitivities for different individual proteins and it has been described that they underestimate low molecular weight proteins, such as the immunoglobulin light chains. Identification of monoclonal immunoglobulin light chains or Bence Jones proteins (BJP) is performed by electrophoresis of proteins in concentrated urine in order to achieve protein levels of mg/L. The aim of this study was the evaluation of the method used in our laboratory to quantify total urine protein according to its sensitivity for the detection of BJP.
Methods: 425 urine samples were evaluated. Minicon B15 concentrators (Millipore Ltd) were used and samples were concentrated 50-fold. Electrophoresis was carried out with the Hydragel protein, Sebia. Determination of total proteins was performed on Cobas c-711 (Roche Diagnostics) with benzethonium chloride method (reference range 0-0.15 g/L). A level of proteins >0.15 g/L was compared with the visualization of protein bands in the electrophoresis pattern.
Results: Monoclonal bands were detected in 35 samples (8.2%) which were later identified with immunofixation. Monoclonal and other protein bands were observed in 19 samples and therefore they were excluded. Monoclonal protein bands without other proteins were observed in 16 samples (3.8%). BJP was identified in 10 of them, BJP plus intact immunoglobulin in 5 of them and intact immunoglobulin just in 1.Total protein quantification was higher than 0,15 g/L in 15 samples (93.7%) and the other one was slightly lower (0,12 g/L) which was BJP.
Discussion: The low sensitivity of the turbidimetric assays for immunoglobulin light chains, could lead to discrepancies between the results of the total protein concentration and the electrophoresis in cases of BJP. In our study we have observed that in any case with monoclonal bands in electrophoresis, the total protein quantification was lower than 0.12 g/L, including the cases of BJP and other cases with very weak monoclonal band in the electrophoresis pattern. Total protein quantification could be a good approach for the initial evaluation of the presence of monoclonal proteins in urine. Further investigations with higher number of samples are needed.

M386
RELEVANCE OF MITOCHONDRIAL UNCOUPLING PROTEINS IN OLDER ADULTS

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Background: Sarcopenia involves loss of muscle mass and strength and, therefore, a reduced physical performance, and is associated with aging. Recently, the association between longevity and the genetic variability of some mitochondrial uncoupling proteins (UCP) has been investigated, demonstrating that UCP play a key role in aging. This is especially the case in muscle and nerve tissues, which are targets of metabolic disorders, and illustrate the fitness of elderly.
Methods: A cohort of 463 subjects of over 70 years of age was randomized from a population study of frailty and dependence (n=1172). The skeletal muscle index (SMI) was determined using the Janssen's formula. According to Janssen criteria, severe sarcopenia has a SMI ≤8.50 kg/m^2 in men and SMI≤5.75 kg/m^2 in women. We also collected data on age, gender, body mass index (BMI), and calorie expenditure. Gene polymorphisms (insertion/deletion of UCP2 exon 8, -866 G/A UCP2, and -55 C/T UCP3) were determined using polymerase chain reaction-restriction fragment length polymorphism techniques.
Results: Eighty subjects (17.7%) were found to meet criteria for severe sarcopenia. Regarding the UCP2 exon 8 polymorphism, 49% (n=230) of the subjects had wild type (del/del), 42% (n=195) heterozygous (del/ins), and 9% (n=38) mutant (ins/ins) genotype. Studies of the -866 G/A revealed 33% (n=153) of the population with wild type (del/del), 54% (n=250) with heterozygous (del/ins), and 13% (n=60) with a mutant (ins/ins) genotype. Analysis of the -55 C/T UCP3 showed that 64% (n=296) presented wild type (C/C), 22% (n=102) heterozygous (C/T), and 14% (n=65) mutant (T/T) genotype. The mutant genotype for the UCP2 exon 8 appeared associated with major. sarcopenia risk (OR 2.88, 95%CI 1.31-6.31). Also, after adjusting for variables (age, gender, BMI, calorie expenditure), elderly ins/ins genotype of UCP2 exon 8 showed had a high risk (OR 2.91, 95%CI 1.10-7.72) for sarcopenia. The regression models did not reveal any significant association between the -866 G/A UCP2 and -55 C/T UCP3 polymorphisms and sarcopenia.
Conclusions: This study revealed an association between sarcopenia and the UCP2 exon 8 polymorphism. These insights prompt further studies of UCP2 as a potential gene candidate for sarcopenia risk in older adults.

M387
URINE OSMOLALITY AT TRAINING CAMP PREDICTS SLOW MUSCLE RECOVERY AFTER SECOND MATCH OF EURO2012 FOOTBALL CHAMPIONSHIP

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Background: Water is one of the most ergogenic substance for athletes enhancing energy production during exercise. Dehydration impairs sports performance. First morning urine osmolality (UO) inversely correlates with hydration. Plasma creatine kinase (CK) is a well established biomarker of muscle damage, widely used in sports medicine to monitor recovery from muscle fatigue after high-force eccentric exercise. We evaluated UO in élite football players to check whether dehydration may predict slow muscle recovery after the match.
Methods: the 26 Ukraine National Team players participating tor the football European Championship (EURO2012) were enrolled in the study. During the pre-competition training camp, we determined UO once, with an hand held digital refractometer (Osmocheck, Vitech Instruments) applying a 1000 mOsm/kg cut-off for dehydration. UO results were not used to modify hydration scheme of any player. At EURO2012, 36 hours after every match, only to the 14 players (including substitutes) who had been in the game, we measured CK activity on capillary blood with POCT dry chemistry analyzer (Reflotron Plus, Roche Diagnostics). The 1000 U/L cut-off was used to detect severe muscle fatigue leading to slow recovery. Results of UO were then statistically evaluated to describe test efficacy in predicting severe fatigue.
Results: Out of 26 enrolled athletes, 7 (27%) showed positive UO (range: 1010-1240 mOsm/kg), and 5 of them were among the14 in the pitch at the second match. CK resulted positive (range: 1017-2901 U/L) in 4 players (prevalence: 29%) and all of them have had positive UO (sensitivity: 100%). Only one player with positive UO did not show positive CK test (UO specificity: 90%). Negative and positive predictive values of UO were 81% and 80%, respectively.
Conclusions: We confirmed that on field evaluation of football players during a top level tournament is effective to monitor muscle fatigue and recovery. Surprisingly, UO is absolutely predictive of slow recovery after the second match detected by CK.

M388
COST-MINIMISATION IN THE TREATMENT OF VITAMIN B-12 DEFICIENCIES: EXPENSIVE IN-VITRO DIAGNOSTICS CAN REDUCE HEALTHCARE SPENDING

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Background: Several studies have shown the comparable clinical effectiveness of intramuscular (IM) injections and high-dose oral supplementation (OS) in treating vitamin B-12 deficiencies. Moreover, OS instead of IM treatment can lead to substantial savings in healthcare costs. Here we examined whether an efficient use of laboratory diagnostics in guiding the management of vitamin B-12 deficiencies and in preventing unnecessary treatment can reduce costs even further. Particular focus is placed on patients with "borderline" vitamin B-12 deficiencies, in whom plasma vitamin B-12 concentrations range between 100 and 200 pmol/L and in which additional laboratory diagnostics, mainly by methylmalonic acid (MMA) is commonly used to guide treatment.
Methods: A cost-minimisation analysis was used to compare the costs associated with the diagnosis and treatment of patients with borderline vitamin B-12 deficiencies. For this analysis we compared the costs for patients that are treated solely on the basis of their vitamin B-12 concentrations to those whose treatment was guided by MMA analysis. Costs and resources taken into account: laboratory analysis of MMA and vitamin B-12, vitamin B-12 medication (IM or OS), pharmacy dispensing fee and administration of injections (by general practitioner).
Results: In patients with borderline vitamin B-12 deficiencies the overall average costs of standard IM treatment are € 210 per patient per year (PPPY). By using MMA analysis to detect functional deficiencies, the unnecessary treatment of 7 out of 10 subjects with "borderline" vitamin B-12 deficiencies can be prevented, thereby reducing the costs PPPY to € 119. Treating with high dose OS allows for even greater savings, whereby the costs of both the standard OS treatment and the MMA guided OS treatment amount to roughly € 79 PPPY.
Conclusions: The use of MMA analysis to guide the diagnosis and treatment of vitamin B-12 deficiencies can enable substantial reductions in healthcare costs. While the exact costs associated with the various options will depend on local prices and protocols, our results show a clear example of how laboratory diagnostics can be used to improve both patient well-being and reduce healthcare spending.

M389
DIAGNOSING THE UNDIAGNOSED: THE ROLE OF LABORATORY TESTING IN THE PREOPERATIVE ASSESSMENT OF CATARACTOUS PATIENTS

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Background: Preoperative laboratory testing is done to predict risk,alter management and improve outcomes. Cataract surgery is a small incision surgery usually performed under local anaesthesia as an outpatient procedure. Some clinicians recommend against the use of routine testing in asymptomatic patients undergoing low-risk procedures while others believe it should be used in hopes of detecting previously undiagnosed conditions. The aim of the study was to examine whether such tests provide a potential benefit to the patients.
Methods: The medical records of 1583 patients [males/females: 784/799, mean age 69 years old (SD 7.3)] who had undergone cataract surgery from 2009 to 2012 were reviewed. All patients had cataract surgery as a day case under local anaesthesia and were medically assessed before the operation. The routine testing included complete blood count, determination of serum glucose, urea, creatinine levels, detection of hepatitis B surface antigen(HBsAg), electrocardiogram and chest X-ray. Patients with known systemic diseases were excluded from the study.
Results: Previously undiagnosed anaemia (Hb <13.5 gr/dL in adult males, <12 gr/dL in adult females) was detected in 19.2% of the patients. The severity of it led to the postponement or cancellation of the surgery in 3 cases. The prevalence of platelet count abnormalities was 4.8%. Pathological values of biochemical parameters indicative of abnormal renal function were found in 13.1%. 11.5% of the patients had impaired fasting glucose levels (100-125 mg/dL) which is considered a pre-diabetic state while 16.6% had glucose concentrations >125 mg/dL. HBsAg was present in 3.7% of the participants. The investigation also revealed 2 cases of previously undiagnosed myocardial infarction,10 of unknown arrhythmia and 1 of lung cancer.
Conclusions: In cataractous patients preoperative investigations are performed to effectively assess the risks and management of anaesthesia and surgery. This routine testing does not seem to have a significant effect on the operative outcomes in asymptomatic patients. Nevertheless, they could be benefited from it as mainly because of age they usually have coexisting diseases reflected on the laboratory results and thus a number of health issues new to them can be detected.

M390
PLASMA GLYCOSAMINOGLYCANS AS POTENTIAL BIOMARKERS IN PHYSIOLOGICAL AGEING

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Background: Structural and functional changes in the ECM (extracellular matrix) components are considered to be among the molecular mechanisms leading to physiological ageing. ECM components remodeling at the tissue level, proceeding as an integral part of ageing process was evaluated by plasma glycosaminoglycans (GAGs) during healthy ageing. Structural diversity of GAGs was assessed by the sulfation pattern of repeating disaccharide units of chondroitin sulfates (CS). Furthermore, since oxidative damage represents the main causes of post-translational modification of matrix molecules, a possible association between plasma GAGs and enzymatic protective system against oxidative damage of polysaccharide chains was also evaluated.
Methods: Blood samples were collected from 177 healthy subjects of both sex, aged from 1 to 86 years, and divided into seven groups according to the proper decade of life. Study participants were rigorously controlled with respect to their health. Total amount of plasma GAGs was quantified by the hexuronic acid assay. The presence of non-sulfated (DDi-0S), 4-monosulfated (DDi-4S) and 6-monosulfated (DDi-6S) disaccharides was determined by HPLC. To assess antioxidant defense system activity the plasma samples were assayed for superoxide dismutase (SOD) and catalase (CT) activity.
Results: During the ageing process plasma GAGs undergo quantitative and qualitative changes. The quantitative changes are reflected by the decreased concentration of total amount of GAGs ($r = -0.44$). The structural study of plasma CS revealed that Di-0S were predominant disaccharide constituents, representing more than 50% of the analyzed disaccharides in all investigated age groups. The Di-0S content of CS increases in aging process ($r = 0.26$) whereas Di-4S remain at the relatively constant level over the lifetime. Decreasing values were found as to Di-6S ($r = -0.34$). Further studies revealed that ageing influence protective enzymatic antioxidant defense system as indicated by increasing with age activity of SOD ($r = 0.46$) and decreasing CT ($r = -0.54$). A significant correlation was also found between total plasma GAGs and both SOD and CT activities.
Conclusions: The analysis of plasma GAGs, owning to its close association with oxidative status could become promising biomarkers of human ageing to date.

M391
OXIDATIVE STRESS AND BREATH CARBON MONOXIDE IN THAI YOUTH CIGARETTE SMOKERS

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Background: Smoking is the greatest risk factor for lung cancer. Smoking behavior still persists in male Thai population, especially in young people. The objective was to assess the association between smoking, serum indices of oxidative stress, and breath carbon monoxide (BCO) levels in youth cigarette smokers.
Methods: Thirty male smokers and 30 non-smokers were enrolled in this study. All subjects were age ranged from 18 to 25 years old. Smokers had smoked cigarettes >1 year and had smoked ≥10 cigarettes per day. Non-smokers had not smoked cigarettes and had not been exposed to tobacco smoke at all, acted as the control group. Malondialdehyde (MDA) was measured as an oxidative stress index by colorimetric method. The imprecision of MDA measurement were <5%. BCO was estimated by a hand held Smokerlyzer and the device was properly calibrated by the manufacture before using. Participants were requested to inhale and hold their breath for 15 seconds before exhaling into the analyzer. A BCO cutoff of ≥7 ppm was used as recommended by the manufacturer.
Results: There was a significantly increase (P <0.001) of MDA levels in cigarette smokers (8.35±3.12 umol/L) when compared to non-smokers (3.92±1.25 umol/L). A positive correlation (r=0.585, P=0.001) was demonstrated between MDA concentration and a high level of BCO in cigarette smokers. Furthermore, the extent of increased in MDA was associated with the duration of smoking (r=0.446, P=0.014).
Conclusion: These results provide possible an evidence of increase in oxidative stress and BCO level in Thais young smokers and can be used to effectively motivate Thai youth smokers to stop cigarette smoking.

M392
REASONS OF WHOLE BLOOD WITHDRAWING IN PREPARATION

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Background: The most important task for Blood Centers is a production of blood components, which guarantees the recipient's safety. Blood, which is taken from donors, is an initial material to produce blood components and already in the moment of gaining it is a subject of a quality control. In the coincidence of analyzing the qualification's procedures, taking blood and laboratory examinations' procedures, it could be a situation in which norms are non-executed. What in consequence lead to blood withdrawing from the production.
Aim: Analysis of pre-production reasons of blood withdrawing from preparation.
Material and methods: Analyzed reasons of whole blood withdrawing in preparation (Blood Preparation Section) from 2007 to 2011. Data for analysis we get from the computer system "Blood Bank".
Results: During the analyzed period to preparation were given 375979 donations of blood, and from it 15628 were withdrawn for different reasons what makes on average 2,81% per year (in 2007 - 2.24%, 2008 - 2.39%, 2009 - 2.71%, 2010 - 2,81%, 2011- 3,88%). Analysis of blood withdrawing in preparation has shown, that the most frequent cause was lipemia - 77%, the second was blood clot in blood donation - 11%, and wrong volume- 5%. The other causes reached about 7% of blood donations withdrawing in preparation.
Conclusions: Education and professional staff training, should have the direct influence for the decreasing numbers of reasons of blood withdrawing from preparation. Blood donors should be paid more attention for the systematic self-control of their health condition.

M393

THE ANALYSIS ON THE FAMILIAL TENDENCY OF TRANSFERRIN SATURATION (TS) IN KOREAN FAMILIES

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Background: This study aims to compare TS level between Korean population and the U.S. Caucasian by using the data of KNHANES V-1 and National Health and Nutrition Examination Survey (NHANES), 2001-2002. In addition, we tried to assess the existence of a familial tendency in Korean's TS level by statistical analysis of parents' and children's TS value.
Methods: Among 8958 KNHANES V-1 subjects, those without anemia and hepatitis B antigen were enrolled in this study. The age distribution of 2946 males was 10-91 years old (mean±SD, 43.3±19.3) and 10-93 years old for 3243 females (44.6±18.6). 557 families with at least one child were included for an analysis for familial tendency and total number of subjects within them was 1907.Each parent was grouped into four quartiles (designated as 1, 2, 3 and 4) based on his or her TS levels and then the mean of paternal and maternal TS four quartile indices (MTQI) were calculated and used for further analysis. Their offspring's mean TS levels (OMTL) among each MTQI group were compared using one-way analysis of variance and post-hoc multiple comparison to show the statistical differences.
Results: The mean TS for Korean male was 40.70±16.41% and 33.41±12.99% for Korean female, and both were statistically higher than the white counterparts (25.88±13.81% and 21.09±10.54%, respectively) (P-value <0.001). The OMTL among the MTQI were as followed with statistically significant differences (P-value<0.001); 30.4±14.7% for 1.0, 32.6±13.3% for 1.5, 35.6±15.8% for 2.0, 39.0±16.7% for 2.5, 39.0±16.0% for 3.0, 41.6±15.6% for 3.5 and 42.1±16.3% for 4.0. The higher the MTQI became, the higher the OMTL were observed. When offspring was subdivided into sex, high OMTL were observed in high MTQI for both male (30.2±16.2%, 33.6±13.1%, 34.0±14.5%, 38.6±17.5%, 39.5±17.1%, 41.9±16.8%, 43.6±13.2%, P-value=0.001) and female (30.7±13.2%, 31.7±13.5%, 37.1±16.8%, 39.5±15.6%, 38.1±14.1%, 41.4±14.7%, 40.3±19.3%, P-value=0.004) offspring.
Conclusion: We confirmed that Koreans have higher TS level compared to Caucasians using the KNHANES V-1 and NHANES 2001-2002. We also showed that TS levels in Koreans have a familial tendency, suggesting the possible role of genetic factors for higher TS level.

M394

OXIDIZED PROTEINS IN NEUTROPHILS OF PATIENTS WITH MODERATE AND SEVERE CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Oxidative stress plays an important role in the pathogenesis of COPD. We supposed that proteins of neutrophils may also be involved in oxidative modification.
Methods: The concentration of advanced oxidation protein products (AOPP) was measured following the protocol of Witko-Sarsat et al. (1996). The level of reactive protein carbonyl derivates was measured following the protocol of Levine R. et al. (1990). The neutrophils were obtained from blood of 45 patients with moderate and severe COPD and 18 healthy subjects. Patients were divided into 2 groups according to the severity. 20 patients with moderate COPD, mixed form (emphysematous and bronchical), exacerbation, respiratory insufficiency of grade 2 were included in first group. 25 patients with severe COPD, mixed form (emphysematous and bronchical), exacerbation, respiratory insufficiency of grade 2 were included in second group. All patients and healthy subjects had received the full information on probable inconveniences and complications at the blood sampling before giving their consent to participate.
Results: There were significant differences between the content of AOPP and protein carbonyl derivatives in neutrophils in COPD patients and the control group (P <0.001). Of note, the level of AOPP was higher in neutrophils of first group compared with ones of the second group by 20-25 times (P <0.001). At the same time the content of protein carbonyl derivatives was significantly lower in neutrophils of first group patients compared with ones of the second group by 1.5-2 times (P< 0.001).
Conclusion: Accumulation of AOPP and protein carbonyl derivatives was significantly pronounced in neutrophils of patients with COPD and was depended on the severity of the disease. On the one hand generation of different aberrant proteins in dependence of severity may be connected with selective sensibility of proteins to direct action of reactive oxygen radicals. On another hand it may be result of defects in the molecular chaperone systems in neutrophils. In any case the accumulation of aberrant proteins in neutrophils may contribute in COPD progression. Detection of AOPP/ protein carbonyl derivatives in neutrophils may be useful for control of COPD severity development.

M395
INSIGHTS INTO THE MOLECULAR MECHANISMS UNDERLYING ANOREXIA NERVOSA

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Background: Obesity (OB) and anorexia nervosa (AN) are two features of the eating disorder spectrum. In fact, fat accumulation has been shown to correlate with systemic oxidative stress in humans and mice; and moreover the production of radical oxygen species has been reported to be increased selectively in adipose tissue of obese mice, accompanied by increased expression of NADPH oxidase and decreased expression of antioxidative enzymes. The purpose of this study was to determine, in vitro, the response to oxidative stress in human primary fibroblasts and pre-adipocytes cultured with sera from OB and AN subjects, and from control (C) individuals.
Methods: Human serum samples were collected from three groups of patients (aged 20-25 years): 1) ten OB women (body mass index [BMI] >40 kg/m^2); 2) ten women with AN (BMI <17.5 kg/m^2); 3) ten healthy sex- and age-matched controls (BMI 20.0-24.9 kg/m^2). Human primary fibroblasts and pre-adipocytes were cultured for 48 h with OB, AN or C sera. Hydrogen peroxide (H2O2), at various concentrations, was added to the cultures for 24 h and cell viability was then determined. We also measured leptin, adiponectin, IGF-1 and IGFBP-3 concentrations by ELISA.
Results: Sera from AN patients increased cell resistance to H2O2 in both cell lines analyzed. Particularly, in human fibroblasts, at an H2O2 concentration of 1300 uM, about 74% of cells treated with AN sera were viable compared to 49% and 46% (P <0.01, P <0.05) of cells treated with OB and C sera respectively. In human pre-adipocytes, at an H2O2 concentration of 200 uM, 65% of cells cultured with AN sera were still viable compared to 34% and 31% (P <0.01) of cells cultured with OB and C sera respectively. As expected, the highest leptin and the lowest adiponectin concentrations (55.9 ng/mL and 9.4 ug/mL, P=0.0001 and 0.002 respectively) were observed in obese sera, whereas IGF-1 and IGFBP-3 concentrations did not differ between the three groups.
Conclusions: Our preliminary data suggest that AN serum contains biochemical compounds able to increase cellular resistance to oxidative stress. Dietary habits could underlie this phenomenon, however further studies are required to understand the molecular mechanisms governing this process.

M396
COTININE EXCRETION IN URINE AFTER SHORT-TERM EXPOSURE TO TOBACCO SMOKE

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Background: Smoking presents one of the most serious health affecting problems in modern society. Passive smoking (PS) is the inhalation of smoke, called second-hand smoke (SHS), or environmental tobacco smoke (ETS), by persons other than the intended 'active' smoker. Up to 75% of nicotine in cigarettes is released into the environment in the form of side stream. SHS causes many of the same diseases as direct smoking, including cardiovascular diseases, lung cancer and respiratory diseases. Cotinine, the nicotine metabolite, is the most specific and sensitive biomarker of exposure to TS.
Methods: We used ELISA method to determine cotinine concentrations in urine of two non-smokers, female (2PS) and male (3PS) which are not exposed to ETS in their everyday life. In our study both of them were exposed to ETS in enclosed space alongside passionate smokers for three hours. Right after the exposure they provided us four urine samples during next 18 hours.
Results: By monitoring cotinine concentration in urine samples we were able to confirm that cotinine could be detected only after some hours from the exposure to TS. It has been shown that the female (0.00, 0.56, 0.86, 1.59 mg/mmol) has higher urine cotinine concentrations than the male (0.00, 0.28, 0.39, 0.32 mg/mmol). We have also proved that cotinine concentration in the male's afternoon urine sample was decreasing, while it was increasing in the female's sample. This could be attributed to many individual, physiological and genetic factors that affect the metabolism and elimination of nicotine. Another possible cause for the increasing cotinine levels in female PS compared to male PS may be that female subject has notably more body fat than the male. Due to nicotine lipophilicity it can accumulate in fatty tissues very well. This could be the cause for the slower degradation and elimination of cotinine.
Conclusions: A PS is at 20-30% higher risk of developing lung cancer than those non-smokers who are not exposed to ETS. A PS may absorb certain amount of nicotine just after a short-term exposure to ETS. It is a fact that should worry us and has also been a major motivation for smoke-free laws in workplaces and indoor public places, as well as some open public spaces.

M397
SELF CONTROL OF IONIZED MAGNESIUM

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Background: Ionized magnesium (iMg), the portion of total magnesium (tMg), is the only physiological active form of Mg, essential for numerous important intracellular biochemical reactions. In an increasing number of conditions it is stated a reduction of iMg, but not tMg.
Methods: The examinations were performed on 91 patients with acute myocardial infarction (AMI), military recruits, 38 soldiers of infantry and 34 soldiers of medical corps, and on 62 smokers and 82 nonsmokers. After anaerobical collection of venous blood serum levels of iMg were analyzed by the AVL 988/4 ion selective analyzer and serum tMg levels were determined colorimetrically by xylidyl blue.
Results: Levels of iMg, contrary to tMg (median value 0.870 mmol/L), were decreased (0.690 mmol/L) immediately after AMI and normalized to the values of reference group only on the tenth day of hospitalization. Levels of tMg were even increased from the third day (0.910 mmol/L), and were 0.935 mmol/L on the tenth day. The soldiers of infantry had decreased iMg levels (P=0.0013), but increased tMg levels (P <0.0001). The soldiers of medical corps had normal iMg levels (P=0.2110), but increased tMg levels (P <0.0001). The smokers had the same iMg levels as nonsmokers (P = 0.8989), but increased tMg levels (P=0.0009).
Conclusions: The levels of tMg were increased in the soldiers of infantry and medical corps, smokers and AMI patients during early hospitalization. Under stress conditions (AMI, military serving, smoking), due to the increased needs of organism for iMg, it is retreated into cells by reducing its concentration in the circulation. The body strives to maintain homeostasis, i.e. physiological level of iMg in blood, by extracting tMg from its deposits and therefore increasing tMg level in blood. Thus, paradoxically, the increased level of tMg in the blood can indicate that the person is under stress.

M398
IDENTIFICATION OF THE SOLUBLE MANNOSE RECEPTOR IN HUMAN SERUM AS A NEW MACROPHAGE-RELATED BIOMARKER

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Background: Macrophages play important roles in the pathogenesis of inflammatory diseases, making macrophage specific proteins in serum very interesting as potential new biomarkers. The mannose receptor (MR, CD206) is expressed primarily by macrophages and dendritic cells, and a soluble functional form has been described in vitro. The aim of the present work was to investigate if a soluble form of the mannose receptor (sMR) is present in human serum and if sMR may be a new biomarker candidate in disease.
Methods: sMR was identified by affinity chromatography and western blotting of human serum, and the identity was confirmed by mass spectrometry MALDI MS/MS (MS). An ELISA assay was established and validated, and used for establishment of reference intervals based on serum samples from healthy individuals (n=217). sMR was also measured in serum from hospitalized patients (n=219), and results were related to clinical diagnosis and routine laboratory results.
Results: sMR was present in serum as a single band of approximately 170 kDa. Tryptic peptides identified by MS showed that the soluble protein covered most of the extracellular domain. A parametric 95% reference interval was established to be 0.10-0.43 mg/L in healthy adults. There was no difference between men and women. More than 50 % of hospitalized patients had concentrations above the upper reference range. Very high levels (up to 6.21 mg/L) were seen in critically ill patients with sepsis and/or severe liver disease. In the patients, sMR correlated strongly to the macrophage specific sCD163 (R^2=0.53, P <0.0001), and to acute phase proteins (erythrocyte sedimentation rate (R^2=0.58, P=0.0002), haptoglobin (log-transformed, R^2=0.58, P=0.01), anti-thrombin (R^2=0.47 (negative), p=0.03), and albumin (R^2=0.41 (negative), P <0.0001).
Conclusion: We have identified a soluble form of the mannose receptor in human serum. The sMR is strongly elevated in various disease states, including sepsis and liver disease, and shows promise as a potential new biomarker.

M399

URINARY DETERMINATION OF 8-OXO-7,8-DIHYDRO-2'-DEOXY-GUANOSINE BY SERIAL LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY

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Background: Reactive oxygen species (ROS) are produced during normal cell life and as a consequence of exposure to oxidizing external agents. ROS attack to DNA may induce potential permanent transmissible alterations. 8-oxo-7,8-dihydro-2'-deoxy-guanosine (8-oxodG) is a biomarker of DNA oxidation. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has demonstrated to be a powerful technique for the quantitative measurement of 8-oxodG in biological specimens. We have developed a LC-MS/MS method where a multi-stationary phase serial chromatography has been employed to obtain a good LC separation allowing the usage of an ion trap MS detector for the measurement of urinary 8-oxodG.
Methods: HPLC separation of urinary 8-oxodG and relative internal standard (IS), chemically oxidized [15N5]8-oxodG, was tested by a single HPLC C18 and a combination of 3 HPLC columns (a cation exchange plus 2 reverse phase C18) together with a 0.5% formic acid, 0.5% formic acid/methanol gradient. Increasing 8-oxodG concentrations have been added to 1:2 diluted urine, containing a fixed amount of IS, to create calibration curves. Mass spectrometry was performed on a HTC Ultra Ion trap. The transition from [M+H]+ 8-oxodG parent ion (m/z 284) to the specific fragment at m/z 168, together with [M+H]+ [15N5]8-oxodG (m/z) transition to the fragment at m/z 173 were analysed.
Results: HPLC with a single C18 demonstrated strong matrix effect: when 177nM 8-oxodG was added to 1:2 diluted urine, MS signal obtained in urine was 9 times weaker compared to the one obtained in water. When the experiment was repeated using the serial HPLC column set, signal reduction was around 1.5 times. Preliminary validation data demonstrated a linear range from 1.77nM to at least 70nM 8-oxodG. Inter-assay CV% for area target compound ranged from 8 to16% depending on target concentration. Limit of quantitation was set at least at 1.77nM. Recovery data, obtained adding 8.8-17.7-70.7nM 8-oxodG to diluted urine, ranged from 94 to 108%.
Conclusions: A serial stationary phase LC approach has demonstrated significative reduction in matrix effect when measuring urinary 8-oxodG by ion trap MS/MS. Preliminary method validation suggests its suitability for analysis of clinical samples.

M400

ASSOCIATION BETWEEN SLC6A4 GENE POLYMORPHISM, GLOBAL DNA METHYLATION STATUS AND LEVELS OF CORTISOL IN SUBJECTS WITH ANXIETY SYMPTOMS

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Background: In recent years, several studies show an association between genetics, epigenetics and affective disorders. The hypotheses of genetic and epigenetic influences on psychopathology, like depression and anxiety disorders, primarily point to an alteration in the expression of key genes in brain homeostasis, producing as a response to cortisol hyperreactivity against stressors. Recent studies have established that individuals with a history of child abuse, and suicide in adulthood, had a pattern of altered DNA methylation of genes that control the response to stress and trauma. Furthermore, the s/s genotype of 5-HTTLPR polymorphism of the SLC6A4 gene has been associated with an increased reactivity to cortisol post-stimulus. In the present study, the presence of 5HTTLPR polymorphism, the global DNA methylation status and its relationship with the hyperreactivity to cortisol and testosterone in individuals with anxiety symptoms were investigated.
Methods: Eighty young men without a history of mental illness were submitted to three assessments to evaluate their psychopathological profile (Test of Hamilton, POMS and PANAS). The exercise was used as stressor factor. The methylation status was evaluated using the colorimetric kit and 5HTTLPR polymorphism was evaluated by PCR. The cortisol and testosterone levels in saliva were analyzed using enzyme immunoassay kits.
Results: When relating the pattern of DNA methylation and overall score on the Hamilton test for anxiety, significant association was found in individuals with the highest score (P=0.027). Similarly for the POMS test in tension-factor anxiety (P=0.034), and negative affect in the PANAS scale (P=0.005). The presence of genotype s/s for 5HTTLPR polymorphism was significantly associated with anxiety symptoms (P=0.032) and increased levels of cortisol in saliva pre (P=0.035) and post (P=0.049) stressor. No significant differences in the response of testosterone.
Conclusions: In summary, our data show an interestingly association between DNA methylation status, the presence of s/s genotype and hyperreactivity to cortisol in individuals with anxiety symptoms, suggesting the involvement of genetic and epigenetic mechanisms in the development of this psychopathology.

M401
EOSINOPHYLIC PLEURAL EFFUSION –A CASE REPORT

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Background: A pleural effusion containing over 10% eosinophils in the cell count is term eosinophilic, it represents around 5-8% of all pleural effusions. It has a varied etiology including post-traumatic, cancer, autoimmune disorders and drug reactions.
Methods: We described the case of a 51 year old male patient with no relevant medical history that presented acutely with pleural effusion after suffering thoracic trauma.
Results: After the patient arrived to the ER the pleural effusion was drained and samples were sent to the ER laboratory; upon examination of the pleural fluid sample under clear field microscopy, and chemical analysis the following results were reported: Pleural Fluid RBC 38000.0/µL [-] WBC 1900.0/µL [0.0- 250.0] Polymorphonuclear 85.0% [-] Mononuclear 15.0% [-]Glucose 107.0 mg/dL [60.0-100.0] Amilase 24.0 UI/L [-]LDH 1262.0UI/L [219.0-439.0] proteines 3.1g/dL [-]
Comments: There is an estimated 42% eosinophilic commponent to the Polymorphonuclear cell count.
Conclusions: The existence of a mechanism that activates local eosinophils or causes their migration after trauma, has been proposed, some evidence in support of this is the observed increase eosinophil counts after pleural puncture. Even when report series suggest that most eosinophilic pleural effusions are of benign prognosis their association with malignancy can not be ruled out without further testing and its finding prompts screening for relevant malignancies. Comprehensive report data is one of the means by which the laboratory scientist can contribute to the multidisciplinary management of the ER patients.

M402
THE ROLE OF BETA–TRACE PROTEIN IN DIAGNOSIS OF CEREBROSPINAL LEAKAGE

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Background: Laboratory assesment of cerebrospinal fluid (CSF) leakage relies on compositional differences between CSF and other body fluid. Beta-trace protein (BTP) is the sacond most abundant protein in CSF after aalbumin. The ratio between CSF and serum concentration amounts to about 35, which is the highest among all CSF specific protein. BTP is an idel marker for the presence of CSF.
Methods: Ventricular CSF,lumbar CSF, samles from nasal secretion, serum were obtained from patients of the Clinic of Neurosurgery. The samples were analyzed according to the manufactures instructions with the Dade-Behring Btp assay Dade-Behring, Marburg, Germany).
Results: The concentrations of BTP in normal lumbar CSF (n=24) were between 7.101- 32.740 mg/L with a median of 14.200 mg/L. CSF/serum BTP ratio was between 10.653-57.041, with a median 30.555. Clinically confirmed cases of CSF rhinorhea (n=12) showed BTP concentrations between 0.971- 16.500 mg/L, with a median of 1.765 mg/L. BTP- nasal secretion/serum ratio was between 1.160-35.870 with a median of 5.380.
Conclusion: BTP is an accurate biomarker for the detection CSF fluid. Determination of BTP in both serum and nasal secretion is recommeded.

M403

MICROORGANISMS INVOLVED IN COMMUNITY-ACQUIRED URINARY TRACT INFECTIONS: TYPES AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS

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Background: Urinary tract infections (UTIs) are one of the most frequent infectious diseases encountered by clinicians worldwide. Due to initiation of antimicrobial therapy in general population without culture and susceptibility tests, antibiotic resistance remains one of the major threats of the present. The aim of this study was to determinate the etiology and antibiotic susceptibility of uropathogens isolated from community-acquired UTIs; study period was from January to November 2012.
Methods: 263 mid stream urine samples obtaind from patiens suspected of urinary tract infections were microscopically evaluated and cultured on specific culture media(Columbia blood agar , chromID CPS and Uricult trio). Strains isolated and identified were tested for antimicrobial sensitivity using Kirby-Bauer disk diffusion method according to CLSI standards.
Results: Of the 263 tested patiens, 75 (28.51%) had urinary infection (mean age 56,8±17,54 (18-86) years); in 9 cases bacteriuria was asymptomatic. Enterobacteriaceae family members were the main pathogens (86,66%), Escherichia coli being the most frequently isolated microorganism (74,66%), followed by Proteus spp. (6,66%) and Klebsiella spp. (5,33%). In few cases (12%) Gram positive bacteria were present (Enterococcus spp. (9,33%) and Staphylococcus spp. (2,66%). The majority (81,33%) of the isolates were from female. E.coli showed low susceptibility to ampicillin, tetracycline and trimethoprim /sulfamethoxazole; the most effective antibiotic for E. coli (>80%) was imipenem, followed by piperacilin/tazobactam and chloramphenicol. Sensitivity to Quinolones (ciprofloxacin, norfloxacin, ofloxacin, nalidixic acid), cephalosporines (cefepime, ceftriaxone, cefoperazone, cefotaxime, ceftazidime) and aminoglycosides (gentamicin and amikacin) was observed in <80%. The most active antibiotic against enterococci was linezolid. Fluoroquinolones were less active on Enterococcus spp.
Conclusions: E. coli remain the most important etiological agent of community aquired urinary tract infections. Regular monitoring is required to obtain important informations about susceptibility patterns of urinary pathogens for optimal empirical treatement of patients with UTIs.

M404

STABILITY OF ROUTINE SERUM CHEMISTRY IN SERUM SEPARATOR TUBE STORED IN REFRIGERATOR

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Background: For some blood chemistry tests, samples may be stored in the laboratory prior to analysis, when a test is added after completion of the original analysis. The aim of this study was to investigate the stability of routine serum chemistry after contact with gel in serum separator tube (SST tube) and stored in refrigerator for 10 days.
Methods: Forty-eight volunteers were recruited and venous blood was collected into SST tube. These primary tubes were centrifuged once and baseline values of routine chemistry tests [Glucose (Glu), blood urea nitrogen (BUN), creatinine (Cre), uric acid, sodium ion (Na+), potassium ion (K+), chloride ion (Cl-), bicarbonate ion (HCO3-), total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), total bilirubin (TB), direct bilirubin (DB), total protein (TP), albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP)] were analyzed within 2 hrs (day 0), then SSTs were stored in refrigerator (2-8oC) and were analyzed periodically at day 2, 4, 6, 8 and 10 of storage. Data were evaluated for both statistical and clinical significance.
Results: Compared to day 0, statistically significant differences of measured values were observed (P <0.05) on the day 2 for uric acid, K+, HDL-C, and DB; on the day 4 for Cre, Na+, Cl-, HCO3-, TC, TP, and ALP; on the day 6 for TG and Alb; on the day 8 for BUN and TB; and on the day 10 for Glu, AST, and ALT. However, all analytes were found clinically insignificant for all 10 days of stogae except uric acid, Na+, Cl-, and HCO3- which clinically significant differences were found on the day 6, 8, 8, and 6, respectively.
Conclusions: When serum was centrifuged and stored in SST tube at 2-8ºC, routine blood chemistries except uric acid, Na+, Cl-, and HCO3- were clinically stable up to 10 days. However, statistically significant differences were found from day 2 of storage.

M405
BLOOD LEVELS OF ETHANOL-CATABOLIZING ENZYMES IN MONGOLIANS

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Background: Excessive alcohol consumption and the high prevalence of related health problems among the adult Mongolian population became a public health concern. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) are the principal enzymes involved in catabolism of ethanol. Individuals who have low activity of these enzymes show low tolerance to alcohol. This fact must be considered in programs aiming to reduce alcohol consumption in the population. Studies show that the prevalence of ineffective alcohol-metabolizing enzymes is high among Asians. No study has been conducted on the serum levels of the enzymes in the Mongolian population to the date.
Methods: Fasting morning blood samples were collected from healthy 307 adults (155 males and 152 females) 25-64 years of age. The alcohol consumption pattern was assessed via questionnaire and the serum levels of ADH and ALDH were determined using the enzyme-linked immunosorbent assay.
Results: The mean serum concentration of ADH was 16.97 ng/mL and of ALDH was 14.91 ng/mL. The inter-individual variations were high for both enzymes, e.g. the serum level of ADH fluctuated from 0.14 ng/mL to 92.04 ng/mL and that of ALDH varied from 0.29 ng/mL to 80.84 ng/mL. There was no statistically significant difference in the level of ADH and ALDH detected between males and females (18.15 ng/mL vs. 15.47 ng/mL, P=0.156 for ADH and 15.73 ng/mL vs. 14.32 ng/mL, P=0.566 for ALDH). The mean ADH and ALDH concentrations of drinkers (17.15 ng/mL and 14.76 ng/mL respectively) did not differ significantly from those of non-drinkers (16.95 ng/mL and 16.00 ng/mL respectively). The levels of ADH and ALDH were higher in participants who engage in frequent drinking, i.e. up to four days per week than in those who drink less than once a month, but the differences were not statistically significant (21.05 ng/mL vs. 16.55 ng/mL, P>0.05 for ADH and 18.30 ng/mL vs. 12.97 ng/mL, P >0.05 for ALDH). The survey results demonstrate that the mean serum concentration of ADH of the surveyed population (16.97 ng/mL) is lower than that of Europeans (59 ng/mL), producing negative effects on health of Mongolians.

M406
THE ANTI-OXIDANT EFFECT OF CO-ENZYME Q10, LYCOPENE AND GRAPE SEED EXTRACT IN THE PREVENTION OF EXPERIMENTALLY INDUCED MYRINGOSCLEROSIS

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Background: Myringosclerosis (MS) is a degenerative healing process of the tympanic membrane. The etiopathogenesis of MS is rather complicated but the relationship between free oxygen radicals and myringosclerosis has been proven in experimental models. The objective of this study was to investigate and compare the possible antioxidant effects of Co-enzyme Q10 (Co-Q10), Lycopene(L) and Grape Seed Extract (GSE) in the prevention of experimentally induced myringosclerosis.
Methods: 40 Wistar albino male rats were bilaterally myringotomized and randomly separated into four groups of 10 animals each: group 1(SF) received 2 ml/kg daily saline as control group, group 2(L) received 200 mg/kg daily L in 2 mL solution, group 3(Co-Q10) received 100 mg/kg daily Co-Q10 in 2 mL solution and group 4(GSE) received 100 mg/kg daily GSE in 2 mL solution. All drugs and saline were administered orally starting from the same day of surgery for 14 days. The occurrence of myringosclerotic plaques was examined otomicroscopically and blood samples for the measurement of anti-oxidative parameters such as superoxide dismutase (SOD), nitric oxide(NO), glutathione peroxidase (GPx) were collected at the end of the 14th day.
Results: Based on otoscopic findings Co-Q10, L and GSE groups revealed statistically significant differences when compared with SF group (P <0.01). SOD levels in SF group were significantly lower than in Co-Q10, L and GSE groups (P <0.05). SOD levels showed statistically significant difference between Co-Q10 and L groups (P <0.05) and L and GSE groups (P <0.05). There was not statistically significant difference of SOD levels between Co-Q10 and GSE groups. MS scores had a negative correlation with SOD levels (r=-0.407). There was no statistically significant difference of the levels of NO and GPx between all groups (P >0.05).
Conclusions: In the present study anti-oxidative parameters were studied to support the otoscopic evaluation of myringosclerosis after systemic administration of Co-Q10, L and GSE. Although the relationship between MS and free radicals had been well documented previously, the present study compared three different antioxidants. More comprehensive clinical researches should undertake in order to obtain further insights.

M407
EVALUATION OF ANTIMICROBIAL POTENTIAL OF ETHANOLIC EXTRACTS FROM SEVERAL EDIBLE MUSHROOM SPECIES

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Background: Resistance to conventional antibiotics often presents as a stumbling block in the medical field today. Mushroom extracts with antimicrobial properties are a viable source of antimicrobial agents obtained from natural sources. The aim of this research was to demonstrate the antimicrobial potential of different extracts from commercial and wild edible mushrooms, with possibilities of their utilization as active ingredients in products that are fortified with antimicrobial activity.
Methods: The extracts were obtained by ethanolic extraction (70% ethanol) from dried fruit bodies of three Pleurotus ostreatus species, Tuber melanosporum, Marasmius oreades and Craterellus cornucopioides. The resulting extracts were lyophilized in a freeze-dryer. In vitro antimicrobial susceptibility tests were performed using different species (strains with pathogenic potential) of microorganisms: Gram-positive and Gram-negative bacteria, as well as yeast, by determination of minimum inhibitory concentration (MIC). Each extract (ethanol extraction and freeze-dried) was incorporated into nutrient agar at different concentrations between 0.2 and 25 mg/mL. Correlation coefficients between the phenolic content and the antimicrobial activity were determined.
Results: The results obtained revealed the antimicrobial potential of these extracts, especially the extracts of wild edible mushroom (T. melanosporum, M. oreades and P. ostreatus). Antimicrobial results indicated that all the tested microorganisms were inhibited in different ways. Among the microbial species used as controls, Candida and Escherichia coli were the most sensitive to these extracts, presenting an MIC of 0.6 and 6.4 mg/mL. The content of phenolics determined in extracts was found to be correlated with antimicrobial activity, depending on the species (0.651–0.921).
Conclusions: The quantity of phenolic compounds in the final extracts, as well as the lyophilization, significantly improved the antimicrobial properties. These forms of the extracts are in agreement with the antioxidant activities, which can be preserved at their maximum potency through freeze-drying.

M408
THE ROLE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE AND GLUTATHIONE S-TRANSFERASE GENE POLYMORPHISMS IN RISK OF SARCOPENIA

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Background: In the last decades, the increase of the elderly population and the interest of the scientific community to determine the impact of sarcopenia in morbidity, has prompted studies to identify genetic polymorphisms associated with aging. However, allelic heterogeneity, epigenetics, incomplete penetrance, and pleiotrophy make that the genetics underlying sarcopenia is complex, and, consequently, the identification of candidate gene polymorphisms is difficult. In this study, the objective was to analyze possible associations between sarcopenia and polymorphisms in the genes encoding endothelial nitric oxide synthase (eNOS) and glutathione S-transferase.
Methods: A cohort of 450 subjects of over 70 years of age was randomized from a population study of frailty and dependence (n=1172). The skeletal muscle index (SMI) was determined using the Janssen's formula. According to Janssen criteria, severe sarcopenia has a SMI ≤8.50kg/m2 in men and SMI ≤5.75 kg/m2 in women. We also collected data on age, gender, body mass index (BMI) and calorie expenditure. Gene polymorphism studies (intron 4 VNTR eNOS, GSTM1, GSTT1) were performed using polymerase chain reaction-restriction fragment length polymorphism techniques.
Results: The mean age was 76.2 years(SD 4.3) and 53% were women. 80 subjects (17.7%) were found to meet criteria for severe sarcopenia. Studies of the polymorphisms in intron 4 of the eNOS gene revealed that 67% (n=301) showed wild type (4a/4a), 30% (n=136) a heterozygous (4a/4b) and 7% (n =13) mutant genotype (4b/4b). 52% (n=234) of the subjects had null GSTM1 genotype and 48% (n=216) had non-null genotype. Regarding GSTT1, 22% (n=99) presented null genotype and 78% (n=351) non-null. The analyzed polymorphisms were not associated with age, gender, BMI and calorie expenditure. Moreover, regression models revealed no significant association between gene polymorphisms and sarcopenia risk.
Conclusions: Our study has found no evidence for associations between gene polymorphisms and sarcopenia. However, the studied population is fairly homogeneous because it is a non-institutionalized elderly cohort, mostly autonomous. Consequently, there is a possible issue of selective survival and elderly who might show a greater association may be dead or disabled.

M409

A REGIONAL AUDIT OF THE INCIDENCE AND FURTHER LABORATORY INVESTIGATIONS OF HYPONATRAEMIA

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Introduction: Hyponatraemia is common in the hospital setting and further biochemistry investigations may be needed to identify the cause.
Aim of the audit: To assess the incidence of hyponatraemia in adults and further investigations laboratories would suggest or recommend.
Method: A questionnaire sent to biochemistry laboratories in the Thames Region of the UK asked them to identify over three months the incidence of their adult inpatients with an initial serum or plasma sodium ranging from below 110 mmol/l to 130 mmol/l,the age and sex of the patients, main sources of the requests, whether they had a low sodium alert range, carried out any reflex testing, suggested or recommended further biochemistry tests and whether their hospital had guidelines for investigating hyponatraemia.
Results: There were 22 responses.The average number of patients with an initial sodium between 121 and 130 mmol/L, 110 and 120 mmol/L and below 110 mmol/L was 4.9%, 0.31% and 0.03% respectively. The average percentage of patients below 50, between 51 and 60,between 61 and 70,between 71 and 80 and over 80 years was 12.6%,10.5%,17.2%, 24.7% and 34.8% respectively. 52.9% of patients were female. 29% of requests were from accident and emergency, 19% from general medicine, 12.3% from general surgery, 10.7% from respiratory medicine and 9.5% from care of the elderly. 21 had a low sodium alert range <120 mmol/L, and 1 <125 mmol/L. 16 didn't reflex proteins and/or lipids to exclude pseudohyponatraemia and 17 didn't request a glucose sample if not previously sent. There was variation as to what further biochemistry tests were suggested or recommended on finding hyponatraemia on the first occasion, a paired (serum and urine) osmolality, spot urine sodium and 9 am cortisol being the three most mentioned. Fewer suggested or recommended thyroid function tests, 13 didn't report on the osmolal gap and 15 didn't issue criteria for diagnosing the syndrome of inappropriate antidiuretic hormone (SIADH). Only 10 had guidelines for investigation of hyponatraemia
Conclusions: The audit highlighted the need for biochemists to work with their clinicians in devising suitable guidelines to ensure appropriate additional investigations are performed to help identify the cause.

M410

PREVALENCE OF ANTINUCLEAR ANTIBODIES (ANA) IN GREEK PATIENTS

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Introduction: ANA detection is an important part of the diagnosis, prognosis and follow up of patients with autoimmune diseases. The aim of this study was to investigate their prevalence in Greek patients, as well as their correlation with patient age and gender.
Materials and methods: We evaluated sera of 3776 patients, 1931 (51,14%) male and 1845 (48,86%) female, aged 18-75 years (mean age 48 years). Sera were obtained from outpatients and inpatients, as part of routine screening for autoantibodies. ANA detection was performed with the ANA-Ease ELISA GD74 Kit (Genesis Diagnostics, UK) on the ETI-LAB DIASORIN fully automated analyzer. All ELISA borderline, weak positive and positive results were run on the indirect immunofluorescence assay (IFA) for result confirmation, ANA titer and fluorescence pattern determination. IFA was performed on Hep-2 substrate slides, (INOVA Diagnostics Inc., San Diego, CA) and read under the fluorescent microscope Zeiss Axiolab.
Results: 266 patients (7,04 %) were positive for ANA (82/4,24% men and 184/9, 97% women). The association between gender and the presence of ANA was examined by means of hypothesis testing. Female patients demonstrated a higher and statistically significant prevalence of ANA positive reactivity (CI 95%). 72 (27,07%) patients had a titer of 1:160, 74 (27,82%) a titer of 1:320, 39 (14,66%) a titer of 1:640 and 81 (30,45%) a titer equal or higher than 1:1280. The most prevalent pattern was the speckled one, observed in 165 (62,03%) samples, followed by the homogeneous in 82 (30,83%), the nucleolar in 8 (3,00%) and the pattern which is localized to the centromere in 11 (4,14%) samples. Homogeneous and speckled patterns appeared at all titers, while nucleolar and localized to the centromere only in titers equal or higher than 1:320. Patients were divided in 4 groups according to age. There was a statistically significant higher frequency of positive ANA in patients aged 31-45 years (96/1357) compared with patients aged 18–30 years (24/600), patients aged 45-60 years (44/726) compared with patients aged 60-75 years (102/1093) and patients aged 60-75 years (102/1093) compared with patients aged 18–30 years (24/600).
Conclusions: ANA prevalence was estimated at 7,04%. Concerning gender, our results show a higher positivity of ANA among females. Sex differences in the prevalence of most autoimmune diseases, with females more commonly affected, are attributed to many factors. We also observed a statistical difference in ANA distribution among age groups. Patients under 45 years of age presented a tendency to a smaller prevalence or ANA, which is in accordance with the data reported in the literature of higher positivity in the elderly, as a result of the loss of autoregulation of the immune system.

M411
EXPERIENCE OF SCREENING FOR SYPHILIS IN WEST TALLINN CENTRAL HOSPITAL

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Background: Syphilis is a sexually transmitted disease caused by the spirochete Treponema pallidum. Diagnosis of Syphilis is more complicated than other diseases as it requires more laboratory input and clinical judgment. Most commonly Syphilis is diagnosed using a combination of treponemal and non-treponemal serological tests. The aim of the study is to evaluate our screening results.
Methods: From January 2009 to June 2012 26616 individuals (26601 adults and 15 newborns) were tested for Syphilis. For screening of Syphilis T. pallidum IgGM was measured by chemiluminescent microparticle immunoassay (CMIA) on Architect ci8200 (Abbott Diagnostics), additional tests were Rapid Plasma Reagin (RPR test, Human) and T. pallidum hemagglutination assay (TPHA, Bio-Rad); confirmatory assays were recomBlot Treponema IgG/IgM (ImmunoBlot, Mikrogen) and INNO-LIA Syphilis Score (Innogenetics). All tests were performed and interpreted following the manufacturers' instructions.
Results: There were 414 (2%) positive screening results out of 26601 adults (T. pallidum IgGM cut-off 1.0 S/CO). Out of these 414 patients, RPR and TPHA were positive in 64 (15%) cases (RPR titer ≥1:16 was in 24 cases and RPR titer ≤1:8 was in 40 cases) and negative in 75 (18%) cases. In 275 (67%) cases TPHA was positive and RPR was negative. In total, Syphilis was diagnosed in 339 cases, of which Early Syphilis in 21 (6%), Late Syphilis (incl. Neurosyphilis) in 42 (12%), Old treated Syphilis in 276 (82%) cases. There were 75 false positive results in patients with pregnancy (n=51), HIV infection (n=7), Lyme disease (n=3) and other diseases (n=14).
Fifteen newborns from fifteen women diagnosed with Syphilis showed antibodies to T. pallidum. Further tests did not confirm any cases of Congenital Syphilis. The antibodies found in the newborns' blood were maternal.
Conclusions: We reported that 75 out of 26601 (0.3%) adults had false positive results in screening tests, for all of them TPHA and RPR were negative. The sensitivity and specificity of screening test CMIA obtained at cut-off >1.0 were 100% and 99.7%, respectively. The present study shows that CMIA is a good choice for screening of Syphilis. The use of RPR, TPHA, ImmunoBlot, as confirmatory assays, is important to avoid false positive results.

M412
GLUTATHIONE S- TRANSFERASE P1 (GSTP1), AS PREDICTIVE BIOMARKER IN PROSTATE CANCER RECURRENCE IN MEN FOLLOWING RADICAL PROSTATECTOMY

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Background: Prostate cancer (PCa), represents the second leading cause of cancer deaths in males in the United States and Western Europe. Without metastasis, PCa is both tolerable and treatable. In our study, we evaluated the association between hypermethylation of serum preoperative gluthatione S-transferase P1 (GSTP1), in men with clinically localized PCa who underwent radical prostatectomy, with prostate-specific antigen (PSA) recurrence on a period of 60 months (5 years), as a predictive biomarker of PCa in men following radical prostatectomy.
Materials and methods: We included a number of 25 men with clinically localized PCa, who underwent radical prostatectomy, and 18 men with negative prostate biopsy. All serum samples were collected before prostate biopsy. PSA recurrence was defined as a single postoperative PSA level ≥0.2 ng/mL. To analyze the methylation status of gene GSTP1 before the surgery intervention we used the methylation-specific PCR (MSP) technique.
Results: From the 25 men who underwent radical prostatectomy , 13 (52%) experienced PSA recurrence within the study period. From the 18 men with negative prostate biopsy only 5 (27.8%) presented GSTP1 hypermethylation.
Conclusions: Our study suggests that preoperative serum GSTP1 may be a used as a predictive biomarker for recurrence, in men with PCa treated with radical prostatectomy.

T001

COST-BENEFIT ANALYSIS OF COPEPTIN COMBINED WITH TROPONIN I FOR EXCLUSION OF ACUTE MYOCARDIAL INFARCTION

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Background: We developed a cost-benefit analysis regarding the use of copeptin for rapid rule-out of acute myocardial infarction (AMI) in the Department of Emergency-Urgency (ED).
Methods: We defined a theoretical decision-tree model for patients arriving to the ED with chest pain and negative ECG. We analysed earlier clinical data from 235 patients on the use of copeptin as a marker for AMI rule out. Evaluation showed that a total number of 113 patients might have been safely discharged with a 100% NPV. In our model, two branches were constructed. First considered the measurement of troponin I and clinical evaluation, second added the measurement of copeptin. We estimated costs of both branches and subsequently performed a differential costs analysis obtaining a benefit/cost rate index. Analysis took into account cost of two biomarkers and a flat fee per patient for the equipment. Health care resources and medical staff were considered: in a first scenario, we valued effective costs for medical staff, monitoring room, exam fees; in a second one we considered a standard flat fee including direct costs and diagnostic tests. Since the number of ruled in patients is equal in the two branches, hospitalization costs showed a sum of zero into the differential analysis.
Results: Cost-benefit analysis showed that the patient pathway using copeptin might have spared medical resources and monitoring room for the 113 patients early and safely ruled out, saving approximately 5 h and 41 min/patient. This could have theoretically resulted in savings of €75/patient, with a cost-benefit ratio of 4.25. Considering the second scenario, potential saving per patient resulted of €125, with a cost benefit ratio of 7.13. This result shows that every euro invested in copeptin may result in a benefit in the range of €4.25 to 7.13 for Hospital.
Conclusion: The early rule out of AMI in patients with chest pain may play an important role to optimize healthcare resources without compromising patient safety. Results highlight the role of copeptin also as a cost-benefit tool. Use of copeptin in this setting may reduce the length of stay in the ED thereby generating savings for the health care system through a better organization of the working time of medical and nursing staff.

T002

S100B BLOOD LEVEL MEASUREMENT TO EXCLUDE CEREBRAL LESIONS AFTER MINOR HEAD INJURY: THE MULTICENTER STIC-S100 FRENCH STUDY

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S100B protein is selectively expressed by astroglial cells, and its measurement in blood is suggested to complete the radiological (cranial computed tomography-CT scan) evaluation for the detection of intracranial lesions after minor head injury (MHI). We aimed to validate S100B as an accurate and valuable screening tool in a large multicenter study, with two complementary objectives: (i) to evaluate whether a second S100B blood level determination 3 hours after the first one would be informative for the management of the patients; (ii) to compare the bioclinical performances of the two commercially available automated methods of measurement of S100B for the screening of patients. We conducted a prospective observational study in 7 Emergency Departments of teaching and general hospitals throughout France. Forty hundred thirty MHI subjects were enrolled; results for serum S100B measurement determined within 3 h after the clinical event (H0) then 3 h later (H3) were compared to that of CT scan performed with 6 h following the medical income. Both the Diasorin and the Roche Diagnostics assays were systematically performed. CT scan revealed intracranial lesions in 6.3% of cases. Median serum S100B levels of the whole cohort were above the normal range whatever the immunoassay considered, and were significantly increased in patients with lesions when compared to patients free of cerebral injury (at least $P < 0.05$). With the cutoff values proposed by the manufacturers (0.15 and 0.10 μg/L for Diasorin and Roche Diagnostics, respectively), cerebral lesions on CT scan were identified with sensitivity and negative predictive value (NPV) of 96.3% and 99.4% (Diasorin, 1 dissonant case), and of 100% and 100% (Roche Diagnostics, no dissonant case). Sensitivity and NPV at H3 appeared lower than those at H0, due to the rapid decrease in S100B levels. A satisfactory correlation was observed between the two methodologies, although results were not interchangeable. WE conclude that serum S100B level on admission of patients with MHI is an accurate and useful screening tool to exclude intracranial lesions. Performing a second late S100B level determination is not informative and useless.

T003

PRESEPSIN (SCD14-ST) AND OTHER INFLAMMATORY MARKERS IN CRITICALLY ILL PATIENTS WITH SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

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Background: 1) To evaluate plasma levels of presepsin in patients with SIRS; 2) to determine whether presepsin could distinguish between infectious and non-infectious SIRS (septic and non septic patients); 3) to assess presepsin as a potential prognostic marker of SIRS; 4) to compare presepsin with other inflammatory markers and the severity of patients' status.
Methods: 84 measurements of 44 patients with SIRS were enrolled in the study. The primary outcome studied was 28-day mortality and differences between septic and non-septic patients. Plasmatic levels of presepsin, procalcitonin (PCT), C-reactive protein (CRP), lactate, leukocyte count and creatinine were analyzed. The clinical status of patients was evaluated with SOFA and APACHE II. Sepsis was diagnosed with clinical signs and confirmation of an evident infect.
Results: AUCs for 28-day mortality were as follows: CRP 0.629, presepsin 0.703, lactate 0.772, PCT 0.855. AUCs for the distinction of septic and non-septic patients: presepsin 0.534, CRP 0.724, lactate 0.798, PCT 0.809. Median values of presepsin were significantly higher in septic shock group compared to SIRS (P=0.047), likewise PCT (P=0.027) and CRP (P=0.009). No correlations between presepsin and SOFA, APACHE II, CRP, lactate, leukocyte count, creatinin were found; however, a significant positive correlation with presepsin was proven for PCT (r=0.418, (P=0,014).
Conclusions: Presepsin has an auxiliary value in the laboratory diagnostics of SIRS and septic shock. More clinical studies are necessary for its routine diagnostic and prognostic use.
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T004

PRESEPSIN: ANALYTICAL PERFORMANCES, REFERENCE VALUES AND EARLY PATTERN OF RELEASE OF A NEW SEPSIS BIOMARKER

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Background: Binding of bacterial antigens (such as lipopolysaccharides or LPS) to the specific membrane receptor CD14 of monocytes/macrophages induces the release of presepsin (soluble CD14 sub-type or sCD14-ST). Circulating concentrations of presepsin are increased in sepsis. We aimed to (1) evaluate main analytical performances of presepsin immunoassay on a PathfastTM analyser (Mitsubishi Chemical, Tokyo, Japan), (2) verify plasmatic presepsin concentrations in a reference population, and (3) study presepsin early release from circulating white blood cells (WBC) after in vitro-stimulation with LPS.
Methods: PathfastTM Presepsin method is a chemiluminescent one-step immunoassay using Magtration® technology (measuring rang from 20 to 20,000 ng/L; reference values announced with a 95e percentile at 327 ng/L). Analytical evaluation was performed according to French protocole for method validation (Société Française de Biologie Clinique). Imprecision was evaluated using two quality controls provided by the manufacturer (CTL-1 and CTL-2), and using an heparinized-plasma pool PP1 obtained from patients' samples. Samples from 50 healthy donors were also analyzed (mean age and temperature: 30±9 years, 36,9±0,3 °C). Study of presepsin release was performed on heparin-whole blood from 4 healthy donors; circulating white blood cells were in contact with 10 ng/mL LPS during the 3 hours of the experiment.
Results: Imprecision coefficients of variation (CV) were <5% at either 282 ng/L (PP1), 820 ng/L (CTL-1) and 2550 ng/L (CTL-2). Linearity of the measuring range was verified from 20 to 4,800 ng/L (sloape=0.991; r^2=0,999; recovery: 89 to 111%). Neither haemolysis (<400 mg/dL) nor LPS (<100 ng/mL) influenced presepsin concentrations. Median plasmatic concentration of presepsin obtained on samples from 50 healthy volunteers was 214 ng/L (interquartile range: 163-258 ng/L; 95e percentile= 346 ng/L). Plasmatic presepsin increases early after LPS contact with WBC (+32% at 1 hour), and reaches 44% at 2 hours.
Conclusions: PathfastTM Presepsin assay presents satisfying analytical performances. Manufacturer's references values are confirmed. In addition, early release of presepsin after LPS stimulation is promising for the use of this new sepsis biomarker in clinical practice.

T005

PROCALCITONIN INCOME FOR THE DIAGNOSIS OF SEPSIS IN SEVERE BURNED PATIENTS

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Background: The systemic inflammatory response syndrome (SIRS) after burns is rather imprecise and nonspecific. Sepsis is the main cause of mortality in severe burn patients. The aim of this study was to evaluate the ability of C-reactive Protein (CRP) and Procalcitonin (PCT) as biomarkers of infection.
Method: During one year 17 severe burns were admitted into the NCCU. The following variables were collected: age, sex, mechanism of injury, the percentage of burned body surface area, the Abbreviated Burn Severity Index (ABSI), and the absence/presence of SIRS and its infectious origin. We analyzed the serum levels of CRP and PCT on admission and every 48 hours.
Results: 157 episodes were analyzed. In 88 of them SIRS was detected and 55 of them had an infectious origin. We found differences in CRP and PCT levels depending on the type of episode analyzed. Both biomarkers discriminated episodes of infection: CRP (AUC 0,69, P <0,0001) and PCT (AUC 0,73, P <0,0001). The PCT levels in the group of more critically ill patients (ABSI> 7) had an AUC of 0,81, P <0,0001. We set the cutoff for PCT on 1,41 ng/mL (sensitivity 81.1%, specificity 71.8%, PPV 57.7%, NPV 88.8%). For every unit increase in PCT levels, there was an OR for infectious origin probability of 1.4, P <0.001.
Conclusion: The determination of PCT focuses the difference between an inflammatory or infectious process, in severe burn patients, in a manner superior to PCR.

T006

ROLE OF S100B PROTEIN AS AN EARLY PREDICTOR OF BRAIN DEATH DEVELOPMENT AFTER SEVERE TRAUMATIC BRAIN INJURY

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Background: To ascertain the role of S100β protein (S100B Pt) as an early predictor of Brain Death (BD) development after a severe Traumatic Brain Injury (TBI).
Methods: During 36 months 144 severe head injury patients were included. The clinical variables were: gender, age, reference GCS after resuscitation, presence of bilateral mydriasis on admission, prehospital hypotension and desaturation, CT findings, presence of other associated injuries, S100B-Pt levels, as well of the final result to Brain Death. Blood samples were obtained as soon as possible on admission and then every 24 h until the fourth day of evolution, unless the patient died earlier.
Results: A total of 16 of the patients 11.1% progressed to BD. Median S100B-Pt levels from the group that evolved to BD were daily higher than non-BD group. Each 1μg/L increased in the S100B-Pt value on admission and in the second sample, showed an Odd Ratio of deterioration to BD of 2.625 (95%CI 1.296-5.316) P=0.07 and 3.742 (95%CI 1.172-11.94) P=0.026, respectively. To detect the cut-off point of S100B-Pt level (on admission and 24h) in those patients with a score of V-VI according to the Marshall-CT-Classification that may evolve to BD, we performed a ROC-analysis (P=0.013 and P=0.007 respectively). The cutoff point of the admission-sample was 0,473 μg/L (sensitivity of 80% and Specificity of 74.5%) and in the 24 hours-sample was 0.371 μg/L (sensitivity of 80% and Specificity of 68.6%).
Conclusion: S100B-Pt may become an early and accurate biomarker to predict deterioration to BD after a severe TBI.

T007

YIELD OF S100B PROTEIN IN PATIENTS WITH MILD TRAUMATIC BRAIN INJURY

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Introduction: This study tested the hypothesis that S100B is a useful screening tool for detecting Intracranial Lesion (IL) in patients with a Glasgow Coma Scale score of 15 after Traumatic Brain Injury (TBI).
Methods: 143 post-TBI patients without a decrease in consciousness (GCS=15) and with at least one neurological symptom (e.g., transitory loss of consciousness, amnesia, headache, dizziness or vomiting) were prospectively included. A blood sample was drawn at 6-h post-TBI. A routine CT scan was obtained within 24 hours post-injury. Diagnostic properties of S100B for IL prediction in CT scan findings were tested using ROC-analysis.
Results: 15 patients (10.5%) had IL. Serum levels were significantly higher in those patients. Significant differences were found between S100B levels and CT scan findings (P=0.007). ROC-analysis showed that S100β is a useful tool for detecting the presence of IL in CT scans (P=0.007). In our series, the best cut-off for S100B is 0.130 μg/L, with 100% sensitivity and 32.81% specificity.
Conclusion: Within the first 6 hours post-TBI, serum S100B seems to be an effective biochemical indicator of IL in patients without a decrease in consciousness. These results indicate that higher S100B cut-off values (0.130 μg/L) substantially improve the clinical relevance of this protein.

T008

ANALYSIS OF DNA CIRCULTAING LEVELS IN SEVERE BURNED PATIENTS

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Background: Circulating DNA has been exhaustively studied in the last years as a potential diagnostic, prognostic and monitorization tool in a variety of clinical situations. Necrosis or cell apoptosis was first postulated to be the origin of plasma circulating DNA and RNA although the exact nature of plasma DNA and RNA still remains unclear. In the critical burned patient cells undergo a process of necrosis as a result of burns. In this way, circulating DNA may be a possible biological marker for critical burned patient.
Methods: 23 critical burned patients admitted to the Intensive Care Unit (ICU) were included in the study. The clinical variables were: gender, age, Abbreviated Burn Severity Index (ABSI), total burned body surface (TBBS) as well of the final result to death. Blood samples were taken 24 hours after the accident in order to study DNA plasma concentration by real time PCR. A group of 18 healthy subjects were analyzed as control group.
Results: Statistically significant differences were found between the levels of circulating DNA of burned patients versus healthy controls (P <0.001). When analyzing mortality, we found differences on it when patients were grouped depending on TBBS (cutoff 40%, P=0.01). In addition, we also found differences on mortality in those patients with a higher degree of third degree burns (P=0.02). The same result is obtained when comparing ABSI (P=0.001). However when we tried to evaluate mortality according to DNA levels among burned patients, we saw that there were no statistically significant differences. Using a logistic regression method we saw each increase of 1% on TBBS on patients, these were more likely to die (Odd Ratio of 1.081; 95% between 1.020-1.147). Similarly, for every point increased on ABSI on admission, the probability of dying increased a 1.935 (95% for 1.152-3.252; P=0.01). We explored the levels of circulating DNA among patients depending on the TBBS, finding no statistically significant differences when stratifying by TBBS 40%.
Conclusion: although DNA circulating levels increase as a consequence of burns in severe burned patients, it is not a useful biomarker for predicting mortality in this group of patients.

T009

MELATONIN: POSITIVE EFFECTS ON HIGH-RISK PATIENTS WITH DESYNCHRONIZED SLEEP-WAKE RHYTHM

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Abstract: Melatonin (MT), a pineal gland hormone, mediates many processes, including the biorhythmic regulation of the organism physiology. The role of MT in the treatment of sleep disturbances, to prevent jet lag or as a part of the sepsis treatment is widely discussed. Circadian rhythm of MT is desynchronized in critically ill pts in intensive care unit (ICU). The restoration of MT levels has been recently proved to be useful. The aims of the investigation are: to confirm that MT is a molecule active in the regulation of sleep/wake rhythm in ICU patients and to compare the differences in the MT pharmacokinetics using different administration route and drug formulation.
Methodology: The clinical effects of long term (5 days) administration of oral MT, as well as the pharmacokinetics profiles as a function of different administration ways (os, os by SLN (solid lipid nanoparticles), transdermal by SLN) have been studied in ICU pts. From the second day of the ICU stay, serial withdrawal were taken to determine both the endogenous and the exogenous plasma MT content, for a total of 20 withdrawal for each patient. Each blood sample was centrifuged and the plasma stored at -20 °C. To determine the MT concentration we used an ELISA kit that includes a pre-purification of the sample by SPE (solid phase extraction) cartridges.
Results: In this study, we have seen that administration of oral MT, is safe, reduces need for analgesic and sedative drugs and shows better neurological status indicators, also restoring the normal circadian rhythm. In patients who have received oral MT, the absorption is rapid: the peak plasma concentration has a median of 30 min and after only 5 min the MT levels were significantly higher than physiological ones. The group treated with transdermal MT presents a delayed peak plasma concentration (4 h).
Conclusions: In this study, we have proved that MT is able to normalize the sleep-wake cycle, to ameliorate the sleep quality and to reduce the number of sedative drugs used in ICU pts. We proved also that transdermal administration by SLN is effective in rising plasma MT levels as well as enteral administration and is more practicable in clinical setting.

T010

SERUM SELENIUM IN PATIENTS WITH HIGH SERUM CRP ADMITTED TO TWO DIFFERENT DEPARTMENTS OF INTENSIVE CARE UNIT

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Background: The selenium (Se) is an essential trace element with antioxidant, immunological, and anti-inflammatory properties. It is essential for maintaining the oxidative balance. Patients in intensive care unit often showed inflammatory reactions with increased inflammatory biomarkers of C-reactive protein (CRP) and leukocyte (WBC) and depletion of trace element selenium. We made a preliminary investigation to see if there was any difference in serum selenium concentration between patients admitted to two different departments of intensive care unit (ICU), the neurosurgical and the internal medicine unit. The patients in the neurosurgical unit had intracranial surgery, and the patients in the internal medicine unit had the presence of infection or organ dysfunction. All the patients had high serum CRP concentration.
Methods: We have examined a total of 14 patients, 7 from neurosurgical ICU and 7 from ICU of internal medicine. We measured their serum selenium concentration by electrothermal atomic absorption spectrometry, CRP by immunoturbidimetry and leukocyte count by hematology analyzer. The results were statistically analyzed by statistical program MedCalc.
Results: The mean serum selenium concentration measured in patients from the internal ICU (34±9 mg/L) was significantly lower than in those from the neurosurgical ICU (74±26 mg/L), $P < 0.05$. There was no significant difference in CRP concentration between two groups of patients, $P > 0.05$. Leukocytes count was significantly lower in patients from the internal ICU (8.7 ± 3.7x109) than in those from the neurosurgical ICU (13.5 ± 2.6x109), $P < 0.05$.
Conclusions: Although the number of patients in this study is very small, these preliminary results indicate that serum selenium concentration might depend on the intensity and duration of acute-phase response, regardless of the high CRP concentration.

T011

EVALUATION OF C-REACTIVE PROTEIN AND PROCALCITONIN TO PREDICT BACTEREMIA IN PATIENTS WITH CHEMOTHERAPY ASSOCIATED FEBRILE NEUTROPENIA IN THE EMERGENCY DEPARTMENT

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Background: Infections remain the mayor cause of serious complications in cancer patients with chemoteraphy-associated febrile neutropenia (FN). Early diagnosis of severe bacterial infection is difficult, and bacteremia is often diagnosed more than 24 h after symptoms appear. Circulating markers of inflammation and/or infection could help to an immediate and proper strategy for this population. We have conducted a study to assess the usefulness of two well known inflammatory markers, C-reactive protein (CRP) and procalcitonin (PCT), as predictors of bacteremia in cancer patients with chemotherapy induced FN.
Methods: 34 episodes of chemotherapy induced FN in 34 cancer adult outpatients (24 with solid tumors and 11 with hematological malignancies) admitted to ED in our hospital were enrolled. PCT and CRP were measured at presentation and venous blood samples for blood cultures were collected. Bacteremia was defined as the presence of viable bacteria in the blood. PCT and PCR levels were measured on a Cobas 411 analyzer using an electrochemiluminiscence inmunoassay and on a Dimension Vista using turbidimetric assay, respectively. Diagnostic accuracy of CRP and PCT for bacteremia was calculated by analyzing the receiver operating characteristic (ROC) curve. All statistical analyses were performed with the use of EPIDAT version 3.1
Results: Bacteremia was identified in 8 episodes of NF (23,5%). Median values of PCT and CRP were significantly higher in bacteremia (+) than in bacteremia (-) [procalcitonin: 0.91 ng/mL (IQR: 0,52-1,50) vs 0,16 ng/mL (IQR: 0,10-0,24)], [C-reactive protein: 120 mg/L (IQR: 65-208) vs 93 mg/L (IQR: 33-140)]. The area under ROC of PCT was 0,863 and that of CRP was 0,668. With a cut-off value of 0.5 ng/mL PCT yielded a diagnostic sensitivity of 75,0%, a specificity of 88,5%, a NPV of 92%, a PPV of 66,7%, a LR (+) of 6,5 and LR (-) of 0,28.
Conclusions: PCT is a useful early diagnostic marker for the detection of bacteremia in chemotherapy associated FN and has better diagnostic accuracy than CRP. It is recommended to establish an early antibiotic therapy strategy to prevent complications of bacterial infection in FN patients with high serum PCT levels in the ED.

T012

INFLAMMATORY RESPONSE IN THORACIC SURGERY USING TWO DIFFERENT ANESTHETICS

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Background: Inflammatory response in surgery is triggered by several factors including surgical injury, ischemia with following reperfusion and also specific factors like one lung ventilation in thoracic surgery. During and after surgery, increased levels of cytokines and other inflammatory parameters can be measured in plasma. Some anesthetics were found to have an immunomodulatory effect, so the choice of anesthetic could influence the inflammatory response in surgery. In our study, we wanted to find out the difference in immunomodulatory effects between anesthetics propofol and sevoflurane during and after thoracic surgery.
Methods: 33 patients were included in the study and either propofol or sevoflurane were used as anesthetics in thoracic surgery. During and after the operation we measured serum levels of inflammatory markers IL-6, IL-8, IL10 and CRP in both groups.
Results: After the operation, we measured a great elevation of IL-8 in propofol group (geometric mean and 95% CI): 14.0 (9.1-21.6) nmol/L compared to preoperative values 6.8 (5.3 – 8.8) nmol/L. In sevoflurane group, the levels of IL-8 increased from 6.2 (4.8-7.9) nmol/L to 7.5 (5.5-10.2) nmol/L. A significant difference was found between the groups in the changes of IL-6 levels during and after the operation. Postoperative levels of IL-6 reached 36.0 (21.5-60.3) nmol/L in propofol group and 20.2 (11.4-36.0) nmol/L in sevoflurane group, preoperative levels being 3.0 (1.9-4.6) nmol/L and 2.5 (1.7-3.9) nmol/L, respectively. For IL-10 levels, a statistically significant postoperative increase was measured in both groups, but the changes were not significantly different between the groups. The increase of CRP was detected in both groups on the first day after surgery.
Conclusions: Inflammatory response that is triggered during thoracic surgery is influenced by anesthesia; immunomodulatory effect depends on the type of anesthetic. Sevoflurane was found to have more pronounced antiinflammatory effect than propofol.

T013

BIOMARKERS POTENTIALLY DETECTING SEPSIS RAPIDLY IN INTENSIVE CARE AND EMERGENCY MEDICINE

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Background: We address sepsis biomarkers, identify gaps in prognostication, and postulate an approach to future diagnostics. Systemic inflammatory response syndrome (SIRS) is defined as temperature <36 or >38 oC, respiratory rate >20 breaths/min, heart rate >90 beats/min, and WBC <4,000/mm3 or >12,000/mm^3 or >10% immature neutrophils. Sepsis is infection plus systemic manifestations; severe sepsis additionally includes organ dysfunction or tissue hypoperfusion. Need for new approaches is evident from unacceptably high mortality.
Methods: Besides clinical signs and symptoms, operationally, sepsis is SIRS, infection, and stochastically manifested high plasma glucose, plasma C-reactive protein (CRP) >2 SD above normal, plasma procalcitonin (PCT) >2 SD above normal, PaO2/FIO2 <300, creatinine increase >0.5 mg/dL, INR >1.5 or aPTT >60 secs, platelet count <100,000/μL, high plasma total bilirubin, and/or hyperlactatemia >1 mmol/L. We analyzed sepsis biomarkers and their changes over time to discover the best approach to improving outcomes.
Results: We synthesized two diagnostic sets suitable for multiplexing. Set 1 comprises available tests: algorithmic lactate, WBC, CRP, PCT, interleukin-6, and lipopolysacharride-binding protein; and Set 2, investigational: cell-free genomic DNA, microRNAs, pancreatic stone protein/regenerating protein (PS/reg), soluble & subtype CD14, type 2 helper T-cell interleukin-33 receptor, soluble triggering receptor expressed in myeloid cells-1, and soluble urokinase-type plasminogen activator receptor. Through gap analysis, we discovered characteristic temporal trends that can improve prognostication and illustrate them with novel visual logistics.
Conclusions: Time is survival. Available biomarkers help, but are not adequate to detect sepsis early enough. Realistically, future sepsis biomarkers must allow synthesis of trends through high sensitivity and dynamic ranges, beat the speed of pathogen-triggered irreversible immunologic cascades, and algorithmically increase care team awareness, in order to prognosticate successive sepsis phases. Extraction of value from multiplex trend monitoring could facilitate earlier pathogen detection, trigger adequate antimicrobial therapy, and improve outcomes.

T014

SERUM ACTIN/GELSOLIN RATIO: A NEW BIOMARKER IN SEPSIS?

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Background: except procalcitonin, the specificity and predictive value of biochemical markers in early diagnosis and monitoring of sepsis is poor. In severe sepsis and septic shock the necrotizing cells release actin in excess (G- and F-actin, respectively) into the circulation where they are eliminated by plasma gelsolin and other actin binding proteins. When the amount of released actin exceeds its elimination capacity, gelsolin concentration decreases and free G-actin levels elevate in the blood. Free actin promotes development of multi organ failure and ARDS in the patients.
Methods: in our study serum actin and gelsolin levels and actin/gelsolin ratios (A/G ratio) were monitored in septic patients for 3 – 6 days (n = 10 patients) and we tried to find correlation between classic laboratory parameters, A/G ratios and outcome of the disease. Sera were electrophoretized by SDS-PAG (by Laemmli) where 10μg protein samples for gelsolin and actin detection were applied. Separated proteins were transferred to nitrocellulose membranes and relative amounts of actin and gelsolin were determined by quantitative enhanced chemiluminescent (ECL) western blot. The signals of the samples were referred to that of an internal standard (serum of a healthy individual) and data were given as ECL ratios. All other parameters were determined by automated routine laboratory methods.
Results: septic patients showed decreased gelsolin levels (20-50% compared to that of the control). Furthermore, when actin ECL ratios were referred to gelsolin ECL ratios, data for surviving patients were only 2-3 times higher than 1,0 (arbitrary unit of control) while non surviving patients showed 8-16 times higher ECL ratio values than that of the control. CRP and procalcitonin data did not correlate closely with survival rates.
Conclusions: Cox regression analysis showed that A/G ratio was a much better predictor for a negative outcome on day 1 than PCT or CRP (P=0,068 vs. P=0,742 and P=0,990, respectively). Our method seems to give complementary and predictive data on the outcome of sepsis and characterizes the overall tissue damage of septic patients.

T015
MONITORING OF BLOOD GAS PARAMETERS IN GENERAL ANESTHESIA

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Background: During general anesthesia besides clinical monitoring it is necessary to control the influence of anesthetics and mechanical ventilation on blood gas exchange.
Methods: In 30 patients we investigated pH and blood gas parameters: pO2, pCO2, saturation of oxygen (SatO2), alveolar to arterial difference for oxygen (AaDO2) and arterial to alveolar oxygen partial pressure ratio (a/A) before, during and after general anesthesia.
Results: We noted a significantly decrease of pH after anesthesia compared to the value obtained during anesthesia (P <0.001). PO2 was significantly elevated during anesthesia, opposite to before and during anesthesia (P <0.001). In contrast pCO2 significantly depleted during anesthesia (P <0.001). We noted a rapid increase of AaDO2 during anesthesia, and significantly decrease a/A in comparison with the values obtained before and after anesthesia. Respiratory alkalosis, higher pO2 and lower pCO2 following mechanical hyperventilation reflects significantly elevated AaDO2 and significantly depleted a/A.
Conclusions: These results suggest that in anesthetized patients with disturbances of the ventilation to perfusion ratio, the increase of mixed venous admixture occurs which finally leads to gas exchange inefficiency.

T016
URINARY OROSOMUCOID IN SEPSIS

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Background: Sepsis is still the leading cause of mortality in intensive patient care. In spite of the fact that 178 serum biochemical markers are known for diagnosis and follow up of septic patients, urinary biomarkers are few. Orosomucoid or α-1-acid glycoprotein (AGP) acute phase protein is filtrated into the urine. The major goal of our study was to decide if urinary AGP levels - as a non invasive novel biomarker in urine - might indicate the existence of sepsis.
Methods: Serum and urine samples of septic patients (n=11, monitored for 5 or 10 days) were collected simultaneously. Samples of 19 healthy controls were similarly obtained. Serum AGP, hsCRP, procalcitonin (PCT), TNF-α, IL6, IL8 were determined by routine automated methods. For urinary AGP analysis we developed a western blot technique with quantitative chemiluminescence (ECL) detection. Luminescence signal of urinary AGP was referred to that of pooled control urine applied as internal standard. Results were expressed as ECL ratios.
Results: Both serum and urinary AGP levels were increased in all samples of septic patients. When patients were individually monitored during the 5- or 10-day period serum AGP levels remained elevated while serum PCT and IL6 showed rapid alterations. The extent of urinary AGP-ECL ratio increase exceeded the serum AGP values (up to 10 fold vs. 2-3 folds). The decrease in urinary AGP levels during the 10-day detection period was a good indicator in assessing the positive outcome of the disease.
Conclusions: Our data suggest that urinary AGP shows a dramatic elevation in sepsis. In order to verify the applicability of urinary AGP measurement in septic patients a commercially available automated urinary AGP determination should be developed and further tested in an extended monitoring period at intensive care units.

T017
OPTIMAL CUTOFF OF PROCALCITONIN FOR PREDICTION OF BACTEREMIA IN PATIENTS WITH SUSPECTED INFECTION IN EMERGENCY DEPARTMENT

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Background: Timely diagnosis and treatment are essential to reduce the morbidity and mortality associated with bacteremia. Blood culture is considered to be the gold standard for detecting the presence of living microorganisms in the bloodstream. A positive blood culture usually provides a definitive diagnosis of bacteremia, and facilitates targeted therapy against the specific pathogens identified. However, blood culture requires several days. In these respects, an ideal marker that is sensitive and specific for detecting bacteremia and the results of which are rapidly obtainable, is required. In this study, we evaluated the diagnostic accuracy of PCT and calculated its optimal cutoff for prediction of bacteriemia, determined by blood culture, in the Emergency Department (ED).
Methods: Subjects enrolled in this study were adult patients (≥ 16 years) admitted to ED because of a suspected infection with at least two or more systemic inflammatory response syndrome (SIRS) criteria and blood culture obtained before antimicrobial treatment. Bacteremia was defined as the presence of viable bacteria in the blood. PCT was measured on a Roche Cobas 414 analyzer (Roche Diagnostics) by electrochemiluminescent immunoassay. Diagnostic accuracy of PCT for bacteremia was calculated with receiver operating characteristic (ROC) analysis. All statistical analyses were performed with the use of EPIDAT version 3.1.
Results: A consecutive serie of 129 patients with suspicion of infection and two or more SIRS criteria were admitted to the ED over a 2-months period. Bacteremia was identified in 19 patients (14,7%). PCT concentrations in bacteremia (+) were significantly higher than those of bacteremia (-) (median: 1,92 (IQR: 0,67-6,96) ng/mL vs. 0,24 (0,12-0,63) ng/ml, P <0.05). Areas under the ROC of PCT for discriminating bacteremia (+) from bacteremia (-) was 0.79 (CI 95%: 0,68-0,89). A PCT level of 0,5 ng/mL yielded a diagnostic sensitivity of 73,7%, a specificity of 65,5%, a PPV of 26,9% and a NVP of 93,5%.
Conclusions: In our hospital, a PCT cuttoff of 0,5 ng/mL, usually recommended to start antibiotic treatment, was a useful marker to rule-out bacteremia in patients with SIRS and suspicion of infection.

T018
HEAT STROKE IN YOUNG MAN

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Case: In July 2011, a 36-year-old man was admitted to the hospital for high fever of 40 °C and delirium. His physical examination revealed permeable airway, oxygen saturation 95%, HR 140 beats/min, BP 90/50 mmHg. Medical history: Studied in psychiatry service by anxious depressive syndrome. Others: hypertension, hypercholesterolemia. His medications included aripiprazole, topiramate, venlafaxine and atorvastatin. Laboratory revealed metabolic acidosis [arterial pH 7.286 (RI, 7.35-7.45)], renal impairment [plasma creatinine 1.78 mg/dL (RI, 0.7-1.2 mg/dL)], thrombocytopenia (platelet count 10 x 103/mm3 (RI, 150-350 103/μl)), rhabdomyolysis (plasma creatine kinase [CK] 3470 U/L (RI,38-175 U/L)), acute liver failure (transaminases [AST] 5630 U/L (RI, 5-37 U/L), [ALT] 7800 U/L (RI, 5-41 U/L)) and myocardial injury (troponine T 3.5 ng/mL (RI, 0-0.1 ng/mL)). With hypotension refractory to high doses of vasoactive drugs, the patient died in this situation, after twelve days of hospitalization.
Discussion: In patients taking neuroleptics who present with fever, the differential diagnosis should include neuroleptic malignant syndrome, heat-related illnesses, and all possible causes of elevated temperature. Of all the possible causes of hyperthermia, the patient was finally diagnosed with heat stroke due to disparate neurological manifestations presenting as delirium, convulsions, coma, accompanied by increased body temperature >40OC in a hot environmental conditions allowed to decide diagnosis. Factors predisposing to the development of heat stroke include advanced age, obesity, cardiovascular disease, lack of acclimatisation to heat and poor physical conditioning. Alcohol and certain drugs, notably antipsychotics, may also increase the risk. Neuroleptics all have varying degrees of anticholinergic and anti-alpha-adrenergic effects. Both impair heat dissipation; anticholinergic properties decrease sweating and alpha-adrenergic blockade causes vasodilation, which may enhance heat absorption from the environment.
Conclusions: Knowledge about the exact underlying mechanisms is not necessarily required since in a clinical and pragmatic perspective, subjects using these drugs have to be considered at higher risk of heat-related diseases and carefully monitored during heat wave periods

T019

EFFECT OF NT-proBNP TESTING FOR THE DIAGNOSIS OF PATIENTS ADMITTED IN THE INTENSIVE CARE DEPARTMENT WITH SYMPTOMS OF ACUTE HEART FAILURE

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Background: N-terminal pro brain natriuretic peptide /NT-proBNP/ is a potent cardiac marker which has shown excellent performance for the diagnosis of acute heart failure /HF/ and left ventricular dysfunction. Diagnosing acute HF is difficult because there are many varied and often non-specific clinical symptoms /dyspnoea, chest pain, fatigue cough/. The diagnosis, therefore, is based on the combined use of patient history, physical examination, ECG, chest X ray and laboratory tests, including NT-proBNP. Thus the challenge is to triage rapidly and accurately patients presenting with dyspnea.
Methods: 146 patients /mean age 68 years, 58% males/ were hospitalized in the Intensive Care Department with complaints of dyspnea. The aim of the study was to examine the relationship between NT-proBNP levels and the symptoms of dyspnea due to HF or other reason The study was realized by chemiluminescent method using SIEMENS DPC IMMULITE 2000 for quantitative measurement of NT-proBNP in heparinized plasma. The most appropriate decision thresholds are 125 pg/mL for patients younger than 75 years old and 450 pg/mL for 75 years or older.
Results: The sensitivity specificity, positive predictive value/PPV/ and negative predictive value/NPV/ were :75%,60% ,18% and 94% respectively. The patients with false positive results of NT-proBNP / 21%/ demonstrated different from HF cardiac pathology. Later 18% of the patients with intermediate values presented symptoms of acute HF.
Conclusions: The measurement of NT-proBNP is recommended in many guidelines as an integral part in the HF diagnostic work-up. This is particularly useful in the assessment of patients with acute dyspnea in the Emergency Departments where NT-proBNP has utility as both a rule-out and rule –in test for HF.

T020

PROTEIN S100β IN SERUM AND URINE MAY PREDICT MORTALITY AFTER SEVERE TRAUMATIC BRAIN INJURY

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Background: S100β protein is a calcium binding protein, released into the bloodstream by astrocytes following a brain injury. Some authors have reported a correlation between serum levels of S100β and severity of brain damage. No clutch there is little information about the predictive value of S100β levels in urine after traumatic brain injury (TBI).
Objective: To evaluate the role of S100β protein in serum and urine in predicting mortality after severe TBI.
Methods: 55 patients who had suffered a severe head injury were prospectively included in our study . Clinical variables collected were: sex, age, score on the Glasgow after resuscitation, CT results, extracranial lesions, score on the Injury Severity Score, and the final diagnosis of death / survival a month post-trauma. Samples of blood and urine levels of S100β value at the time of admission, 24, 48, 72 and 96 h.
Results: 18.2% of patients died a within a month of the trauma. S100β levels (both serum and urine) were significantly higher in those who died than in survivors. ROC analysis showed that S100β protein determination at 24 hours after a severe TBI can predict mortality (AUC: 0.958 to serum AUC: 0.778 for urine). The following setpoints were established : 0.461 mg/L for serum, 0.025 mg/L for urine, with a sensitivity of 90% in both cases and a specificity of 88.4% in the case of serum, 62.8% in the case of urine.
Conclusion: The determination of both serum and urinary levels of S100β protein acts as a sensitive and specific marker for the early detection of mortality after severe TBI.

T021

DIAGNOSTIC AND PROGNOSTIC VALUE OF suPAR IN PATIENTS WITH SEPSIS IN COMPARISON TO PRESEPSIN AND PROCALCITONIN

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Background: Urokinase plasminogen activator receptor (uPAR) is expressed on the cell membrane of various cell types. After cleavage induced by immune activation its soluble form (suPAR) is released into the circulation. suPAR concentrations are increased in critical ill patients especially with infectious diseases and sepsis. First evidence suggested that suPAR may serve as a prognostic marker. We compared suPAR with the biomarkers presepsin and procalcitonin and with the APACHE II score in patients presenting with sepsis in the emergency room (ER).
Methods: suPAR, presepsin (PRE), procalcitonin (PCT) and APACHE II score were determined at admission in 69 patients with sepsis admitted to the ER. Primary endpoint was death within 30 days. The combined endpoint “major adverse event” (MAE) consisted of at least either the primary or at least one of the secondary endpoints (intensive care, mechanical ventilation or dialysis).
Results: 41, 18 and 10 patients had sepsis, severe sepsis and septic shock, respectively. PRE, PCT and APACHE II score differed highly significant between patients with sepsis and septic shock (P-values were 0.0028, 0.01 and <0.0001, respectively) whereas the difference of suPAR was only slightly significant (P=0.0752). The 30-day mortality was 27.5%, ranging from 7.3% in sepsis to 44% in severe sepsis and 80% in septic shock. Receiver operating curve (ROC) analysis for discrimination between survivors and non-survivors revealed AUC values of 0.883, 0.727, 0.568 and 0.835 for PRE, suPAR, PCT and APACHE II score, respectively. AUC values for prediction of need for dialysis were 0.808, 0.792 and 0.672 for PRE, suPAR and PCT, respectively. PRE demonstrated a stronger relationship with 30-day MAE compared with suPAR and procalcitonin (AUC: 0.753, 0.615, 0.610), respectively.
Conclusion: The prognostic accuracy of suPAR was superior to PCT but not to PRE. Although suPAR provided reliable prognosis and prediction of 30-day mortality, the diagnostic accuracy of PRE was superior to PCT and suPAR as well as to the APACHE score for prediction of outcome (mortality and MAEs) including additional procedures like dialysis or mechanical ventilation. PRE was also superior in discrimination between sepsis, severe sepsis and septic shock.

T022

DIAGNOSIS OF SEPSIS AND MONITORING OF WEANING FROM MECHANICAL VENTILATION IN CRITICALL ILL PATIENTS BY PATHFAST PRESEPSIN

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Background: The role of biomarkers is not yet established in patients who require mechanical ventilation (MV) for non-surgical acute diseases. We intended to examine the diagnostic and prognostic value of the new sepsis marker presepsin during weaning from MV.
Methods: Plasma samples were obtained at 4 time points (1: shortly after intubation, 2: immediately before weaning, 3: shortly after extubation, 4: before discharge) from 120 patients with non-surgical acute diseases (mean age 67.9; 77.5% males) at the intensive care unit (ICU) who underwent MV. Presepsin was determined using the PATHFAST Presepsin assay. Patients were followed throughout their hospital stay until patients reached the endpoint (death) or until discharge.
Results: 38 (31.7%) patients died during follow-up. The presepsin levels (medians) in survivors and non-survivors were 1098 and 1609 pg/mL, respectively (P=0.04). 16 (13.3%) patients developed sepsis. 9 patients with sepsis died, demonstrating a significant higher mortality rate of 56.3% compared to 31.7% of the total study group (P <0.00001). Presepsin differed highly significant between non-septic and septic patients (median values: 1098 (95% CI: 886-1263) and 3185 (95% CI: 1734-3904) pg/mL, respectively, P=0.0004). ROC analysis for discrimination between sepsis and non-sepsis revealed an AUC of 0.893 (sensitivity 85.7%, specificity 84.0%, cutoff 1965 pg/mL). The median values of presepsin at the time points 1 to 4 during the weaning process were increasing in patients with sepsis from 3185 (IQR: 1727-3905) to 5703 (IQR: 2764-6815) pg/mL, respectively. In patients without sepsis the presepsin concentrations remained below 1600 pg/ml.
Conclusions: Weaning success is lower in patients with sepsis. Our results showed that development of sepsis during weaning from MV was associated with a higher mortality risk. Therefore, diagnosis of sepsis during weaning is very important. The new sepsis biomarker presepsin distingished patients who develop sepsis and those who do not during weaning with high diagnostic accuracy at the earliest time point already shortly after intubation. PATHFAST Presepsin allows the determination within 15 min from whole blood. This assay might be useful to monitor weaning from MV at the point-of-care.

T023

TIME-PROFILE KINETICS OF SERUM VEGF, IL-6, AND MCP-1 IN TRAUMATIC BRAIN INJURY

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Background: Cytokines and growth factor VEGF play a role in neuroinflammation, promoting angiogenesis and vascular stability after brain injury. Methods: The patients suffered from brain trauma (n=22) divided into group I with space-occupying lesion (SOL, expansive brain contusions, n=8) and group II without expansive contusions (n=14) were investigated by biochemical (measurement by cytokine and growth factors array on Evidence Investigator, Randox) and histological methods (immunohistochemical investigation with antibodies anti-CD68, anti-PSGL, anti-VEGF (Chemicon).
Statistical methods: data were tested by the unpaired t-test with Welch's correction, differences at p< 0.05 were considered as statistically significant.
Results: In 80% of all patients were found higher levels of IL-4, 6, 8, 10, MCP-1 from 1st day post-trauma above serum levels of published data from healthy volunteers. In terms of serum values time-profile development, the significant increase of IL-6 values (133.5±42.7 vs. 359.2±85.1 ng/L, P <0.04) was found in group I (SOL) from 1st day up to 2nd-3rd day, and significant decrease of MCP-1 values (853.3±155.7 vs. 334.5±83.9 ng/L, P <0.01) was found in group II from 1st day up to 2nd-3rd day. With respect of all data, there was a correlation between IL-6 and MCP-1 serum values (proved by Deming model of linear regression, P <0.05). In terms of growth factors serum level, the highest values of VEGF (303.0±55.6 vs. 155.4±33.8 ng/L, P <0.03) were found in group II as compared to group I (SOL), reaching the peak 1339.4 ng/L in patient with diffuse axonal injury. The relevant decrease of VEGF values was revealed in 2 patients who died, and significant increase was proved in group II (138.1±38.8 vs. 358.8±95 ng/L, P <0.05) from 1st day up to 2nd-3rd day. Immunohistochemistry demonstrated signs of inflammatory response of CD-68 and PSGL-positive microglial cells following injury in patient of group I submitted to neurosurgery, and local immunoreactivity with anti-VEGF was observed in astroglial cells.
Conclusion: IL-6, MCP-1,and VEGF has a different time-profile kinetics in patient with, and without expansive traumatic brain contusions. Acknowledgements: Our work was supported by Research Project VZMZ 00064165.

T024

CYTOKINE AND GROWTH FACTOR ARRAY IN MONITORING OF INFLAMMATORY RESPONSE IN SURGERY PATIENTS

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Background: Cytokines are useful biochemical parameters in surgery patients for predicting infected necrosis in acute pancreatitis, and associated systemic complications in patients with acute pancreatitis, and for predicting multiple organ failure in multiple trauma patients.
Subject and methods: The patients (n=14) suffered from pancreatitis, stomach necrosis, and multiple trauma were enrolled into our study dealing with utilization of cytokine and growht factor array for monitoring of inflammatory response (CTK HS array, Randox). Patients with pancreatitis were divided according to Balthasar classification. Statistical methods: data were tested by the unpaired t-test with Welch's correction, differences at P <0.05 were considered as statistically significant.
Results: In terms of the draw a comparison of the time-profile kinetics, there is early increase of IL-6 (184.4±51.6 ng/L) before CRP (128.3±49.6 mg/L) in the first day above reference interval. With respect to all data, there was a non-significant correlation between IL-6 and CRP serum values (proved by Deming model of linear regression, P <0.3). In pancreatitis stratified according to Balthazar classification: C-E (based on the extent of pancreatic inflammation and gland necrosis) were proved higher value of IL-10 (5.56± 1.6 vs. 1.42±0.2 ng/L, P <0.04), IL-8 (93.08±18.5 vs. 44.81±12.1ng/L, P <0.05), IL-6 (254.0±46.01 vs.75.46±27.5, P <0,007), and non-significant differences of MCP-1 (419.2±67.8 vs. 428.6±68.3 ng/L) compared to patients with Balthazar classification: B. The levels of TNF alpha were monitored in patients with sepsis (due to endocarditis, or stomach necrosis) or fever of unknown origin in multiple trauma (29.11±3.8 vs. 10.48±1.1 ng/L, P <0.002) compared to other patients.
Conclusion: Cytokines are promising marker of inflammatory response, particularly in pancreatitis.
Acknowledgements: Our work was supported by Research Project VZMZ 00064165.

T025

DIAGNOSTIC YIELD OF SERIAL MEASUREMENTS OF C-REACTIVE PROTEIN AND PROCALCITONIN

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Background: Serial measurement of the biomarkers C-reactive protein (CRP) and procalcitonin (PCT) are used in the monitoring of infection and response to therapy. The reference change value (RCV) is the minimum change required before two consecutive results are considered different enough to influence clinical decision, taking into account the intra-individual and analytical variability. The aim of the study was to examine the proportion of CRP and PCT results that revealed clinically significant changes when repeated at different testing intervals in clinical practice.
Methods: All CRP and PCT results of patients over a period of 6 months, were extracted from the laboratory information system. Only patients who had their CRP and PCT results serially measured within 1 to 5 days were included in the analysis. The RCV was calculated as 2.8 (CVa2+CVw2) 0.5(preceding result of the patient)/100, where CVa was the analytical coefficient of variation and CVw was the intra-individual biological variation. CVa was derived from quality control values over the period of analysis and was 2.4% for CRP and 4.4% for PCT. CVw was derived from literature and was 32% for CRP and 16% for PCT. The proportion (%) of significantly changed results was calculated by dividing the number of significantly changed results by the total number of measurements at the testing interval and multiplied by 100%.
Results: A total of 3733 CRP measurements and 790 PCT measurements were performed within one to five days. At a testing interval of one day, only 6.1% of CRP serial measurements had significant changes. In contrast, 25.8% of serial measurements of PCT had significant changes at a testing interval of one day. The proportion of CRP and PCT results that revealed clinically significant changes increased with longer testing intervals. For CRP, 9% of results were significantly changed at two days compared to 14.9% at five days. For PCT, 43.5% of results were significantly changed at two days compared to 58.2% at five days.
Conclusions: Serial measurements of PCT had greater diagnostic yield than serial measurements of CRP. Serial measurements of PCT and CRP at a testing interval of one day had poor diagnostic yield compared to longer testing intervals of two to five days.

T026

PROCALCITONIN AND C-REACTIVE PROTEIN IN THE DIAGNOSIS OF BLOODSTREAM BACTERIAL INFECTIONS

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Background: In the diagnosis of bloodstream bacterial infections, bacterial culture remains the gold standard. Total white cell count (WCC), percentage of neutrophils (%N) and C-reactive protein (CRP) are commonly used biomarkers of bacterial infection. Procalcitonin (PCT) is a precursor peptide from the hormone calcitonin, and has been shown to be a useful biomarker in bacterial sepsis. The aim of the study was to compare PCT with CRP, WCC and %N in the diagnosis of bacterial sepsis.
Methods: WCC, %N, and CRP with PCT were compared to blood cultures in patients admitted to our hospital over a period of one month. Patients who had WCC, N, CRP, PCT and bacterial cultures taken on the same day were selected for analysis. The specificity and sensitivity of PCT were compared with those of CRP, WCC and N. Receiver operator curves (ROC) were drawn and the area under the curves (AUC) were calculated.
Results: A total of 160 results were obtained. Of these, 23 cultures were positive for bacteria. The ages of the patients ranged from 2 to 99. Ninety-six were male and 67 female. Sixty percent were Chinese, 13% Malays, 13% Indians and 13% were from other races. PCT had a better sensitivity and specificity than CRP, WCC and %N with an AUC of 0.819 for PCT, 0.651 for CRP, 0.605 for WCC and 0.626 for %N respectively. The combined use of CRP and PCT did not improve the sensitivity and specificity compared to PCT alone. (AUC 0.803 for CRP and PCT). At a cut-off of 0.5 mcg/L, PCT had a positive predictive value (PPV) of 0.262 and a negative predictive value (NPV) of 0.969 for predicting bacterial sepsis. At a cut-off of 10 mg/L, CRP had a PPV of 0.153 and an NPV of 0.918 for predicting bacterial sepsis.
Conclusions: PCT was superior to CRP, WCC and %N in the diagnosis of bacterial sepsis. The combination of PCT with CRP did not improve the diagnosis of bacterial sepsis compared to PCT alone.

T027
CELL-FREE DNA AND THE PROGNOSIS OF MILD TBI PATIENTS

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Introduction: Circulating cell free DNA levels are increased after trauma injury. This study tested the hypothesis that Circulating cell free DNA levels are useful to predict the outcome of patients who have already suffer a non severe Traumatic Brain Injury (TBI).
Material and methods: Cell free DNA was measured on 89 post-mild TBI patients who had a Glasgow Coma Scale up to 9 points at the moment of the admission by real time PCR. A blood sample was drawn at 6-hour post-TBI. A routine CT scan was obtained within 24 hours post-injury. The competence of cell free DNA to distinguish patients with worse prognosis was probe with CT scan findings and compared with severe TBI results from others studies.
Results: Cell-free DNA levels were lower in mild TBI patients samples compared with severe ones (P=0.0005). Comparing with control group, we found also notable differences (P=0.0001). In 13 mild TBI patients (those who were hospitalized more than 24 h), the decrease of cell-free DNA levels were reduce in the mild TBI group compared with severe one, although significant differences were also found (P=0.05).
Conclusions: In summary we showed that mild TBI is associated both with lower cell-free DNA at the moment of the admission and with better prognosis than severe TBI. A more rapid decrease of cell-free DNA exhibit more desirable outcomes.

T028
BASE EXCESS/DEFICIT LEVELS IN PATIENTS WITH INTRA-ABDOMINAL HYPERTENSION

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Intra-abdominal pressure (IAP) is a harbinger of intra-abdominal mischief, and its measurement is cheap, simple to perform, and reproducible. Intra-abdominal hypertension (IAH), especially grades 3 and 4 (IAP >20 mmHg), occurs in over a third of patients and is associated with an increase in intra-abdominal sepsis, bleeding, renal failure, and death. IAH has adverse effects on cardiovascular, respiratory, renal, gastrointestinal and neurological function. IAH and abdominal compartment syndrome are associated with significant morbidity and mortality. The reduction of IAP is a cornerstone of breaking the series of pathophysiological changes that triggered others, and resulting in a poor outcome for the patients. The two most commonly used markers in assessing resuscitation remain base deficit and lactate. A significant base deficit has been a marker of mortality in many studies. The aim of this study is to identify the base excess (BE)/ base deficit (BD) levels in patients with intra-abdominal hypertension.
Materials and methods: prospective study for the period from March 2011 to June 2012 on 44 patients aged >18 years undergoing major abdominal surgery and hospitalized for more than 72 hours in the intensive care unit of University Hospital in Stara Zagora. Demographic characteristics: N=44, age 65 ± 5.2 years, Gender (male / female): 33/10; BMI = 26.6±3.5 kg/m²; SOFA score 6.1±2.6. Patients were divided into 4 groups according to the value of IAP and WSACS guidelines - group A - normotensive with IAP up to 11mmHg, group B with IAP from 12 to 15mmHg, group C with a pressure of 16 to 20 mmHg, and Group D with IAP from 20 to 25 mmHg. The base excess (BE)/ base deficit (BD) levels were tested whit blood – gas analyzer “ABL basic 800” – Radiometer and Siemens Rapidpoint 350.
Results: The obtained data were processed statistically, using level of significance P <0.05. Analysis of the results showed that patients with second and third degree abdominal hypertension have significantly higher BD levels.
Conclusion: Significantly higher levels of BD in patients with high IAH correlate with the severity of pathophysiological changes and may serve as a marker for the extent of the damage to come.

T029

DIAGNOSTIC VALUE OF ENDOTOXIN ACTIVITY ASSAY IN PATIENTS WITH SEVERE SEPSIS AFTER CARDIAC SURGERY

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Background: Endotoxin activates the release of cytokines and other biologically active components. Developing of systemic inflammatory response syndrome leads to disturbances in membrane permeability together with multiple organ dysfunction syndrome and, often, death. Therefore, clinicians require a precise laboratory diagnostic tool for endotoxin detection for the timely initiation of specific treatment. Quantitative techniques for the measurement of endotoxin levels in blood have been known for more than 40 years and are based on the application of the LAL endotoxin assay (Limulus Amebocyte Lysate). Recently, a new method for assessing endotoxin concentration in blood has been developed – the Endotoxin Activity Assay (EAA). EAA is based on the principle that endotoxin binds to antiendotoxin antibodies (IgM) and is then delivered to neutrophils via complement receptors. In the presence of zymosan and luminol, neutrophils undergo a respiratory burst accompanied by light emission. The light produced is quantified by a chemiluminometer, and its intensity is proportional to the amount of endotoxin.
Aim: To evaluate the prognostic value of endotoxin activity assay (EAA) in adult patients with suspected or proven severe sepsis after cardiac surgery.
Methods: Blood samples taken from 81 patients immediately after the diagnosis of severe sepsis were tested with the EAA. Patients were divided into 3 groups: low (<0.4, n=20), moderate (0.4-0.6, n=35) and high (≥0.6, n=26) EAA levels.
Results: Gram-negative bacteraemia was found in 19/55 (35%) of cases with EAA <0.6 and in 11/26 (42%) of cases with higher EAA, P=0.67. Mortality at 28 days in Groups 1, 2 and 3 was 20%, 43% and 54%, respectively. Patients with an EAA higher than 0.65 had a higher 28-day mortality than those with lower EAA values (18/26 – 69% vs. 19/55 – 34.5%; P=0.0072). ROC analysis for the prediction of 28-day mortality revealed an AUC for APACHE II scores, EAA and PCT of 0.81, 0.73 and 0.66, respectively.
Conclusions: EAA might be useful for recognising patients who have an increased risk of mortality due to severe sepsis.

T030

COMPARISON OF SERUM PROCALCITONIN (PCT) AND C-REACTIVE PROTEIN (CRP) IN 1263 MEDICO-SURGICAL HOSPITAL PATIENTS

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Serum CRP is generally accepted laboratory marker of acute phase reaction of different etiology including infection and sepsis. There is also some evidence that procalcitonin is more specific for bacterial infection with serum level rising and falling rapidly. Aim of the study was to compare serum CRP, procalcitonin and white blood cell (WBC) in samples of hospital patients.
Material and methods: Measurements of serum CRP (immunoenzyme technique Vitros), procalcitonin (ELFS Vidas) and WBC (Sysmex XT 2000) were performed once with no relation to clinical symptoms, but according to clinical need specified by clinicians. 1263 medico-surgical patients: 56,1% from medical, 33,4%, from surgical, 10,5% from intensive care units were included. They were divided into groups according to CRP mg/L: AI < 10, (4% of population) AII 11- 20 (5%) AIII 21-50 (19%) AIV > 50 (72%) and WBC BI <10x109/l (41% of population), BII 11-20 (45%), BIII >21 (14%). Statistical analysis was performed using Pearson's test.
Results: In the whole population mean PCT was 7,36±23,63 ng/mL and above normal(>0,1 ng/mL) in 670 cases /53%/. CRP 140±114 mg/L and above normal (>10 mg/L) in 1207 /96%/ patients. In group AI, in 82% subjects PCT was also normal. With increasing of CRP PCT raised and in group AIV was increased in 59%. In all groups weak positive correlation CRP/PCT was observed /p <0,05/. The WBC in the whole population was 13,3±8.9x109/l and above normal (>10x109/l) in 58,7% cases. In the group BI increased PCT was in 59% subjects. There were no correlation WBC/PCT in this group. In the group BIII 77% cases showed abnormal PCT and only weak positive correlation WBC/PCT was found.
Conclusions: There is no close relations either between CRP/PCT or WBC/PCT. Abnormal serum CRP almost in the whole population and PCT only in 53% is in favor of suggestion that PCT is independent factor most likely standing for bacterial infection and in the medico-surgical patients there are many other causes for acute phase reaction response manifested by increase of serum CRP. No significant relation WBC/ PCT was proved.

T031
MACRO TSH: MYTH OR REALITY?

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We examined a case of a 59 year old man with a remarkably elevated thyroid-stimulating hormone (TSH) level, measured by a chemiluminescence immunoassay (Centaur-Siemens), but with no specific symptoms of hypothyroidism. Levels of free T3 and free T4 were normal and the autoimmune antibodies, anti-tyroglobulin and anti-peroxidase had borderline values. We performed a literature review and found a very few small casuistic documented over the last 10 years. Several different additional TSH assays were tested: by chemiluminescence imunoassays (Abbot, IMMULITE-Siemens, VISTA-Siemens), by immunometric immunoassay (Vitros ECi/ECiQ). All of the automated test results gave similar results (see table1), with TSH above the upper limit of sensibility for the methodology used. Patients with high TSH and normal levels of Free T3 and FreeT4, without clinical signs or symptoms of hypothyroidism are considered as having an immunological interference by heterophile antibodies or that we are in the presence of a form of macro-TSH consisting of an immune complex bound to the anti-TSH autoantibody. We used Protein G Agarose Gel (Roche) and polyethylene glycol precipitation test (PEG) methods, to do the separation of TSH from the complex. There may be more patients with macro-TSH than expected. Unexpected values should be investigated using other methods of analysis.

T032
PLASMA FREE METANEPHRINES ANALYSIS USING SPE-LC-MS/MS IN CLINICAL LABORATORY: DEVELOPMENT AND VALIDATION METHOD

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Background. Pheochromocytomas are characterized by hypertension due to excessive secretion of catecholamines, which are then metabolized to metanephrines, including metanephrine (MN) and normetanephrine (NMN). The measurement of plasma free metanephrines (Pmets) has been shown to be a highly sensitive test. Traditionally Pmets measurement has been done by HPLC with electrochemical detection, enzyme immunoassays and gas chromatography-mass spectrometry. Liquid chromatography-mass spectrometry (LC-MS/MS) can resolve many of the analytical limitations of the previous methods. Recently LC-MS/MS methods have been proposed for the analysis of Pmets and the main challenge for these analysis is to develop an efficient sample pre-treatment. Our aim was to develop and to validate a SPE-LC-MS/MS method for Pmets, suitable for a routine clinical laboratory.
Methods. After SPE with weak cation exchange cartridge, chromatographic separation of Pmets was achieved by use of a HILIC Silica column (2.1x30 mm, 3 μm). MN, NMN, d3-MN and d3-NMN were detected in the MRM mode using the specific transitions m/z 166->134, 180->148, 169->137, 183->151, respectively, with positive electrospray ionization. Linearity, imprecision and recovery were evaluated. Fifteen plasma samples from healthy volunteers and one plasma sample from a patient with pheochromocytoma were analyzed.
Results. Calibration curves were linear throughout the studied ranges (MN: 0.12-1.98 nmol/L; NMN 0.13-5.26 nmol/L) with correlation coefficient >0.995 for both analytes. LLOQ were 0.12 for MN and 0.13 nmol/L for NMN. The intra and inter-day imprecisions (n=10) were <10% in 5 samples (MN: 0.14 -0.64 nmol/L; NMN: 0.39-1.52 nmol/L). Recoveries were 91±9% and 99±9%, for MN and NMN, respectively (n=3). MN and NMN levels were 0.13±0.05 nmol/L and 0.52±0.25 nmol/L in 15 healthy volunteers and 11.88 nmol/L and 61.79 nmol/L in the patient with pheochromocytoma, respectively.
Conclusions. The proposed method demonstrated satisfactory analytical performances for Pmets. The influence of blood collection mode and the different plasma storage conditions have to be investigated, together to validate the reference ranges. Considering the obtained data, the method can be suitable for the application in a routine clinical laboratory.

T033

USE OF URINARY STEROID PROFILING FOR DIAGNOSING DIFFERENT VIRILIZING DISEASES

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Background: An increased androgen production and virilization may be caused by a number of different diseases, e.g. adrenocortical carcinoma, a highly malignant tumor, deficiencies of certain steroid converting enzymes, particularly 21-hydroxylase deficiency (congenital adrenal hyperplasia), Cushing's syndrome and polycystic ovary syndrome. Since some of these diseases require immediate treatment, it is important that they can be readily diagnosed by methods in clinical routine use. In our laboratory we use urinary steroid profiling for diagnosing such diseases and the potential of the method is demonstrated.
Methods: Urine samples (24-hour collections) were from patients of various age with symptoms of virilization and/or hirsutism. Metabolic steroid profiles in urine were analyzed by gas-liquid chromatography (GLC) and gas chromatography-mass spectrometry (GC/MS) following extraction, hydrolysis/solvolysis of steroid conjugates, purification and derivatization (MO-TMS). Twenty-one urinary steroids are routinely quantified by GLC and new steroids are identified by GC/MS.
Results: The results show that the urinary steroid profiles of patients with adrenocortical carcinoma and enzyme deficiencies, 21-hydroxylase deficiency being most frequent, are usually very characteristic and easy to recognize (diagnose) and so is also the case with Cushing's syndrome. Steroid profiles of patients with polycystic ovary syndrome (PCOS) and some other virilizing disorders demonstrate an increased production of testosterone. The method can be used for diagnosing children with precocious puberty. Since the former diseases generally need immediate treatment, it is important that they are readily diagnosed.
Conclusions: Urinary steroid profiling is a highly specific and sensitive analytical tool for diagnosing malignant adrenocortical tumors and various enzyme deficiencies, diseases that usually cause virilization and require immediate treatment. For patients with other virilizing disorders steroid profiling provides information about normal and increased production rates of testosterone. Steroid profiling is also useful for following-up treatment of such diseases.

T034

COMPARISON OF FOUR COMMERCIALLY AVAILABLE THYROXINE (T4) IMMUNOASSAYS TO LC-MS/MS IN SERUM FROM NONPREGNANT AND PREGNANT FEMALES

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Background: Monitoring thyroid function during pregnancy is important for both maternal and fetal health. Many free thyroxine (FT4) immunoassays are affected by binding protein concentrations. Thus, total thyroxine (T4) may be used to monitor thyroid function during pregnancy but limited data are available regarding the performance of T4 immunoassays in pregnant women. The goal of this study was to compare four T4 immunoassays to an LC/MS-MS method.
Methods: De-identified residual waste serum from physician-ordered clinical tests was obtained from females who were not pregnant (n=53), 1st trimester (n=50) and 2nd trimester (n=50) of pregnancy. Total T4 was measured by LC-MS/MS and four T4 assays: Abbott Architect (Abbott Diagnostics), Beckman Coulter Access (DxI) (Beckman Coulter, Inc.), Siemens Centaur (Siemens Healthcare Diagnostics) and Roche Elecsys (Cobas e601) (Roche Diagnostics). Thyroxine-binding globulin (TBG) was measured using the Siemens Immulite assay. Results from each assay were compared and linear regression analyses were performed.
Results: All immunoassays yielded T4 results that were lower than those obtained using LC-MS/MS and the magnitude of under-recovery increased in 1st and 2nd trimester samples. The percentage of results differing by >20% or 1.0 mcg/dL compared to LC-MS/MS in nonpregnant, 1st trimester and 2nd trimester serum samples, respectively, were: Abbott (0%, 6%, 38%); Beckman Coulter Access (9%, 42%, 56%); Roche Elecsys (0%, 0%, 14%); and Siemens Centaur (0%, 0%, 16%). Linear regression analysis of TBG versus T4 concentration yielded LC-MS/MS T4 = 0.16(TBG). Slopes were lower for assays where T4 under-recovered in 1st and 2nd trimester samples.
Conclusions: All immunoassays demonstrated varying degrees of under-recovery of T4 in 2nd trimester serum samples. Two assays demonstrated under-recovery of >20% in more than 35% of 2nd trimester samples when compared to LC-MS/MS. The under-recovery of T4 was associated with increasing TBG concentrations suggesting that there may be inefficient dissociation of T4 from TBG. Laboratories and clinicians should be aware that the performance of T4 immunoassays varies when measuring T4 in samples with increased TBG concentrations, such as those found in pregnant females.

T035

MEASUREMENT OF DEHYDROEPIANDROSTERONE SULPHATE (DHEAS): A COMPARISON OF ISOTOPE-DILUTION LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (ID-LC-MS/MS) AND SEVEN CURRENTLY AVAILABLE COMMERCIAL IMMUNOASSAYS

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Background: Dehydroepiandrosterone sulphate (DHEAS) is an important marker of the adrenal gland. Its assessment is required in several adrenal diseases, such as adrenal tumours, adrenal insufficiency and congenital adrenal hyperplasia. Most clinical laboratories assess DHEAS using commercially available immunoassays. The aim of the present study was to develop an ID-LC-MS/MS method for the assessment of DHEAS in serum and to use this method to investigate the accuracy of currently available commercial DHEAS assays.
Methods: Our ID-LC-MS/MS method consisted of a sample preparation with addition of deuterated internal standard ([2H6]DHEAS) to the serum samples and a protein precipitation using acetonitrile, where after the samples were analyzed using a symbiosis online SPE system (Spark Holland, Emmen, the Netherlands) coupled to a Quattro Premier XE tandem mass spectrometer (Waters Corp., Milford, MA). Intra-assay CV was 4.1%. Seven currently available DHEAS assays were compared to our ID-LC-MS/MS method by assessing 75 serum samples (concentration range 0.06- 20.6 μmol/L measured by ID-LC-MS/MS) by each method as well as performing recovery experiments and dilution series. Data obtained in the present study were also compared to data of three EQAS's.
Results: Three methods agreed well with ID-LC-MS/MS (R between 0.93 and 0.99 and slopes ranging from 0.92 to 1.07) and showed good recoveries (range 76-115%). Four methods showed standardization problems (slopes were 0.84, 1.14, 1.20 and 1.28) and recoveries in these methods were 61-85%, 103-126%, 115-136% and 126-142%, respectively. Intra-assay coefficient of variation were <5.5% in six methods; one assay had an unacceptably high intra-assay coefficient of variation of 18%. Linearity was good in all methods. Our data are in agreement with data obtained in three EQAS's.
Conclusion: Some of the currently available DHEAS methods show standardization problems and/or a high variation. These problems have potentially adverse clinical consequences. We advise the manufacturers to improve their assays and laboratorians to scrutinize the DHEAS method they employ.

T036

PITUITARY SOMATOTROPES IN OVARIECTOMIZED RATS AFTER TREATMENT WITH COMBINED LHRH AND ESTRADIOL

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Background: Estradiol is important hormone which controls the secretory activity of hormone producing cells in the rat female pituitaries. Luteinizing hormone-releasing hormone (LHRH), found in hypothalamus, primarily controls the release of gonadotropic hormones from the anterior pituitary gland, but also affects some other pituitary cells. In the present study, the influence of estradiol dipropionate (EDP), combined with LHRH, on the morphology and secretory activity of pituitary somatotropes, in ovarictomized (ovx) Wistar rat females, was studied.
Methods: Female Wistar rats were ovx at 12 weeks of age. Animals were divided into two groups, each comprising seven females. The first group of ovx females received EDP (i.p. 250 μg) daily for 4 weeks, combined with LHRH (i.p. 25 μg), during the last three days of the fourth week after the surgery. The second group were ovx controls, who i.p. received the adeqate volume of sterile olive oil and saline during the same period. All females were sacrificed 24 h after the last injection. Pituitary somatotropes were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Serum concentrations of growth hormone in control and EDP+LHRH treated female rats were measured by the hGH-Delfia kit.
Results: The absolute and relative pituitary weights in EDP+LHRH treated females were significantly increased (P <0.05) by 229.7 and 273.7% respectively, in comparison with the controls. Immunopositive pituitary somatotropes in the control rats were round to pyramidal in shape, usually located in close proximity to the blood vessels. In the EDP+LHRH treted ovx females somatotropes were elongated, irregularly shaped, with more intensely stained cytoplasm. Concentration of growth hormone in the serum of EDP+LHRH treated ovx females was significantly increased (P <0.05) by 60.0%, compared to the Ovx controls.
Conclusions: Our findings show that EDP+LHRH application has caused the change in the morphology and stimulated hormone secretion of pituitary somatotropes, in ovx females.

T037

DETERMINATION OF SALIVARY CORTISOL WITH CHEMILUMINESCENT METHOD AND CONFIRMATION BY MASS SPECTROMETRY IN HYPERCORTISOLISM

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Background and purpose of the study: The determination of night salivary cortisol (NCS) is useful in screening for hypercortisolism. A number of recent studies have highlighted the potentiality of this test in the screening of Cushing's syndrome (1) and different methods have been introduced as an alternative to RIA, with very different characteristics and cut-off. The aim of this study is to evaluate the level of NCS measured with an automated chemiluminescent method (CLIA) and with mass spectrometry (LC-MS/MS), which is considered the gold standard, as confirmation.
Materials and methods: From 01 Jan 2012 to 30 June 2012, 36 saliva samples were collected, at 11 pm, by Salivette® (Sarsted Ltd, UK) from healthy volunteers and 10 saliva samples from patients with Cushing's syndrome, followed by the Clinical Endocrinology of Hospital "Umberto I" of Ancona. The samples were analyzed with both chemiluminescent (Beckman Coulter Access) and LC-MS/MS methods.
Results: The healthy controls showed significantly lower values of NCS compared to Cushing in LC-MS/MS (0.89±0,37 ng/mL versus 3.18±1.86 ng/mL; P <0.001). A similar result was obtained using the CLIA method (0.40±0.22 ng/mL versus 1.43±0.66 ng/mL P <0.001). The diagnostic performance of the overall method LC-MS/MS [AUC 0.94 (0.86 to 1.02)] was slightly higher than that the CLIA method [AUC 0.89 (0.71 to 1.07)]. The cut-off with higher sensitivity and specificity was calculated by the ROC curve and was 1.2 ng/ mL in LC-MS/MS and 0.58 μg/dL in CLIA. The CLIA method showed a sensitivity of 90% and a specificity of 84.8%. The values in CLIA correlated significantly with those in L-MS/MS (R:0.9).
Conclusions: Despite the numerical limitation of the samples analyzed, to our knowledge is the first study that uses a method CLIA confirmed by LC-MS/ MS. Our data confirm that an appropriate cut-off is highly influenced by the method used. Therefore, the methods discussed (LC-MS/MS and CLIA) may have a similar performance, however attention must be given in order to redefined the cut-off in each laboratory. The introduction of automated method will reduce the sanitary cost and improve the waiting times of reports.

T038

IL-6 CIRCULATING LEVELS DO NOT CORRELATE WITH SERUM HORMONAL MARKERS OF OVARIAN FOLLICULAR IMPAIRMENT

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Background: The involvement of pro-inflammatory cytokines in endometriosis is well documented. Growing evidences plead for their role in the growth and development of ovarian follicle. We aimed to evaluate the relationship between serum levels of AMH (as a marker of ovarian reserve) and IL-6 and to assess the value of circulating pro-inflammatory factor in infertility.
Materials and methods: 45 women (controls and infertility – with and without PCOS, aged 20-44 years) were investigated. Hormonal levels (gonadotrophines, estradiol, testosterone, PRL, AMH) and IL-6 in serum samples in the early follicular phase of the menstrual cycle were measured. Patients with hyperprolactinemia or thyroid diseases were excluded. AMH was measured by Elisa (BeckmanCoulter), the other hormones by electrochemiluminiscence (Cobas e600, Roche) and IL-6 by immunochemiluminiscence (BeckmanCoulter).
Results: IL-6 serum levels are similar in control and infertile women (mean±SD: 2,37±1,19, 1,77±0,94 pg/mL, P=0,11). There was no significant difference between serum levels of AMH and IL-6, neither in infertility group, nor in controls (r=0.12, P=0,9 and r=0,48, P=0,5, respectively). AMH and testosterone strongly correlate in women with infertility (P=0,0002, r=0,63), and weaker in controls (P=0,002, r=0,32). IL-6 and testosterone were negatively correlated in patients with infertility and SOPC (r=-0,18, P <0,01).
Conclusion: Consistent with the role of IL-6 in reproductive pathophysiology we conclude that local proinflammatory factors are involved in initiation and progression of folliculogenesis diseases rather than circulating ones.

T039

TESTOSTERONE CONCENTRATIONS IN MEN WITH FINASTERIDE ADVERSE SIDE-EFFECTS OVER SIX-MONTHS AFTER DRUG DISCONTINUATION

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Background: Finasteride is an inhibitor of the 5-alpha-reductase enzyme, thus, it provokes a reduction of the conversion of testosterone (T) to the more active hormone dihydrotestosterone (DHT). Finasteride is widely and successfully used for reduction of benign prostatic hyperplasia, usually at 5 mg/die. Additionally, Finasteride is used against male pattern hair loss (androgenic alopecia), usually at 1 mg/die. Some studies reported sexual dysfunction during and/or after use of Finasteride. However, long term effects studies are scanty; in general these results require confirmation and a deeper evaluation of reasons producing long-term persistence of sexual dysfunctions is warranted. We aimed to characterize the hormonal profile of 9 patients with persistent sexual dysfunction after Finasteride discontinuation.
Methods: Nine men (36±5 years old) who assumed Finasteride at dosage of 1 mg/die and presenting adverse effects including erectile dysfunction and loss of libido at over 6 months of drug discontinuation were enrolled. For comparison, 30 healthy matched male controls were examined. Total testosterone, free testosterone, and available testosterone, sexual hormone binding globulin (SHBG), estradiol (E2), and progesterone concentrations were evaluated in morning serum of all subjects.
Results: Total testosterone concentrations (P <0.01), free testosterone (P=0.01) and available testosterone (P <0.01) were all lower in cases than in healthy controls. Contrariwise, SHBG and E2 were not different in the 2 groups (both P > 0.05). Interestingly, the ratio of T/E was lower in cases than in healthy controls, P <0.01. Additionally, progesterone concentrations were lower in patients with long term adverse effects than in untreated healthy controls (P <0.01).
Conclusions: Our study highlighted persistent low levels of testosterone as total, free and available forms, in subjects with sexual dysfunction over six months after Finasteride wash-out. Concerning evidence suggests a permanent impairment of the endocrine system producing testosterone in some young men who assumed Finasteride at low dosage.

T040

PREVALENCE OF THYROID DISORDER AND THE EFFECT OF MEDICATION ON BODY MASS INDEX AND PLASMA LIPID LEVEL AMONG HYPOTHYROID SUBJECTS

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Background: Lack of adequate data masks the real picture of thyroid disorder which is highly prevalent in different parts of Nepal. Results on improvement of hyperlipidemia and increased Body Mass Index (BMI) to healthy level with thyroid replacement therapy is still not conclusive, thus requiring further study. The objective of this study was to determine the one year prevalence of thyroid disorder among Nepalese subjects attending thyroid clinic at tertiary health center in Eastern Nepal, and to determine pre-medication and post-medication BMI and plasma lipid level in hypothyroid subjects.
Methods: A total of 1854 subjects attending thyroid clinic from January to December 2011 at BPKIHS, Dharan in Eastern Nepal, were included. Fasting plasma Total Cholesterol (TC), Triacylglycerol (TAG), LDL-Cholesterol (LDL-C) and HDL-Cholesterol (HDL-C) along with BMI were measured before medication and at 4 and 7 month after medication in 304 subjects with hypothyroidism (overt and subclinical) meeting criteria of TSH≥10 mIU/L (reference range 0.4-6.2 mIU/L) with subnormal or normal free T3 and T4. Necessary statistical tests were applied and p value <0.05 was deemed statistically significant.
Results: One year prevalence of thyroid disorder was 32.95% (hypothyroidism 25.51% and hyperthyroidism 7.44%) with female predominance and within the age group 20-70 years. In 304 hypothyroid subjects, before medication, abnormal increase in BMI above 25 kg/m^2 and plasma TC, TAG, LDL-C was highly significant with reduction in HDL-C. After medication (L-thyroxin), with the reduction of TSH level towards normal and normalization of fT3 and fT4, there was significant improvement of BMI, TC and TAG towards healthy level. Although not significant, LDL-C was reduced and there was slight improvement in the level of HDL-C. Significant positive correlation of TSH level with BMI and plasma lipid (TC, TAG, LDL-C) was seen before and after medication.
Conclusion: This study shows high prevalence of thyroid disorder with female predominance and hypothyroidism being the most common condition. Hyperlipidemia and increase in BMI seen during hypothyroidism where TSH level is elevated improves towards healthy level after medication as TSH level improves towards euthyroid state.

T041

MOLECULAR BIOLOGY INVESTIGATION OF SOMATOSTATIN AND ESTROGEN RECEPTORS IN CLINICALLY NON-FUNCTIONING PITUITARY TUMORS

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Background: Non-functioning pituitary adenomas (NFAs) comprise about 20% of all pituitary adenomas. Because of supra- or parasellar extension of the tumor, transsphenoidal neurosurgery is not very often successful and frequently leaving tumor remnants which can regrow again. This is the reason for development of a new treatment such as biological medicines (somatostatine analogues-SAs, dopamine agonists-DAs and estrogen receptor modulators). The effect of these medicines is mediated by receptors on the surface of cells. Our aim was to determine the somatostatin (SSTR1, 2, 3, 4 and 5) and estrogen receptor 1 (ER1) profile in 69 patients with clinical NFA during transsphenoidal surgery.
Methods: Operated tissue samples were immediately submerged in RNAlater Tissue Protect (Qiagen) and transported to the laboratory. RNA was isolated by Trizol Reagent (Invitrogen) and transcribed to cDNA by SuperScript III (Invitrogen). The expression of the receptors was determined by quantitative real time PCR on RotorGene 6000 (Corbett). Plasmid DNA was used to construct the calibration curve. Results were normalized to beta-glucoronidase housekeeping gene.
Results: With the exception of SSTR5, all receptors were expressed in 69 examined tumor samples. SSTR 5 was detectable only in 58%. Median relative quantification values were: 63.8% for SSTR1, 55.4% for SSTR2, 19.6% for SSTR3, 26.1% for SSTR4, 1.8% for SSTR5, and 75.4% for ER1.
Conclusions: Nowadays SAs are commonly used in the treatment of acromegaly and neuroendocrine tumors. Newly patients with Cushings disease who can not undergo surgery and patients with postoperative residue are treated by SA (pasireotid). The NFAs treatment of choice is still neurosurgery which often fails because of tumor recurrence. The new medicines could prevent the tumor regrowth after operation or help with tumor shrinkage. Because of variable expression of somatostatin, dopamine and estrogen receptors and variable affinity of these receptors for the new drugs, it is necessary to determine the receptor expression profile in each NFA before the treatment with biological medicines. This study was supported by Charles University Grant Agency, no. 723912 and by Internal Grant Agency of the Czech Ministry of Health, no. NT 11344-4/2010.

T042

GLUCOSE CHALLENGE TEST THRESHOLD VALUES IN SCREENING FOR GESTATIONAL DIABETES IN A SPANISH POPULATION

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Background: Gestational diabetes mellitus(GDM) is a common medical complication and metabolic disorder in pregnancy,occurring in 1-14% of patients depending on the population described and the criteria used for diagnosis.It is associated with an increased incidence of fetal macrosomia,preeclampsia,and caesarean.Type 2 diabetes develops in 30-50% of women with gestational diabetes. The aim of this study is determine the most sensitive and highest value positive predictive in the diagnosis of gestational diabetes in patients with pregnancies of 24-28 weeks.
Materials and methods: Retrospective analysis of all singleton pregnancies screened for GDM during 2months at Hospital de Cabueñes was conducted.Sensitivity and specificity of the 50g glucose loading test was calculated for different cut-off values in our population. A subsequent 3 h 100 g OGTT for confirmation was carried out if screened positive.GDM was diagnosed using the Carpenter and Coustan diagnostic criteria (ADA) with two or more of the following venous plasma glucose values to meet or exceed: FBS >95 mg/dL, 1hour>180 mg/dL, 2 hour>155 mg/dL, 3hour >140 mg/dL. The receiver-operator characteristic (ROC) curve was used to identify the cut-off value of GCT for detecting GDM, using the MedCalc statistical package.
Results: 117pregnant women were analyzed. In 36 (29.9%), with plasma glucose level of more than 140 mg/dL, a 100g OGTT was performed.Eleven of them were biochemically diagnosed with GDM using the ADA criteria (two or more points above the cutoff) (9.4%). The best cut-off point in 50g OGTT for detecting GDM was 149 mg/dL, with a sensitivity and specificity of 100% and 81.1% respectively (AUC =0.946, 95%CI: 0.888-0.979). The best cut-off point in 50g OGTT for detecting impaired glucose tolerance (IGT) (only one point above the cutoff) was 139 mg/dL, with a sensitivity and specificity of 100% and 83.5% respectively (AUC=0.927, 95%CI: 0.863-0.967).
Conlusion: The elevation of current 50g OGTT cutoff (140 mg/dL), that involves making 100g OGTT curve to a higher value,to 150 mg/dL without losing sensitivity,would allow that this uncomfortable and expensive test can be omitted in a significant number of pregnant,especially for those patients with no risk factors.

T043

FIRST TRIMESTER MATERNAL SERUM PREGNANCY-ASSOCIATED PLASMA PROTEIN-A AS PREDICTORS OF DIABETES GESTATIONAL

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Backgroundnd: Gestational diabetes (GDM) complicates 3-5%of gestations and is a risk for pregnancy related maternal and neonatal morbidity.Furthermore mothers who have had GDM are at risk for having type 2 diabetes develop later in life. As currently recommended by the ADA,serum-screening for GDM typically is done at 24-28 weeks of gestation and this timing leaves only a brief window for implementing interventions designed to improve outcome.
Materials and Methods: In an observational study conducted over duration 2 months,we performed a 50 mg oral glucose challenge test (OGTT) in all pregnant women at 24–28 weeks of gestation with laboratory test in our hospital.Women with plasma glucose level of ≥140 mg/dL subsequently underwent 100gOGTT. We analyzed if first-trimester basal glucose, maternal free β-human chorionic gonadotropin (β-hCG), pregnancy-associated plasma proteinA (PAPP-A) and other classic biochemical serum markers levels (uric acid, creatinine, urea, AST, ALT, GGT, ALP, bilirubin, iron and ferritin) were different in women who did and did not develop subsequent GDM.
Results: We performed a 50g OGTT in 117 pregnant women.In 36 (30.8%) with plasma glucose level of more than 140 mg/dL,a 100mg OGTT was performed. Eleven of them were biochemically diagnosed with GDM (two or more points above the cutoff) (9.4%).This is a preliminary study;data currently were being expanded by our laboratory including a greater number of pregnant women to make the data more robust. We found no relationship between first trimester of pregnancy levels of BHCG, fasting glucose, uric acid, creatinine, urea, AST, ALT, GGT, ALP, bilirubin, iron and ferritin with subsequent GDM. The only serum biochemical marker analyzed in the first trimester of pregnancy that was associated with the subsequent development of GDM was PAPP-A [(Spearman's coefficient of rank correlation r= -0,250 (P=0,0073)]. First-trimester PAPP-A levels were lower in subjects who developed GDM than in control subjects, median 0,630 [interquartile range 0,52–0,93] vs. 1,29 [0,63–2,16] UI/mL (P=,0196).
Conclusion: Despite the previously found by other authors,we found no relationship between levels of PAPP-A or basal glucose with the risk of developing GDM. In contrast, we observe the existence of a weak association between decreasing concentrations of PAPP-A and this pathology

T044

TYPE 3 POLYENDOCRINE SINDROME IN A FAMILY FROM PAKISTAN

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Background: In type 3 Poly Endocrine syndrome (PAS-3), autoimmune thyroiditis occurs with other organ-specific autoimmune disease, but not with autoimmune adrenalitis. In this report we descrine a family from Pakistan in which mother and three daughters were affected by a PAS-3.
Methods: We studied a family from Pakistan Father MA age 48, mother KN aged 44, three daughters MM age 20, MH age 16 and MA age 14 and a son MG age 18. These subjects were tested for thyroids function, metabolic function, adrenal function, autoimmune disease.
Results: In this family the mother and four children presented autoimmune thyroiditis: hypothyroidism with presence of anti thyroid auto antibodies and high TSH serum concentration. KN, MM, MH and MA presented celiac disease with positivity for anti transglutaminase and anti endomysium auto antibodies. Moreover KN presented alopecia and MM presented a Sjogren syndrome with positivity for anti nuclear auto antibodies (granular pattern) and for auto antibodies against nuclear extractable antigens (SSA and SSB). No diabetes or pernicious anemia were observed. Adrenal and Pituitary function were normal.
Conclusions: The concept of polyglandular failure is not new, having achieved recognition as early as in the 19th century. In 1853, Thomas Addison first described the clinical and pathological features of adrenocortical failure in patients who also appeared to have pernicious anemia. PAS were classified in 1980, by Neufeld and Blizzard in three type: PAS 3, in contrast to PAS 1 and 2, does not involve the adrenal cortex. PAS 3 can be further classified into the following 3 subcategories: PAS 3A autoimmune thyroiditis with immune-mediated diabetes mellitus, PAS 3B autoimmune thyroiditis with PA, PAS 3C autoimmune thyroiditis with other organ-specific autoimmune disease such as celiac disease. PAS 3C is an uncommon disease, in literature there is only a previous report of a PAS 3C in monozygotic twins. In this family from Pakistan we observed a PAS 3C in the four female members: mother and three daughters while father was apparently unaffected and the son presented autoimmune thyroiditis alone.

T045

INSULIN RESISTANCE, DYSLIPIDEMIA AND C-REACTIVE PROTEIN LEVELS IN WOMAN WITH POLYCYSTIC OVARY SYNDROME

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Background: Women with polycystic ovary syndrome (PCOS) carry a number of cardiovascular diseases (CAD) risk factors, such are insulin resistance (IR) and dyslipidemia. Aim of this study was to evaluate IR, lipid levels and high sensitive C-reactive protein (hs-CRP) in patients with PCOS.
Methods: Samples were collected from 181 patients with PCOS and 40 healthy, age matched female subjects. Oral glucose tolerance test (OGTT) was performed, fasting and 2 h insulin and glucose were measured. In fasting samples LH, FSH, androstenedion, testosterone, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol and hs-CRP were analyzed. Anthropometric measurements were performed and insulin resistance was defined by homeostasis model assessment (HOMA-IR) and Gutt at al.`s insulin sensitivity index (ISI 0,120).
Results: Patients with PCOS had significantly higher testosterone, androstenedion, fasting and 2 h insulin and glucose levels. Triglycerides levels (0.99 ±0.36 and 0.80 ±0.36 mmol/L, P <0.05) and hs CRP (0.74±0.37 and 0.74±0.37 mg/L, P <0.001) were higher and HDL-cholesterol (1.31±0.29 and 1.45±0.22 mmol/L, P <0.05) concentrations lower in PCOS patients versus controls. Insulin resistance was found in 42.5% (77/181) PCOS patients defined by HOMA-IR. IR-PCOS had significantly higher triglycerides, hs-CRP, fasting and 2 h insulin and lower HDL-cholesterol levels than non-IR PCOS patients. ISI 0,120 and HOMA IR levels were inversely related to each other (r= -0.52, P <0.001). ISI 0,120 were lower in IR-PCOS (43.7±6.5) compared to non-IR PCOS (59.3±15.8, P <0.05) and controls (72.2±23.5, P <0.001). Body mass index was not significantly different among all three groups.
Conclusions: Few studies have examined CRP levels in woman with PCOS and results are not consistent. In our study higher levels of hs-CRP, together with higher triglyceride and lower HDL-cholesterol concentrations contribute to greater risk of CAD in PCOS patients. These CAD risk factors were higher in IR-PCOS compared to non-IR PCOS. Clinical strategies aimed at reducing IR may prevent early atherosclerosis in this population.

T046

HYPOTHYROIDISM IN FEMALE POPULATION IN OHRID - STRUGA REGION IN MACEDONIA

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Background: One of the most frequent diseases of thyroid gland present in female is hypothyroidism. It is characterized as condition of insufficient concentration of thyroid hormones T3 and T4 in serum. These hormones are important for development, growth and in metabolism regulation. Hypothyroidism can be typically recognized by high concentration of TSH in blood serum but it is not always the case. In our country we do not have official data for participation and presence of hypothyroidism, and because of that, we do this study to see the frequency and presence of hypothyroidism in females in our region (Ohrid –Srtuga). Here we display our results for percentage participation of patients with hypothyroidism in stand of the other thyroid diseases, derived from diagnoses which were carried out in our private medical institution and laboratory.
Methods: The diagnostic assessment of the thyroid gland done by thyreodologist was consisted of physical exam, thorough anamnesis of the patients (local difficulties, weakness, appetite, thirst, sweating, anxiety, sleeping, intolerability of hot/cold, heart trouble, breathing under strain, hair loss, nails, languor, voice and speech, menses, body weight, tremor, hypercinetosis, skin, pulse, blood pressure, reflex, eye changes, pigmentation, swellings, goiter size, symmetry, scope, hardness, noise, vascularity). Thyroid ultrasound exam was done on every each of the patients and thyroid hormonal status was done be measuring the TSH and FT4 in blood serum (Abbott, Axsym). For processing of obtained values for concentration of THS and FT4, age of the patient and gender, and diagnosis, was used SPSS program for statistical data processing (IBM SPSS version 19).
Results: In a period of one year (01.2011-02.2012) in our laboratory were examined 826 patients, from which 473 were excluded due to insufficient data. Were analyzed 353 patient from which 87,5% were female at age 13 to 85 year old. From all of the set diagnoses, 20,5% were diagnosed with hypothyroidism.
Conclusions: As predicted at the start of the study, we can conclude that hypothyroidism in our region is present in high percentage with emphasize on female population corresponding with the results of the National Committee for Iodine Deficiency for 2010, 24,1%.

T047
THE IMPACT OF TRH-TESTING IN INFERTILE PATIENTS

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Background: Latent subclinical hypothyroidism represents a particular problem in the treatment of infertility since thyroid disorders may cause infertility. Latent subclinical hypothyroidism is diagnosed only after the TRH-testing. The survey intended the evaluation if the subclinical hypothyroidism has an impact in the treatment of woman infertility.
Material and methods: The study includes 343 during the period January 2011- June 2012 women with infertility problems which had the normal T3, T4, TSH and Prolactine values. The first sampling was made in the morning and then Antepan® nasal spray has been applied in two streams. The second and third sampling was made upon 30 and 60 minutes after spraying. For the determination of T3, T4, TSH and Prolactine we used ECLIA method on Roche Elecsys2010 Analyzer.
Results: According to the values of the TSH, 60 min after application of Antepan® we have divided the patients in 2 groups. 1st group included 294 patients with TSH 60' after stimulation values <30 mIU/l (83.9%), T3 mean=1.84 nmol/L SD=0.29, T4 mean=114.5 nmol/l, SD=5.13, TSH before stimulation mean=1.74 mIU/l, SD=0.26, TSH 30'after stimulation mean=16.8 mIU/l, SD=0.79, TSH 60' after stimulation mean=15.6 mIU/l, SD=0.80, Prolactine mean = 12.3 ng/ml SD=5.45. And the 2nd group with 49 patients with TSH III values >30 mIU/l, (14.3%), T3 mean=1.73 nmol/L SD=0.11, T4 mean=109.2 nmol/l, SD=10.64, TSH before stimulation mean=3.40 mIU/l, SD=0.26, TSH 30'after stimulation mean=43.7 mIU/l, SD=4.36, TSH 60' after stimulation mean=41.3 mIU/l, SD=13.6, Prolactine mean = 12.6 ng/ml SD=1.16. P <001.
Conclusions: We found that there are a considerable number of patients (14.3%) with the subclinical hypothyroidism which will benefit with L-thyroxin therapy. Administration of thyroid gland hormones will improve the pregnancy rate in infertile women.

T048
A RAPID AND SELECTIVE METHOD FOR THE MEASUREMENT OF TESTOSTERONE IN HUMAN SERUM IN 10 SECONDS USING LASER DIODE THERMAL DESORPTION-DIFFERENTIAL ION MOBILITY SPECTROMETRY-TANDEM MASS SPECTROMETRY (LDTD-DMS-MS/MS)

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Background: For Research Use Only. Not For Use In Diagnostic Procedures. It has been well established that liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides excellent accuracy, precision and sensitivity for measurements of steroids in biological matrices compared to traditional techniques such as immunoassays, which may suffer from cross-reactivity. However, a limitation of LC-MS/MS for steroid research is the comparatively low throughput of the measurements, due to the need for chromatographic separations.
Methods: In this work we present a rapid method for the measurement of testosterone in human serum using a combination of Laser Diode Thermal Desorption (LDTD) ionization, differential ion mobility spectrometry, and tandem mass spectrometry. LDTD ionization enables rapid sample analysis of less than 10 seconds per sample. The use of the SelexION™ differential ion mobility spectrometry (DMS) device filters out potential interferences prior to detection by tandem mass spectrometry, ensuring that the presence of isobaric interferences in the sample will not result in overestimation of testosterone levels, and therefore eliminating the need for liquid chromatography separation. Sample preparation consisted of a simple liquid-liquid extraction of serum or plasma, followed by dry-down and reconstitution of the sample in a mixture of methanol and water. 5uL of the final sample extract was spotted and dried in a 96-well LazWell plate prior to analysis by LDTD-DMS-MS/MS.
Results: To confirm the validity of the method, a comparison study was performed by analysing a set of 24 anonymized serum samples (i) by LC-MS/MS, and (ii) by LDTD-DMS-MS/MS. The measured concentrations varied by less than 10% (accuracies ranged from 90-110%) for the two methods across the entire sample set. The method exhibited a linear response over the concentration range from 0.1 ng/mL to 100 ng/mL of testosterone, with %CV <14% at the Lower Limit of Quantitation and %CV <6% across the remainder of the concentration range.
Conclusions: The use of Laser Diode Thermal Desorption ionization combined with differential ion mobility spectrometry-tandem mass spectrometry (LDTD-DMS-MS/MS) has enabled the rapid analysis of testosterone in human serum, in less than 10 seconds.

T049

DETERMINATION OF URINARY METANEPHRINE, NORMETANEPHRINE AND METHOXYMETANEPHRINE BY LIQUID CHROMATOGRAPHY-ELECTROSPRAY TANDEM MASS SPECTROMETRY

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Background: The aim of this work was to develop and validate a method for the determination of metanephrine (M), normetanephrine (NM) and methoxymetanephrine (METHO) in urine by liquid chromatography-tandem mass spectrometry (LCMS-MS) on the Triple Quad TQ 5500 from AB SCIEX. In fact, the determination of M and NM concentrations is used in clinical diagnosis of pheochromocytoma, a rare but potentially fatal tumor arising primarily from the chromaffin cells of the adrenal medulla.
Methods: The samples were made of 24 hours acidified urines after centrifugation. Sample preparation was performed by hydrolysing and purifying by extraction column. After that, labeled M, NM and METHO were added as internal standard. Samples were analysed by liquid chromatography-electrospray tandem mass spectrometry. We determined the repeatability, reproducibility, accuracy profile and recovery on pooling urines samples from 9 volunteers analysed in triple run.
Results: The results of the precision evaluation are shown in table. The repeatability did not exceed 8.4% for M, 6.8% for NM and 10.8% for METHO. The concentration range was 71-781 μg/24h, 71-853 μg/24h and 20-854 μg/24h for the M, NM and METHO respectively. The total precision did not exceed 12.5%, 11.8% and 8.8% for M, NM and METHO. The limit of quantification (LOQ) were 33.77 μg/24h, 14.49 μg/24h and 19.81 μg/24H for M, NM and METHO respectively. The accuracy varied from 99.69 to 100.2% for a range of 71 to 781 μg/24h, from 93.32 to 100.2% for a range of 71-853 μg/24h and from 99.85 to 100.6% for the range 20-854 μg/24h for M, NM and METHO respectively. The recovery were 99.96% (95% CI for the mean: 96.5-103.4), 99.75% (96.5-102.9) and 100.08 (95.97-104.2) for the M, NM and METHO respectively.
Conclusions: We have successfully developed and validated an LCMS-MS method to determine urinary M, NM and METHO on the TQ 5500 from AB SCIEX. It represents a convincing alternative to the HPLC method for a faster and reliable measurement of urinary M, NM and METHO.

T050

INFLUENCE OF AGE AND SEX ON TSH FOR DIAGNOSIS OF HYPOTHYROIDISM

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Introduction: Reference ranges for classifying a patient as hyper-, normo- and hypothyroid vary from one laboratory to another, due to the techniques employed, regional factors (race, iodine), but there's also the variables of age and sex patient not usually consider. Article Thyroid Volume 21,1,2011,5-11 Boucai et al, published equations that adjust normal ranges based on these variables.
Objective: Evaluate these equations and determine if there are significant changes to normal.
Material and Methods: We studied 2521 outpatients that have requested TSH and antithyroid antibodies (thyroid peroxidase and thyroglobulin antibodies) from the health center throughout the year 2011. Determine if necessary the hormone thyroxine (T4) and triiodothyronine hormone (T3). Assays TSH, T3 and T4 are provided by chemiluminescent microparticle immunoassay (CMIA) in ARCHITECT i2000. Antithyroid antibodies by ELISA InmunoCAP250. Our reference range for TSH is between 0.35 and 4.94 mUI/mL, so adapting the equations described in the article are obtained to study the following equations: TSH (2.5)=0.35+0.00073* age-0.031 *SexTSH (97.5)=4.94+0.05 * age -0.223 *Sex considering age in years and sex 0 for men and 1 for female
Results: The design equations is developed with patients with negative antithyroid antibodies, our population studied have only antithyroid antibodies negative 1513 (60%) patients. With our classification reference range of thyroid function are 120 hyperthyroid patients (7.9%), normothyroid are 1109 (73.3%) and hypothyroid are 284 (18.8%) Applying the equations to study the following results: 122 (8.1%) hyperthyroid, 1288 (85.1%) normothyroid and 103 (6.8%) hypothyroid. We have 181 patients, corresponding to 12% of the total and 63.7% of patients classified as hypothyroid, which would be reclassified to normothyroid.
Conclusions: These equations to determine new TSH reference ranges are attractive because they allow us to dispense with the verification of results, enlargement of new assays, such as T4, T3 or antibodies. It significantly reduces the number of patients presenting negative antithyroid antibodies were classified as hypothyroid due to having a TSH above the normal range. But these patients presented normal thyroid function according to their age and sex

T051

CALCIUM RECOVERS THE GROWTH HORMONE-PRODUCING CELLS IN OVARIECTOMIZED RATS

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Background: In addition to hormones, secretory processes in anterior pituitary cells are controlled by regulatory molecules such as Ca2+ ions, vitamins, metabolites and growth factors. Calcium is an essential mineral involved in the most metabolic processes and phosphate salts of calcium provide mechanical rigidity to the bones and teeth, where 99% of the body's calcium resides. This study was designed to evaluate the morphometric and secreting characteristics of growth hormone-(GH-) producing cells in the anterior pituitaries of ovariectomized (ovx) and calcium glucoheptonate treated ovariectomized female rats (ovx+Ca).
Methods: Female Wistar rats were ovx at 12 weeks of age. Animals were divided into three groups, each comprising seven females. The first group contained ovx rats, treated with saline, daily for 4 weeks. The second group of ovx females received i.m. calcium glucoheptonate (Ca; 11.4mg) following the same regimee. The third group were intact controls, who, like the ovx group, received the adeqate volume of saline during the same period. All females were sacrificed 24 h after the last injection. GH-producing cells were studied using the peroxidase-antiperoxidase (PAP) immunohistochemical procedure. Serum concentrations of GH in all groups were measured by the hGH-Delfia kit.
Results: The absolute pituitary weights were significantly increased (P <0.05) in ovx and ovx+Ca treated females, by 16.2% and 17.6% respectively, compared to the intact controls. The relative pituitary weights were also significantly increased (P <0.05) in ovx and ovx+Ca treated females, by 14% and 20% respectively, in comparison with the intact control rats. Immunohistochemically labelled GH cells in intact control rat pituitaries were ovoid to pyramidal in shape, usually located along the blood vessels. In the ovx and Ca treted ovx females GH cells were longer, irregularly shaped, with more intensely stained cytoplasm. Concentration of GH hormone in the serum of ovx and ovx+Ca treated females was significantly decreased (P <0.05) by 75.8% and 12.9% respectively, compared to the intact controls.
Conclusions: Our findings presented here show that multiple application of Ca to ovx rat females results in a recovery of the secretory activity of pituitary GH cells.

T052

ROUTINE SERUM STEROID PROFILING USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY BASED IVD REAGENT KIT

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Background: The measurement of a complete steroid profile rather than individual analytes is a powerful tool in the diagnosis of steroid metabolism disorders. The immunoassays are widely used in clinical laboratories for the quantitative measurement of steroids, but these methods are not very specific (cross-reactivity and matrix interferences). We validated an isotopic dilution liquid chromatography–tandem mass spectrometry method for the simultaneous measurement of 11-deoxycortisol, 17α-hydroxyprogesterone, androstenedione, corticosterone, cortisol, dehydroepiandrosterone sulfate, progesterone and testosterone in serum, based on IVD reagent kit from Perkin Elmer (Wallac OY,Turku, Finland). We tested the advantages of steroid profiling measurement in 123 pediatric patients (84 healthy subjects and 39 with endocrine pathologies).
Methods: A 96-well plate based assay involves a simple sample preparation, where 100 μl of serum were spiked with stable-isotope-labeled internal standards (ISs) and extracted by protein precipitation. This method uses an ultra-performance liquid chromatography system and a tandem mass spectrometer equipped with electrospray ionization (ESI) source (Acquity UPLC with TQ detector WATERS, Milford-USA). Analytes were separated on UPLC® with Acquity column, during 11 min gradient run and they were revealed by Multiple Reaction Monitoring (MRM) analysis.
Results: The preliminar results obtained in terms of accuracy, precision and lower limit of quantification (LLOQ), allows the use of this method for a routinely measurement of a wide steroid profile. The assay was linear over each analyte concentration range with all correlation coefficients (r2) >0.99. The intra-assay precision (n=2) was <15% for all steroids escluded testosterone and progesterone (<20%).
Conclusions: the newly standardized LC-MS/MS assay in kit-format allows the simultaneous determination of a wide steroid profile by a single run, in clinically relevant ranges, both in pediatric and adult age with good sensitivity, accuracy and precision. The importance of steroid profile in clinical diagnosis and the limit of routine immunoassay at low concentration were also highlighted. The easy sample preparation makes this method suitable for routine use in clinical laboratories.

T053
SCREENING AND AGE DISTRIBUTION OF PROSTATE-SPECIFIC ANTIGEN IN GENERAL PRACTICE

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Background: The symptoms and signs of prostate cancer usually manifest after is too late to cure the condition. General practitioners (GPs) are ideally suited to diagnose the disease early and need to know how to identify the patients. Prostate-specific antigen (PSA) is a well-known tumor marker for prostate cancer. The aim of this study was to undertake PSA testing and to determine its age distribution among healthy men, aged 40 to 79 years.
Methods: 100 men followed up by GPs from Health Centre of Soxos, were invited to participated in a prostate screening protocol, consisting of measurement of serum PSA and rectal examination (DRE). At the time of the study all subjects had no evidence or history of prostate cancer/surgery/lower urinary tract symptoms. Subjects were grouped into four age groups: group I 40-50 years old (n=25), group II 50-60 years old (n=25), group III 60-70 years old (n=25) and group IV >70 years old (n=25) years. Subjects with abnormal DRE and or PSA ≥4.0 ng/mL were referred to urologists for further evaluation.
Results: 8 subjects (8%) had PSA levels ≥4.0 ng/mL. The proportion of the patients with PSA levels ≥4.0 ng/mL in the screening groups was determined as follows: group I (0/25, 0%), group II (1/25, 4%), group III (1/25, 4%) group IV (6/25, 24%) (P <0,05). The mean PSA level was detected as follows: group I (1.7 ng/mL), group II (2.6 ng/mL), group III (3.1 ng/mL), group IV (5.8 ng/mL).
Conclusions: Our findings indicate that serum PSA levels correlate with age. Patients with PSA ≥4.0 ng/mL should seek additional testing.

T054
THYROID DISEASE IN THE ELDERLY POPULATION FROM NORTHERN GREECE

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Background: Several changes in thyroid hormone secretion, metabolism and action occur with the increase in age. In elderly, diagnosis of thyroid disorders is often complicated due to chronic non-thyroidal illness and medication therapy. The objective of the present study was to find out the prevalence of subclinical hyporthyroidism / hyperthyroidism in older people, living in urban areas from northern Greece.
Methods: 90 patients (45 men/55 women) with median age 63,5 ± 6,8, followed up in primary care settings (Health Centre of Soxos), were avalaible for analysis of TSH, FT3 and FT4. At the time of the study all patients were clinically euthyroid and noone had a history of recent infection or other illness or received any medication that could affect thyroid function. Serum samples were obtained and levels of TSH, FT3 and FT4 were measured (Roche, Modular E170). Subclinical hyperthyroidism was definied when TSH was abnormal low (<0,27 μIU/ml) and FT3 (3,1-6,8 pmol/lit) and FT4 (12-22,0 pmol/lit) were present at normal levels. Subclinical hyporthyroidism was definied when TSH was abnormal high (>4,20 μIU/ml) and FT3 and FT4 were present at normal levels.
Results:Subclinical hyperthyroidism was detected in 3/90 (3,3%) patients [1/45 men (2,2%) and 2/55 women (12.2%)]. Subclinical hypothyroidism was detected in 11/90 (9,2%) patients [2/45 men (4,4%) and 9/55 women (P <0,05) (16,3%)]. Hyperthyroidism was not detected and 3/55 (5.4%) women presented with hypothyroidism.
Conclusions. Thyroid abnormalities were not unsual in the elderly. Therefore serum levels of TSH, FT3 and FT4 should be considered in evaluation in elderly subjects.

T055

DOES FERRITIN AND LEPTIN LEVEL IN YOUNG WOMEN WITH POLYCYSTIC OVARY SYNDROME DEPEND ON THE NUTRITIONAL STATUS?

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Background: Polycystic ovary syndrome (PCOS) is one of the pathological conditions associated with inflammation and oxidative stress. Ferritin was considered as one of the inflammatory reactants or markers of oxidative status. The question remains whether elevated serum ferritin levels representing iron overload in patients with PCOS are the cause or consequence of insulin resistance existing in these subjects. The aim of this study was to test the hypothesis that serum ferritin, like the leptin levels in young women with polycystic ovary syndrome depend on the nutritional status.
Methods: Study included 54 female patients, in the age of 18 to 40 years, with proven PCOS (among whom 32 lean, with body mass index-BMI <25 kg/m^2, and 22 overweight or obese, with BMI>25 kg/m^2) and 46 healthy control female patients, in the age of 20 to 36 years (among whom 29 lean and 17 overweight or obese). PCOS was diagnosed based on well-known clinic, biochemical and ultrasonographic criteria. Ferritin was measured by immunochemiluminescence method, while leptin was measured in blood samples by sandwich enzyme-linked immunosorbent assay (ELISA).
Results: In all the subjects included in this investigation there was a strong positive correlation between leptin and ferritin levels (r=0.429, P <0.001), as well as between leptin and BMI (r=0.930, P <0.001) and ferritin and BMI (r=0.467, P <0.001). However, while leptin levels differed significantly between lean and overweight healthy control (P <0.001) and PCOS (P <0.001) subjects, this could not be demonstrated for ferritin. Ferritin levels did not differ significantly neither between lean and overweight healthy control, nor between lean and overweight PCOS subjects. At the same time, ferritin levels were significantly higher in lean subjects with PCOS than in lean healthy control subjects (p=0.025), as well as in overweight patients with PCOS than in overweight healthy control subjects (P=0.018).
Conclusions: Results of this investigation have suggested that while leptin levels depend almost solely on nutritional status, ferritin levels were determined by some additional factors in young women with PCOS. Role of inflammation, oxidative stress and/or insulin resistance in this context has to be determined in future investigations.

T056

CHRONIC MAGNESIUM SUPPLEMENTATION INFLUENCES BASAL BLOOD LEVEL OF CORTISOL AND TESTOSTERONE/CORTISOL RATIO IN RUGBY PLAYERS

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Background: There is a special interest during last years on the effects that certain diet supplements may have on endocrine responses of physically active persons during intense exercise and competition. Magnesium ion is known to influence the secretion of certain steroid hormones and by its role in the pathophysiology of physical exercise. The purpose of this study was to investigate if a four-week administration of magnesium oral supplement affects basal serum values of ACTH, cortisol (C), FSH, LH, testosterone (T), and T/C ratio in male rugby amateur players.
Methods: Blood samples were collected from 13 rugby players (22.9±4.6 years) before and 28 days after magnesium oral supplementation (2x250 mg of Magnesium 250mg ®, Natural Wealth®, NBTY Inc.). Serum levels of hormones were measured using Beckman Coulter immunoassays on Access® 2 analyzer. Statistical significance between hormone levels obtained prior and after magnesium administration was calculated using Student's t-test.
Results: Even though there was no change in the serum level of ACTH, our study showed significant reduction in C level (P <0.01) after Mg supplementation. Analysis of pituitary-gonadal axis hormones levels didn't show any statistically significant changes apart from trend in reduction LH level (P=0.062). The T/C ratio was significantly increased (P <0.01) comparing to values measured prior magnesium supplementation.
Conclusion: Results of our study imply that oral magnesium supplementation in persons regularly subjected to intense physical and psychological stress, reduces basal level of stress hormone cortisol without affecting level of pituitary ACTH. This change in cortisol level is mainly responsible for an increase in T/C ratio, which is known to be significantly diminished after intense physical exercise.

T057

ENDOCRINE RESPONSE AND HEMATOLOGY STATUS IN MALE SEDENTARY STUDENTS FOLLOWING FOUR-WEEK ORAL ADMINISTRATION OF MAGNESIUM SUPPLEMENT

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Background: Sleep deprivation, malnutrition and lack of physical activity are contemporary stress related factors present in student population. Recently, magnesium (Mg) supplement therapy has been reported to affect hormonal imbalance and hematology parameters variations observed in intense physical exercise. In this study, we aimed to examine whether magnesium supplement therapy influences hormonal and hematological status of male sedentary students.
Methods: Thirteen healthy male students of Belgrade University, age 22.7±0.5 years (mean±SD) participated in the study. Exclusion criteria were recreative physical activity more then two times per week. Blood samples were collected before and 4 weeks after supplementation with 2x250 mg of Magnesium 250 mg ®, Natural Wealth®, NBTY Inc. Serum levels of FSH, LH, testosterone, ACTH, and cortisol were measured using Beckman Coulter immunoassays on Access® 2 analyzer. Hematological parameters: total red blood cells (RBC), hemoglobin (HB), white blood cells (WBC) and WBC differential, were determined using Beckman Coulter® LH750. Statistical significance between corresponding values prior and after Mg supplementation was calculated using the paired Student's t-test and P-value less then 0.05 was considered significant.
Results: Despite the fact that there was no change in the serum level of ACTH, our trial showed statistically significant ($P < 0.05$) decrease in serum cortisol levels after Mg supplementation. Analysis of pituitary-gonadal axis hormones did not show any significant change. A noteworthy increase in total RBC count ($P < 0.001$) was found, and although the total WBC count was unchanged, statistically significant decrease in percentage of neutrophils ($P < 0.05$), and a trend in lymphocytes percentage increase ($P = 0.064$) were observed.
Conclusion: The results presented here suggest that chronic oral magnesium supplementation in male sedentary students reduces serum level of stress hormone cortisol and may have a beneficial effect on some hematological parameters.

T058

ABNORMAL THYROID FUNCTION TESTS IN CLINICALLY HEALTHY INDIVIDUALS

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Background: Recent development of medical science and technologies contributed vastly to the diagnosis of health disorders. This was, however, accompanied by a significant "side effect": many physicians began to rely on more and more specific tests and underestimate the significance of medical history, examination, and environmental factors. The aim of the study was to evaluate the significance of specific laboratory tests as compared to physical examination and standard laboratory tests in patients with specific risk factors.
Methods: Thyroid function tests including thyroid antibodies, TSH, free serum T4 and T3 were performed in 170 individuals on active military service, 162 male and 8 female, aged 22 to 43 yrs (mean age 30.04 yrs). All of them were subjects to regular annual medical examinations which included, but were not limited to, physical examination, basic urine, haematology, and biochemistry tests. Samples were taken at 06:30-08:00 a.m. after an overnight fast and rest. Tests were performed in central laboratory via hemiluminiscent analysis by Immunolite 2000 DPC equipment.
Results: Test results showed abnormal values in 30.00% of the tested population (51 patients). The most predominant abnormality was high free T3 which occurred in 32 (18.82%) of the subjects followed by low free T4 in 11 subjects (6.74%). In 37 of the cases (29 with high T3 and 8 with low T4) all other tests were within normal limits. Higher than expected was also the incidence of abnormal values of TSH – 9 cases (5.29%), in 6 (3.53%) of which TSH was elevated, and in 3 (1.76%) TSH was below lower limits of normal.
Conclusions: The authors conclude that the extremely high incidence of thyroid function tests abnormalities is probably influenced by some factors specific for the tested population such as stressful professional environment in combination with mild to moderate iodine deficiency. Normal physical exams and standard laboratory tests indicate the necessity of regular specific thyroid function tests in this population. The later should be interpreted with caution, in consideration with all possible environmental, professional, and lifestyle factors.

T059

IDENTIFYING OPTIMAL AGE-RELATED REFERENCE RANGES DURING HORMONE REPLACEMENT THERAPY IN AGING MALES

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Background: Andropause is a condition resulting from biological changes experienced by men during mid-life and beyond. Symptoms are generally attributed to a gradual decline in testosterone levels. However, in addition to testosterone, the changing levels of additional hormones may impact the symptoms experienced. These symptoms include changes in libido and sexual function, weight gain, loss of muscle mass, gynecomastia, sleep and mood disorders. Hormone replacement therapy for the management of symptoms of andropause is gaining acceptance in the medical community. Reference ranges for hormones are available, but may differ from the levels that are optimal for the reduction of symptoms, particularly following the implementation of hormone replacement therapy. The purpose of this study was to determine the optimal range for free and total testosterone, estradiol, progesterone, and DHEA-S in order to alleviate the symptoms of andropause.
Methods: 100 males between the ages of 40 and 65 years with no acute disease were assessed by physical exam and symptom questionnaire. Blood was drawn at first visit and 3-6 months following hormone replacement. Symptoms assessed included sexual dysfunction, loss of muscle mass, sleep disorders, fatigue, weight gain, depression and/or anxiety. The following assays were measured before and after treatment: Testosterone, Free Testosterone, Estradiol, Progesterone, and DHEA-S. All assays were measured by chemiluminescence immunoassays using the Beckman Coulter Access® 2. Free testosterone was calculated using the Vermillion calculator.
Results: Hormone measurement ranges after treatment and resolution of symptoms: Free testosterone 150-300 pg/mL, Total testosterone >350 ng/dL, Estradiol 25-50 pg/mL, Progesterone 0.5-1.5 ng/mL, DHEA-S 350-450 ug/dL.
Conclusions: The utilization of optimal hormone ranges may assist in the management of the symptoms of andropause in aging men.

T060

ASSESSING THE STEROID PROFILE IN KLINEFELTER SYNDROME: THE IMPORTANCE OF THE CHOICE OF RELIABLE LABORATORY ASSAY

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Background: scientific evidence on steroid profile and functional deficit of Leydig Cells (LC) in patients affected by Klinefelter Syndrome (KS) are not exhaustive and rather controversial. Gonadotropin (hCG) stimulation has been used to assess the steroid profile and to investigate the enzymatic blocks in KS, but the low accuracy of immunometric assays (IA) is the main cause of uncertain conclusions.
Objective: to investigate steroid serum levels in KS before and after hCG stimulation comparing IA to LC-MS/MS and to define the functional deficit of LC possibly clarifying which step of steroidogenesis is defective. In this abstract we focus only on the results of the comparison between different assays for the main steroids.
Methods: prospective interventional trial conducted among 7 patients with genetic diagnosis of KS without androgen replacement who underwent blood sampling at 8 a.m. after overnight fast and after an intramuscular dose of 5000 IU of hCG for 6 consecutive days. Plasma steroids and gonadotropins have been measured, steroids levels have been evaluated with routine IA and with LC-MS/MS.
Results: progesterone (P) and 17-hydroxyprogesterone (17OHP) increased in the first day after hCG stimulation, P and 17OHP immunoassay overestimated in the lower and higher range respectively. IA and LC-MS/MS showed consistently that androstenedione (A) was not responsive to hCG stimulation but IA was much inaccurate. Testosterone (T) levels enhanced one day later, IA was well correlated to LC-MS/MS and slightly overestimating at higher levels. DHEA (LC-MS/MS) and DHEAS (IA) remain unchanged demonstrating an adrenal derivation; hCG stimulation didn't modify Cortisol (F) secretion, with good accuracy of IA.
Conclusions: this study demonstrated that LC in KS are responsive to hCG stimulation, the Delta4 pathway is preserved, and that the adrenal gland is not responsive. LC-MS/MS has been demonstrated to be much more reliable than IA whose sensitivity at low level range is inadequate to measure P and 17OHP in male subjects. Results of A are absolutely unreliable. IA have been demonstrated to be reliable to test T and F. LC-MS/MS should be implemented as routine assay for P, 17OHP and A, as recommended by different organizations including the Endocrine Society.

T061

ASSOCIATION OF THE ADIPONECTIN GENE RS1501299 VARIANT WITH POLYCYSTIC OVARY SYNDROME IN CHILEAN WOMEN

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Background: Adiponectin, encoded by ADIPOQ gene, has anti-atherogenic properties and reduced serum adiponectin levels are associated with cardiovascular disease. Recently, we demonstrated that polymorphisms of adiponectin gene (ADIPOQ) are associated to coronary artery disease (CAD) in Chilean subjects. In addition, we observed that the Chilean women with Polycystic Ovary Syndrome (PCOS) presented a high risk for CAD. Thus, the aim of the present study was to evaluate the possible association between the rs1501299 (276A>C) polymorphism of ADIPOQ gene with Polycystic ovary syndrome in Chilean women. Methods: We designed a case-control study, analyzing a total of 200 women. The case group was comprised of 100 adult women in reproductive age, not related and diagnosed with PCOS, from Hernán Henríquez Aravena Hospital of Temuco city (Chile). The control group was conformed by 100 women of similar age range, no related, without a diagnosis of PCOS, with normal menstrual cycles and no signs of hirsutism, acne or alopecia and no hormonal treatment. The rs1501299 polymorphism of ADIPOQ gene was determined by using polymerase chain reaction-restrict fragment length polymorphism (PCR-RFLP). Results: The genotype distribution of the 276A>C polymorphism was in Hardy-Weinberg equilibrium. PCOS patients exhibited a high frequency of C allele when compared to controls (0.76 vs. 0.16; P <0.001). The OR for PCOS associated to C mutated allele was 16.6 (95%CI: 8.2-33.6) confirming the association observed. Conclusion: Our data show that the rs1501299 ADIPOQ gene polymorphism is associated to PCOS in the studied women.

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T062

THE PREVALENCE OF POLYCYSTIC OVARIAN SYNDROME AMONG REPRODUCTIVE FEMALES AT QATAR UNIVERSITY: A CROSS-SECTIONAL STUDY

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Back ground: Polycystic Ovarian Syndrome (PCOS) is found to be at least 6-10% prevalent all over the world. It is the most common endocrine pathology among females of reproductive age. PCOS is associated with chronic menstrual irregularities, anovulatory infertility and hyperandrogenemia manifested clinically with oligomenorrhoea, hirsutism and acne. No data about the incidence of the PCOS or its metabolic features are known in state of Qatar among young reproductive females. The aim of this study is to highlight the incidence of PCOS and its metabolic features among Qatar university female students.
Methods: A population of 121 female students was volunteered from Qatar University from March until Dec 2011. The age group of the participants was between 18-25 years.tations of hyperandrogenism and Modified Ferriman-Galleyway scores for hirsutism was calculated. 5 ml of fasting blood samples were drawn from each subject in early morning for analysis of testosterone, DHEAS, SHBG, progesterone, estradiol, insulin, prolactin, TSH and glucose.
Results: The results showed that, the Rotterdam criteria being the widest definition, gives the highest incidence of PCOS (25.83%) followed by Androgen Excess Society criteria, AES (20.83%) and National Institute of Health criteria, NIH (19.17%), respectively. The incidence of PCOS being highest among Asian (43.4%), followed by African (22.6%), Qatari (16.1%) and European (12.9%) female students, in order. Testosterone and irregular menstrual cycle are highest observed parameters in 77% of PCOS cases.
Conclusion: The incidence of PCOS is relatively high among student females at Qatar University of 17-25 years of age by different criteria. There is a need for Qatari population to be aware of this syndrome and its complications.

T063
PERFORMANCE EVALUATION OF A NEW ECLIA ASSAY FOR DETERMINATION OF ANTI-TSH RECEPTORS AUTOANTIBODIES

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Graves Disease (GD) is an autoimmune thyroid pathology that causes hyperthyroidism, has its laboratorial diagnosis made by measuring the existence of antibodies against TSH receptor (TRAbs). Traditionally this measurement was performed with a timing-consuming and expensive technique involving cell cultures, not suitable for modern hospital laboratories. In the past years techniques evolved to approaches that use monoclonal antibodies and RIA assays, which requires special laboratories, equipment and personnel to perform. Recently, a new alternative using electrochemiluminesence came into marked and promises to deliver similar results in a much faster way.
The goal to this work is to determine TRAb levels among a Graves' Disease population (new cases and under treatment) and compare those to a control group, both followed at the Outpatient Clinic at Oporto Medical Centre, using a RIA assay (RiaRSR TRAb CT, RSR Limited) and the new ECLIA assay (Elecsys Anti-TSHR, Roche Diagnostics). Sensibility and specificity of the ECLIA assay using the provided cut-offs were established. Recording and data analysis was performed using SPSS 20.0 (descriptive statistics, Spearman correlation coefficient, significance level α=0,05). Patients with GD were mostly women (40 women; 12 men), median 42,3±2,2. Control group was also mainly consisted of women (38 women; 15 men), median 53,13±3,0. Results allowed us to establish a 95% specificity and 93% sensibility for the ECLIA assay regarding GD laboratorial diagnosis. The correlation between TRAb levels obtained either using RIA and ECLIA was established using Spearman coefficient and a strong positive correlation was noted r=0,858, n=105, P <0,001. We also noted that for every patient having a RIA undetermined result (those with measurements between 1-1,5 UI/L inclusive; n=4), when tested using ECLIA they were positive. We conclude that there is a fair correlation between the RIA and ECLIA methods. Using the provided ECLIA cutoff, sensibility and specitivity values we established slightly lower values for both specificity and sensitivity. Finally, our results suggest this new ECLIA kit for determining TRAbs may be used to provide accurate results in a shorter time than RIA and can be performed at a more broad range of laboratories.

T064
UNUSUAL CAUSE OF DELAYED PUBERTY

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Background: Delayed puberty occurs only in about 3% of children with constitutional delay of growth and puberty being the commonest cause. Congenital deficiency of pituitary hormones is not common but a recognised cause of delayed puberty with heterogeneous clinical presentation.
Methods and Results: We present a case of a 16 year old Indian boy who was referred for investigations of poorly developed secondary sexual characteristics, excessive weight gain and headaches. His maternal uncle was reported to have delayed puberty. He was obese with BMI of 37.8 and of short stature. His height (154 cm) was below the 3rd centile for his age. His Tanner stage was G2 and P2. He was found to have colour blindness. He had low testosterone of 6.1 nmol/L (adult ref. range: 7.6 -27.7 nmol/L) with low normal gonadotrophin concentrations The X-ray of his wrist confirmed bone age of 15 year old, Spinal- X-ray showed anterior wedging of Th10-12. Insulin stress test revealed lack of growth hormone response to insulin infusion. MRI of brain showed hypoplastic anterior pituitary with absent stalk and ectopic posterior pituitary in keeping with pituitary dwarfism.
Conclusions: Pituitary hypoplasia is a rare congenital disorder involving the adenohypophysis, neurohypophysis and often the pituitary stalk. Failure of hypothalamic axons and surrounding venous plexus to connect with adenohypophysis results in incomplete growth of the pituitary gland. Ectopia of the posterior pituitary is also a rare congenital anomaly. Patients with absent pituitary stalk present with multiple hormonal deficiencies while those with pituitary stalk have isolated growth hormone deficiency.

T065
DIAGNOSTIC CAPACITY OF AMH AND INHIBIN B

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Background: The fertility of mature woman reduces with the increasing of age. It is due to the gradually drying up of eggs in the gonads. Anti-Mullerian hormone (AMH) is a reliable marker for valuation of ovarian reserve, together with the reduction of Inhibin B, increasing of FSH and the decreased number of antral follicles, evaluated by ultrasound. The aim of our study is to present the diagnostic possibilities and practical application of AMH and Inhibin B
Methods: 303 samples of women in age 20 to 53 were examined. The analysis of AMH and Inhibin B is performed with manual ELISA-method, Beckman Coulter reagents, calibrators and controls. The examination of LH, FSH, E2 is performed with ECLIA of Cobas 6000 with reagents and calibrators of Roche and BioRad controls.
Results: The results of 303 samples analyzed showed that 52.8% were in the reference area and 47.2% were outliers in one or more of the studied parameters. The results out of the reference range for the group, showed the following distribution: increase of AMH or in combination with increased LH/FSH ratio in 12.6% of cases, decrease of AMH or in combination with decreased Inhibin B, E2 or with increased FSH in 72.7% of cases, an isolated reduction of Inhibin B in 14.7%. The distribution of results is presented also by age groups.
Conclusions: AMH and Inhibin B are markers for assessing reproductive function as AMH is a better marker for the evaluation of the ovarian reserve. Its decrease precedes the reduction in Inhibin B and the increase in FSH. AMH is not affected by the phase of menstrual cycle, while Inhibin B is the highest in the follicular phase and then decreased – it must be examined between 3-5 days of the menstrual cycle. Of importance is the determination of AMH in assisted reproductive technologies such as marker for determining ovarian response in controlled ovarian stimulation. This facilitates the identification of women who will not respond to stimulation as well as those who will develop ovarian hyperstimulation syndrome.

T066
HOLOTRANSCOBALAMIN AS AN EARLIER INDICATOR OF VITAMIN B12 DEFICIENCY IN THYROGASTRIC SYNDROME

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Background: The thyrogastric syndrome is the autoimmune association of Hashimoto thyroiditis and atrophic gastritis which occurs in 14% of autoimmune thyroiditis (AIT) and is often undiagnosed. Holotranscobalamin (HoloTC) is the biologically active form of vitamin B12 and represents only 20% of total vitamin B12. HoloTC is believed to be an earlier detection marker of vitamin B12 deficiency (then total vitamin B12) which is especially frequent when gastritis manifestations are present. In this study, we want to compare two markers to determine the vitamin B12 status of our population: total vitamin B12 (Roche) and HoloTC (Abbott).
Methods: A total of 119 patients have been separate in 3 groups: “controls” (n=35), “autoimmune thyroiditis” (n=60) and “thyrogastric syndrom” (n=24). We measured serum HoloTC (Abbott) and total vitamin B12 (Roche). We excluded the patients with estimated glomerular filtration rate (eGFR) calculated using MDRD equation <30 mL/min/1.73m2 because HoloTC concentrations may be affected by renal function. Most patients underwent TSH, FT4, FT3, ATPO, ATG, TBII, parietal cell autoantibodies (PCA), intrinsic factor antibodies (IFA) and gastrin determinations to determine their auto-immune status. All patients supplemented with vitamin B12 (per os or IM) were excluded of the study.
Results: We determined that the correlation between HoloTC and total vitamin B12 HoloTC was significantly but not strongly associated with total vitamin B12 (r=0,5414; P <0,0001). The vitamin B12 (both total and HoloTC) concentration was significantly (P <0,0001) lower in “thyrogastric syndrom” group that prove there is vitamin B12 malabsorption during gastritis. However, more patients were deficient with HoloTC (73,9%) than with total vitamin B12 (47,8%) in the “thyrogastric syndrome” group. So, total vitamin B12 is not low in all low HoloTC.
Conclusions: HoloTC is as suitable as total vitamin B12 for diagnosis of vitamin B12 deficiency and tends to be lower than total vitamin B12 in thyrogastric patient. So, these patients would be more quickly supplemented if their vitamin B12 status is determined by HoloTC.

T067

ICAM1 K469E AND E-SELECTIN S128R POLYMORPHISMS COULD PREDISPOSE TO INCREASED AUTOANTIBODY PRODUCTION AND TSH SUPPRESSION IN GRAVES' DISEASE

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Background The etiopathogenesis of Graves' disease (GD) has not been clearly elucidated although the role of chronical inflammation and endothelial dysfunction has been established. Adhesion molecules such as intercellular adhesion molecule 1 (ICAM1), vascular cell adhesion molecule 1 (VCAM1), and E-selectin are secreted from vascular endothelium and promote accummulation of leukocytes in damaged endothelial areas. This study examined the possible association of ICAM1 (G241R and K469E), VCAM1 (T-1591C and T-833C), and E-selectin (S128R) single nucleotide polymorphisms (SNPs) with the occurence of GD. Methods ICAM1 (G241R and K469E), VCAM1 (T-1591C and T-833C), and E-selectin (S128R) SNPs in DNA from peripheral blood leukocytes of 171 patients with GD and 259 healthy controls were investigated by real-time PCR combined with melting curve analysis using fluorescence-labeled hybridization probes. Results We did not find significant differences in the distributions of studied polymorphisms, nor in the haplotype frequencies between patients with GD and healthy control. However, the ani-TPO levels in E-selectin 128R allele carrying subjects (SR+RR) were higher than S128S genotype ($P < 0.05$). In addition, the decline of TSH levels was more prominent in ICAM1 469 E carrying subjects (KE+EE) in comparison with wild homozygotes ($P < 0.05$).
Conclusions Although there is not assosiation between ICAM1 (G241R and K469E), VCAM1(T-1591C and T-833C), and E-selectin (S128R) SNPs and susceptibility to GD, higher anti-TPO in E-selectin 128 SR+RR, and lower TSH in ICAM1 469 KE+EE subjects suspect that these genotypes are prone to increased antithyroid autoantibody production with more accentuated TSH suppression in GD. Further studies with a larger cohort, analyzing other polymorphisms in ICAM, VCAM1 and E-selectin genes are necessary to support our observations.

T068

QUALITY INDICATORS TO MONITOR THE REJECTED OUTPATIENTS' SAMPLES

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Introduction. The sample collection is an activity that can be performed inside and outside of the laboratory walls (LW) and literature reports that laboratory personnel submitted significantly fewer rejected samples than others. The implementation of quality indicators (QIs), is becoming essential to monitor and improve the activities affecting the samples suitability. The aim of this work is to describe the results, from 2009 to October 2012, of QIs used to monitor and evaluate the errors concerning the outpatients samples collected in different phlebotomy sites: centralized hospital laboratory, public peripheral places, other hospitals, geriatric centers, public and private health clinics.
Methods. Eight different QIs have been identified on the basis of the rejection causes: clotting, haemolysis, insufficient volume, inadequate blood sample-anticoagulant volume ratio, lost/not received, inappropriate containers, data lack about diuresis volume. Collected data have been analysed to identify the improvements or worsening trends during the years after implemented improvement actions (sending of periodic information sheets and training course concerning the main causes of samples rejection and the correct procedures to be used).
Results. The results concerning some QIs are described as an example. The number of rejected samples (and percentage on total number) was: 2009: 506 (0.060%); 2010: 697 (0.064%); 2011: 778 (0.07%); 2012: 758 (0.08%). The main causes, calculated on total rejected samples, (average from 2009 to 2012) were: clotting (38%); inadequate blood sample-anticoagulant volume ratio (28%); inappropriate containers (16%). The public peripheral places showed the higher percentage of errors and a decreasing during the years (from 2009 to 2012): clotting, 55.25%-26.12%; inadequate blood sample-anticoagulant volume ratio, 39.64%-10.62%; inappropriate containers, 65.77%-42.86%.
Conclusions. The critical prerequisite to improve the activities performed outside of LW is dependent upon the degree of laboratory supervision and monitoring the used procedures. The implemented corrective actions during the years have been effective for same QIs, but in many cases, the critical patient typology makes it difficult to achieve further improvements.

T069

HETEROPHILIC ANTIBODIES INTERFERENCE IN TSH ASSAY: AN INTERESTING CLINICAL CASE

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Background: It is well known that immunoassays may have interference of different types depending on the method used, which can cause both false negatives and false positives and the laboratory should be aware of the possibility to produce incongruent results, causing negative outcomes for patients.
Objective: To present the clinical case of DF, a woman aged 39 , followed by the Eating Disorders Center for Anorexia Nervosa (NOCSAE, Modena). Suspecting hypothiroidism (asthenia and bradycardia) TSH FT3 FT4 were evaluated (TSH= 45,2 μIU/ml, FT3= 2 pg/mL, FT4= 8 pg/mL) and a therapy with Tyroxine replacement was set. After six months the values were incongruent both with the functional relationship of TSH vs. free thyroid hormones and the therapy set (TSH= 37,2 μIU/ml, FT3= 9 pg/mL, FT4= 34 pg/mL).
Methods: The results led to suspect the presence of interference in the TSH assay (Abbott) so we decided: 1) to perform dilution sample test (1:50, 1:100) 2) to use alternative methods (Roche, Siemens Healthcare) and 3) to pretreat samples using HBT tube (Heterophilic Blocking Tube) in order to eliminate interference of heterophilic antibodies.
Results: It was demonstrated that: 1) the test dilution was not sufficient to reduce the interference (TSH = 8,5μIU/ml) 2) other methods highlighted the differences which confirmed the presence of interferents 3) the use of tubes HBT confirmed that the interference was due to the presence of heterophilic antibodies in the serum of the patient (TSH = 0,01 μIU/ml). The therapy with Thyroxine was immediately suspended.
Conclusions: The analysis of this clinical significant case confirmed that a close communication and collaboration between clinicians and the laboratory is necessary to identify incongruent results, in this case potential interference that might not otherwise be detected, especially when co-patologies are so significant and could hidden clinical symptoms. By working together, we can identify false-positive results caused by heterophilic antibodies and have a profound impact on the clinical management of patients.

T070

THE EFFECTS OF PNEUMATIC TUBE SYSTEM ON SOME LABORATORY PARAMETERS

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Background: Pneumatic tube systems (PTSs), have been installed in hospitals to transport blood specimens from the phlebotomy site to the core laboratory. The aim of this study was to evaluate the possible changes to the serum levels of some biochemical and hormonal parameters due to PTS.
Methods: Forty healthy blood donors were involved. A total of 10 mL of blood from each donor was collected into five Vacuette clot activator tubes. Of the five specimens, No. 1 (protected with sponge-rubber insert) and No. 2 (transported without insert) were transported by the PTS which is the longest site to the core laboratory. No. 3 (protected with sponge-rubber insert) and No. 4 (transported without insert) were transported by the PTS from the phlebotomy unit for outpatients of the hospital to the core laboratory. No.5 was hand carried from the phlebotomy unit for outpatients of the hospital to the core laboratory in fifteen minutes. From all these samples, serum levels of sodium (Na), potassium (K), chlorine (Cl), magnesium (Mg), calcium (Ca), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), thyroid stimulating hormone (TSH) were assayed by Roche Diagnostics (RD) Modular System and E170 using original RD kits.
Results: K, Mg, LDH and TSH levels were significantly different in PTS carried groups when compared with hand carried group (Group 5) ($P < 0.001$). Also LDH levels were affected from transport capsules; the levels were significantly different in tubes protected with sponge-rubber insert when compared with samples transported without protection.
Conclusion: As a conclusion, each hospital should test their pneumatic tube system in order to determine the effects of transport systems on the accuracy of laboratory test results.

T071
FALSE HYPEREOSINOPHILIA IN THE PRESENCE OF NEUTROPHILIC LEUKOCYTOSIS

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Introduction: Nowadays one of the most frequent haematology lab checks is the differential count. All automated analysers perform it with an excellent precision, better than microscopic count, at least in most cases. Examining automatic scattergram of one of the routine inpatients we noticed a possible misclassification between polymorphonuclear (PMN) and eosinophil (EO) populations. Thus we decided to peruse the case.
Methods: EDTA-anticoagulated blood collected in Beckton-Dickinson tubes was analyzed on Beckman-Coulter LH-780 for total and differential leukocyte count. Differential was carried out on a dedicated channel applying to more than 8000 cells VCS Coulter principle (Volume, Conductivity and Scatter) integrating cell volume, inner complexity, inner granularity and membrane structure measures. With these three different technologies it was possible to assess all of the five leukocyte populations. Afterwards blood smear was investigated in bright field transmission microscopy (1000X) after May-Grünwald-Giemsa staining.
Results: Instrumental analysis revealed a differential scattergram in which PMN and EO populations were not properly parted but merging into a single continuous cloud of cellular signals giving the following results: total leukocytes 25.3x109/L, PMN 60.3% (15.3x109/L), EO 31.8% (8.1x109/L). The portion of the cloud related to PMN population, incorrectly classified as EO by instrumental software, had a lower mean volume if compared with mean volume of a normal EO population. Blood film examination showed a large number of PMN containing a lot of thin vacuoles (about 20 thin and regular vacuoles in each cell) and some gross basophilic cytoplasmic granules, probably for this reason misclassified as EO by the software. Microscopic count yielded PMN 94% and EO 0%. Out of the mass of PMN, vacuolated ones were about 46% (46/100).
Conclusions: Nucleated PMN (with empty spaces due to an increased spontaneous phagocytic activity) are not unusual in acute infectious processes like septicaemia, condition affecting the patient under examination. However the feasibility of automated cellular count must not preclude the possibility of instrumental errors, hence it is quite necessary to revise instrumental results by properly trained staff.

T072
THE ANALYSIS PROCESS OF DNA SEQUENCING BASED TESTS FROM FFPE TUMOUR TISSUES FOR TARGETED THERAPIES IN CANCER MANAGEMENT: DOKUZ EYLUL UNIVERSITY MOLECULAR ONCOLOGY LABORATORY EXPERIENCE

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Background: Biomarkers and targeted therapies have made personalised cancer management possible. Targeted therapies may block tumour progression, metastasis & angiogenesis. Validated molecular biomarkers are used to evaluate the prognosis of cancer or efficacy of chemotherapeutic agents. Molecular biomarker tests are presented in guidelines for determine the effectiveness of chemotherapy. The vast majority of GISTs carries activating KIT gene mutations. KIT mutations are prognostic and predictive molecular biomarker for gastrointestinal stromal tumours (GIST) which are the most common mesenchymal tumours of the gastrointestinal tract. KIT mutation status is correlated with clinical prognosis and response to treatment.
Methods: KIT gene mutation tests from formalin fixed paraffin embedded (FFPE) GISTs are performed in many routine laboratories worldwide. Several protocols have been published because mutational testing should meet the highest standard for quality assurance. There is no specific methodology which is recommended. DNA sequencing, PCR-array or high resolution amplicon melting analysis based methods are used in routine practice. DNA sequencing is the most common technique. In our routine molecular oncology laboratory, KIT gene mutation test of 96 patients' were performed by classic DNA sequencing which is gold standard for mutation testing.
Results: In our routine laboratory, the consequence of KIT gene mutation analyses in 96 cases diagnosed as GIST: 6,25% cases showed exon 9 mutation, 37,5% cases showed exon 11 mutation, 11,46% cases showed 13 mutation and 2,08% cases showed exon 17 mutations. 7,29% cases' DNA quantity and quality was inadequate for molecular analysis. 5,21% cases' sections (10micron) were not reach our laboratory from pathology department.
Discussion: This molecular test has been applied on FFPE tissues. Reliable test results are related on adequate tumour specimen, sample preparation processing (fixatives etc.) and other analyses procedures. Unsuitable treatment of tissues may cause DNA extraction, PCR or DNA sequencing failure. When tumour cells comprise <70% of the cell population in heterozygous tumour specimens, it may result false negative results.

T073

THE ANALYSIS PROCESS OF DNA SEQUENCING BASED TESTS FROM FFPE TUMOUR TISSUES FOR TARGETED THERAPIES IN CANCER MANAGEMENT: DOKUZ EYLUL UNIVERSITY MOLECULAR ONCOLOGY LABORATORY EXPERIENCE

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Background: Biomarkers and targeted therapies have made personalised cancer management possible. Targeted therapies may block tumour progression, metastasis & angiogenesis. Validated molecular biomarkers are used to evaluate the prognosis of cancer or efficacy of chemotherapeutic agents. Molecular biomarker tests are presented in guidelines for determine the effectiveness of chemotherapy. The vast majority of GISTs carries activating KIT gene mutations. KIT mutations are prognostic and predictive molecular biomarker for gastrointestinal stromal tumours (GIST) which are the most common mesenchymal tumours of the gastrointestinal tract. KIT mutation status is correlated with clinical prognosis and response to treatment.
Methods: KIT gene mutation tests from formalin fixed paraffin embedded (FFPE) GISTs are performed in many routine laboratories worldwide. Several protocols have been published because mutational testing should meet the highest standard for quality assurance. There is no specific methodology which is recommended. DNA sequencing, PCR-array or high resolution amplicon melting analysis based methods are used in routine practice. DNA sequencing is the most common technique. In our routine molecular oncology laboratory, KIT gene mutation test of 96 patients' were performed by classic DNA sequencing which is gold standard for mutation testing.
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Discussion: This molecular test has been validated on FFPE tissues. Reliable test results are related on adequate tumour specimen, sample preparation processing (fixatives etc.) and other analyses procedures. Unsuitable treatment of tissues may cause DNA extraction, PCR or DNA sequencing failure. When tumour cells comprise <70% of the cell population in heterozygous tumour specimens, it may result false negative results.

T074

FREQUENCY OF HAEMOLYSIS, JAUNDICE, LIPAEMIA IN BLOOD GAS TEST AT ST GERARDO HOSPITAL

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Background: Blood gas analysis is a critical test performed in laboratories or on bed side in Intensive Care Units (ICUs) on whole blood which can provide useful parameters for the study of acid-base and electrolyte balance, and gas exchanges. The haemolysis is the main cause of pre-analytical errors in fact increasing or decreasing different analytes can determine an incorrect interpretation of the results and a low degree of reliability. Unfortunately blood gas analyser can not detect the possible presence of haemolysis and other interfering (jaundice and lipaemia) at the same way of modern multiparameter analyzers by the determination of serum indices. The aim of this study was to investigate and determine the frequency of these interfering agents paying particular attention to haemolysis in all samples of blood gasses belonging to the central laboratory and ICUs (general, neurosurgery and cardiac surgery).
Methods: For 33 days we collected 1119 of blood gas samples from central laboratory and ICUs; we considered suitable for the study 942 samples: we excluded coagulated samples or samples with volume <0.5 mL. After blood gas analysis we transferred samples into secondary tubes and after centrifugation at 1500g per 5 min serum indices on Cobas C6000 CE (Roche Diagnostics, Germany) were performed. The statistical analysis was performed by chi-square test using MINISTAT 2.1 program.
Results: The overall frequency of haemolysis is equal to 6% for 652 samples belonging to the central laboratory. If we looked at the origin department, frequency of haemolysis appears to be greater for emergency department, 14% ($P < 0.001$) and paediatric ward, 10% ($P < 0.01$) compared to the phlebotomy department samples (2%), taken as reference; frequency of haemolysed samples in General ICU is significantly lower than that of Central Laboratory ($P < 0.03$). Secondarily it was observed that 14% of samples tested are jaundiced and 12% are lipemic, data very similar to those found in the literature.
Conclusion: Due to high frequency of haemolysis it is mandatory that the builders implement inside a blood gas measurement system the automatic detection of the main interfering agents.

T075

CAN THE INCOMPLETE SERUM SEPARATION ON GEL TUBE VACUTAINERS LEAD TO THE DIAGNOSIS OF MULTIPLE MYELOMA?

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Background: Gel vacutainers have become common in clinical labs and have made analysis and storage of samples easier; eliminating the need for transferring of serum into secondary tubes The basic principle is gradient density centrifugation using the thixotropic property of the gel, where it forms a barrier separating serum from the cells. Occasionally incomplete separation is seen in some samples where the gel packed cells fail to go below the gel. There are very few studies conducted on this phenomenon and some studies have suggested that incomplete separation of serum occur in patients with paraproteinemia particularly multiple myeloma (MM).
Methods: Hence we conducted a prospective study on the relationship between incomplete serum separation on gel tubes and paraproteinemia. This was done to identify whether incomplete gel separation was associated with increased total protein. The gel tubes used in our study was BD- SST.
Results: This study was done for a period of two years. Incomplete gel separation was seen in a total 14 gel tube samples out of a total of 198000 samples (0.007%).In 4 samples the incomplete separation was corrected on repeat sample collection. In 10 patient samples we observed incomplete separation even on repeat sampling. Raised total protein with altered albumin to globulin ratio was seen in those samples. Serum Protein Electrophoresis (SPEP) confirmed the presence of M bands in all the 10 cases and subsequently multiple myeloma was confirmed with bone marrow aspiration. The immunoglobulin subtypes with immunofixation were: Ig A (5/10), Ig M (4/10) and biclonal type with Ig M and Ig G (1/10).
Conclusions: Our study shows that incomplete separation on gel tubes is very commonly associated with paraproteinemia like multiple myeloma. The increase in paraprotein component possibly increases the viscosity of the sample leading to inhibition in separation. Clinical laboratorians need to be aware of this and should estimate the total protein and albumin reflectively in those patients showing incomplete separation. Patients having increased total protein and altered albumin globulin ratio should be followed up with SPEP. Clinical correlations and interaction with treating physicians might lead to early diagnosis in such patients.

T076

LEAN- SIX SIGMA PROJECT CHARTER IN A HOSPITAL LABORATORY

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Background: To pursue lean - six sigma project charter in a hospital laboratory, in order to eliminate error in process design through verification of tests and to improve Turn around Time (TAT) through process improvement.
Methods: Our current state value stream maps (VSM) identified opportunities to use Lean - Six Sigma strategies in our process flow. Therefore, we designed the laboratory process flow according to DMAIC (Define, Measure, Analyze, Improve and Control) flow. In the define phase, the tools we used were project charter, SIPOC (Supplier, Input, Process, Output, Customer), CTQ (Critical to Quality) tree. In the measure phase, we used prioritization matrix and process capability. Here we calculated DPMO (defects per million opportunities) and expressed the value as a sigma rating. In the analyze phase, tools used were root cause analysis. In the improve phase, we used brainstorming, decision analysis matrix. In the control phase, the tools were control charts, audits etc. In our laboratory, Lean- six sigma strategies were applied in process improvement (TAT compliance) and in process design (verification of all tests). Verification of all the tests were done [with particular reference to Thyroid Stimulating Hormone (TSH)] by verifying analytical accuracy and precision, inter-assay and intra-assay variations, and reportable range i.e., (i) Analytical measurement range (AMR) and Clinically reportable range (CRR) as well as sigma metrics.
Results: In our process improvement case study, we found that after receiving the STAT sample (with particular reference to Troponin I), both the non value added (NVA) times and value added (VA) times were around 45 minutes. So we eliminated NVA steps and our current TAT came down to 45 minutes from 1.5 hrs. In two year time period our monthly TAT were improved from 74.7% compliance, 50450 Defects per million opportunities (DPMO) and 3.14 Sigma in August, 2010 to 99.4% compliance, 1270 DPMO and 6.72 Sigma in Dec, 2012. With Reference to TSH, the Sigma Metrics of TSH have increased considerably; as we have achieved >9 Sigma in (1/10/2012-6/12/2012) from 3.78 sigma (1/1/2011- 31/03/2011).
Conclusions: Lean -Six Sigma Project Charter in the laboratory ensures that accurate and precise results are reported in a clinically relevant TAT.

T077

STRONG NEGATIVE INTERFERENCE OF ETHAMSYLATE (DICYNONE) IN SERUM CREATININE QUANTIFICATION VIA ENZYMATIC ASSAY USING TRINDER REACTION

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Background: With discrepancies encountered as early as the verification of enzymatic method for quantification of serum creatinine, our research pointed to a later confirmed interference caused by a compound called ethamsylate present in commonly used antihemorrhagic drug Dicynone.
Methods: We measured 44 samples from 14 patients of various age and sex. Blood was taken from patients before and 15 min after the application of Dicynone (10 patients) and from patients who had quit the therapy including Dicynone 6, 12, 18 and 24 hours after the last application of the drug (3 patients). For sample measurement, we used our routine laboratory diagnostics of human serum cobas c501 analyser with quantification kits Creatinine Plus Ver.2 (enzymatic with Trinder reaction) and Creatinine Jaffé Gen.2 of Roche diagnostics company.
Results: The levels of concentration of creatinine in 10 patients varied from 40 μmol/L to 445 μmol/L (before application of Dicynone) and were reduced only to 52 % in average (range 34-83%) of their original values in blood taken 15 min after the drug application via the enzymatic assay. In contrast to this, results of compensated Jaffé method yielded consistent values in all samples (average deviation 0.7% from original values). We therefore carried on with our research and collected samples of blood taken from three patients who stopped receiving the therapy with Dicynone. Two of these patients had results comparable by both creatinine assays and in accordance with the clinical status in samples taken after 12 hours after the last drug dose, while the last one had comparable results in sample taken just after 6 hours.
Conclusions: Considering the strong negative interference of ethamsylate in enzymatic assay using Trinder reaction for creatinine quantification, blood from patients with prescribed Dicynone should be taken at least 12 hours after the last application of the drug for obtaining the correct creatinine values.

T078

THE EFFECT OF ELECTRONIC REQUESTING ON PRE-ANALYTICAL ERRORS IN PRIMARY CARE

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Background: Pre-analytical variables are common across all laboratories and can negatively impact on patient care. The introduction of electronic requesting has been proposed to reduce these errors. The aim of this study was to review the impact of electronic requesting in Primary Care on the number of pre-analytical errors seen by the laboratory.
Methods: Error data was reviewed during two six-month periods, pre- and post implementation of Primary Care electronic requesting. The outcome measures related to: the correct information on the sample tube (patient name, unique patient ID number, date of collection); the correct sample type received and the availability of a clinical history.
Results: There was a marked decrease in the number of pre-analytical errors following the introduction of electronic requesting (2764 (1.3% of total requests) pre implementation versus 498 (0.2% of total requests) post implementation, P <0.001). There was an improvement in the quality of information provided, with 76 requests received without clinical information prior to electronic requesting and just one post implementation (P <0.001). A similar pattern was seen with regard to date and time of sample collection being provided. This was missed on 373 requests prior to electronic requesting and just 2 post implementation (P <0.001).
Conclusions: The introduction of electronic requesting in Primary Care can reduce the number of pre-analytical errors and can improve the quality of information received on each request.

T079
DETERMINATION OF HEMOLYSIS INDEX IN BIOCHEMICAL TESTS

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Background: The presence of hemolysis in biological samples can produce mistakes in the accuracy of the measurement of certain biochemical parameters. The hemolysis index (HI), which is automated in most analyzers, quantifies the interference by hemoglobin, contributing to objectivity and rapidity in the detection of interferences. Our aim was to evaluate if the limits below which the possible interference is considered not relevant, are appropriate for the biochemical parameters studied.
Methods: Serum values for potassium (K) (n=1709), lactate-dehydrogenase (LDH) (n=456) and aspartate-aminotransferase (AST) (n=844) were collected, being the reference values 5.5 mmol/L for K, 378 U/L for LDH and 34 U/L for AST. Two cut-offs for HI were considered, dividing the patients in four groups: HI <45, ≥45, <140 and ≥140 mg/dL of hemoglobin. For every parameter and group the percentage of patients with values below and over the reference value was calculated. The determinations were performed in an autoanalyzer Advia 1800 (Siemens), by spectrofotometry for LDH and AST, and by potenciometry for potassium.
Results: In the group with HI <45, there were 98% of normal values for potassium and 2% of high ones; 71% of normal values for LDH and 29% of high ones; and 85% of normal values for AST and 15% of high ones. In the group with HI ≥45, we found 85% of normal values for potassium and 15% of high ones; 9% of normal values for LDH and 91% of high ones; and 51% of normal values for AST and 49% of high ones. In the group with HI <140, we found 98% of normal values for potassium and 2% of high ones; 66% of normal values for LDH and 34% of high ones; and 83% of normal values for AST and 17% of high ones. In the group with HI ≥140, there were 65% of normal values for potassium and 35% of high ones; 100% of high values for LDH; and 41% of normal values for AST, and 59% of high ones.
Conclusions: When the cut-off was ≥45 no increase in the number of elevated values was observed in any case, but for LDH. However, for a cut-off ≥140 in all the parameters it could observed an increase in the percentage of higher values, specially for LDH. This tool enables to improve the quality of the measurement and to minimize the incorrect analytical results, with a minimal cost.

T080
STABILITY OF C-PEPTIDE IN BLOOD SPECIMENS AFTER DELAYED PROCESSING

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Abstract: In the process of biosynthesis of insulin,the C-peptide is formed as a by-product togheter with insulin by the proteolytic cleavage of the precursor molecule proinsulin. Measurements of C-peptide,insuline and glucose are used as an aid in the differential diagnosis of hypoglucemia (factitious hypoglycaemia and hypoglycaemia caused by hyperinsulinism) to ensure an appropriate management and therapy of patients. To quantify the endogenous insulin secretion, C-peptide is measured basally,after fasting and after stimulation and suppression tests. Due to high prevalence of endogenous anti-insulin antibodies C-peptide concentrations reflect the endogenous pancreatic insulin secretion more reliably in insulin-treated diabetics than the levels of insulin itself. Measured of C-peptide may therefore be an aid in the assessment of a residual β-cell function in the early stages of type-1 diabetes mellitus and for the differential diagnosis of latent autoimmune diabetes of adults and type-2 diabetes.
Materials and methods: Were processed 60 serum samples by electrochemiluminescence immunoassay:autoanalyzer Cobas e411 (Roche Diagnostics). All samples were taken in the morning after fasting for at least 8 hours. The samples were processed with different conditions of temperature and time before freezing: immediate centrifugation and freezing (group 1 reference); sample at room temperature ≥2 h, centrifugation and frozen until analysis (group 2 study). We found the coefficient of variation of the technique (2 consecutive determinations of each sample) and compared with that obtained between 2 groups using MedCalc® software statistical analysis: Student t-statistic for paired data.
Results: The mean C-Peptide reference group was 2.92 pg/mL (95%CI 2.30 to 3.54) while C-Peptide in the study group was 2.89 pg/mL (95%CI 2.29 to 3.50) (P=0.252).The coefficient of variation of the technique was of 1.49% and between 2 groups was 1.88% (P=0.179)
Conclusions: According to the data extraction,for the determination of C-Peptide is unnecessary the immediate centrifugation and subsequent freezing.The determination may be made in the same serum tube used to analyze the different analytical parameters,the avoiding pre-analytical errors,saving time and expenditure rationalization

T081

HOW TO MIX BLOOD COLLECTION TUBES, A COMPARATIVE EVALUATION OF THE GME LABSYSTEMS T-SWING AUTOMATED TUBE MIXER VS. MANUAL MIXING DURING PHLEBOTOMY

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Background: Mixing of blood collection tubes is an important process which if not done correctly has the potential to induce platelet clumping, hemolysis, and other sample artifacts. In general, recommendations for mixing are poorly adhered to. The GME Labsystems T-Swing is an automated sample mixer, mixing each sample by a specified number of inversions, either 4 or 8. This study evaluated the T-Swing mixer for effects on sample quality, key analytes and for ease of use at phlebotomy.
Method: The performance of the mixer was compared against recommended manual mixing by the phlebotomist at the time of sample collection. Samples were collected from apparently healthy individuals in the correct order (one discard tube followed by twelve BD Vacutainer® Blood collection tubes, Citrate (3), EDTA (3), BD SST™II (3), BD PST™II (3). One set was mixed by the phlebotomist according to the manufacturer's recommendations and the other two sets using the T-Swing mixer. With one of these sets the tubes were mixed either 4 or 8 times (whichever was closest to Manufacturer's recommendations) and all tubes in the third set were mixed 4 times. The appropriate samples were analysed for routine coagulation (PT & aPTT), haematology (FBC) and biochemistry (Electrolytes, Creatinine, Glucose, Urea & LDH). The results were compared for each sample set, along with visual observations of the samples. In addition, the phlebotomist commented on the ease of use of the T-Swing, in comparison to manual sample mixing.
Results: Clinical equivalence was demonstrated for all visual observations and analyte comparisons. There was no increased incidence of haemolysis in serum or plasma as measured through Potassium (bias BD SST™ II <0.5%, BD PST™ II <3.0%) and LDH (bias BD SST™ II <1.0%, BD PST™ II <7.0%) in any of the tube sets. All EDTA tubes showed no platelet clumping (bias <1.0% for 4 or 8 mixes) indicating effective mixing. Comments from the phlebotomist were positive.
Conclusions: Clinical equivalence was demonstrated between the automated methods and the manual mixing method, with no increase in the incidence of haemolysis or platelet clumping. The data suggest that four automated inversions is equivalent to recommended number of manual inversions, and the T-Swing is easy to use.

T082

CAUSES OF HAEMOLYSIS IN BLOOD GAS SAMPLES AT ST GERARDO HOSPITAL

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Background. The major interference in blood gas analysis is due to haemolysis (6% in our Central Laboratory). Only few studies have investigated the possible causes or contributing factors. The aim of our study was to focus on same of these possible causes such as volume of sample, time of collection, modes of carriage and storage in all samples of blood gases belonging to the Central Laboratory and Intensive Care Units, ICUs (General, Neurosurgery and Cardiac Surgery).
Methods. For 33 days we collected 1119 of blood gas samples from Central Laboratory and ICUs. We considered suitable for the study 942 samples: we excluded coagulated samples or samples with volume <0.5 mL. After blood gas analysis we transferred samples into secondary tubes and after centrifugation at 1500g per 5 min serum indices on Cobas C6000 CE (Roche Diagnostics, Germany) were performed. We consider different sample volumes (>0.5 and <1.0 mL, ≥1.0 and ≤2.0 mL, >2.0 mL, time of collection (8-20 h or 20-8 h) and modes of carriage (manual or with pneumatic tube) and storage (at room temperature, RT or at +4°C). The statistical analysis was performed by chi-square test using MINISTAT 2.1 program.
Results. Low volume sample (>0.5 and <1.0 mL, ≥1.0 and ≤2.0 mL) increased significantly (P <0.01) the haemolysis rate in the samples belonging to the Central Laboratory and Neurosurgery and Cardiac Surgery ICU. Storage at RT increased haemolysis (the frequency is almost double comparated to +4°C storage) even if the difference was not significant. The same increasing was observed for carriage with pneumatic tube vs manual but without significant difference. No particular difference was found if we looked at time of collection.
Conclusions. Our data suggest that low volume samples increase the frequency of haemolysis which can be explained with an impaired ratio between blood and anticoagulant. In other hands, RT storage and carriage with pneumatic tube induce haemolysis by mechanical way. At the end, this study underlines the need to revise the sampling procedures for blood gas analysis for all departments of the hospital.

T083
CORRECTING LABORATORY RESULTS FOR THE EFFECT OF INTERFERENCES – A GUM APPROACH

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Background: For some laboratory tests potential interferents are quantified so that the laboratory can provide a consistent response. An example is the effect of in-vitro haemolysis on serum potassium. A common response is to withhold results once a limit of the interferent concentration is reached which may cause significant interference. This approach has the effect of allowing the addition of a known maximal bias to results. An alternative approach is to correct for the effect of the interference with the aim of releasing more results. In this paper I analyse the effect of correction for interferences on ability to release results.
Methods: A model was developed in Microsoft Excel to predict the amount of interferent which would produce more than a fixed percentage of results beyond acceptable limits with and without correction for the effect of the interferent. An example using potassium (K, in mmol/L) and haemolysis, (measured as Haemoglobin, Hb, in mg/dL) is presented. The following inputs were included (K,Hb example): true value (5.0); uncertainty of the measurement (CVa=3%); slope of the effect of the interferent (0.004); uncertainty of the slope (CV=20%); maximal allowable error (0.5 mmol/L) and maximal percentage erroneous results (5%). The inputs to the model were varied and the maximal allowable interferent concentration determined for corrected and uncorrected results.
Results: The model demonstrated that in all cases tested the use of results corrected for the effect of the interferent allowed results to be released at a higher interferent concentration compared with uncorrected results. Using K/Hb example the allowable haemolysis without correction is 56 mg/dL compared with 250 mg/dL with correction for haemolysis.
Conclusions: The issuing of results corrected for the effect of interference is superior to uncorrected results in order to avoid releasing erroneous results. This can be considered an application of a principle from the Guide to the Expression of Uncertainty of Measurement (GUM) which is to remove known bias if possible and add the uncertainty of the process of removing the bias to the uncertainty of the result.

T084
A PILOT SURVEY ON CLINICIANS' ATTITUDE TOWARD THE CLINICAL PATHOLOGIST AND LABORATORY CONSULTATION UTILIZATION AT A HOSPITAL LABORATORY IN NAIROBI

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Background: The role of the clinical pathologist is not properly defined nor utilized in many hospitals. The Aga Khan University Hospital Nairobi offers a postgraduate degree leading to masters in clinical pathology that has produced a growing pool of pathologists whose role is still being defined.
Objectives: We designed a pilot survey aimed to determine clinicians' attitude to the role of clinical pathologists and current utilization of laboratory consultation services.
Methods: consecutive sampling was used to select clinicians from the various departments. A questionnaire was administered to capture doctors' attitude toward clinical pathologists and laboratory consultations in the preceding 12 months.
Results: 50 questionnaires were sent out via print copy and the online tool Survey Monkey©. We received 36 responses, giving a response rate of 72%. 79% of respondents reported consulting a clinical pathologist in the past 12 months with majority [39%] having done so only 2 - 5 times. 23 % consulted on average once a month while 17% had not consulted at all in the past 12 months. Of reported consults majority were directed at microbiology [33%] followed by haematology and routine chemistry [both at 21%]. 97% of respondents felt that clinical pathologists were needed in their clinical area while 86% felt that more face to face interaction would be useful. 11% preferred phone consultations. While 72% of respondents felt that input on choice of test prior to requesting would be useful, 82% reported that in practice while looking up what test to request for a specific case their first query would not be directed to a clinical pathologist but a clinical colleague, the internet or a textbook.
Conclusions: Our pilot survey revealed a positive attitude toward the clinical pathologist and some understanding of their role but actual utilization of consultation service is poor. More interaction is desired by the clinicians. We also found that consultation demand varies between the various units with very few consults in serology and transfusion. A wider reaching survey would likely explore this and provide useful feedback to our pathologists on how to best serve their biggest clients – the clinicians.

T085

DOES THE IMPLEMENTATION OF PNEUMATIC TUBE DELIVERY SYSTEMS IMPACT PATIENT RESULTS?

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Background: The preanalytical phase is a critical phase for laboratories. It has been shown to be responsible for the majority of laboratory errors that could influence patient results. During the last decade the implementation of pneumatic tube systems (PTS) to carry blood specimen from wards to labs has been accelerated, in order to improve in particular Turn Around Time (TAT). Moreover compliance with ISO 15189 for French laboratories requires them to monitor sample transportation. Due to these recent advances we conducted a review to understand where PTS is an appropriate solution for sample transport.
Material and method: We compiled a systematic review more than 30 publications including CLSI recommendations, literature, and PTS Suppliers data. Inclusion criteria were: • Blood specimen transportation system (Venous and Arterial): PTS vs. handling for chemistry and hematology parameters.
• PTS specification used by Hospital (suppliers, speed, acceleration, length)• Information about patients (Healthy and Pathologic)• Description about collection device used (Tube : Volume ,Serum/plasma and Arterial Syringe)• Recent Articles: after year 2000
Results: Few publications validate PTS without having observed result changes. Several adverse events are described which could interfere with the patient outcomes. For blood gas, the carriage by PTS could produce spurious elevated result pO2: 115.6 mmHg on syringe without air bubbles and hand transported vs 180,5 mmHg on a syringe with air bubble through PTS and transported through PTS. For venous blood, several cases of significant pseudo hyperkalemia were encountered in particular with CLL chronic lymphocytic leukemia patients (ex: K result with PTS: 6.6 mmol/L vs 3.2 mmol/L hand transportation), LDH, ASAT results could also be affected. Hemolysis could be encountered if the PTS is defective or if tubes are under filled. The platelet function could also impact by transport through PTS
Conclusion: PTS is a convenient and reliable solution for clinical laboratories. However some vigilance should be required. Considering each PTS is unique, an evaluation at the implementation should be done, with regular control. Laboratories and clinicians should be aware that some patients with hematopatholgies could produce spurious results. As consequence each PTS user should implement specific guidelines to minimize variability as a result of their use.

T086

SODIUM FLUORIDE VACUUM TUBES VALIDATION: AN ESSENTIAL TOOL TO GUARANTEE THE PATIENT SAFETY

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Background: All potential sources of nonconformities need to be identified, and the relevant improvements must be set up in order to allow validation of the laboratory processes. Sometimes the in-vitro diagnostic devices (e.g. blood collection vacuum tubes) are received by the laboratory without previous validation; therefore when the quality laboratory managers decide to start using new devices or to change the brand, a validation procedure should be done as recommended by ISO 15189 document.
Objective: The aim of this study was to validate four different kinds of sodium fluoride vacuum tubes to guarantee the patient safety.
Methods: Blood specimens from 19 volunteers were collected in four different sodium fluoride vacuum tubes (Tube I: Vacutainer® FX 5 mg / 4 mg, Tube II: Vacuntainer® NaF 6mg Na2EDTA 12mg, Tube III: Venosafe® FX and Tube IV: Vacuo-Care® NaF/OxK). Glucose and lactate were performed on the same instrument cobas® 6000 < c501 > module. The significance of the differences among samples (fluoride vacuum tubes) was assessed by Friedman test and Wilcoxon ranked-pairs test after checking for normality. The level of statistical significance was set at $P < 0.05$.
Results: The median concentration and interquartile range of glucose and lactate are respectively: Tube I 84.0 mg/dL [79.0-89.0 mg/dL] and 1.15 mg/dL [1.00-1.72 mg/dL]; Tube II 87.5 mg/dL [79.8-92.0 mg/dL] and 1.05 mg/dL [0.88-1.70 mg/dL]; Tube III 82.0 mg/dL [78.0-88.2 mg/dL] and 1.35 mg/dL [1.10-1.92 mg/dL], Tube IV 81.5 mg/dL [74.8-85.2 mg/dL] and 1.65 mg/dL [1.45-2.20 mg/dL]. No significant difference was observed only for glucose when Tube I vs. Tube III was compared ($P=0.11$).
Discussion and Conclusion: The results of the present validation not allow the laboratory or hospital managers to choose indifferently among the above brands of sodium fluoride vacuum tubes. Changes in vacuum tubes without validation could caring physicians unaware of the real patient situation might abstain from appropriate treatments as a consequence of change in sodium fluoride vacuum tubes brands. We suggest that every laboratory management should both standardize the procedures and frequently evaluate the quality of in-vitro diagnostic devices to guarantee the patient safety as recommended by ISO 15189 document.

T087
RECORDING, MONITORING AND MANAGING PRE-ANALYTICAL ISSUES IN A METROPOLITAN UNIVERSITY HOSPITAL

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Background: Errors in laboratory testing process may have an adverse impacts on patient safety and care. The pre-analytical phase is responsible for ~70% of these errors. In this study we present the experience in assessing the frequency of the most common pre-analytical issues in our university hospital by monitoring their trend over time and comparing, when possible, data with goals suggested in literature. The impact of corrective actions, if any, was also checked.
Methods: A comprehensive retrospective analysis of the pre-analytical nonconformities (NC) recorded through laboratory information system over a 5-year (2007-2011) time span was undertaken. Retrieved data were evaluated on a yearly basis for NC type and then for type of sample and for involved laboratory section and hospital department.
Results: The relatively most frequent NC was the test request without the corresponding sample, accounting on average for 2.3% of all requested tests. Hemolysis occurred in 1.15% (mean value over 5 years) of requested tests, affecting ~20,000 determinations per year, mostly interesting clinical wards taking care of critically ill patients, i.e. neonatology, oncology and emergency department. Clotted and not sufficient samples showed a significant reduction over time, induced by the change of the analytical system measuring erythrocyte sedimentation rate and the corresponding replacement of sodium citrate rectangular tubes by more reliable K3EDTA round tubes, easier to fill in and mix cup. NC related to samples conveyed at wrong temperature, both for tests requiring transportation in ice bath (i.e. ammonium) or at 37 °C temperature (i.e. cryoglobulins), were also relative frequent.
Conclusions: Our results show that recording, monitoring and critically evaluating pre-analytical issues in laboratory testing process is mandatory for providing a good laboratory service, permitting to identify the causes of NC and to apply corrective interventions that may help to reduce their incidence.

T088
PRE-ANALYTICAL ERRORS: AN EXPERIENCE FROM A UNIVERSITY HOSPITAL IN A DEVELOPING COUNTRY

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Background: Laboratories are faced with challenges of preventing laboratory errors. Some of these errors can have a significant impact on patient management. This is a bigger problem in developing countries since majority of the laboratories are not accredited. Pre-analytical errors contribute to 60-70% of all errors occurring in the clinical laboratory. We carried out an audit to determine type and frequency of pre-analytical errors in our laboratory. This is a first critical step in trying to curb this challenge.
Methods: This was a laboratory based audit to assess the common pre-analytical errors among samples received in our laboratory from our satellite centres and other institutions. The data on all the samples rejected for a period of 20 months was obtained from 1.01. 2011 - 31.08.2012 from a sample rejection book. Data on date of rejection, reason for rejection, lab section affected patient identification and whether the rejection could affect patient management was collected. Data on the total number of tests requested from the aforementioned centres was obtained from our Lab information system and summarized.
Results: A total of 60,720 tests were received over the study period with a total of 166 samples rejected which represented an average rate of 0.27% with month to month variation ranging from 0.18-0.50%. The most common pre-analytical errors were collection of sample in the wrong tube or collecting the wrong type of sample in 43.3% of the cases.18.7% were mislabeled while 7.8% of all rejected samples lacked any form of identification. Hemolysed/clotted samples were at 9.6% while insufficient specimens were 13.2%. Microbiology section had the highest rates of rejection at 0.40%, Histology at a rate of 0.22% and Haematology 0.26%. Some of the patient had their samples for the same test rejected more than once.
Conclusions: Most of the errors reported are easily preventable. More training is needed on good laboratory practice among staff that collects and handles patient samples. This will go a long way in helping reduce the pre-analytical errors.

T089

INTRODUCING RFID IN THE BLOOD GAS PROCESS HELPS REDUCING PREANALYTICAL ERRORS

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Background: Unlike many other medical process, activities in laboratory medicine are precisely defined and therefore they are more controllable than a procedure or treatment in an emergency department. A standard laboratory process is usually divided in three stage (preanalytical, analytical postanalytical) and in each of them we find a lot of events that can cause errors. Most of errors in Laboratory still comes from the preanalytical phase. A good control of the preanalytical phase could reduce a lot of laboratory errors. Information Technologies today can be helpful to us only if we put under control the entire process and organization: this means not only evaluating the total testing process, but also focusing on possible errors that could translate into adverse events for patients.
Methods: After introducing Quality assurance in the control of POCT (Point of Care Testing) blood gas analyzers in our hospital implementing ISO Internal Standards series (22870), procedures for Risk Assessment and Information Technology, we made a risk assessment with FMEA (Failure mode and effects analysis) method to focusing on the preanalytical errors in identifying two types of errors in using POCT instruments: patient identification and sample identification. We introduced RFId (Radio Frequency Identification) Technology in our system to evaluate his utility in reducing preanalytical errors.
Results: An analysis was made in comparing the risks for three possible scenarios: uncontrolled Blood gas POCT; Blood gas POCT under the control of the laboratory with complete integration of the results into LIS, but with samples/patients identification via the use of a bar-code; Blood gas POCT with the introduction of the RFId. Estimated error of Identification in manual procedures is reported in literature as an average error every 100 characters. In the case of the RfiD at the moment there are no data in literature so we gave the same error we have with a bar code control read automatically, or one error in 10 million characters. In our case we tested 350 sample of Blood gases analysis and we have no errors of Identification reported. Instead the use of a bar code.
Conclusions: Analyzing preliminary results of our risk analysis we can demonstrate that the use of RFId guarantee the reduction of preanalytical errors. But wristbands with a patient identification barcode is not enough to guarantee the reduction of preanalytical errors in Identification of sample and patient, and it is not sufficient to avoid mismatching patient and sample ID overall when the patient identification is read not at the bedside of the patient. Even in this case it is possible to mismatch results. Only the use of RFId showed the best traceability of the system and the lowest risk of errors and it allows to provide to the laboratory in real time a series of informations (therapy, temperature of patient, time of execution of the collection) that the reading of a simple barcode does not allow.

T090

THE USE OF A CENTRAL LAB WORKSTATION IN THE CONTROL OF POCT INSTRUMENTS IN HOSPITAL: A REVIEW OF 6 YEAR OF WORK ON QUALITY ASSURANCE AND POCT MANAGEMENT

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Background: More and more in complex environments ,the use of POCT is a convenient way to perform diagnostics at the bed side. The CLIA regulations, in section §493.1256, require each laboratory to implement "control procedures that monitor the accuracy and precision of the complete analytic process and detect errors that occur due to test system failure, adverse environmental conditions, and operator performance. The CLIA and ISO standards are based on a quality management system (QMS) approach that includes widely accepted good laboratory and error-prevention practices and incorporate essential elements for management, technical guidance, and structure of the entire testing process. In our institutions we have started many years ago and in this document we are reporting our experience in managing remotely the blood gas testing spread out throughout our Hospital.
Methods: After introducing Quality assurance in the control of POCT blood gas analyzers in our hospital implementing ISO Internal Standards series (22870), procedures for Risk Assessment and Information Technology, we made a risk assessment to focusing on the errors in using POCT instruments for Blood Gas Analysis. All the systems and instruments of blood gas analysis (Rapidpoint 405 and Rapidlab 1265 – Siemens Healthcare diagnostics), were monitored, either in Laboratory than in several department, with an automatic system of Quality control and a Central Lab monitoring system, on each parameter of Blood gas analysis (pH, pCO2, pO2, Ca++, Na+, K+, Cl-,Hb, and CO-OX, Glu, NBil). Each cartridge of AQC were provided with aqueous control materials on three different level [Low (acidosis) – Medium (normal) – High (alkalosis)]. Each processes either of calibration or of Quality control were monitored in continous, from the Central lab workstation (Rapidlab).
Results: All the instruments showed, during those 6 years of uninterrupted work an excellent performance, with an excellent imprecision of measurement of pH and electrolytes (SD between 0.005 an 1 – and CV between 0.1 and 1.5), and very good performances for pO2, pCO2 CO-OX and glucose. Better performances were obtained with the instruments of RP405 series. Blood gas POCT under the control of the laboratory with complete integration of the results into LIS. All calibration processes not adequate, and all Automatic Quality Controls out of range were immediately signed from the Central Lab Workstation and corrected from the POCT coordinator on line.
Conclusions: Analyzing the results we can demonstrate that with the use of a work station in monitoring each instrument for Blood-Gas Analysis we could reduce errors, and guarantee the better performance of the instruments. Above results can be achieved thanks to regular collection of most frequent analytical errors e, more importantly, modifying the attitudes of personnel through dedicated and efficient training.

T091
PATIENT SAFETY AND SELECTION OF NECESSARY ANALYSES

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Background: It frequently occurs that big and unjustified demands, not for specific parameters but for "all and everything", are sent to laboratory and on the basis of the results obtained conclusions are made. Many physicians beginners beside the necessary often ask for numerous unnecessary parameters, probably led by the idea "not to miss something". However, physicians with more experience and knowledge require smaller number of parameters but they require specific parameters, in other words, those parameters that will give them useful information. The question is: Is the patient's health safe with a small number of parameters?
Methods: During the normal course of routine work in the laboratory of Codra Hospital within a period of one month 609 patients have been treated. Biochemical parameters were performed on the machine Dimension Xpand plus (Siemens), and imunnochemical on Axsym (Abbott) and Immulite 1000 (Siemens).
Results: The patients were divided into 9 groups according to the requests: 222 patients (36.4%) had a request for 1 parameter; 69 patients (11.3%) had a request for 2 parameters; 51 patients (8.4%) had a request for 3 parameters; 21 patients (3.4%) had a request for 4 parameters; 27 patients (4.4%) had a request for 5 parameters; 87 patients (14.3%) had a request for 6 -10 parameters; 63 patients (10.3%) had a request for 11-15 parameters; 60 patients (9.9%) had a request for 16-20 parameters; 9 patients (1.5%) had a request for 21-26 parameters.In patients who were done with one parameter it was mainly about control. Patients who were done with up to 10 parameters were sent on routine inspections by physician specialists, and patients that were done with from 10 to 20 parameters were part of detailed diagnostic procedures. Patients who had from 21 to 26 parameters were patients with personal requirements.
Conclusions: Health security may be obtained with relativelly small number of parameters which are well selected. The number of parameters required is inversely proportional with the level of expertise. When you ask for parameters, there should be, clearly defined question and the parameters should give you the answer.

T092
USE OF A RAPID SERUM TUBE AT A HEMODIALYSIS CENTER DISTANT FROM THE LABORATORY: BENEFIT OF IMPROVING SAMPLE QUALITY IN PHOSPHO-CALCIUM METABOLISM FOLLOW-UP

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Background: Hemodialysis patients need frequent analytical monitoring and blood drawn is usually carried out during dialysis session. Measurement of parathyroid hormone (PTH) is essential in the follow-up of phospho-calcium metabolism in these patients. However, the implementation of clinical and therapeutic recommendations based on PTH levels is hampered by the frequent occurrence of laboratory errors related to preanalytical phase. Promptly centrifugation and cold storage of samples improves PTH stability, but usually results in low sample quality due to late fibrin formation. A thrombin additive in serum tubes may shorten coagulation process and improve quality of samples. This may result in a special benefit for hemodialysis centers remote from the laboratory, where on-site centrifugation is performed.
Objectives: To validate the use of Rapid Serum Tube (RST, Beckton Dickinson) for the biochemical follow-up of phospho-calcium metabolism (PTH, vitamin D, calcium, phosphorus, ions) in hemodialysis patients.
Methods: In order to compare clotting times, 8 samples from 5 volunteers were collected, 4 on RST and 4 on Serum Separation Tube (SST), and stored at 0 ºC until centrifugation at 15, 30, 60 and 120 min. In a second stage, we studied samples (obtained in RST and SST tubes) from 37 patients of a hemodialysis centre distant from the laboratory, assessing sample quality (in terms of fibrin remains) and comparability (CLSI EP9) of results of phospho-calcium metabolism magnitudes.
Results: In the first step, samples from RST showed clotting times shorter than 30 min, compared with more than two hours in SST tubes. In hemodialysis patients, sample quality improved markedly on RST tubes (8% fibrin remains) vs SST (35%). Biochemical parameters were comparable in both tubes (differences did not exceed the limit recommended for systematic error at decision levels)
Conclusions: The use of RST tubes improves the quality of serum samples that need promptly centrifugation and cold storage, reducing the number of preanalytical errors due to fibrin formation, without significant effects on biochemical parameters of phospho-calcium metabolism. This may demonstrate special benefits when samples are drawn in locations distant from the laboratory.

T093
IFCC PROJECT "MODEL OF QUALITY INDICATORS": A METHOD FOR RESULTS EVALUATION

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Background: The IFCC project on Quality Indicators (QIs) started in the 2009 with the aim of implementing a Model of Quality Indicators to be used in clinical laboratories over the world for monitoring and improving all steps of the total testing process. The ultimate goal of the project is, therefore, to decrease the error rates and improve patient services in laboratory testing. Since 2012 the working phase is ongoing and an External Quality Assurance Program has been implemented. Currently, several laboratories are using the proposed QIs; they are regularly collecting and reporting their data and are receiving a report that highlights their data compared with those from all other laboratories. The aim of this work is to describe the information provided in the report and the method used for evaluating laboratories results.
Methods: The results collected from laboratories participating in the project have been processed and statistical data have been calculated (mean, median, etc.). Concerning the QIs that measure the number of defects, the sigma value and its confidence range have been calculated in relation to the laboratory result, group of results and all results. Graphics that describe the trend and distribution of laboratories results and sigma values are provided too.
Results: To evaluate an indicator, different measures can be needed. In particular, as an example, the sigma range of the different measures, concerning the QIs of pre- and post-analytical phase, is reported: patient identification (7 different measures): 4.4-5.1; request input (7): 3.5-4.4; sample collection (6): 4.8-5.4; sample transport (5): 3.7-5.4; sample acceptance (13): 3.3-5.0; timeliness of result reporting (2): 4.2-4.6; accuracy of result reporting (2): 3.8-5.1.
Conclusions: The reports provide a picture of the state-of-the-art and allow laboratories to: monitor their performance trend; know their sigma values and the related need for improvements; define the priorities; compare their data with those of other laboratories stimulating effective benchmarking activities; and share the improvement projects with all other participants.

T094
RIGHT QUALITY CONTROL PLAN THROUGH RISK MANAGEMENT

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Background: Medical Laboratories require a quality plan to ensure diagnostic test results are scientifically relevant, accurate, and timely for effective patient care. Laboratory failures of operations, processes, procedures and practices can adversely impact the quality of laboratory test results and present a risk of harm to the patients. Through risk management, laboratory process failures are controlled, and activities monitored to meet the defined accreditation standards.
Methods: A quality control plan: QCP: with comprehensive approach to the entire path of work flow was developed at Military Hospital Medical Laboratory with risk management principles adapted from ISO 14971. The QCP encompasses developing process charts and all inclusive quality control tools. The risk management process first identified and eliminated causes of process and system failures before implementing measures to correct and prevent failures (occurrence, severity and detection). Secondly, vital risk analysis threshold data was gathered for estimating failure mode probabilities from regulatory & accreditation authorities, equipment information from diagnostic manufacturers, laboratory operation qualification reports, staff competency, publication reports and consultation with clinicians.
Results: Process maps for total measuring process were prepared to reduce risk to patient safety, and improve processes and prevent errors. Evidence of improvement was observed by: Reduction of Blood culture contamination from 9% to 1.8%, communication of critical results improved from 40% to 95%, STAT test TAT reduced from 75 minutes to 50 minutes. Analytical process precision performance using CAP validated control materials reduced instrument precision outliers by 90% and analytical process BIAS standardized for accuracy by comparison to reference materials and methods for Lipids, Vitamin D, coagulation indices, Creatinine, testosterone, Estradiol and Urine Albumin.
Conclusions: Quality control through Risk management improved processes and performance, with significant decrease in defects and errors throughout the entire laboratory path of workflow activities in accordance with accreditation requirements.

T095

IMPROVING OUTPATIENT SAFETY BY A MDRD-GUIDED ADJUSTMENT ON DRUG DOSAGE BY COMMUNITY PHARMACISTS

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Background: Pharmacokinetics of many drugs are influenced by a decreased kidney function. When regular drug dosages are used, a decreased kidney function therefore might result in high or even toxic drug levels in patients and possibly even in hospitalization. Based on decreased kidney function the community pharmacists can advise to adjust the drug dose, cease medication or change choice of the type of medication. However actual lab results, such as MDRD (estimated Glomerular Filtration Rate, eGFR), estimating kidney function, are often not known to the community pharmacy that is dispensing medication to outpatients.
Methods: The kidney function of outpatients was estimated using the MDRD-equation (mL/min) in a central laboratory in the Tilburg Area. An excel-file reporting all outpatients with a MDRD-value ≤50 mL/min was uploaded once a week to a secured website. For each patient, the website redirected this value to the patients community pharmacy. The pharmacy registered this MDRD-value and checked if the patient was using drugs that are contraindicated to use in case of a renal impairment. In consultation with the general practitioner the community pharmacist could decide to intervene. 20 community pharmacies in the region Tilburg participated in this project.
Results: In a five months period the number of patients with a decreased kidney function that was known to the community pharmacy increased from 1000 to 2500. This resulted in 160 interventions, such as ceasing medication, changing the type of medication or dose adjustment. 133 interventions took place during the time that the patient was using the drug, 12 interventions took place at the moment of prescribing a new drug, and 15 took place at the the moment of renewing a prescription.
Conclusions: The 160 MDRD-guided interventions on drug therapy provided the community pharmacists with the possibility of more accurate drug dosing. This approach improves patient safety pro-actively, not only improving the patient's quality of life but also reducing the burden of healthcare expenses.

T096

THE LABORATORY TRANSITION FROM MANUAL TO BAR-CODING AT GERTRUDE'S CHILDREN'S HOSPITAL NAIROBI –KENYA FOR YEAR 2012

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Background: In the 21st century in the era of electronic documentation, many hospitals all over the world still struggle with patient identification protocols in trying to improve patient safety. From the traditional pen and paper to the automated bar code still errors occur in the field of laboratory medicine. Implications of poor sample identification will always lead to long turnaround times, delayed interventions, results reporting errors, misdiagnosis, inappropriate treatment with deadly consequences. The present observational retrospective study was a managerial exercise, an attempt to know the trends of comprehensive data capture during a transition period of systems change from manual to bar coding method in our clinical laboratory.
Objectives of the study/audit: 1. Establish the percentage number of bar-coded samples 2. To check the progress of transition to automation.
Methods: The study area is in a medium sized hospital in Nairobi –Gertrude's Children's Hospital. This was done by retrospective random sampling of the data on samples received in the month of August 2012 for one week and the month of October 2012 for a period of one week in hematology and biochemistry sections.
Results: Of all the samples analyzed, (n=1420), 51.4% (n=732) were bar-coded and therefore had all the verifiers while 48.4% (n=688) were handwritten or manual. The transition from manual to automation was at 50% .Only 3% (n=44) of the manual samples had all verifiers documented, the rest lacked one or two verifiers but met the minimum criteria of a well labeled sample.
Conclusions: The target was to reach 100%; however this was not achieved due to cost implications and Poor acceptance by the staff. The 3% of well labeled manual sample means 97% of manual samples could be a source of errors in the laboratory .The laboratory is an important line of defense against error that can result in patient harm. By establishing processes to accurately label and Identify samples; laboratory staff can significantly reduce the incidence of misidentified, lost or unusable samples, and the related negative consequences, including redraws, misdiagnosis and inappropriate treatment. All laboratories should have set up minimum acceptance criteria for all samples they receive.

T097
PRE-ANALYTICAL ERROR RATES: IMPACT OF PROVIDING FEEDBACK TO LABORATORY USERS

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Background: Pre-analytical errors have a significant impact on patient care. To date there are no generally agreed criteria for categorisation of these errors. Data collection can be difficult and improvement targets hard to identify. A continuous improvement process is likely to be a more successful strategy than short-term interventions.
Methods: We developed a system for automated error collection utilising the laboratory information system (LIMS). Data is collected by querying the LIMS and processed using database and spreadsheet programs. Error categories are based on the Australian Key Incident Monitoring & Management Systems (KIMMS). Immediate feedback of inadequate specimen collection is provided to requestors by issuing results identifying errors that have occurred. In addition, summary data are issued in monthly reports to users.
Results: Monthly data collection allowed reporting for the 3 monthly KIMMS returns as well as regular analysis. Haemolysis rates varied during the data collection period due to changes in data collection and handling and this had a sizeable impact on the overall data. Overall (excluding haemolysis) pre-analytical monthly error rates declined from 2057 (Nov 2011, 4.1%) to 1916 (Oct 2012, 3.75%). Labelling errors decreased during the study (0.30% to 0.21%) whilst other error categories such as insufficient and no sample received showed little change over time. During the latter half of the analysis, we held feedback meetings with divisional managers and phlebotomists to review the data and propose recommendations for change.
Conclusions: Use of the LIMS ensures full data capture and is more robust than manual and retrospective data collection. The haemolysis rate fluctuated due to the introduction of automated measurement rather than visual estimation. Labelling errors may have changed due to the changes in the use of computerised ordercomms for requesting. Despite the provision of immediate feedback, error rates for sample collection remained largely stable. This indicates that more effective intervention and reinforcement than simple data provision is required. Although feedback meetings resulted in greater awareness, their impact remains to be reflected in manager-led interventions.

T098
ECONOMIC RESULTS OF THYROID REFLEX TESTING APPLICATION

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Background. The appropriateness of laboratory testing is an important component of healthcare quality. Reflex testing, as the measurement of further tests when the initial test meets established thresholds, is an approach capable of improving laboratory service by avoiding the performing of clinically unnecessary tests. A further result of this operational change is the economic return, that would be interesting to quantify.
Methods. Our Laboratory adopted reflex testing for all thyroid test orders since May 2006. By protocol, thyroid stimulating hormone (TSH) was the only mandatory initial test, with few clinical exceptions; the reflex added tests (free tyroxine and triiodothyronine) were generated for TSH result falling outside the 0.35 – 4.30 μUI/mL range. The process was based on an algorithm running automatically on the laboratory information system, to set a standardized process. The total reduction of free thyroid hormone tests, generated by process change, was estimated on the basis of percentages for free thyroid hormones related to TSH, observed in the period before intervention without order restriction. The economic result evaluated the reagent cost saving and the costs for updating the information system.
Results. In year 2005 the laboratory performed 37,166 TSH tests: the FT4/TSH and FT3/TSH ratios were 0.77 and 0.65, respectively. After the protocol application, from June 2006 to May 2012, the mean ratio decreased to 0.25 for FT4/TSH and to 0.11 for FT3/TSH. The thyroid reflex testing protocol saved a total of 212,132 FT4 tests and 223,351 FT3 tests over 6 years, in a frame of TSH testing increase. The net gain over the period was 601,050 € (630,150 € of savings in analytical reagents, minus 29,100 € of information system costs).
Conclusions. The application of thyroid reflex testing protocol produced a considerable reduction in thyroid test orders for clinically irrelevant tests. This approach yielded a large economy over the years. The protocol application on health information system warranted consistency and certainty of results even with the observed increase of thyroid testing.

T099

THE COST OF POOR SAMPLE QUALITY: ASSESSING THE FINANCIAL IMPACT OF SAMPLE REJECTION AND RECOLLECTION IN HEALTHCARE INSITUTIONS

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Objectives: To model the financial impact for the healthcare institution of specimen rejection due to preanalytical errors in the laboratory testing phase, using institution specific data.
Methods: As part of BD Laboratory Consulting Services™ a cost of poor quality model was developed with a consultant health economist in order to enable an estimation of the opportunity cost associated with poor sample quality to be assessed. Critical data such as number of beds; overall budget; number of patients of different types seen each year; and number of sample rejections, as well as qualitative data, eg. probable impact of sample rejection were collected by interviewing institution staff from multiple country institutions (N=10). The data were then entered into the model to calculate the possible financial impact of laboratory sample rejections. The model separates patients into different groups according to the likely effects of having a sample rejected, the probability that the rejection would have a low, medium or high impact and the consequences on institution time and resources. The overall consequence, or opportunity cost, was expressed as patient treatment time and financial cost.
Results: The size of the institutions ranged from 326 to 1,200 beds, with total operating costs varying between €41 million and €1.1 billion. The number of blood tests per month was between 37,000 and 458,000 and of these between 0.09% and 2% were rejected (mean 0.93%). The total cost of specimen rejection ranged from €22k to €5.9 million per annum (mean €1.9 million), equating to a percentage of total operating costs from 0.1% to 1.2% (mean 0.4%). The estimated cost per patient for a sample rejection was from €29 to €349 (mean €171).
Conclusions: A customisable model which allows institutions to estimate, using local data and assumptions, the effect of sample rejection on patient treatment and cost. Results can be compared to those from other institutions to benchmark performance and assumptions. The results demonstrated that a reduction in the number of rejected samples due to preanalytical errors could lead to significant cost savings for most institutions.

T100

OPTIMIZATION OF DOWNTIME FOR ROCHE COBAS 8000 MAINTENANCE

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Introduction: In our laboratory, routine and emergency analyses are performed on Cobas 8000 modular analyzer (Roche Diagnostics), that is composed by a pre-analytic module (MPA) linked to two operative lines, CCE and CC. Aim of the study was to organize the instrument daily maintenance procedures in order to reduce the downtime of the CCE line. Two types of samples are analyzed on Cobas: serum samples for the measurement of drugs, bile acids, C-Reactive Protein and immunochemistry parameters, and heparinized plasma samples for the clinical chemistry assays. The system needs some daily, weekly and fortnightly preset maintenances, that requires a machine downtime for a long period of time. The CCE line, composed by C-702(ISE), C-502 and E-602, is operative 24 hours 24 and 7days out of 7; for this reason it was necessary to optimize the downtime that are made up by other analytic procedures; in fact, during daily maintenance clinical chemistry tests are processed on CC line, which lacks the E-602 module, while emergency immunochemistry tests (CK-MB ,TNT-hs, NT-proBNP, β-HCG) are analyzed on Cobas 411.
Results: Analyzing the criticisms we have taken appropriate corrective action introducing new operating modes, different from those suggested by Roche, so obtaining a considerable reduction of waiting times. Our daily maintenance procedure is essential for a significant gain of time on the E-602 module operation. Excluding one module at a time is possible to perform individual maintenance without necessarily resorting to total block of the line. In this way it minimizes the times of downtime from about 1h/30 to less than 15 minutes for E-602 module, and about 35 minutes for C-702, ISE, C-502 modules.
Conclusions: This new operating procedure considerably cut down the time of maintenance thus improving the TAT of our analyses. It is always important to critically accept the suggestions of the manufactures that require a practical customization.

T101
PRE-ANALYTICAL PHASE: A CHALLANGE TO MODERN LABORATORY MEDICINE

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Introduction: In recent years the need to convey in few hospital centers the large majority of biological samples in order to provide cost effective service was aroused. Quality issues need to be considered: pre-analytical , analytical and post analytical phase could be affected, however pre-analytical phase is not easy monitored. The Azienda Sanitaria 10 (Florence, Italy) adopted an "hub and spoke" model and all the outpatient biological samples from the Florentine area conveyed in the San Giovanni di Dio General Laboratory. The following issues needed to be addressed: transfer to the laboratory (mean distance 15 km); standardization of sampling procedures; communication.
Method: A systematic approach was undertaken and tool such as Total Quality Management, Process reengineering, Lean and Six- sigma were adopted to develop a strategy covering the three mentioned critical areas. As first step the transfer to central laboratory was planned and a standard time schedule and route established; written sampling procedures were prepared and discussed with all the professionals involved; a permanent course was organized to maintain the knowledge, finally a specific software was produced as a mean to monitor pre-analytical phase. The software (named PRAMLab) allows the registration of transfer and sampling errors and, automatically, sends, a warning e-mail to the outpatient center.
Results: The turnaround time of collecting and delivering samples is appropriate and allows a constant workflow in the central laboratory; the PRAMLab statistical analysis shows a decrease of 1% sampling errors in tubes for hematological tests and a decrease of 1.3% in tubes for coagulation tests (June and September 2012 data). Such percentage becomes notable bearing in mind the laboratory total number of results (7x106 /year) and when considering that such errors involve asking an outpatient to repeat the procedure.
Discussion: Modern Laboratory Medicine needs to face major organization changes. Tools traditionally used in other branch of science, mainly engineering, are gaining an important role in planning such changes and in maintaining the focus on quality of performances. Moreover, leadership and communication skills are needed to mediate any transformation.

T102
A MATHEMATICAL APPROACH AND APPROPRIATE SOFTWARE FOR THE REGISTRATION OF QUALITY INDICATORS IN TRANSFUSION MEDICINE ENABLING QUALITY COMPARISONS AMONG BLOOD-SERVICES

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Background: The request for minimal-risk blood and blood-products has led during the last 15 years, to an immense progress in R&D and practice of Transfusion Medicine (TM). Quality indicators (QIs) constitute a method providing for the necessary proof of the level of quality performance of each individual Blood Establishment or Service. The purpose of this project was a Mathematical approach of this method and the development of appropriate software for the registration of Quality Indicators in Transfusion Medicine and further ensuring Blood quality and enabling comparisons among Blood-Services.
Methods: We have adopted the most recent and comprehensive list of QIs, produced this year by the Working Party on Quality Management (WPQM) of the International Society of Blood Transfusion (ISBT) and including presently 36 indicators. Starting from this point we have established a finite-dimension (currently 36) Vector-Space (R36), including the presently selected representative Quality Indicators in Transfusion Medicine. Each set of Quality Indicators from a Blood Service constitutes a vector BBxxxxx = (QI1, QI2, …, QI36), whereas xxxxx represents the unique classifier that identifies every collection facility worldwide, according to the ISBT 128 identification standard (e.g. G1517 identifies the Welsh Blood Service, Wales, UK). The Vector-Space dimensions can be modified by adding or omitting QIs and virtually all Blood Services worldwide could belong to the Vector-Space or a subspace with the same or other dimensions.
Results: Our custom developed software is supporting the documentation of the existing each time Q Is, appropriate quadratic or individual-QIs based Vector-Metrics can be defined on demand thus, allowing for the calculation of an appropriate Metric related "order", enabling overall, partial and individual Q Is-based quality comparisons among various and numerous Blood Establishments, providing flexible and objective concrete proofs that expected degrees of quality have been missed, met or even exceeded.
Conclusions: The system is presently tested off-line, within a "virtual-world" environment, by employing imaginary, however realistic data. An official Web-service, if necessary, should be put under the international auspice (ISBT, WHO etc.).

T103

THE EMERGING NECESSITY OF INCREASED MATHEMATICAL COMPLEXITY IN LABORATORY MEDICINE AS REFLECTED ON RECENTLY FILED RELEVANT INDUSTRIAL PROPERTY DOCUMENTS

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Background: The employment of modern equipment and methods in Laboratory Medicine (LM), during the last two decades, starting with Flow Cytometry and reaching Biosensors, Microarrays, Nanotechnology and Surface-enhanced/Matrix-assisted LASER desorption ionization/ time-of-flight/mass spectrometry (SELDI/MALDI-TOF-MS), produce an immense volume of data, requiring the employment of complex Mathematical Algorithms and the associated Software, in order to approximate the multifarious reality of the Biological procedures under investigation. It is the purpose of this paper to outline the emerging necessity of increased Mathematical Complexity in Laboratory Medicine, as reflected on recently filed relevant Industrial Property documents (IP-Docs).
Methods: We have retrieved and assessed hundreds of IP-Docs, by employing the on-line esp@cenet search-engine of the European Patent Office, concerning Molecular and Microscopical Complexity, that are corresponding to the two major components of LM involved.
Results: The first category has resulted in 16 groups of Algorithms employed, such as Machine Learning, Dynamic Programming, Hidden Markov Models, Exhaustive Search etc. and the second category has resulted in 13 groups of Algorithms employed, such as Geometrical Transformations, Wavelet based Image Processing, morphological Image Processing, Image Digitization etc. among the filed Patent applications. It should be taken into account that some of the employed algorithms in very recent IP-docs (US 2012/0185177: "Harnessing high throughput sequencing for multiplexed specimen analysis", 19 July 2012) have their origin in Eratosthenes sieve (3rd Century B.C.) and the Chinese Mathematicians (3rd – 5th Century AD). However, decoding the complex Biological data does not automatically produce a definite Diagnosis, since the employment of another one appropriate intermediate mathematical Medical Decision supporting Algorithm is usually necessary.
Conclusions: The synergy of Mathematics with Laboratory Medicine promotes the development towards individualized Patient Diagnosis and Treatment, on the common basis of routine genotyping, if necessary. It seems that finally Mathematics, Science and Biomedical Technology start building up a new mature bilateral relation to Medicine.

T104

AUTOMATED INDIRECT IMMUNOFLUORESCENCE EVALUATION OF ANTINUCLEAR AUTOANTIBODIES ON HEp-2 CELLS

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Background: The evaluation of ANA screening using indirect immunofluorescence (IIF) on human epithelial (HEp-2) cells is still carried out visually by laboratory technicians, thus being time-consuming, subjective and prone to interobserver variability. In the current study, we evaluated the performance of a novel system (EUROPattern) compared to visual IIF interpretation.
Methods: Two sample collectives were examined, consisting in total of 351 serum samples originating from different referral laboratories. ANA detection was performed by IIF using HEp-2 cells. The complete incubation process was carried out manually. Then, the IIF slides were subjected to automated immunofluorescence microscopy, with the system taking focused images of all reaction fields. Subsequently, using the same images, the fluorescence patterns were evaluated in two ways: (i) by EUROPattern and (ii) visually by two independently working laboratory technicians.
Results: There was an agreement of 99.4% (κ=0.984) between the visual and automated approach regarding positive/negative discrimination. The analytical sensitivity and specificity of EUROPattern amounted to 100% and 97.5%. The overall efficiency of automated main pattern recognition was 94.0% and varied for the different patterns, declining in the following order: centromeres, nuclear dots (100%) > negative (97.5%) > nucleolar (95.6%) > speckled (94.6%) > cytoplasmic (93.1%) > homogenous (81.8%). In 21 out of 351 (6.0%) sera, the main pattern was not recognized. EUROPattern is a computer-aided diagnostic system, meaning that in daily laboratory routine all automatically retrieved results have to be validated by the laboratory staff based on the fluorescence images, accomplishing the desired 100% agreement with visual reading.
Conclusions: EUROPattern proved to be very sensitive, which is the prerequisite for handing over the first step in ANA screening to an automated detection system. EUROPattern also proved to be highly efficient in sorting out negatives and providing good pattern recognition. This half-automated laboratory process is now less time-consuming and less error-prone than purely visual reading and helps clinical laboratories to standardize IIF-based ANA diagnostics.

T105
IMPLEMENTATION OF A NEW LABORATORY INFORMATION SYSTEM [LIS]

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Background: The four clinical laboratories of the central laboratory have different analytical laboratory equipment. The first step towards restructuring the laboratory medicine was made by implementing a new single Laboratory Information System (LIS) at the central laboratry. In order to create master data it was necessary to carry out comparative measurements: 41 methods had been compared. 35 patient probes for each method have been measured parallel at Dimension [Fa. Siemens Healthcare Diagnostics GmbH] and at MOULAR [Fa. Roche Diagnostics Deutschland GmbH]. Pearson's Correlation Coefficient, Passing-Bablok-Correlation and Bland-Altman-Blot were used to evaluate the results mathematically.
Methods and results: It was possible to use identical master data for 30 methods: [alanin aminotransferase, albumin, aspartat aminotransferase, total bilirubin, calcium, total cholesterol, HDL-cholesterol, LDL-cholesterol, creatine kinase, creatinin, c-reactive protein, iron, total protein, ferritin, fibrinogen, γ-glutamyltransferase, gentamycin, glucose, HbA1c, uric acid, urea, potassium, lactate, lactate dehydrogenase, lipase, magnesium, myoglobin, sodium, inorganic phosphorus und triglycerides]. 11 methods required different master data: [alcalic phosphatase, amylase, direct bilirubin, cholinesterase, chlorid, digitoxin, lipase, myoglobin, theophyllin, transferrin and TSH].
Conclusions: The comparative measurements and their statistical evaluation were of vital assistance in deciding whether to use identical or separate master data for a method in the new Laboratory Information System [LIS].

T106
STATISTICAL INVESTIGATION OF TRANSFUSION OF BLOOD PRODUCTS IN THE HOSPITALS OF A HOSPITAL ASSOCIATION FROM 2005 TO 2010

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Background: This research project investigated the transfusion of blood products in the hospitals of a hospital association from 2005 to 2010. The hospital association is a clinical in a hospital network composed by four units, with a total of 1.200 beds. This complex comprehends wards for Internal Medicine, Surgery, Orthopaedics and Traumatology, Gynaecology and Obstetrics, Paediatrics, Psychiatry, Ophthalmology, Oto-Rhino-Laryngology and Urology.
Methods: The clinics of the four hospitals of the hospital association were combined to the following three medical disciplines: a) Predominantly internal working disciplines [Internal Medicine; Paediatrics, Psychiatry, Ophthalmology, Oto-Rhino-Laryngology]; b) Predominantly surgical working disciplines [Surgery, Orthopaedics and Traumatology, Gynaecology and Obstetrics, Urology] and c) Intensive care. The transfusion of the following blood products was statistically evaluated from 2005 to 2010: heterologous and autologous red blood cells (RBCs); heterologous and autologous fresh frozen plasma (FFP); platelets; prothrombine complex (PPSB), Antithrombin III (ATIII) and Fibrinogen.
Results: In all three disciplines an increase in the transfusion of heterologous RBCs was observed from 2005 to 2010. For instance was the increase from 2005 to 2010 in Hospital A 26 percent; in Hospital B 295 percent, in Hospital C 3 percent and in hospital D 32 percent. The transfusion of autologous RBCs continuously decreased during that period. For instance was the decrease from 2005 to 2010 in Hospital C 87 percent and in Hospital D 64 percent. In contrast, the transfusion of platelets increased in all three medical disciplines. For instance was the increase from 2005 to 2010 in Hospital A 146 percent, in Hospital B 40 percent, in Hospital C 132 percent and in Hospital D over 500 percent. The transfusion of PPSB was different in the 4 Hospitals. For instance from 2005 to 2010 in Hospital A was a decrease of 6 percent, in Hospital B a decrease of 70 percent and in Hospital C a decrease of 34 percent. Only Hospital D shows an increase of 96 percent in transfusion of platelets. While transfusions of Fibrinogen increased, the number of transfusions of ATIII significantly decreased. For instance was the decrease from 2005 to 2010 in Hospital A 31 percent; in Hospital C 63 percent and in hospital D 92 percent.
Conclusions: On the one hand, the results of this investigation reflect the new guidelines in transfusion medicine. On the other hand, the results also provide information about the modified and new implemented methods of treatment in most disciplines of the hospitals of the hospital association.

T107
CLINICAL-ECONOMIC EFFECTIVENESS OF IMMUNOASSAY IN PATIENTS WITH CHRONIC INFECTIOUS AND INFLAMMATORY DISEASES

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Purpose and Objectives: To carry out clinical-economic effectiveness of laboratory immunological assays in patients with infectious and inflammatory diseases.
Materials and methods: 80 patients with chronic infection and inflammatory diseases who passed immunogram (group 1) were examined and 500 out-patients that did not conduct immunological studies (group 2) were retrospectively analyzed. Evaluation of immune status included the determination of the absolute and relative rate of lymphocytes and their subpopulations, the definition functional activity of leukocytes, levels of immunoglobulins, level of circulating immune complexes, phagocyte number and phagocyte index. Methods of clinical and economic analysis: the «cost-effectiveness ratio» (CER) and the «incremental cost – effectiveness ratio» (CER incr). Direct costs were calculated as the total cost of diagnostics and treatment of patients within a year; indirect - as the basis of costs in period of the patient's disability according the municipal values of salary and payments on disability sheets. A criterion of efficiency was calculated as percentage of patients with recurrence-free period for a year.
Results: The direct costs of diagnosis and treatment using immunological methods is higher, whereas the indirect costs is more favorable. Direct costs in group 1 were 10,692 rubles, in group 2-7370 rubles, while indirect costs–3894 rubles and 11,680 rubles, respectively. Percentage of patients with recurrence-free period for a year in group 1 was 17% in group 2 - 3%, P <0,05. CER in group 1 was 858 rubles, 7,4 fold less than in group 2 (6350 rubles). CER incr showed that in order to achieve more efficiency units (1% of patients with recurrence-free period for 1 year) using immunological methods is only 237,3 rubles, that is comparable to the threshold of economic viability in a national scale.
Conclusion: The use of an integrated approach with immunological methods provides significant economic benefits while improving the quality of care for patients with chronic infectious and inflammatory diseases.

T108
ELECTRONIC HEALTH RECORD: CODING OF LABORATORY TESTS USING STANDARD SYSTEMATIC NOMENCLATURE

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Background: Clinical labs display a wide variety of formats for both receiving requests and issuing results, and therefore coding is required for their use in the Electronic Health Record (EHR). Our objective is to evaluate the standard systematic nomenclature recommendations to specify which is the best adapted to the functional design of the Lab Test Module (LTM) of the Andalusian EHR (Diraya).
Methods: The Coding and Nomenclature Group (CNG) of the Andalusian Health Service was created for this task and is composed of specialists from the different clinical lab areas. The codes to be reviewed had to contain systematic rules for naming any lab test, specifying: Where it is performed (sample), what is performed (determination) and how it is done (method). The strengths and weaknesses of each system were studied, as well as their adaptation to the specific needs of the LTM.
Results: The lab test coding systems most used internationally are IUPAC/IFCC and LOINC. The coding structures of the two systems collect similar data although in different order. The main characteristics of both systems are summarised as follows: IUPAC: Suitable for transmitting information both electronically and between professionals, based on international terminology consensus, limits the use of abbreviations and recommends the use of the International System of Units. LOINC: Suitable for transmitting electronic information but difficult to understand due to the use of abbreviations. Uses proprietary terminology and does not assess the use of the International System of Units. Of the two systems, only IUPAC follows European recommendations for nomenclature and results for lab tests (ENV1614:1995 and ENV12435:1995), this being recommended in the ISO15189:2007 standard.
Conclusions: In light of the results obtained in the review, the CNG selected the IUPAC recommendations as the standard to be followed for coding clinical lab tests in LTM. As a strong point of the IUPAC standard, we would highlight that, both in form and content, it is always based on the recommendations of international bodies. The current CNG database, based on IUPAC but standardising all that is not specified in the international standard, has about 12000 records between coded tests and methods.

T109

FUNCTIONAL DESIGN OF THE LABORATORY TEST MODULE FOR THE ANDALUSIAN ELECTRONIC HEALTH RECORD ("DIRAYA" PROJECT)

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Background: The "Diraya" electronic health record integrates all health information of the Andalusian Public Health System (APHS). A Lab Test Module (LTM) has been developed to integrate all lab information (44 hospitals, 1500 health centres, 8.3 million citizens). It aims are: That a lab test can be requested from any APHS centre, that the patient can go to the public lab of your choice, and that the results are available at any EHR access point, regardless of the requesting centre or lab performing the analysis.
Methods: The functional design is based on: A common coding scheme for transmitting the information that respects the differing nomenclature of each lab; two coding levels, a clinical one and another for labs, developed by the andalusian Coding and Nomenclature Group (CNG) based on the IUPAC-IFCC standard; traceability of the whole process (request, preanalytical phase, sample collection, reception in the lab and sending of results); and possibility of connecting to any Lab Information System (LIS) without changing the internal work protocol of each lab.
Results: We have been working on the LTM's functional design for four years. At the same time, the CNG database was being created. We realised the pilot test in primary care over two years in the North Cordoba Health Area (NCHA). Currently the LTM is connected to five different LIS and is operative in all APHS primary healthcare. LTM has processed more than 2.5 millions of lab requests. Now is being implemented in hospital outpatient consultation to continue in hospital inpatient areas and emergency rooms. LTM maintains traceability with the method used, as well as the reference ranges of each laboratory, facilitating interpretation of the results regardless of where they were obtained.
Conclusions: The LTM makes it possible to connect any LIS and facilitates user access to any APHS lab. The success of a project like this requires: A clear functional scope and an adequate strategic plan in the health administration; a functional design under the responsibility of clinical lab professionals who contribute knowledge about the entire lab request process; and a pilot testing period that is long enough to guarantee the absence of functional errors before undertaking new implementations.

T110

IMPLEMENTATION OF THE LABORATORY TEST MODULE OF THE ELECTRONIC HEALTH RECORD IN THE NORTH CORDOBA HEALTH AREA (ANDALUSIA, SPAIN)

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Background: The laboratory test module (LTM) is one of the most important functionalities in the electronic health record (EHR) because of the large volume of information clinical laboratories generate. Our aim is to assess quality indicators after implementation of the LTM in order to assess the potential benefits contributed by the LTM to the EHR and know the subjective opinion of professionals to see the LTM's level of acceptance in the daily work routine.
Methods: We compared different quality indicators before and after LTM implementation in primary care (43 extraction points) of North Cordoba Health Area (NCHA). These indicators collect information about the pre-test phase, patient security, response time (RT) and economic impact. To assess the opinion of LTM users, an anonymous survey of the professionals was conducted.
Results: Before the LTM, 20% of requests lacked a diagnosis, 6% lacked a record number and 49% lacked the traceability of the extraction (professional taking the sample, date, time). With the LTM, we have reduced these errors to 0% and the identification is unequivocal through the Andalusian unique medical record number. Manual programming errors in the laboratory information system and loss of results have disappeared. Before the LTM, results were printed at the end of the work day and distributed the following day, implying a minimum RT of 48 h. With the LTM, p80 of requests receive the first result <1 h after reception in the laboratory, and p90 receive them in <1.3 h; p70 of requests receive complete results the same day (<5.4 h). The savings in paper consumption for the reports has been over 1,500,000 sheets yearly. In the satisfaction survey, professionals highlighted the following aspects: speed, security and results available from any centre, with 93% of them recommending use of the LTM.
Conclusions: The possibility of requesting and receiving results using specific EHR modules is one of the features most demanded by professionals. The elimination of errors and improvement in response time represent significant advances in care quality. The results obtained in our study conclude that implementation of the LTM in the NCHA has meant a quantitative and qualitative improvement for both professionals and patients.

T111
POINT OF CARE TESTING AND LABORATORY MEDICINE: WHICH EVIDENCE? A SYSTEMATIC REVIEW.

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Point of Care testing (POCT) had rapid technological development and their use is widespread in clinical laboratories to assure reduction of turnarountime (TAT) and rapid patient management in some clinical settings where it is important to take quick decisions. Until now the works about the POCT have focused the attention on the reliability of the technology used and their analytical accuracy. The use of POCT to provide a more efficient healthcare is become important, because the devices provide more services with less expense to improve a healthcare systems centered on patients. Purpose: To conduct a systematic review of the evidence to support POCT, focusing on clinical outcomes.
Methods: We searched in Medline. Two independent reviewers assessed the eligibility, extracted study details and assessed the methodological quality of studies.
Results: We analyzed 109 studies for five POCT types: neonatal bilirubin, troponin, blood gas analysis, intraoperative parathyroid hormone and procalcitonin. Most of the included studies (75%) were comparative, only 7 (6%) are RCTs, and 25 studies considered important patient's outcomes. Most of the included studies were about correlation between POCT and laboratory, 31% were studies about diagnostic accuracy and 24% studies evaluated the impact of the POCT on the clinical practice. Only 3 studies reported economic evaluation. Moreover the incomplete outcome data were not adequately addressed. We have performed meta-analysis about TAT and LOS for troponin POCT.
Conclusions: POCT reduced the time taken to make decision on patient management but the clinical outcomes have never been adequately evaluated. Our work shows that, although POCT has the potential to provide beneficial patient outcome, further studies may be required.

T112
AUTOMATED PRE-ANALYTICS CHALLENGES MANUAL WORK: DOES IT SUCCEED?

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Background: Pre-analytical phase is the longest and the most critical part of the laboratory process. It can be divided in procedures made out of the laboratory and in practices within the laboratory in which participate not only laboratory staff but also other health care personnel. Use of the automated pre-analytical system enables the automation of all stages of sample preparation, and thus the error reduce in the pre-analytical stage as well as the number of employees needed. The aim of this study was to verify whether automated pre-analytical system can replace human resource.
Methods: In our core laboratory, which annually makes more than 2.1 million analyses, we have been using Modular pre – analytics evo (MPA) system since 1st June 2011. We have observed the number of errors caused by the solely work of MPA, the number of employees needed as well as the turnaround time (TAT) before and after using the MPA. Only 8, 5 ml standardized gel separator test tubes were considered.
Result: The total of 3576 samples, we found 57 (1,5%) errors. 21% errors caused by insufficient volume of the sample and 79% errors caused by the presence of micro clots in the primary tube. TAT has been reduced by 46% and the number of employees by three.
Conclusion: The use of appropriate automated pre-analytic system significantly reduces the time as well as the number of laboratory personnel required for the sample preparation. Aliquoting failures are fully avoided as well as the accidents during the transport of samples to the analyzer. The limitations of this MPA system are quantity and quality of the sample. Errors caused by insufficient volume of sample can be avoided by previous selection and review of the primary tubes. The existence of micro clots can be successfully detected by the air pressure aspiration system and whereby the analytical failures are avoided. Thus failures are registered with description actions for their solving. Therefore education and involvement of the laboratory personnel in the work of MPA systems are necessary for delivery of an adequate sample to the particular workplaces.

T113
USEFULNESS OF A NEW TECHNOLOGY IN THE STUDY OF CELIAC DISEASE

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Introduction: Currently, laboratory's first step in the study of coeliac disease (CD) is based on the measurement of anti-human tissue transglutaminase IgA (t-TG IgA), involving the joint determination of IgA immunoglobulin (IgA) to avoid false negatives errors in patients with IgA deficiency. Determination of t-TG IgA by chemiluminescence (CLIA) using a ZENIT RA Analyzer was implanted a year ago in our laboratory. This technique also provides information on the same analysis about total IgA concentration by a conversion factor: t- TG IgA 0.8 AU/mL = 25 mg/dL. Results <0.8 AU/mL suggest the possibility of "IgA deficiency".
Objective: Assessing economic improvements and technical management time in a sample of patients, once implemented the technique in our laboratory.
Material and methods: Concentrations of t-TG IgA and IgA we reviewed in samples taken over 3 months in patients who requested EC study. 1517 determinations of patients aged between 298 days and 87 years. In this sample of patients was performed t-TG IgA (Zenit RA. Menarini Diagnostics) and IgA (BNII. Siemens). The cutoff was set at 1.1 AU/mL to increase the sensitivity of the technique.
Results: The 3.62% (n=55) of patients had values ≤1.1 AU/mL, of them 11 patients had levels below the reference values of IgA, not find any IgA deficiency. The implementation of this technique provided the possibility to avoid the 96.38% (n=1462) of the determinations of IgA, which resulted a 7,904.13/year of savings, besides the technical handling time avoiding the realization IgA.
Conclusions: Implementing this technique has resulted in considerable savings in our laboratory wich optimizes our financial and technical resources. If the cut off had remained at 0.8 AU/mL had only made 7 IgA determinations. By raising the cutoff to 1.1 AU/mL increased the sensitivity of the technique, which allowed to perform only the 3.62% of the IgA requested EC studies.

T114
DESIGNING AND IMPLEMENTING TRAINING PROGRAM FOR LABORATORY TECHNICIANS IN A TERTIARY CARE CENTRE OF CENTRAL NEPAL

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Background: Though referred as different names viz laboratory technician, laboratory technologist, clinical laboratory scientists the prime responsibilities of these health care professionals is to perform biochemical, hematological, microbiological and immunological tests in various samples of a clinical laboratory. These professionals generally prepare specimens for examination, count cells, and look for abnormal cells in blood and body fluids. The consistent advances in technology require technicians to stay informed and current on the equipment in their lab and within their field. So, it was thought that the training the technicians will be important to keep them updated regarding recent advances in laboratory medicine, refresh them in terms of the routine work they perform and overall improve the quality of the laboratory reports.
Methods: The study was conducted at the college of medical sciences teaching. All the 19 technicians at the CMS-TH central diagnostic laboratory were involved in the study. Out of 19 participants 3 with MSc MLT were used as trainers. Whereas, 3 participants have BMLT degree, 6 have CMLT and 7 have basic laboratory assistant training. Pre intervention and post intervention results were taken with the help of structured questionnaire. A total number of 25 h were taken to complete the training module. The module included 13 different topics related to general laboratory practice, Hematology, Biochemistry, Microbiology. A group discussion session and session on Quality Control were also included in the module.
Results: Wilcoxon Signed Ranks Test shows that there is significant positive change in the results after intervention. Mann-Whitney and Kruskal Wallis tests showed that the results were independent of the age and the qualification of the technicians.
Conclusion: In conclusion, it was observed that continuous laboratory education is important in laboratory setup of developing countries.

T115

APPLICATION OF THE CRITICAL VALUES POLICY IN AN INTER-DISTRICT CENTRALIZED CLINICAL DIAGNOSTICAL LABORATORY

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Background: In compliance with the ISO 15189 standards of quality and regulations for centralized clinical diagnostic laboratories (CDL) in Saint-Petersburg, Russia, a CDL should have approved procedures of urgent notifications of clinicians in cases when values of test results are alarming or critical, as they represent a potentially life-threatening pathophysiologic state that requires immediate intervention.Methods. Statistical analysis of test results.
Results: CDL in outpatients' clinic № 34 in Saint-Petersburg performs tests for 30 different medical establishments. In 2011 we made nearly 1300000 tests, of which 1100 (0,084%) had critical values (CVs). The distribution of alarming and critical results between tests was as following: 36% - urine protein in pregnant women, 30% - clinical chemistry, 23% - immunochemistry, 8% - hematology, 3% - coagulation. When we tried to analyse these results we found out that there are no official Russian standards for CVs in urine, and blood CVs are much overrated. According to the GLP we determined our own CVs for common tests. Now we notify customers of such results as >14 mmol/L for glucose, >0.3 g/L for urine protein in pregnant women, <50 g/L for hemoglobin, <5 mkmol/L for iron, >double normal values for creatinine, creatine kinase, bilirubine and transaminases. All test results are validated by doctors. In case of alarming results we send messages to customer establishments 3 times a day. When test results are of critical values we make urgent telephone calls to attending doctors. Last year we introduced into our clinic a medical information system (MIS), which allows clinicians to obtain results online in real-time directly from the laboratory information system, and see not only critical values, but also results of accompanying tests. Now we have installed MIS to all our customers.
Conclusion. Critical values are an important patient safety indicator. Notification of attending doctors of critical test values is an essential procedure. The policy of CVs implemented in clinic № 34 keeps the laboratory in collaboration with clinicians, even when tests are performed in an outlying medical establishment.

T116

SPIDIA-RNA: FIRST EXTERNAL QUALITY ASSESSMENT FOR THE PRE-ANALYTICAL PHASE OF BLOOD SAMPLES USED FOR RNA BASED ANALYSES

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Background: The diagnostic use of in vitro molecular assays can be limited by the lack of guidelines for collection, handling, stabilization and storage of patient specimens. One of the goal of the EC funded project SPIDIA (Standardisation and improvement of pre-analytical procedures for in vitro diagnostics, www.spidia.eu) was the implementation of a pan-European External Quality Assessment (EQA).
Methods: 102 laboratories were recruited for the EQA by the European Federation of Laboratory Medicine (EFLM) support,. They received two blood samples: in EDTA tube (unstabilized blood) or in PAXgene® Blood RNA tube (stabilized blood) and performed RNA extraction according to their procedure providing details. At the SPIDIA facility, the survey data were classified, and the extracted RNA samples were evaluated for purity, yield (by spectrophotometric measurements), integrity (RIN), stability (by RT-qPCR of GAPDH, IL1B, IL8 and c-fos the gene expression), and the presence of interfering substances affecting RT-qPCR assays (by Kineret® software). All participants received a report comparing the performance of the RNA to the other participants and each RNA quality parameter was classified as "in control", "warning", "out of control" and "missing" by consensus mean analysis.
Results: From the survey data, the most variable parameters were: the volume of collected blood, the time and storage temperature between blood collection and RNA extraction. Analysing the results of quality parameters we observed the distribution of purity, yield, and presence of assay interference in agreement with expected values. The RNA Integrity Number (RIN) values distribution was much wider than expected value, so we had an "in control" classification even for partly degraded RNA. Even if RIN values below 5 significantly correlated with a reduction of GAPDH expression levels. Assuming the presence of at least two quality parameters "out of control" as an indication of a critical performance of the laboratory, 33% of the laboratories were included in this group.
Conclusions: The results of this study will be the basis for implementing a second pan-European EQA and the results of both EQAs will be pooled and will provide the basis for the implementation of evidence-based.

T117

APPROPRIATENESS PRE-PRE-ANALYTICAL QUALITY CONTROL REPORT AND PROCEDURE FOR COMPARING TEST REQUESTING BETWEEN ORGANIZATIONS

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Background: Identifying inadequacies in the use of diagnostic tests is part of the pathologist tasks. For this purpose, the first step would be to examine the use of tests in one's laboratory as compared with others, to examine overspending and to optimize the use of testing. The aim of the study was to show the procedure, and also the indicator results report to be used to compare requesting variability between organizations.
Methods: A call for data was posted on a website (www.redconlab.es). Spanish laboratories willing to participate in the study were invited to fill an enrollment form and submit their results on-line. With data collected, 2 types of appropriateness indicators were calculated: request rate per 1000 inhabitants and related requesting ratios. A frequency histogram and a box plot were constructed for each indicator. A results report was prepared; each report had 1 sheet for every indicator. As an example, results related to iron and ferritin requesting by General Practitioners are showed.
Results: We obtained production statistics for year 2010 from laboratories at 37 distinct hospitals from different regions all over Spain. The results report was sent to each participating laboratory indicating graphically their individual results as compared to the others. A media of 131.7 per 1000 inhabitants for iron and 130.9 per 1000 inhabitants for ferritin was showed. Regarding the related requesting ratios (Iron/Ferritin) 1.025 was obtained.
Conclusion: We have showed a practical and effective methodology for identification and monitoring of pre-pre-analytical test requesting behaviour taking advantage of the data from every laboratory database. An appropriateness pre-pre-analytical quality control report based on appropriateness indicators has been designed and sent to every laboratory to visualize at a glance the individual requesting situation when compared with others. It can be used for future efficient feedback between different laboratories, or intra-laboratory over time, to monitor requesting after corrective measures establishment.

T118

AN AUDIT OF IN-HOSPITAL VITAMIN D REQUESTING AND RESULTS; IMPLICATIONS FOR DEMAND MANAGEMENT

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Introduction: Laboratories have reported increasing numbers of requests for 25-hydroxy vitamin D (VitD) levels. This has a significant impact on managing workload within the laboratory. Our hospital has 3-sites, each with its own biochemistry laboratory and is currently processing ~20,000 VitD in-hospital requests/annum. We issued local intranet-based guidelines to prevent repeat requesting within 3 months, previously. We thus examined VitD requests and results to determine sources of requests, frequency of repeat requests and trends in results over a 3-month period.
Methods: Data on requests originating from one hospital site (without renal services) were retrieved from Dec 2011 to March 2012. VitD results were recorded (measured using Abbott Architect immunoassay and if >150 nmol/L confirmed with liquid chromatography tandem mass spectrometry). Levels were classified as severely deficient (<25 nmol/L), deficient (25-50 nmol/L), insufficient (50-70 nmol/L), replete (70-150 nmol/L) or possibly toxic (>150 nmol/L). Origin of requests were categorised as in-patient or out-patient, and by specialty.
Results: 1971 requests were processed. 57% of requests originated from adult medical specialties. The top 5 requesting departments were, paediatrics (22%), endocrinology (13.7%), HIV medicine (13.4%), rheumatology (10.2%) & obesity (7%). 78% were out-patient clinic requests. 20% of requests were repeated at least once in the 3-month period. 60% of results were severely deficient or deficient, 21% replete and 0.8% possibly toxic. Median VitD level was 43 nmol/L (28-67 inter-quartile range), but was significantly different across specialties, with lower levels in paediatric, geriatric and HIV medicine ($P < 0.05$).
Conclusion: There is widespread requesting of VitD across all specialties within the hospital. A significant proportion of patients are VitD deficient. A large number of requests originate from HIV and paediatric services. Increasing requests from specialist services may reflect the need for specific guidance in these areas. There is still a significant repeat-requesting rate, despite local guidance. Effective demand management will require targeted education and guidance as well as electronic decision support to prevent repeat testing, in keeping with guidelines.

T119
HOW TO PROCESS BLOOD SAMPLES FASTER IN A ROUTINE CLINICAL BIOCHEMISTRY LAB

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Background: Inspired by the results obtained over a multi-year period by the Clinical Biochemistry Department at Gentofte Hospital (DK), we set out to implement a similar project in our department in a much shorter time frame. The goal is to analyse 90% of samples from in-hospital patients within an hour from reception time in the laboratory.
Method: We are focusing on samples from in-hospital patients and selected analyses within the areas of hematology, coagulation and general biochemistry.
Results and discussion: The project is divided into different stages: 1) extraction and analysis of data, 2) changes of the daily routines in the lab, 3) emphasis and attention on implementing the project from the lab management. A flexible system was developed using Microsoft Excel 2010 and the add-on programme PowerPivot containing several years of data. Cumulative charts were created for monitoring the effects of the different changes in the daily work routines. We focused on simple and easy-to-manage changes in the lab such as continuous activity at the work station and keeping a steady flow of samples. Before commencing the project about 60-65 % of samples from in-hospital patients were analysed within 1 hour from reception time in the lab. After two months of working on the project, an increase to almost 80% was observed and after an additional 2 months it was possible to analyse 90% of the samples within 1 hour.
Conclusions: During the summer of 2011 a project was initiated with the aim of processing blood samples in a faster manner at the Department of Clinical Chemistry at Frederiksberg Hospital. In the first months a major effect was observed and it was possible to analyse 90% of samples from in-hospital patients within an hour from reception time in the laboratory. A year later, the project was finished and the changes were implemented into the daily work routines. We still monitor response times closely and have been succesfull in expanding the amount of analyses included.

T120
LABORATORY INFORMATION SYSTEM IN MEDICAL DIAGNOSTIC LABORATORIES IN AN URBAN CITY IN WESTERN NIGERIA

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Background: Laboratory information system (LIS) is a class of software that receives processes, and stores information generated by laboratory processes. These systems often interface with analytical instruments. Its application has reduced errors in all phases of the testing process, as well as reduced turn-around time (TAT) resulting in improved quality of laboratory test results. Therefore, the aim of this study was to determine the use of LIS in diagnostic laboratories.
Methods: This was a descriptive study of diagnostic laboratories in Lagos, an urban city in western Nigeria, using interviewer-administered questionnaires and visual inspection of Laboratory information system facilities.
Results: Ten laboratories (7 private and 3 public) were studied, and six (60%) had functional LIS. Of the remaining 4, 2 (20%) were unaware of LIS, while the other 2 (20%) claimed it was too expensive to implement. Three different types of LIS were identified in the six laboratories. Two had features supporting preanalytical (patient and physician demographics, order entry, sample integrity check), analytical (programming analysis, quality control), and postanalytical (result entry, reporting) phases, while one had features supporting only the preanalytical phase. The 6 laboratories with LIS had a shorter average TAT of 5-10 min, 5-15 min, and <2 min for the preanalytical, analytical and postanalytical phases respectively for emergency samples; compared to those without LIS where the emergency samples' average TAT was 10-30 min, 30-120 min, 30-60 min for the preanalytical, analytical, and postanalytical phases respectively. The 6 LIS-laboratories claimed that LIS was inexpensive to implement. However, they had challenges with frequent internet down-time and power outages.
Conclusion: LIS with varying applications are in use due to its advantages of limiting errors, especially preanalytical, and reducing TAT. Despite the perceived notion that LIS is costly, laboratories implementing it disproved this. Awareness, constant power supply and improved internet connection is required to maximize the benefits of LIS.

T121
THE FIRST NATIONWIDE MULTICENTRE REFERENCE INTERVAL STUDY IN TURKEY AND THE FINDINGS

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Background: The Committee of Reference Intervals and Decision Limits (C-RIDL) within the International Federation of Clinical Chemistry (IFCC) is currently coordinating the worldwide multicentre study on reference values by use of the common protocol. Since studies to determine reference intervals (RIs) in Turkey have been few and limited, we collaborated in the study to establish RIs in Turkish population for commonly tested biochemical analytes and to explore sources of variations in reference values including regionality.
Methods: Blood samples were collected from healthy individuals according to the IFCC/C-RIDL protocol at 28 participating laboratories from seven areas in Turkey (≥400 samples from each area,3188 in all). The serum specimens were sent to the central laboratory in Uludag University in Bursa where 26 biochemical tests were collectively measured. As a cross-check study, the participating laboratories measured a part of the samples (n=20-25) for the same tests and the results were compared by linear regression analysis. RIs were calculated by parametric method using the modified Box-Cox formula and compared with the values calculated by non-parametric method. Three-level nested ANOVA was used to quantitate variation (SD) of test results due to sex, age and region. A ratio of SD for a given factor over residual SD (SDR) exceeding 0.3 was considered significant. Multiple regression analysis (MRA) was performed for each sex to analyze association of test results with age, BMI and region.
Results: No apparent regionality exceeding SDR>0.3 was observed in any of the analytes. Significant sex- and age-related differences were observed in 9 analytes (HDL-C, ALT, etc) and in 5 analytes (TG, LDL-C, etc), respectively. RIs determined by parametric method were mostly narrower than those calculated by non-parametric method. By MRA, BMI-related changes were observed for UA and ALT in male, for TG, UA, HDL-C and ALP in female after adjustment for age and region. The cross-check analysis revealed that the test results were well standardized among the laboratories.
Conclusions: With lack of regional differences and well standardized status of test results, the RIs derived from this nationwide multicentre study can be used in common for the Turkish population.

T122
APPLICATION OF SIGMA METRICS FOR THE ASSESSMENT OF QUALITY ASSURANCE IN CLINICAL BIOCHEMISTRY LABORATORY IN BHAVNAGAR

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Introduction: Ensuring quality of laboratory services is the need of present time in the field of health care which required quality planning, quality control (QC), quality assessment (QA) and quality improvement. Keeping in mind the usefulness of six sigma concept, which is an evolution in quality management that is being widely implemented in the corporate world and is also considered as the universal measure of quality. Health care sector can be benefited by implementation of Six sigma for quality assurance, which provides a general methodology to describe performance on sigma scale and it also provides a more quantitative framework for evaluating process performance and more objective evidence for its improvement. We aimed to gauge our laboratory performance by sigma metrics.
Material & Method: In the present study, internal and external quality control data were analyzed retrospectively for the period from June to September 2012. Laboratory mean, standard deviation, coefficient of variation and bias were calculated for the selected parameters (Glucose, Total protein, Cholesterol, Triglyceride, HDL, LDL, SGPT, SGOT, ALP, Amylase and Uric acid). Sigma was calculated for level I & II of IQC.
Results: Satisfactory sigma values (≥3) were elicited for blood glucose, ALP, Total protein, Triglyceride, HDL, uric acid and amylase while, SGPT, SGOT and cholesterol performed poorly on the sigma scale.
Conclusion: The findings of our exercise emphasize the need for detailed evaluation and adoption of quality measures in order to improve six sigma standards for all the analytical processes. Although Six Sigma provides benefits over prior approaches to quality management, it also creates newer challenges for laboratory practitioners.

T123
TURNAROUND TIME IN EMERGENCIES

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Background: The total response time (RT) is an indicator of laboratory quality, which affects diagnosis and treatment. It is defined as the interval between the arrival time of a sample at the laboratory and the time of clinical validation. Our goal was to evaluate the RT for emergency analytical biochemistry, studying the workflow and the preanalytical, analytical, postanalytical and total phases, in order to plan possible actions to improve the new work procedure recently implemented.
Methods: We analysed the data of the STAT requests of 6 days, and separated them into three periods: 1 (8-10 h), 2 (10-12 h) y 3 (12-14 h), representing different workloads. RT was collected (in minutes) for each period and phase. The data were processed with Excel 2010.
Results: There was an average of 18 samples per day, with 4.5, 7.8 and 5.7 for period 1, 2 and 3 respectively. The average number of determinations per sample was 9. 42% of samples were tested for six determinations (glucose, urea, creatinine, sodium, potassium and chloride). The average RT was 33.76, 15, 12 and 60.76 minutes for preanalytical, analytical, postanalytical and total phases, respectively. For periods 1, 2 and 3 the average total RT was 85.59, 56.11 and 47.47 minutes, respectively.
Conclusions: During the periods when our emergency samples were mixed with routine Hospitalization samples (8-10 h) and Primary Health Care samples (10-12 h), the total RT was higher, mostly because of the time spent in the preanalytical phase. We have to analyse which factors influence this phase and prioritize urgent samples in order to adapt to health care needs.

T124
CLOSE MONITORING OF TEMPERATURE AND TIMING OF SAMPLE TRANSPORT IN A LARGE HOSPITAL IN SUMMER TIME

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Background: Handling and intra-hospital transfer of biological samples can affect analytical results, given the temperature variation and the time delay itself. Standardization of transport methods and temperature monitoring need to be pursued, particularly in centralized laboratory, where long distance may need to be covered. We decided to investigate timing delay and temperature variation occurring from blood draw moment and sample centrifugation; we conduct a survey in summer and we valuated the possible advantages of a new transport box that allows time and temperature monitoring.
Methods: Careggi hospital is located in several buildings with an area of over 2 square Kms. Internal biological transport is assured by cars on request for emergency and scheduled for routine samples. The survey was performed monitoring time and temperature of routine delivery from 4 different units care in august 2012. Three clinical wards were located in the same building nearby the laboratory (200 m) and the forth in a structure more distant (1 Km). The latter and two of the previous three used specific transport box (H-BIN Biotransport, Becton Dickinson UK) and the samples were tracked by a monitoring system (BD T&T, Becton Dickinson UK). The forth unit carried on with the standard transport system becoming the naïve condition reference. A total amounts of 219 shipments were monitored, 169 employing specific transport box and 50 using traditional bag provided by a thermometer for temperature registrations.
Results: Overall median transfer time was 51 min (range 30–123 min); noticeably no difference was found between buildings location. Mean shipments temperatures resulted 26.0 °C and 27.3 °C with and without transport box respectively ($P < 0.01$).
Discussion: The close monitoring of sample shipments allows to verify the quality of pre-analytical phase and to underline possible drawbacks in samples transfer. The use of specifically designed transport boxes resulted in a closer temperature control even if the transport temperature does not appear to be a major problem. Ninetieth percentile of transport time is a criticism in our pre analytical phase; a fixed limit of shipment time can be used to avoid the analysis of sensible tests with significant pre analytical improvements

T125

STRATEGIES TO IMPROVE CLINICAL LABORATORY OUTCOMES THROUGH BALANCED SCORECARD MANAGEMENT SYSTEM: TEN YEARS EXPERIENCE.

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Background: Balanced Scorecard (BSC) is a management system, the necessary tool to achieve Vision through four perspective objectives with their related indicators, their goals and strategic initiatives. The aim was to show how the use of a BSC management system, by applying strategies oriented to customer needs, improved laboratory performance in terms of turnaround time (TAT).
Methods: It was decided to establish TAT improvement as a Laboratory strategic line in Personalized Chemistry Unit. TAT key performance indicator (KPI) was devised as the percentage of certain key tests, the most requested tests per unit analyzer and/or knowledge area (Thyrotropin, Hepatitis C virus antibody, Carcinoembryonic Antigen, Carbohydrate antigen 19-9), that were verified the same day of the phlebotomy before 15 p.m. To plan the strategy for TAT improvement, laboratory work groups were defining over time strategic objectives and indicators with specific targets, to be obtained through initiative strategies. Objectives were established depending on the different requesters. KPIs targets were, accordingly, established over time. Also, the number of test request modifications, were measured to check if the personalized attention was maintained over time in the Personalized Chemistry Unit: number of tests that were added by the laboratory staff to the physician request and number of requested tests that were cancelled, or replaced by a more appropriate one.
Results: In October 2003, as strategy initiative, report based on post analytical TAT KPIs was hold in the laboratory intranet, being established a 95% target value before 15h for every primary care KPI, and a 99% target KPI value when related to inpatients. In January 2008 a KPI target value of 90% before 12 h was established for inpatients. TAT improved over time and number of modified requested tests was maintained. Clinician satisfaction improved from 7 to 8 since 2004.
Conclusions: We have showed how laboratory performance can be enhanced, through a KPI strategy using BSC as a management system. Improving laboratory performance can be achieved using business models, through communication and teamwork, which are the basis for the improvement of patient management and health care system efficiency.

T126

CONTROL AND CHECK OF PROCESS IN THE MULTILAB: THE ANSWERS IN THE DASHBOARD

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Since 2005 in Modena has been build a multi laboratory serving 7 hospitals and over 700.000 citizens.The multilab consist in 4 different laboratory,logically concentric,for type of service provided that ranges from a panel of health-supporting tests,up to all the possible test that can be delivered.The management of the multilab,has highlighted the need of a management tool streamlined,intuitive and flexible which enables the retrieve of immediate information,from the macro events up to details of a single event with real-time checks of the production efficiency of the laboratories themselves.
Aims:In order to solve the problem,we develop an ICT solution,trying to realize a single framework,in which different information could be linked in one logical point of view.The software is a web application developed using SpagoBi, an open source platform of business intelligence,where all have been reorganized in order to make them available in a fast and intuitive way.It collect and connect data from Laboratory Information System,from warehouse management system and from a software used to manage medical devices.
Result:The dashboard provides information both in graphical and tabular views,allows an outlook from the macro department data,down to the analytical activities carried out by a laboratory field,provides different types of queries allowing comparison of the results in different time frames.In real time it was possible to verify the operational effect induced by the application of appropriateness rules applied in prescribing laboratory tests,both in terms of reduction of activity per prescriber that real savings of consumables.In addition,the dashboard allowed the monitoring timing and amount of samples processed and to suggest corrective methods to eliminate any “bottlenecks” optimizing the distribution network and delivery of materials. The development of IT solution in laboratories is now consolidated. Available data are numerous,but collecting and correlate them in order to have a benefit in a short time is sometimes a difficult obstacle,especially for multi-laboratories. This dashboard gave us a concrete answer to the management of the laboratory,providing significant improvement in terms of efficiency and effectiveness based on objective data.

T127

OPTIONS FOR IMPROVING TURNAROUND: COMPARISON OF POINT-OF-CARE AND RAPID SERUM TUBE FOR CHEMISTRY TESTS

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Background: Meeting clinical turnaround demands, particularly for high-acuity patient areas, is a common problem for laboratories. A variety of options exist for shortening turnaround, including point-of-care (POC) tests and use of collection tubes containing thrombin to accelerate clotting (rapid serum tubes, RST). Each option must be weighed in terms of cost, feasibility, validation, and reliability of results.
Methods: 3 high-acuity areas at our 244-bed hospital initiated POC testing for select needs: blood creatinine (radiology), PT/INR (emergency department, ED), and a limited chemistry panel (intensive care unit, ICU). At the same time, 3 areas (ED and 2 chemotherapy infusion units, AIC and H/O) replaced standard serum gel tubes with RST for chemistry tests.
Results: The POC creatinine test showed a small (<0.5 mg/dL) systematic positive bias relative to the core lab test; to address this, all POC creatinines resulting in low glomerular filtration (GFR) estimates must be confirmed by the core lab test. Despite this, the number of stat creatinine requests from Radiology has decreased dramatically as the majority of results do not alter interpretation of the GFR. Validation of the PT/INR POC instrument was also problematic: use in the ED is considered off-label and requires nursing to obtain informed consent. The additional workload on busy ED nurses delayed patient accrual, with only 8 of the 20 required patients consented 9 months after the start of validation. RST implementation required substantial up-front work by the laboratory to validate accuracy, reference ranges, and stability, plus additional ongoing work to validate both RST and standard serum for all new tests. RST use also required education of nurses performing line draws. The RST improved turnaround immediately: 82% of serum potassium results met turnaround in the two months after RSTs were introduced in AIC, compared to only 39% in the two months prior to implementation.
Conclusions: After implementation, both POC and RST have improved turnaround and provider satisfaction. Costs and the burden on the lab are higher for both POC and RST use, but for high-acuity areas the benefits to patient care and provider workflow justify the increased cost.

T128

ESTABLISHMENT OF AUTOMATED MONITORING PROGRAM FOR TRANSFUSIONAL IRON OVERLOAD USING ELECTRONIC MEDICAL RECORDING SYSTEM

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Frequent blood transfusion because of chronic hematopoietic dysfunction, such as aplastic anemia and myelodysplastic syndrome, causes iron overload and leads to various organ failure in patients with anemia. To assess the implementation of iron chelation therapy, we analyzed actual situation of transfusional iron overload during a five-year period from January 2007 to December 2011 at Saga University Hospital. Four hundred and nineteen patients who received over 20 units of annual total erythrocyte transfusion were analyzed. Fifteen people received chelation therapy with Deferasirox during the study period and only four patients initiated the chelation therapy according to the medical guide for transfusional iron overload, probably due to the difficulty of precise recognition for total units of erythrocyte transfusion on a patient and the delay for understanding of the status of iron overload. In this regards, we established a new comprehensive program on electronic medical recording system in our Hospital. This program consists of three phases including calculation of total units of erythrocyte transfusion on a patient, automatic ordering of a ferritin test and displaying the results of serum ferritin level to attending physician. We report here, an automated and integrated program for managing transfusional iron overload using electronic medical recording system.

T129
REVISION OF LABORATORY TEST PANELS IN HOSPITAL SETTING

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Background: The laboratory test panels for hospitalized patients, as predetermined group of diagnostic tests to help diagnosis and follow-up, have been widespread for the last 30 years, favoured by the availability of multi-parametric analyzers. The health information systems allow to order test panels in a just few steps. This approach, irrespective of individual patient conditions, is recognized as a possible source of inappropriateness or unnecessary testing, as well of cost growth. The laboratory staff may help clinicians to direct this system towards evidence-based laboratory medicine, keeping its ease and speed.
Methods: Our Laboratory activated a permanent working group for testing behaviour, including clinicians from medical, surgical and anesthesiological departments. The group mission was the overall revision of several laboratory test panels accumulated over the years, in order to achieve better adherence to current clinical guidelines and diagnostic protocols.
Results: The working group stated no. 5 new laboratory test panels to be performed in different medical conditions. A basic panel was defined for medical department, including 21 tests. Moreover the group established the standard laboratory test panel for preoperative anesthesiological evaluation, including 11 tests. Finally, a complete protocol for management of acute pancreatitis was identified, with 23 tests at admission, 9 tests at routine control on 2^{nd} and 7^{th} days, and 9 tests before and after endoscopic retrograde cholangiopancreatography. Consequently no. 79 corresponding active panels were deleted, as redundant or inappropriate, to encourage the order of patient-oriented and disease-specific tests.
Conclusions: Test panels are a tool to reduce the variability in laboratory testing orders among clinicians, but a peer management of laboratory test ordering is needed, involving laboratory professionals and clinicians. This cooperation may improve the efficiency and cost effectiveness of suggested test panels.

T130
APPLICATION OF LEAN AND AUTOMATION TO IMPROVE STAT LAB TURNAROUND TIME

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Background: National Cheng Kung University Hospital is a 1130-bed teaching hospital serving southern community in Taiwan. Staffs were limited to handle the highly complexed processes of STAT testing including automation and manual examination. Over 700 sample volumes and 3500 assays per day needs to be reported within 1 hr at 95% turnaround time (TAT). This study aimed to simplify and standardize the existing processes and to cope with increasing workload using a combination of lean study and automation system.
Methods: Phase 1 of the project involved a lean assessment, using process mapping of both specimen flow and related operator activities, in order to identify waste in existing processes. We redesigned the workflow and operator activities by using Ortho EnGen™ automation and 2 VITROS systems. Phase 2 of the project involved construction within the facility and redesigned analytical area to be moved closer to specimen reception.
Results: Our results indicated that lean assessment in phase 1 identified wastes in a large portion of time where specimens were waiting between process steps and a long distance of specimens delivery between reception to analysis. The combination of redesigned workflow and implementation of automation reduced 67% process steps and 67% manual dilutions. After phase 2, overall TAT was improved by 3.2% (from 94.3% to 97.5%). The average TAT of critical assay glucose and potassium were decreased by 25% (from 40 to 30 minutes) and 37% (from 49 to 31 minutes), respectively.
Conclusions: Performing the lean component prior to automation may be essential to avoid automating bad processes. In this study, the combination of lean and automation has markedly reduced process steps, manual dilutions and improved TAT for making prompt clinical decision in STAT laboratory.

T131

ASSESSING THE KNOWLEDGE OF JUNIOR MEDICAL STAFF OF BIOCHEMISTRY TESTS AND THEIR INTERPRETATION

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Biochemistry laboratories in the UK have seen a steadily rising workload and it is often the most junior doctor who requests biochemistry tests so it is important that they have an understanding of their relevance both in order to reduce inappropriate requesting,in ensuring proper interpretation of the results obtained and better patient management. To assess their knowledge,a quiz was held after the first four months in post where they were asked to respond either true or false to a number of biochemical tests and their use in a number of clinical scenarios. These included the value of serum protein electrophoresis, the use of creatine kinase and troponin in suspected myocardial infarction, value of the commonly requested tumour markers AFP, CA125, CEA, CA15-3 and PSA, causes of hyperkalaemia and hypophosphataemia, advantages of measuring C reactive protein (CRP) over the erythrocyte sedimentation rate (ESR), value of measuring certain therapeutic drugs,interpretation of the short synacthen test (SST), interpreting liver function tests,distinguishing biochemically between sodium and water depletion and pre renal from renal failure,assessing the severity of acute pancreatitis using both biochemical and haematological criteria, biochemical changes in metabolic bone disease,biochemical testing in establishing the cause of hyponatraemia,criteria for diagnosing the syndrome of inappropriate antidiuretic hormone secretion (SIADH) and the appropriate use of autoantibodies. In general the highest average scores were for those tests where guidelines were available for junior medical staff such as the appropriate use of tumour markers and of autoantibodies. There were disappointing average scores for questions relating to CRP versus ESR,use of creatine kinase and troponin in suspected myocardial infarction,interpretation of the SST,distinguishing pre renal from renal failure and sodium from water depletion, establishing the cause of hyponatraemia and criteria for diagnosing SIADH. Feedback to the quiz was positive and it is intended that further guidelines will be made available to help junior medical staff to make more appropriate use of the biochemistry tests available to them.

T132

HAPLOGROUP ANALYSIS OF THE RISK ASSOCIATED WITH APOE PROMOTER POLYMORPHISMS (-219T/G, -491A/T AND -427T/C) AND ALZHEIMER DISEASE IN A TUNISIAN POPULATION

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Background: Alzheimer's disease (AD), in its sporadic form, is a paradigmatic model of complex diseases resulting from the interaction between genetic and non-genetic factors such as environmental ones. The most important risk is conferred by the e4 allele of Apolipoprotein E (APOEe4). However, since APOEe4 is neither necessary nor sufficient for the development of the disease; it is believed that other genetic factors modulate its risk, either alone or in conjunction with APOEe4. Several studies analyzing the APOE promoter polymorphisms have produced contradictory results, probably due to differences in the intrinsic genetic background of the populations studied. In this study, we have analyzed three APOE promoter polymorphisms (-491A/T, -219T/G and -427T/C) in order to evaluate the major haplotype associated with AD in a Tunisian population.
Methods: Our study included 85 patients and 90 controls recruited at the consultation of memory of the Neurology Department. The diagnosis of AD was selected according to the DSM IV and NINCDS-ADRDA. All patients underwent neurological and neuropsychological examinations, and brain imaging. Genomic DNA was extracted from peripheral blood leukocytes by the phenol/chloroform protocol and the salting out procedure. Genotyping was performed using the PCR restriction fragment length polymorphism (PCR-RFLP) method.
Results: The frequency of the T allele of -491A/T polymorphism resulted higher in AD than in controls (45.30% vs 32.78%). In the analysis of -219T/G and -427T/C polymorphisms no difference was found between AD and controls. The ATG haplotype resulted the most represented in both AD and controls (34.1% and 32.8%, respectively), followed in decreasing order by the ATT haplotype (16.5% vs 30%, respectively), TTT haplotype (28.2% vs 14.4%) and TTG haplotype (11.2% vs 15%). In AD or in controls, all the other haplotypes showed a frequency lower than 10%.
Conclusion: This study provides additional evidence that the APOE gene plays an important role in the development of AD. However, future studies on larger cohort of AD might further increase the power of the analysis and confirm the possible risk/protective effects associated with these polymorphisms/haplotypes and their complex interaction.

T133
ANTI-LIPID PEROXIDATION AND HEPATOPROTECTIVE EFFECTS OF CORCHORUS OLITORIUS EXTRACT AND FRACTIONS

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Background: Nature is and will still serve as the man's primary source for the cure of his ailments. In West Africa,Corchorus olitorius leaves (Jute plant) are commonly consumed leafy vegetable speculated to have several medicinal values. This study investigated the anti-oxidative efficiency,anti–lipid peroxidation with the hepatoprotective potential of the extract and fractions of Corchorus olitorius leaves in-vivo and in-vitro.
Methods: Phenol and Flavonoid contents of the extracts prepared in appropriate solvents were measured by Folin Ciocalteu and Aluminium chloride assays. Hydroxyl radicals scavenging effect and Malondialdehyde (MDA) levels were determined using deoxyribose assay and the reaction of MDA with Thiobarbituric acid. Enzyme assays were done using standardized methods by the International Federation of Clinical Chemistry while the cholesterol determination was based on the quantification of cholesterol released from enzyme hydrolysis and oxidation of cholesteryl esters.
Results: The extract and fractions inhibited the generation of hydroxyl radical and lipid peroxide respectively. Rats orally exposed to the methanol extract for 21 days at 50, 100, 150 and 200 mg/kg body weight significantly (P <0.05) reduced MDA in concentration dependent manner 2.02,1.34,0.76 and 0.02 mg/ml respectively. Interestingly at the same concentrations, significant increases in the tissue level of glutathione (GSH) were observed (52.80, 68.7, 81.80 and 88.80 μg/mL). Administration of the extract (100 mg/kg) also significantly (P <0.05) reduced sodium arsenite induced hepatotoxicity in rats, as judged from the serum and tissue activity of marker enzymes (Alanine aminotransferase, Aspartate aminotransferase and Gamma glutamyl transferase). Compared with the intoxicated animals, the extract reduced cholesterol concentrations. Extract and fractions contained various amount of phenol and flavonoids from (10-130 mg/g) and (12-65 mg/g) respectively. Conclusions: Diets rich in polyphenols are epidemiologically associated with lower risk of some neurological age-related diseases in humans. This correlates with the ability of the extract to reduce MDA and cholesterol level,boost GSH and shows hepatoprotective ability. This extract may be explored as therapeutic agent in future

T134
THE RELATIONSHIP BETWEEN ONE-CARBON METABOLISM AND HEAVY METALS IN ALZHEIMER'S DISEASE

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Background: In many studies, elevated plasma homocysteine levels were demonstrated in Alzheimer's disease as a result of one-carbon metabolism impairment. In the same patient group, elevated plasma and brain heavy metals which may affect the vitamin B12 using, were also demonstrated but these changes were not clarified in the pathogenesis of the disease and relationship between each other.
Methods: For this purpose, we measured serum vitamin B12, folat, urine methylmalonic acid (uMMA), plasma total homocysteine (tHcy), amino acids, mercury, cadmium, aluminum, selenium, and arsenic in 62 Alzheimer patients and 25 controls. Alzheimer group was separated into two groups according to the minimental state examination (MMSE) test scores.
Results: In the Alzheimer group, tHcy, serum cysteine, serine, glycine, glutamate, glutamine, proline, histidine, ornitine and mercury levels were found to be statistically higher than the control group (P <0.05). Also folate, selenium, cadmium levels and CK enzyme activities were found to be statistically lower than the control group (P <0.05). In Alzheimer group, serum folate levels were negatively correlated with serum cysteine, serine, proline, glutamate, glutamine and ornitine levels. tHcy levels of patients (15.3±7.3 μM)were positively correlated with serum cysteine, glycine and ornitine levels (P <0.05). Serum cysteine, serine, glycine, glutamate, glutamine, proline, histidine and ornitine levels were statistically higher (P <0.05) and the CK enzyme activity was lower (P <0.01) especially in the severe Alzheimer patients (MMSE score <16). Also in those patients, serum phenylalanine, asparagine and lysine levels were found to be statistically higher than the others (P <0.05) . There was not any statistically significant relationship between blood metal element levels and one carbon metabolism parameters in patient group.
Conclusions: These findings demonstrated an impaired one-carbon metabolism which is not related to heavy metals in patients with Alzheimer's disease.

T135

HYDRATED C(60) FULLERENE AMELIORATE GENE EXPRESSION PROFILE OF TRPM2 AND TRPM7 DISTURBED BY HYPERHOMOCYSTEINEMIA

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Background: HHcy caused apoptozis brain and cardiovascular tissue by induced oxidative stress. Transient receptor potential melastatin 2 (TRPM2) and TRPM7 channels are calcium channels and activated oxidative stress. These channels expressed in brain, heart and vessel and important role in H(2)O(2) induced neural injury and apoptosis. Due to marked antioxidant activity, C60 fullerene, the third natural allotropic form of carbon, and some of its water-soluble chemical derivatives have recently gained considerable attention as promising candidates for many biomedical applications, in particular, at neurodegenerative states. We aimed to examine the effects on TRPM2 and TRPM7 gene expression of homocysteine and C60.
Methods: C57BL/6 J. mice were divided into four groups of 10 animals each: (1) control group, (2) C60HyFn-treated group, (3) methionine group and (4) C60HyFn-treated methionine group. HHcy was induced by methionine administiration. After the animals were decapitated at the end of the 5th week, the blood was collected, brain, heart and aorts were removed. TRPM2 and TRPM7 gene expression were detected Real time PCR. Apoptosis in brain, heart and aorts were assessed by TUNEL staining and DNA fragmentation methods.
Results: TRPM2 and TRPM7 gene expression are significantly increased HHCy group. C60HyFn significantly decreased serum levels of homocysteine, reversed Hcy induced oxidative stress, decreased DNA fragmentation, apoptozis and TRPM2 and TRPM7 gene expression in the mouse brains, hearts and blood vessels.
Conclusions: Our results provide preliminary indication that Hhcy may be increased TRPM2 and TRPM7 expresion in examined tissues by induced oxidative stress. C60HyFn decreased TRPM2 and TRPM7 gene expression in examined tissues. This protection is most likely mediated by C60HyFn-induced up-regulation of antioxidant capability of tissues. In conclusion, we have presented for the first time substantial evidence that administration of C60HyFn significantly reduces homocysteine level in blood, oxidative stres induced apoptozis, TRPM2 and TRPM7 gene expresion and Hhcy may increased TRPM2 and TRPM7 gene expression.

T136

CONCORDANCE BETWEEN DETERMINATION OF OLIGOCLONAL BANDS IN CEREBROSPINAL FLUID, FREE KAPPA INDEX AND THE INTRATHECAL SYNTHESIS IN PATIENTS WITH INFLAMMATION OF THE CENTRAL NERVOUS SYSTEM

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Background: In inflammatory diseases of the central nervous system (CNS), like multiple sclerosis (MS), a highly restricted number of clones of B cells are activated within the CNS in cerebrospinal fluid (CSF) and they are transformed into immunoglobulin-secreting plasma cells.The measurement of oligloclonal bands (OCBs) in CSF is based on that premise because OCBs are the best biological marker for predicting clinical MS in patients with clinical symptoms. Besides the determination of OCBs in CSF, other important parameters for a possible diagnosis of inflammatory diseases of the CNS are the Tibbling index, the Tourtellote index and the free kappa index. In this study we relate the indices with the presence or absence of OCBs and age because these indices are influenced by age.
Methods: We studied 66 patients from different hospitals in Spain between November 2011 and February 2012. We obtained a blood and CSF sample from each patient who met the study criteria (patient with suspected CNS inflammatory disease). With the CSF and serum samples, we performed a quantitative analysis of IgG, IgA and IgM by nephelometry (BNII Siemens) and the qualitative analysis of OCBs by isoelectric focusing and transfer followed by immunodetection. The free light chains (FLCs) were measured by FREELITE (The Binding Site) turbidimetric assay. Tibbling index, Tourtellotte index and K index was determined for every patient. Patients were divided into 2 groups based on the presence (n=22) or absence of OCBs (n=44). Mann Whitney-U test was used for the comparison of median. ROC curve was plotted for every index.
Results: K index (111,02 vs. 17,37), Tibbling index (0,69 vs.0,52) and Tourtellotte index (4,41 vs -1,73) were significantly elevated in MS than in patients with other inflammatory disease of the CNS (P <0,001). The area under curve (AUC) was highest for Tibbling index (0,892) followed by Tourtellotte index (0,805) and K index (0,778) respectively.
Conclusion: The study of the CSF parameters in association with clinical data, help us to diagnose MS. K index, Tibbling index and Tourtellotte index differ significantly between patients with MS than other inflammatory disease of the CNS. Tibbling index has a very good diagnostic value in the MS than the other indices.

T137
CLINICAL UTILITY OF IMMUNOGLOBULIN FREE LIGHT CHAINS MEASUREMENT IN CSF

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Background: intrathecal immunoglobulin synthesis (IIS) detection is the best Cerebrospinal Fluid (CSF) marker for the diagnosis of inflammatory process of the central nervous system (CNS) and CSF Isoelectric focusing (IEF) with IgG immunodetection is the gold standard. Immunoglobulin light chains are synthesized in a higher rate than heavy chains; free light chains (FLC) are secreted in blood and pass in little amount the CSF-blood barrier. Since in case of IIS there is also an increase of FLC in CSF, FCL can be used as markers. It has been shown that CSF FLC has the same sensitivity as CSF IgG IEF, however, i) some studies compare FLC to electrophoresis (not to IEF) or quantify only free kappa; ii) there are differences in reporting i.e., CSF FLC absolute value vs. CSF FLC index. To assess the clinical value of SCF FLC we studied 24 patients with definite diagnosis of Multiple Sclerosis and 54 patients with non inflammatory disease in CNS.
Methods: IEF was performed in agarose macrogel, blotting and IgG immunodetection. Free Kappa (FK) and free Lambda (FL) chains were measured with Binding Site reagents on Delta nephelometer (Radim). CSF FK or FL were evaluated as FLC Index (FLC CSF/serum / Albumin CSF/serum), or absolute value and validated by ROC curves.
Results: both absolute value and indices were different in the two populations (P <0.0001). The FK Index cut off value 7.82 had 95% sensitivity and 98% specificity. The FK value cut off 0.56mg/L had 92% sensitivity, 93% specificity. The FL index cut off 4.36 had 83% sensitivity and 98% specificity. The FL value cut off 0.31 mg/L had 75% sensitivity, 94% specificity. Summing FK and FL indices, the cut off value 12.41 reached 100% sensitivity, 100% specificity. Summing FK and FL values, the cut off 0.92 mg/L had 92% sensitivity, 94% specificity. IEF had 96% Sensitivity, 94% specificity.
Conclusions: FLC indices perform better than total values, showing that CSF-blood barrier and serum values affect CSF values. Adding FK and FL indices, we obtained excellent results, showing that both FK and FL are necessary for diagnosis. We confirm that CSF FLC perform as IEF. CSF FLC is a promising test for clinical use, as it is automated and data are shortly available, while IEF is time consuming and operator-dependent.

T138
AMINOACID PROFILE IN PLASMA DURING THE ACUTE PHASE OF STROKE

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Background: In the last years, researchers have found early markers that can facilitate the rapid classification of the type of cerebral vascular disease and benefit from the most appropriate therapy in each case to minimize possible side effects. Our objective was to compare plasma amino acid profile (AAP) changes in patients with acute cerebral vascular disease with healthy controls and to determinate if such pattern provides information about etiology of stroke.
Methods: Fyfty healthy subjects and 103 patients served on Urgent care facility and diagnosed (<3.5 h of evolution) with acute cerebrovascular disease by Neurology unit of Complejo Hospitalario Universitario de Albacete were enrolled in a prospective case-control study. Protocol of stroke included neurological assessment on admission and complementary examinations (radiological, ultrasound and laboratory for classifying the subtype etiological stroke). Subsequently, plasma levels of aspartic acid (ASP), glutamic acid (GLU), serine (SER), glutamine (GLN), histidine (HIS), glycine (GLY), threonine (THR), arginine (ARG), taurine (TAU), alanine (ALA), tyrosine (TYR), phenylalanine (PHE), isoleucine (ILEU), valine (VAL) and gamma-aminobutyric acid (GABA) were determined by High Performance Liquid Chromatography.
Results: The study included 78 patients with ischemic stroke and 25 patients with hemorrhagic stroke. The analysis of aminoacids levels (μM) showed that the stroke patients displayed a different AAP of GLU, HIS, GLY, THR, ARG, TAU and ALA respected to control subjects. The plasmatic concentration detected in stroke patients were 123.7±6.5; 65.3±2.1; 168.6±8.1; 94.2±6.1; 54.3±3.1; 80.4±3.8 and 293.3±19 respectively, while control showed levels of 96.3±2.6; 76.7±1.6; 210±9.8; 127±3.7; 69±3.8; 70.2±2.4 and 345±9.5. Statistical analysis by U-Mann Whitney Test reveal these differences to be significant (P <0.05). It is noteworthy that the study detected diferences in AAP between ischemic (GLU: 140±6.7; HIS: 68.5 ±2.1 and PHE: 53.6±4.5) and Hemorrhagic (GLU: 107±6.5; HIS: 58.9±2.9 and PHE: 44.6±1.9) patients.
Conclusion: Together, our findings strongly suggest that the determination of aminoacids pattern in plasma could determine at very early-term between ischemic and hemorrahgic stroke.

T139

SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR AND MYELOPEROXIDASE LEVELS IN OBSTRUCTIVE SLEEP APNEA SYNDROME

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Background: Obstructive sleep apnea syndrome (OSAS) is characterized by recurrent respiratory disorders in the upper airways during sleep. The presence of both systemic and airway inflammation has been suggested in OSAS by increased levels of inflammatory biomarkers in the circulation. Previous reports have shown a positive relationship between severity of OSAS and inflammatory markers. Myeloperoxidase (MPO) is an enzyme stored in azurophilic granules of polymorphonuclear neutrophils and macrophages and released into extracellular fluid in the setting of inflammatory process. Soluble tumor necrosis factor receptor (sTNF-R) is released by proteolysis of the cell-bound receptor under the control of inflammatory cytokines, T cell activation, and by TNF-α itself. There is no study in the literature investigating the both MPO and sTNF-R levels in OSAS patients. The aim of our study is to assess the clinical utility of serum MPO and sTNF-R levels as markers of inflammation in OSAS.
Methods: Serum MPO levels were measured by spectrometric method. sTNF-R levels were studied with ELISA method. 59 patients with OSAS, diagnosed with polysomnograhpy (Apnea-Hypopnea index (AHI) >15 events/hour), and 26 healthy volunteers were joined in our study. Samples were collected after overnight fasting.
Results: Patients with obstructive sleep apnea had a higher incidence of hypertension and body mass index. Serum MPO levels (43.2±21.65 U/L vs 30.44±8.058 U/L, P=0.0046) were significantly higher in the OSAS patients compared with the healthy controls. Serum sTNF-R levels also (2.379±1.209 ng/mL vs 1.086±0.86 ng/mL, P=0.0001) significantly higher in the OSAS patients compared with the healthy controls. MPO levels were significantly correlated with AHI (P=0.031). There was no significant correlation between MPO and sTNF-R levels in the OSAS patients.
Conclusions: OSAS leads to an increase in inflammatory markers. Our data suggest that elevated systemic inflammation may contribute to the disrupted sleep quality in OSAS patients. Therefore, it would be advisable to measure MPO and sTNF-R levels in OSAS patients to determine the level of inflammation.

T140

DECREASED HEME OXYGENASE ACTIVITY IN PATIENTS WITH ALZHEIMER'S DISEASE

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Background: Heme Oxygenase (HO) enzyme is responsible for the degradation of heme to biliverdin and also yields metabolically active molecules, free iron and carbon monoxide. HO has a special importance for aging of lipid-rich central nervous system and various neurodegenerative disorders including Alzheimer's Disease (AD). Our aim was to evaluate HO activities in leukocytes of patients with AD.
Methods: Leukocyte HO enzyme activities were measured by kinetic fluorometric method based on the determination of bilirubin produced from HO- biliverdin reductase coupled enzyme system in 32 AD patients and 30 healthy age and sex matched controls.
Results: HO activity in patient group were significantly lower than those in the control group (0.53±0.31 nmol/hour/mg protein and 1.19±0.83 nmol/hour/mg protein, respectively; P=0.001). In ROC analysis a cut-off value of 0.572 nmol/hour/mg protein was found as giving 66% for sensitivity and 63% of specificity. Positive and negative predictive values for that cut-off value were 65% and 63%, respectively.
Conclusions: To date this is the first study showing lower HO activities in AD patients. The decrease in HO activity may contribute to the progression of the AD by lowering carbon monoxide levels which also found as a neuroactive molecule.

T141
VALPROATE MONOTHERAPY MAY INCREASE SERUM REELIN

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Background: Reelin is a secreted extracellular matrix glycoprotein. He has been suggested to be implicated in pathogenesis of numerous brain diseases. It is known that level of the reelin has been found to be significantly lower in schizophrenia. Contrary, there is no direct evidence between action of reelin and epilepsy. It is assumed that antiepileptic drugs, which are used to treat seizures in epilepsy, may have a secondary influence on other disorders mainly by unknown mechanisms.
Methods: In our study, we investigated the effects of valproic acid (VPA), one of the mostly applied antiepileptic drugs, on reelin, secreted protein which is accused for many brain disorders. After long-term treatment with VPA (10-15mg/kg per day) we determinate level of VPA in serum of 15 young children (age 6.1±1.1 years) and in 12 adult persons (age 31.4±11.5 years) by chemoluminescent method. Simultaneously we determinate level of serum reelin by enzyme-linked immunosorbent assay and these results we compared mutually and with two age appropriate control groups (healthy children and adult). Statistical significance of differences was estimated using the Student's t test. The results are expressed as mean±SD.
Results: Our results showed significant increase in reelin values in patiens with VPA therapy vs. control group (115.55±56.55 ng/mL vs. 52.46±24.23 ng/mL; $p < 0.01$). There is no significant change in reelin between adult and young patients, and also there is no significant correlation between values of reelin and level of valproic acid.
Conclusion: Our results showed a similar answer of different age groups on reelin protein during VPA therapy – increase level of reelin. It may may be a consequence of VPA impact and may contribute to additional understanding to mechanism of valproic acid action and their relationship to reelin. Cellular and molecular mechanisms by which reelin influences on schizophrenia may potentially include anti-epileptogenic therapy as additional therapy in other brain disorders.

T142
ROLE OF KALLIKREIN 6 (KLK6) IN THE DIFFERENTIAL DIAGNOSIS OF DEMENTIA

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Background: Dementia is global loss of cognitive functions. Based on the pathobiochemical process, there are two major types of dementia: Alzheimer's disease (AD) and vascular dementia (VAD). The prodromal phase of dementia is defined as mild cognitive impairment (MCI). Proper recognition of the dementia etiology is necessary in order to assure early and effecitive treatment. Human kallikrein 6 (KLK6) is a serine protease highly expressed in brain. The aim of this pilot study was to evaluate the role of KLK6 as a serum biomarker for differential diagnosis of dementia.
Methods: We recruited 28 patients with AD (18 females, range 56-84 years) and 27 patients with VAD (13 females, range 58-89 years) from the University Department of Neurology. The control group consisted of 11 cognitively healthy individuals (9 females, range 57-78 years) and 14 individuals diagnosed with MCI (10 females, range 53-79 years). The concentration of KLK6 was measured by an ELISA method.
Results: Mann-Whitney test for independent samples was used for statistical analysis of the data. A significant difference in KLK6 concentration was found between AD (median 3.20; range 2.83-3.63 ng/mL) vs. VAD (median 2.54; range 2.25-2.86 ng/mL) ($P=0.004$) patients and in AD (3.20; 2.83-3.63 ng/mL) vs. MCI group (2.61; 2.36-2.74 ng/mL) ($P=0.0145$). KLK6 values were significantly higher in the control group of cognitively healthy individuals (2.97; 2.64-4.38 ng/mL) vs. VAD patients (2.54; 2.25-2.86 ng/mL) ($P=0.0478$).
Conclusions: AD patients display significantly higher serum concentration of KLK6 than VAD patients. More extensive studies are needed to asses clinical utility of KLK6 in the differential diagnosis of dementia.

T143

DETERMINATION OF κFLC AND κ INDEX IN CEREBROSPINAL FLUID: A VALID ALTERNATIVE IN MONITORING INTRATHECAL IMMUNOGLOBULIN SYNTHESIS

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Background: Intrathecal immunoglobulin synthesis is observed in several disorders of the central nervous system, but its detection by current laboratory tests is relatively insensitive and operator depending. We assessed the diagnostic accuracy of an assay for κ free light chain (κFLC) in cerebrospinal fluid (CSF), in serum and compared it with traditional test.
Methods: κFLC were measured by nephelometry in CSF/serum pairs from 45 patients. The samples were grouped according to the presence (n=16) or absence (n=29) of oligoclonal bands evaluated by isoelectrofocusing method. A ROC curve for kFLC concentrations in CSF, kFLC CSF/serum ratio and κ Index (κliquor/ κserum/ Qalbumina x 1000) for blood-CSF barrier function evaluation were performed. Tentatively a cut-off value for kFLC in CSF and for k Index was calculated.
Results: The areas under ROC curves were 0.916 (95% confidence interval, 0.860–0.972) for κFLC concentrations in CSF, 0.944 (0.901–0.987) for κFLC CSF/serum ratio and 0.972 (0.950–0.994), for k Index respectively. Based on the ROC curve the proposed cut-off was 0.5 mg/L for κFLC in CSF and 5 for κ Index. Using this approach would have been misclassified five patients considering κFLC in CSF and only two using κ Index.
Conclusions: κFLC concentrations in CSF and κFLC CSF/serum ratio, identified oligoclonal bands with high specificity and sensitivity, but κ Index seems to be more accurate parameter. Our data indicate that nephelometric assay for κFLCs in CSF reliably detect intrathecal immunoglobulin synthesis. This automated, quantitative and fast method could simplify the diagnostic procedure for CSF analysis. A more comprehensive study is on-going in order to validate cut-offs, to compare patients' clinical diseases and to assess prognostic value of κFLC in patient's CSF with neurodegenerative pathologies.

T144

ASSESSMENT OF S100B AND NSE SERUM LEVELS IN AN ANIMAL MODEL OF INTRACRANIAL HYPERTENSION

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Introduction: Some authors have described the role of Neuron Specific Enolase and S100B protein when predicting mortality after brain injury. In this study we analyze these biomarkers serum levels in an animal model of Intracraneal Hypertension (ICHT).
Methods: This research project was supervised and approved by the animal experimentation committee of our center before its start. They meet all ethical standards for research and legal requirements established by the relevant legislation (Directive 2010/63/EU). The model was performed in Wistar male rats. Animals were anesthetized by intraperitoneal injection of a preparation comprising ketamine hydrochloride 50 mg, 2cc xylazine and atropine 1 mg. Heart rate and breathing were monitored during the procedure. The animals were covered with thermal blankets and a thermal support was used in order to guarantee a temperature of 37 °C and thus prevent the development of hypothermia during the procedure. There will be two study groups: A: rats that were subjected to ICHT model; B (Sham): rats that will not be exposed to the stimulus of ICHT. Five blood samples were taken, unless the animal died before: previous to procedure, just after the procedure, 20 min, 40 min after the procedure and once the animal dies after the injection of a lethal dose of ketamine.
Results: We studied a total of 12 rats, divided into an experimental group of 8 rats and a control group of 4. The distribution of baseline serum levels of NSE and S100B is the same in the experimental and control groups, with the differences between the averages of -0.068 ng/mL (95% CI: -0.158, 0.023), P = 0.128 for basal NSE and -0.383 (95% CI: -0.929, 0.163) for S100B, P = 0.149. Differences were found between the experimental and control group, so that the distribution of the protein concentration of NSE and S100B is the same in both groups (P <0.015). In the experimental group differences were found in the serum levels of both proteins among different points studied (P <0.001), but not in the control group.
Conclusion: Serum NSE and S100B protein levels are raised in Wistar rat Intracraneal Hypertension model.

T145

DEVELOPMENT OF METHODS TO IMPROVE THE EARLY DIAGNOSIS OF DEMENTIA IN BIOLOGICAL FLUIDS

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Background: The clinical syndrome called dementia consists of an acquired impairment of memory and cognition that dramatically affects patient 's life and whose diagnosis occurs when the disease is full-blown. The continuous increase of the disease incidence and the lack of effective therapies have stimulated the search for early markers for discrimination and prevention of this pathology. In this contest we faced two studies : a) based on the analysis of CSF proteomic content by MALDI Profiling in patients with idiopathic Normal Pressure Hydrocephalus (iNPH) subjected to tap test which may represent a unique reversible form of neuronal injury and recovery, and Alzheimer's Disease (AD) to discriminate a neurodegenerative disorder from a disease with a likely vascular pathogenesis, b) to set-up a method for Neuroserpin (NS) quantitation in AD patients. NS is a member of the serpin family more abundant in CSF in AD patients and its quantitation at femtomole level by mass spectrometry could overcome the often erratic and difficult ELISA test.
Methods: 10 CSF from AD and 10 from iNPH patients were profiled by MALDI-MS for the detection of protein patterns which discriminate the two forms of dementia. Statistics was performed by ClinProTools (Wilcoxon test P <0.05, PCA analysis and AUC >0.800). For NS quantization, the SELDI Antibody technology was employed and the LOD and linearity of the method were evaluated.
Results: MALDI Profiling allowed to discriminate iNPH from AD patients through the presence of 18 differentially changed peaks (16 over and 2 underexpressed in AD) in the acquisition range of 4-34 kDa. On the other hand, up to now the NS quantitation set-up allowed to reach the detection limit of 6.7 fmol/uL with a concentration linearity ranging from 3 pmol/uL to 71 fmol/uL (R2=0.99).
Conclusions: Our results show the significativity of CSF profiling encouraging the employment of larger cohorts of samples and adopting selective depletion methods in order to search for further differentially changed peaks originating from low abundant protein species. Furthermore, the set-up of NS quantitation appears promising for being adopted and extended to other bilogical fluids like serum or plasma in order to develope non-invasive tests for dementia diagnosis.

T146

DE NOVO MITOCHONDRIAL DNA ALTERATION IN CHILD WITH COMPLEX NEUROLOGICAL COMPROMISSION

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Background: A wide spectrum of neurological and neuromuscular human diseases have been associated with mitochondrial DNA (mtDNA) variations, causing defects of oxidative phosphorylation. These dysfunctions affect preferentially tissues with high energy demands and give arise to several degenerative disorders such as optic neuropathy, cerebellar ataxia, movement disorders, dementia, muscle weakness and deafness. The extremely heterogeneous clinical phenotype is due to the involved tissue, to specific mtDNA mutations and their heteroplasmic level, but also to nuclear DNA alterations, environmental and epigenetic factors. In this study we investigated a child affected by a complex neurological disease whose clinical features were suggestive of a mitochondrial involvement.
Methods: mtDNA from proband, her healthy relatives (grandmother, mother and two sisters) and 80 controls were collected and studied by sequencing. The enzymatic activity of specific respiratory chain complex was tested on lymphocytes by spectrophotometric assay. Bioinformatic analysis was performed to predict the pathogenicity of the detected variants.
Results: In all subjects we detected 11 known polymorphisms, whereas 1 novel heteroplasmic variant in complex I [ND5:12514G>A (E60K)] was present only in the proband and in her grandmother and absent in controls. The bioinformatics predicted the novel variant to be deleterious. Further, spectrophotometric assay of complex I activity was lower both in the proband and in her relatives than in the controls.
Conclusions: We report a novel mtDNA variant detected in a patient affected by a complex neurological disease. The reduction of complex I respiratory chain activity associated to this variant suggests it could exert a pathogenic role in the disease.

T147

SERUM ISCHEMIA MODIFIED ALBUMIN AND OXIDATIVE STRESS PARAMETERS IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA SYNDROME

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Background: Obstructive sleep apnea syndrome (OSAS) is an increasing major health concern affecting approximately 5% in the adult population. The main pathophysiologic feature in OSAS is characterized by the repeatly cessation of the respiration during sleep due to the obstruction of the upper airway. Several studies show that OSAS causes to increase cardiovascular morbidity and mortality. The increment of the systemic oxidative stress in OSAS has been considered as a major pathogenic mechanism of cardiovascular disease. Several studies assessed only the oxidative stress and/or lipid peroxidation but there is no information about serum ischemia modified albumin (IMA) and there is limited information about serum Advanced Oxidation Protein Product (AOPP) in OSAS. Therefore, we aimed to evaluate the status of AOPP, IMA and other oxidative stress markers in OSAS.
Methods: 59 patients with OSAS, diagnosed with polysomnograhpy (Apnea-Hypopnea index (AHI) >15 events/hour), and 26 healthy volunteers were enrolled in our study. Samples were collected after overnight fasting. IMA is studied with enzyme immunoassay method. AOPP, Total Oxidative Status (TOS), Total Antioxidative Capacity (TAC), Paraoxonase (PON) levels were measured with spectrophotometric methods.
Results: TAC values were significantly lower (1,836±0,54 mmol/L vs 2,085±0,36 mmol/L, P <0,05) and TOS values were significantly higher (63,21±30,5 μmol/L vs 34,5±6,3 μmol/L, p <0,0001) in the OSAS group compared to the controls. TOS values were significantly correlated with AHI (P=0,023). IMA and PON values weren't different between OSAS and controls (P >0,05). Although AOPP levels were higher in OSAS than controls (59,42±45,8 vs 42,41±34,36 umol/L), the difference wasn't statistically significant (P=0,09).
Conclusion: OSAS causes oxidative stress and decreased TAC values in the OSAS patients demonstrating impaired anti-oxidant defenses. Although we found an incresed oxidative stress in OSAS patients compared to the controls, IMA and AOPP values weren't significantly different between the groups. Other studies has reported increased lipid peroxidation in OSAS. In this respect, we suggest the systemic oxidative stress in OSAS may influence lipid parameters more than protein parameters.

T148

PHOSPHOLIPASE A2 AND PROSTAGLANDIN E2 LEVELS IN INDIVIDUALS WITH MULTIPLE SCLEROSIS

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Background: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system which presents with plaque formation and neuro-degeneration of axons. During inflammatory activation C20:4n-6 is released from immune cell membrane phospholipids by the enzyme phospholipase A2 (PLA2) for the synthesis of pro-inflammatory eicosanoid such as prostaglandin E2 (PGE2). The objective of this study was to investigate the relationship between C20:4n-6, PLA2 and PGE2 concentrations in patient with MS during inflammatory active and inactive stages as measured by C-reactive protein (CRP).
Methods: Enzyme-linked immunosorbent assays (ELISAs) were used to determine PLA2 and PGE2 concentrations in 31 patients with MS and 30 control subjects. The peripheral blood mononuclear cell (PBMC) membrane fatty acid concentrations were measured by gas chromatography and CRP by nephelometry in a routine Chemical Pathology laboratory.
Results: PGE2 was significantly higher in patients with MS than in controls. PLA2 showed a significant positive correlation with the CRP in patients with MS (R = 0.64; P=0.0001), but not in control subjects (R = 0.28; P=0.1302). There was a significant inverse correlation between PLA2 and C20:4n-6 in PBMC membranes from patients with MS (R =-0.42; P=0.0401) as well as between PGE2 and PC C20:4n-6 in PBMCs from patients with MS (R =-0.43; P=0.0352).
Conclusions: The inverse correlation between PLA2 and fatty acid C20: 4n-6 in PBMC membranes from patients with MS with subsequent increased production of PGE2 may suggest that reported decreases in C20: 4n-6 may have been the result of an on-going immune process in these patients. These results taken together demonstrate a link between PLA2, C20:4n-6 and PGE2 production in patients with MS.

T149
REFERENCE RANGE FOR MUSCLE MITOCHONDRIAL COENZYME Q10

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Background. We have developed and validated an accurate liquid chromatography tandem mass spectrometry (LC-MS/MS) method for determination of mitochondrial coenzyme Q10 (CoQ10). As CoQ10 deficiency is one of the few oxidative phosphorylation (OXPHOS) defects that can be treated, we aimed to establish mitochondrial CoQ10 reference range for interpretation of results.
Methods. We used left-over mitochondria isolated from vastus lateralis muscle samples of patients (n=166, median age 27.5 yrs, range 4 d – 80 yrs) suspected for OXPHOS defect. The samples were spiked with CoQ10-[2H6], extracted and CoQ10 (nmol/g wet weight) quantified by LC-MS/MS. Biochemical OXPHOS analysis and citrate synthase (CS) activity [nmol/(min x g wet weight)] were also determined. Reference range for CoQ10/CS was established from the patient data according to the guidelines of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (CLSI C28-A3c).
Results. The mitochondrial CoQ10 content in muscle from men (median 48 nmol/g wet weight, 95% CI 37-56 nmol/g wet weight, n=80) was higher (P=0.0168) than that in women (median 32 nmol/g wet weight, 95% CI 26-40 nmol/g wet weight, n=86). Furthermore, there were significant differences in mitochondrial CoQ10 content (nmol/g wet weight) with age. However, when CoQ10 content was normalized to CS activity of the sample, no differences between the genders or various age groups existed. The mean CoQ10/CS ratio (1/min) of all samples was 7.34 x e-3 (95% CI 7.00 - 7.68 x e-3). For establishing the reference range we used results from patient samples with no evidence for an OXPHOS defect by biochemical analysis (n=116). We suggest a CoQ10/CS reference range (1/min) of 3.9 - 11.6 x e-3 for combined genders and ages.
Conclusions. No differences between the genders or age groups exist when mitochondrial CoQ10 content is calculated as a ratio between CoQ10 and the generally accepted mitochondrial marker CS. The established reference range for vastus lateralis muscle mitochondrial CoQ10 is likely to improve diagnosis of mitochondrial diseases and treatment of patients with CoQ10 deficiency.

T150
A SENSITIVE AND SELECTIVE LC-ION MOBILITY-MASS SPECTROMETRIC ANALYSIS OF ALLOPREGNANOLONE AND ITS ISOMERS IN HUMAN PLASMA OR SERUM

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Background: There is growing interest in the therapeutic potential of gabaergic neuroactive steroid compounds, and the 3-alpha metabolites of progesterone, testosterone, deoxycortisol and androstenedione have been shown to have potent anxiolytic, analgesic, antiseizure, and neuroprotective effects in animal models and to activate GABA-A receptors. The most studied of these has been allopregnanolone. However, understanding of the physiological role of these compounds has been limited by the difficulty of measuring these compounds in biological samples. Currently only GC/MS assays with labor intensive extraction steps have adequate sensitivity to measure these compounds in biological samples and only a few specialized academic laboratories have the expertise to conduct these measurements. We propose to develop the capacity to use LC-MS/MS to measure GABAergic neurosteroid compounds in biological samples to enable the identification of biomarkers of disease risk, predictors of treatment response, and new therapeutic targets.
Methods: The challenges for LC-MS/MS analysis of allopregnanolone are its poor ionization efficiency, and the presence of numerous isobaric interferences in biological samples, including its isomer pregnanolone. To overcome these challenges, ion mobility separation was combined with conventional LC-MS/MS detection using an AB SCIEX triple quadrupole mass spectrometer equipped with the SelexION™ ion mobility device. The method employed liquid-liquid extraction of 100 μL serum or plasma. After extraction, the sample was derivatized using a commercially available quaternary aminooxy reagent.
Results: Separation of allopregnanolone and its isomer pregnanolone was achieved using a Phenomenex Kinetex C18 2.1x100 mm column. The calibration range is from 5 pg/mL to 100 ng/mL in serum or plasma, with inter- and intra-day precision less than 10%, and accuracy between 90-110%. The recovery is 98%, and the limit of detection is 50fg for allopregnanolone and pregnanolone. Plasma samples from 'normal', pregnant, and postpartum women were analysed using this method.
Conclusions: An LC-MS/MS method has been developed for the detection of allopreganolone in human serum or plasma at <5 pg/mL.

T151
ROLE OF KAPPA FREE LIGHT CHAINS IN PATIENTS WITH SUSPECTED MULTIPLE SCLEROSIS

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Background: A clinically isolated syndrome (CIS) may be the initial presentation of multiple sclerosis (MS). MS initiates with a first attack or a clinically isolated syndrome (CIS, and it usually consists in optic neuritis, brainstem syndrome or spinal cord disorder. Recently it has been shown that kappa light chains measurements in CSF have an important role in predicting CIS evolution to MS. We aim to study the benefits of k light chain in the diagnosis and management of MS
Methods: Kappa free light chains were quantified in serum and cerebrospinal fluid (CSF) by nephelometry using polyclonal antibodies based assay (Freelite.The Binding Site). Serum and CSF Albumin, IgG and IgM were also quantified by nephelemetry (BNII. Siemens) and IgG Oligoclonal bands (OCB) were performed by isoelectrofocusing (SEBIA).
Results: We studied 29 samples from patients with suspected MS and with positive OCB. When we compared the number of samples with k CSF values > 0,53 mg/L, the previously described cut point to predict CIS evolution to MS, 23/29 (79%) of the patients presented altered k CSF values vs 25/29 (86%) of patients that presented altered k index vs 19/29 (65%) of patients with an altered IgG Index. From all the OCB studied, 2 patients presented a “mirror” pattern, 1 patient presented at least 2 OCB, 1 patient presented at least 1 OCB and all the remaining patients were positive by OCB. The patients presenting “mirror” OCB patterns were finally diagnosed as a Creutzfeldt Jakob disease and a transient ischemic attack (TIA) and presented normal values for IgG and k indexes and for k CSF. The patient that presented with at least 2 OCB pattern, was finally diagnosed of cardiovascular accident and also presented normal k CSF and IgG and k indexes. However, there was one patient with a MS diagnosis that presented only 1 OCB, normally not referred as MS and that presented altered k CSF and Igk indexes. All the patients except one that had clear OCB presented altered k CSF and k index values.
Discussion: Due to the impact that a delay in a MS diagnostic might have in these patients, the use of k index and the quantification of the direct concentration of k in CSF may be an important help added to OCB and magnetic resonance imaging (MRI) in the diagnostic of

T152
THE ASSOCIATION OF PLASMA CYTOKINE CONCENTRATION WITH STROKE SEVERITY

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Background: Cerebral ischaemia initiates an inflammatory response in the brain and periphery. Inflammation may play an important role in acute ischemic stroke. Experimental and clinical data suggest that post-stroke inflammatory responses are complex cascade phenomena, which may have detrimental or beneficial effects on outcome. Study purposed to establish the correlation between plasma inflammatory cytokine concentration and severity of stroke.
Methods: 53 patients with acute ischemic stroke have been investigated. All of them were admitted to hospital within 24 h after stroke onset. Severity of stroke was assessed by National Institute Health Stroke Scale (NIHSS). Serum samples for cytokine measurements were collected on admission. Plasma levels of inflamatory cytokines (IL-1β, IL-6, TNF-α, IL-10) determined using the enzyme-linked immunosorbent assay (ELISA).
Results: On the first day since the development of ischemic stroke plasma level of IL-6 was positively correlated with the score on a scale of neurological disorders NIHSS ($r = 0.65$ at $P < 0.01$) and plasma level of IL-10 correlated inversely ($r=-0.74$ at $P < 0.05$).
Conclusions: Determining the level of markers of inflammation, including cytokines IL-6, IL-10 in peripheral blood on the first day since the development of ischemic stroke can serve as an additional diagnostic criterion for assessing the severity of stroke.

T153

NEUROPROTECTIVE ROLE OF 17B-ESTRADIOL ADMINISTRATION ON ALTERED AGE RELATED NEURONAL PARAMETERS IN FEMALE RATS

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Background: During normal aging, brain experiences structural, molecular, and functional alterations. Aging in females and males is considered as the end of natural protection against age related diseases like osteoporosis, coronary heart disease, diabetes, Alzheimer's disease and Parkinson's disease. Protection from age-related disorders is provided by several factors, including estrogens. These changes increase during menopausal condition in females when the level of estradiol is decreased.
Objective: The objective of this study was to observe the changes in activities of superoxide dismutase (SOD), glutathione S-transferase (GST), Ca2+ATPase, intracellular calcium levels, DNA degradation and glucose transporter 4 (GLUT4) expression occurring in brains of female albino Wistar rats of 3 months (young), 12 months (adult) and 24 months (old) age groups, and to see whether these changes are restored to normal levels after exogenous administration of 17β estradiol (E2).
Methods: The aged rats (12 and 24 months old) (n= 8 for each group) were given subcutaneous injection of E2 (0.1 μg/g body weight) daily for one month. After 30 days of hormone treatment, experimental animals of all the groups were sacrificed and brains were isolated for further study. GLUT4 expression in cerebral cortex was study with RT-PCR, Western blot and immunohistochemistry.
Results: The results obtained in the present work revealed that normal aging was associated with significant increases in calcium levels, at 12 months ($P < 0.01$) and 24 months ($P < 0.001$) when compared with 3 month control groups. Genomic DNA degradation and laddering pattern increases in the brains of aging female rats, and a significant ($P < 0.001$) decrease in SOD and GST activities and Ca2+ATPase ($P < 0.01$) at 24 months when compared with 3 month control animals. There was 45% decrease in GLUT4 mRNA expression levels at 24 months. Densitometric measurements showed that with aging there was a significantly decreased of 40% ($P < 0.01$) and 58% ($P < 0.01$) n the expression of GLUT4 proetin in the cortex of 12 months and 24 months aged rats. E2 treatment significantly increased the GLUT 4 levels ($P < 0.001$) in 12 and 24 months. There was a significant decrease of 41% ($P < 0.01$) in the GLUT4 immunoreactivity of the cortex of 24 months aged rats as compared to 3 months controls. E2 treated rats respectively and restored the GLUT 4 levels nearly to control values. Our data showed that exogenous administration of E2 brought these changes to near normalcy in aging female rats.
Conclusions: It can therefore be concluded that E2's beneficial effects seemed to arise from its antioxidant and antilipidperoxidative effects, implying an overall neuroprotective and anti-aging action. The results of this study will be useful for pharmacological modification of the aging process and applying new strategies for control of age related disorders.

T154

CEREBROSPINAL FLUID PURINE NUCLEOTIDE DEGRADATION PRODUCTS IN PATIENTS WITH MULTIPLE SCLEROSIS IN RELATION TO SERUM ANTIOXIDANT CAPACITY

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Background: In the pathogenesis of demyelinating diseases including multiple sclerosis (MS) plays an important role oxidative stress. Increased energy requirements of axons and mitochondria failure associated with subsequent depletion of macroergic phosphate is one of the causes of axonal degeneration and disability in MS. In a previous study we found that MS patients have an increased degradation of purine nucleotides in the CNS. In this context, we are curious as to whether the ongoing inflammatory process in the CNS in MS patients with early stage of the disease will be reflected in purine nucleotide metabolism and antioxidant protection of organism and if is there any association with alterations in serum levels coenzyme Q10 (CoQ10), which is an indicator of bioenergic state and also has antioxidant properties.
Methods: Serum concentrations of alpha- and gamma-tocopherol, beta-carotene, CoQ10, TBARS and cerebrospinal fluid (CSF) levels of degradation products of purine nucleotides (adenosine, inosine, hypoxanthine, xanthine, uric acid) were measured using HPLC. Samples were obtained from 23 MS patients (average age 35.6±9.3) in the early stage of the disease.
Results: The analyzed group of MS patients was characterized with reduced antioxidant capacity of serum (beta-carotene, alpha- and gamma–tocopherol and CoQ10), increased lipid peroxidation (increased serum levels of TBARS 5.0±0.7 μmol/L vs. ≤4.5 μmol/L) and increased catabolism of purine nucleotides. All MS patients showed the intrathecal IgG production and the presence of oligoclonal bands in CSF. Serum levels of CoQ10 were positively correlated with serum alpha-tocopherol ($r=0.438$; $P=0.05$) and CSF levels of adenosine ($r=0.396$; $P=0.06$) and xanthine ($r=0.416$; $P=0.04$). Decreased levels of gamma-tocopherol positively correlated with turnover of xanthine to uric acid ($r=0.587$; $P=0.003$). MS patients with lower serum levels of beta-carotene were characterized by increased intrathecal IgG production and increased lipid peroxidation.
Conclusions: Results suggest participation of CoQ10 in the regeneration of alpha-tocopherol and possible impact CoQ10, gamma-tocopherol and beta-carotene on bioenergetics of reparative remyelinating process that take place in the early stages of this disease.

T155
THE ACTIVITY OF ALCOHOL DEHYDROGENASE ISOENZYMES AND ALDEHYDE DEHYDROGENASE IN THE BRAIN TUMOR

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Background: Some findings suggest that alcohol consumption increases the risk of brain tumor consistent with a dose-response relationship. Human brain contains three classes of alcohol dehydrogenase (ADH) isoenzymes: classes I, III and IV and possess also aldehyde dehydrogenase (ALDH) activity. These isoenzymes of ADH participate in the metabolism of many biological substances such as retinol or serotonin. ADH is responsible for the metabolism of ethanol but it has not been definitively demonstrated to play a significant role in brain. ALDH plays the crucial role in the further oxidation of ethanol-derived acetaldehyde in the brain cells. The aim of this study was to compare of the metabolism in brain cancer tissue and normal brain tissue by measerment ADH isoenzymes and ALDH activities in these tissues.
Methods: Biopsy specimens of brain tumor (glioblastoma) and healthy tissues were taken during resection of brain carcinoma from 52 patients (33 males, 23 females, 46-74 years). Class I and II ADH isoenzymes and ALDH were measured by fluorometric method using the specific substrates. The activity of class III ADH was measured by photometric method with n–octanol and class IV with m–nitrobenzaldehyde as a substrate.
Results: We have found that the activity of class I ADH was significantly higher in tumor tissues (0.431 nmol/min/mg protein) than in healthy cells (0.346 nmol/min/mg protein). The other tested classes of ADH had tendency to higher level activity in cancer than in normal tissue. The total activity of alcohol dehydrogenase was significantly higher (about 27%) in brain cancer than in healthy ones (2.485 vs. 1.798 nmol/min/mg protein). The total activity of ALDH was not statistically significantly higher in cancer tissues than that in healthy.
Conclusion: The ADH activity is significantly higher in brain tumor cells than in normal tissue and therefore might be a factor of metabolic changes in low mature cance

T156
ASSESSMENT SPECIFIC ENOLASE AS A PROGNOSTIC MARKER OF CARDIAC ARREST

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Background: Cerebral ischemia is the main consequence after cardiac arrest and largely determines the final prognosis of the patient, causing severe brain damage, persistent vegetative state or death.Neuron-specific enolase (NSE) has been evaluated in cases of head trauma and stroke, and studies also show its clinical utility as a predictor of neurological status between 12 and 48 h after cardiac arrest.
Objective: Evaluate the determination of NSE as a marker of neurological status after cardiac arrest.
Methods: 24 patients admitted to our hospital for cardiac arrest were selected.We excluded patients with head trauma, hemorrhagic processes,neoplasms associated with elevated NSE or hemodialysis.The NSE was determined in serum 24-48 hours after the cardiac arrest, by electrochemiluminescence. We stablished two groups according to GOS scale: group 1 (unfavorable outcome) included GOS 1 and 2, group 2 (favorable outcome) included GOS 3-5.Both groups were compared on the variables sex, time of cardiac arrest and extraction time of NSE,considering significant P <0.05.The groups were compared by “t” Student, Mann-Whitney and Fisher exact test (SPSS 15.0).The cut-off for predicting poor outcome was calculated by ROC curve.
Results: Of the 24 patients evaluated, 15 (62.5%) died (GOS 1) and 3 (12.5%) remained in a vegetative state (GOS 2). Of the 6 remaining patients, 1 (4.2%) had severe disability (GOS 3), 3 (12.5%) moderate disability (GOS 4) and 2 (8.3%) had a favorable outcome (GOS 5).The groups did not show statistically significant differences in terms of sex,time cardiac arrest and extraction time of NSE. NSE levels were significantly higher in group 1 (median 65.42, interquartile range (IQR) 38.10 to 147.15) than in group 2 (median 19.61, IQR 15.08 to 23.80); P=0.001).The area under the curve was 0.94 (CI 95% 0.85 to 1.03). The optimal cut-off was at 25.85 ng/mL (sensitivity 88.9%, specificity 100%).
Conclusions:NSE levels measured in 24-48 h after cardiac arrest are significantly higher in patients with poor prognosis. Given our small sample size and in a preliminary way, we can state that the determination of NSE is a useful marker of neurological status and,combined with other methods, could be useful in assessing the prognosis of these patients.

T157

OXYSTEROLS AND CHOLESTEROL METABOLISM IN NEURODEGENERATION. EVIDENCE FOR ANABOLIC IMPAIRMENT

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Background: Brain cholesterol is involved in cell membrane structure and synaptogenesis. Impairment of brain cholesterol metabolism was described in neurodegenerative diseases, as Alzheimer (AD), Huntington (HD), and Multiple Sclerosis (MS). Since the blood-brain barrier (BBB) efficiently prevents cholesterol uptake from the circulation into the brain, de novo synthesis is responsible for almost all cholesterol present there. Excess of cholesterol is converted into 24S-hydrocycholesterol (24OHC) by neuronal specific cholesterol 24-hydroxylase (CYP46A1). 24OHC decrease the formation of amyloid, contrasted by 27-hydroxycholesterol (27OHC), which brain levels depend by BBB function. In case of AD altered distribution of membrane cholesterol is associated with higher formation and deposition of amyloid.
Material and methods: Oxysterols 24OHC and 27OHC, cholesterol precursor lathosterol, desmosterol and lanosterol were measured by isotope dilution mass spectrometry in plasma collected from patients affected by several neurodegenerative diseases.
Results: In HD patients significantly reduced levels of circulating oxysterols and precursor sterols were related to mutated huntingtin interference with LXR-SREBP pathway. In AD patients, 24OHC was found reduced proportionally to the degree of brain atrophy. Cholesterol synthesis was increased in aging individuals who developed AD in more advanced age. In Pantothenate kinase 2 (PANK2) deficiency patients, reduced levels of CoA synthesis were found related to a general impairment in cholesterol and fatty acids synthesis with reduced plasma oxysterols and sterols.
Discussion: A systematic metabolic approach provides new information in studies of pathogenesis, biomarkers discovering, and therapeutic strategy definition. The combined study of oxysterols and sterols in plasma collected from patients revealed in all neurodegenerative diseases alteration of whole body cholesterol homeostasis, oxidative stress, disturbance of brain cholesterol turnover.

T158

METABOLIC CONSEQUENCES OF COENZYME A DEFICIENCY IN PATIENTS WITH PANK2 MUTATIONS

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Background: Pantothenate kinase-associated neurodegeneration (PKAN) is a rare, inborn error of metabolism characterized by iron accumulation in the basal ganglia, dystonia, and dysarthria. Mutations in pantothenate kinase 2 (PANK2), the rate-limiting enzyme in mitochondrial coenzyme A biosynthesis, are the only known genetic causes of this disorder.
Material and methods: Plasma levels of pantothenate, lactate, alpha- and beta- hydroxyl-butyrate, alanine, valine, leucine, isoleucine, myristic, palmitic, oleic and stearic acids, bile acids, cholesterol precursor lathosterol and lanosterol, oxysterols (27-hydroxycholesterol and 7a-hydroxycholesterol) were analysed by isotope dilution gas-chromatography mass spectrometry on plasma collected from 14 genetically defined patients and 20 age matched controls with a Perkin Elmer Clarus 600 GCMS system.
Results: Pantothenate levels were higher in patients with premature stop mutations in PANK2 ($P < 0.03$). Increased lactate ($P < 0.001$) and alpha-hydroxy-butyrate ($P = 0.02$) were elevated in PKAN patients according with possible mitochondrial dysfunction. Alanine ($p = 0.03$), valine ($P = 0.006$) isoleucine ($P = 0.02$) were reduced suggesting both hypercathabolic situation and reduced aminoacid absorption. Reduced levels of lathosterol and lanosterol (both $P < 0.001$) (cholesterol precursors marker of whole body cholesterol synthesis) and reduced levels of bile acids precursor 27-hydroxycholesterol ($P < 0.001$) were also observed together with impaired bile acids conjugation. Plasma fatty acids were found reduced ($P < 0.01$). Studies in patient-derived fibroblasts confirmed reduced cholesterol synthesis and CoA-depending pathways.
Conclusion: Metabolic profiling is an appropriate approach to study biochemical pathogenesis of inherited diseases and to discover possible therapeutic targets and biomarkers for the disease diagnosis.

T159
VASCULITIC NEUROPATHY AND PLASMA VEGF LEVELS

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Background: Vascular endothelial growth factor (VEGF), secreted from endothelial cells and pericytes in response to hypoxia. It induces angiogenesis and microvascular hyperpermeability. We observe serum VEGF concentrations in some patients with with vasculitic neuropathy.
Methods: Plasma VEGF was measured, using GenWay's human VEGF ELISA Kit which is based on standard sandwich enzyme-linked immune-sorbent assay technology. Human VEGF specific-specific monoclonal antibodies are precoated onto 96-well plates. The human specific detection polyclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. The VEGF levels were measured in 5 patients with vasculitic neuropathy and 8 healthy controls.
Results: Plasma VEGF was higher in subjects with vasculitic neuropathy as compared to controls. In control group we obtain VEGF levels from 9.8 pg/mL up to 15 pg/mL with average of 11.6 pg/mL for males; 8.9 pg/mL up to 14.5 pg/mL with average of 11.2 pg/mL for females. In female patients with vasculitic neuropathy the plasma VEGF levels were between 79.9 pg/mL and 111.2 pg/mL; with average of 92.375 pg/mL. We had one case with vasculitic neuropathy in men, which plasma VEGF level was 102.9 pg/mL.
Conclusions: The results from our study indicate that plasma VEGF levels are significantly associated with vasculitic neuropathy and may be used to predict this disease.

T160
ANTI–AQUAPORIN - 4 ANTIBODIES : CLINICAL IMPORTANCE

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Background: Neuromyelitis optica (NMO) is a demyelinating disease of the central nervous system. Early discrimination between multiple sclerosis and NMO is crucial , as treatment may differ considerably. Antibodies known as NMO-IgG from their initial description cause a characteristic colouring of the Virchow-Robin's space along the small arterioles in the grey and white matter in immunofluorescence on CNS tissue. The protein aquaporin - 4 (AQP4) was later identified as the target antigen.
Methods: AQP4 antibodies were detected by indirect immunofluorescence assay using a neurology mosaic test (Neurology Mosaic 17 – Euroimmun, Germany) consisting of five different substrates : HEK cells transfected with AQP4, non transfected HEK cells, primate cerebellum, cerebrum and optic nerve tissue sections. We investigated 46 serum samples from patients followed up to Neuroscience Department (NMO-spectrum diseases and other neurological diseases).
Results: AQP4 antibodies were detected in 3 of 46 cases. These 3 cases were diagnosed as NMO, according with Wingerchuk criteria. 3/46 cases had doubtful pattern , but were not NMO. 40/46 cases were negatives and were not NMO.
Conclusions: The indirect immunofluorescence biochip mosaic assay allows the detection of antibodies in routine screening and is very sensitive. AQP4 antibodies represent a highly specific and reproducible method to discriminate between NMO and other demyelinating disorders in particular optico-spinal form of MS. This test not only enables a reliable distinction between NMO and MS, but also facilitates differential diagnosis concerning autoimmune diseases affecting the CNS. This ability to distinguish is particularly important when patients presenting with myelitis have concomitant serological findings suggesting systemic autoimmunity, which poses diagnostic challenges to clinicians in many cases. This standardized and reproducible assay improves the detection of AQP4 antibodies and allows to better define NMO cases with also the possibility of monitoring disease activity with AQP4 antibodies.

T161
HYPERHOMOCYSTEINEMIA IN A GROUP OF PATIENTS WITH ISCHEMIC STROKE

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Increased plasma total homocysteine concentration is independent risk factor for cardiovascular and many other diseases. This study examines whether there is an association between homocysteine levels and ischemic stroke. Homocysteine concentrations were measured in group of patients with ischemic stroke. 90 patients (51 male and 39 female) from Clinic of Neurology, Clinical Center of Vojvodina, and 50 volunteers from control group were included in investigation. Patients were divided in tree groups. Patients with TIA (transitory ischemic attack), group with ischemic stroke confirmed with magnetic resonance (MRA) and patients with recurrent stroke. Homocysteine was determined at the admission in the hospital and in a group with high homocysteine concentrations one mount after the first determination. Homocysteine level was determined using the fluorescence polarization immunoassay FPIA on Abbott AxSym Analyzer. In a control group median value of homocysteine concentration was 9,89 μmol/l (SD=1,93; 95% CI: 9,33-10,44). Elevated homocysteine levels were determine in 26, 7% of patient with ischemic stroke while only 10% of participants from the control group had homocysteine concentration above 12 μmol/l. Men with ischemic stroke had significantly increased homocysteine level (P=0,05) with median value of 11, 64μmol/l (SD=4,3; 95%CI: 10,43-12,85) while the women was not characterized by significantly higher homocysteine concentrations. Median value of homocysteine concentration in the group of patients with TIA and in the group of patients with ischemic stroke was 9,06 μmol/L (SD=1,98; 95% CI:7,54-10,6), and 10,91 μmol/L (SD=3,81; 95% CI: 10,0-11,8) respectively. There were no significantly difference between the group with stroke and recurrent stroke. The highest median value of homocysteine concentrations was in the group of patient with history of myocardial infarction 13,1 μmol/L (SD=6,09 with a range 6,34-18,2). Our results show an association between homocysteine levels and stroke only in men group of patients with the highest concentration in group of patients with history of myocardial infarction.

T162
LOWER ENZYME ACTIVITIES OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) IN PATIENTS WITH FIRST EPISODE PSYCHOSIS AND SCHIZOPHRENIA INDEPENDENT OF MTHFR C677T AND A1298C MUTATIONS

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Background: Methylenetetrahydrofolate reductase (MTHFR) which provides methylfolates for the methylation of homocysteine is one of the key enzymes of one carbon metabolism (OCM). In this study, we aimed to evaluate the impact of MTHFR enzyme activity on OCM which is effected in neuropsychiatric diseases.
Methods: Thirty three first episode psychosis (FEP) patients, 30 chronic schizophrenia (CS) individuals and 24 healthy controls were involved to the study. We analyzed total plasma homocysteine (tHcy), erythrocyte folate (EF), serum folate, serum vitamin B12, urine methylmalonic acid (uMMA), serum/urinary amino acids and erythrocyte folic acid sub-groups. MTHFR C677T and A1298C mutations were identified and the enzyme activity in leukocytes was measured by high performance liquid chromatography (HPLC) method and an optimized automated photometric method.
Results: Lower enzyme activities were found in FEP and CS patients without mutations compared with controls (99±42, 131±50 and 174±64 nmol/min/g protein, respectively). tHcy and uMMA levels were significantly higher (P <0.05) and serum folate and EF levels were significantly decreased in the patient groups than the control group (P <0.01). Also serum and urine alanine, glycine, serine, threonine levels and percentages of the 5-methyl tetrahydrofolate-two-glutamates (5-MTHF-Glu2) were significantly higher in FEP group. Serum methionine levels were significantly lower in the CS group (P <0.05). MTHFR enzyme activity was positively-correlated with 5-MTHF-Glu4 and 5-formyl-Glu4, negatively correlated with tHcy levels in CS group and was positively correlated with uMMA levels in FEP group.
Conclusions: To the best of our knowledge, this is the first study in the literature measuring MTHFR enzyme activity by an automated photometric method in addition to the most common mutations of the MTHFR gene in our study populations. Lower MTHFR activities in FEP and CS patients might indicate the contribution of this enzyme both in the beginning and the progression of schizophrenia. Furthermore the reasons of decreased MTHFR enzyme activities which is not related to the common genetic mutations of the enzyme in our patient groups should be further investigated.

T163

THE RELATIONSHIP BETWEEN THE NUMBER OF CEREBROSPINAL FLUID IGG OLIGOCLONAL BANDS AND SERUM ANTIOXIDANTS LEVELS

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Background. Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) associated with a pathological autoimmune reaction against CNS myelin. Typical biochemical findings in cerebrospinal fluid (CSF) of MS patients, intrathecal production of immunoglobulin IgG, is reflected by presence of oligoclonal IgG bands (OCBs). Two or more OCBs detected after separation of CSF proteins and not demonstrable in corresponding serum reflect a local B-cell response accompanying CNS inflammation. In the pathogenesis of demyelinating diseases including MS an important role plays oxidative stress. In this regard the aim of this study was to assess whether the ongoing inflammatory process in the CNS in patients with MS will be reflected in serum levels of some antioxidants and in the relation between OCBs number and serum levels of antioxidants.
Methods. We examined CSF and serum specimens from 28 MS patients (aged 17-49, mean 37.3±7.6 years). All the patients were positive for CSF OCBs. OCBs were detected by isoelectric focusing (IBF). Total protein, albumin and IgG in serum and CSF were measured by nephelometry. Intrathecal IgG production (RIG) was calculated according to Reiber and Felgenhauer (1987). Serum antioxidants alpha- and gamma-tocopherol, beta-carotene and coenzyme Q10 were analyzed by HPLC- UV method. CSF cells were analyzed by microscopy.
Results. We found negative correlation between the number of CSF OCB and serum levels of beta-carotene (r=-0.437; P=0.02) and positive correlation between the number of CSF OCB and gamma-tocopherol (r=0.508; P=0.006), CSF levels of IgG (r=0.657; P=0.0001), IgG index (r=0.901; P=0.0001), RIG (r=0.744; P=0.0001) and the number of CSF mononuclear (r=0.508; P=0.05). The mean plasma levels of coenzyme Q10 (39.3% MS patients), gamma –tocopherol (75% MS patients) and beta-carotene (42.8% MS patients) were reduced.
Conclusions. The results of this study suggest decreased serum antioxidant levels in patients with multiple sclerosis. Serum beta-carotene concentrations in MS patients are negative associated with intrathecal IgG synthesis. The reduced antioxidant reserve may play important role in an early pathogenic mechanism in inflammatory demyelinization in this disease.

T164

GSTM1 AND GSTT1 GENETIC POLYMORPHISM, AND ANTHROPOLOGICAL AND OXIDATIVE STRESS BIOMARKERS IN SUBJECTS WITH PTSD - A PILOT STUDY

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Background. Posttraumatic stress disorder (PTSD) is a complex anxiety disorder caused by a traumatic experience. It is over represented in combat veterans from Croatian homeland war. The glutathione S-transferases (GSTs) belong to a group of major detoxifying enzymes responsible for eliminating of oxidative stress products. It is hypothesized that GSTs genetic isoforms together with oxidative stress biomarkers might contribute in pathogenesis of PTSD.
Methods. 134 male combat veterans from Croatian homeland war were included in the study, 121 with PTSD diagnosed according to Diagnostic and Statistical Manual of Mental Disorders (DSM-IV), and 13 control subjects. Concentrations of serum lipid parameters were determined with standard enzymatic methods. Gluthatione peroxidase (GPx) and superoxide dismutase (SOD) catalytic concentrations were determined in hemolysates of erythrocytes by standard spectrophotometric methods. Anthropometric data were collected. GSTM1 and GSTT1 were determined by multiplex PCR.
Results. GSTM1 genotype frequencies (functional vs. deleted) were 95 vs. 26 in PTSD, and 9 vs. 4 in controls, respectively, (P=0.487). GSTT1 genotype frequencies (functional vs. deleted) were 97 vs. 22 in PTSD, and 9 vs.4 in controls, respectively, (P=0.487). Waist-to-hip ratio (WHR), SOD and GPx were significantly lower in PTSD subjects (P=0.040, P <0.001 and P=0.001, respectively). There was a significant difference in WHR values between GSTM1 genotype subgroups in subjects with PTSD (P=0.001). WHR-median in subjects with at least one GTSM1 functional allele was 0.94 (25th and 75th percentiles were 0.89 and 0.97), while in subjects with both deleted alleles WHR median was 1.01 (25th and 75th percentiles were 0.89 and 1.03). Spearman rank correlation test showed significant correlations in subjects with PTSD for WHR values and GSTM1 genotypes (rho=-0.597, p <0.001), SOD and GPx (rho=0.503, P <0.001) and low correlation between GPx and MDA (rho=-0.274, P=0.054).
Conclusions. The results indicate that GSTs genetic polymorphism might be associated with development of PTSD by altering the values of oxidative stress markers. It is evidenced that different genetic, anthropometric and biochemical markers might be involved in pathogenesis of PTSD.

T165

CORRELATION BETWEEN SEVERITY OF PSYCHOPATHOLOGY AND SERUM CYTOKINES LEVELS IN SCHIZOPHRENIC PATIENTS

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Background: Schizophrenia (SCH) has been associated with altered levels of various cytokines and their receptors. Current research indicates possible association between cytokine alterations and severity of psychopathology. However results are often inconsistent. Therefore, the aim of our study was to investigate the correlation between serum levels of Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α) and severity of psychopathology in patients with SCH, in acute and remission phase of illness.
Method: Serum levels of IL-6 and TNF-α in acute exacerbation and in remission phase were measured in 43 SCH inpatients by enzyme-linked immunosorbent assay (ELISA). The severity of psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS). All patients fulfilled DSM-IV criteria for SCH.
Results: Serum TNF-α level, in the acute phase of illness, was negatively correlated to the positive PANSS score (P=0.048, P=0.303). Correlations between severity of psychopathology measured by positive, negative and total PANSS scores and cytokine levels (IL-6 and TNF-α) were not detected, regardless the phase of illness.
Conclusion: Our results suggest that the severity of psychopathology and the phase of illness could be an important trait influencing the immune status of patients with SCH. Further prospective studies are needed to investigate biological mechanisms that lie behind this complex phenomenon.

T166

INITIAL EVALUATION OF A NEW ANALYTICAL PLATFORM FOR THE ANALYSIS OF Aβ-42 AND TAU BIOMARKERS IN CEREBROSPINAL FLUID

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Background: Evaluation of cerebrospinal fluid (CSF) biomarkers in Alzheimer's disease (AD) is becoming increasingly more important to increase ante-mortem diagnosis of the disease, ensuring proper management of the patient. The use of such markers for clinical purposes, in conjunction with potential disease-modifying therapies requires assays that can deliver high analytical and clinical performance. Two biomarkers, beta amyloid1-42 and tau protein, are shown to correlate with disease progression, and numerous research use only (RUO) tests are available. We report on the VITROS® Immunodiagnostics Products Aβ-42 and Tau assays currently under development for the VITROS® 3600 System with the objectives of high precision, reproducibility, sensitivity, specificity and ease of use.
Methods: Performance was evaluated using two reagent lots to test clinical samples. Twelve CSF samples were tested in duplicate for five days with both lots. In addition, 60 clinical samples comprised of 30 controls and 30 AD patients were tested to assess the ability of the assays to discriminate between the normal and AD states. The same 60 clinical samples were also tested using RUO CSF AB-42 and Tau assays.
Results: The mean tau levels in the 12 CSF samples tested for precision ranged from 319 to 1303 pg/mL, and the mean Aβ-42 levels for the same samples ranged from 292 to 588 pg/mL. The maximum inter assay imprecision for all samples was 3.0% and 5.7% for tau and Aβ-42, respectively. ROC analysis of the samples yielded a cutoff of 379 and 913 pg/mL for Aβ-42 and tau respectively. Sensitivity and specificity was 90% and 50% for Aβ-42 and 96.7% and 93.3% for tau. The tau: Aβ-42 ratio of 3.05 resulted in 100% sensitivity and 96.7% specificity. Using the same cohort, the RUO CSF Tau and AB-42 assays resulted in a sensitivity and specificity of 100% and 96.7% for tau and 86.7 and 73.3% for Aβ42, respectively.
Conclusion: The VITROS® AB-42 and Tau assays in development for tau and Aβ-42 proteins have demonstrated good precision over two lots of reagents and shown good discrimination between AD and control patients. The fully automated format and ability to perform these assays in a clinical laboratory should make testing for these important markers more reliable and routine.

T167

THE DETERMINATION OF SERUM HOMOCYSTEINE AND LIPID LEVELS AT PATIENTS WITH ISCHEMIC STROKE AND VASCULAR DEMENTIA

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Background: The aim of our study was to investigate serum concentration of homocysteine and lipids at patients with ischemic stroke and vascular dementia.
Methods: The study covered 600 patients, 200 with the first ischemic stroke during the acute phase, 200 with vascular dementia and 200 healthy subjects. We determined concentrations of homocysteine, creatinine, cholesterol, triglycerides HDL cholesterol and LDL cholesterol in patients' serum. Homocysteine concentration in serum was measured by AxSYM apparatus of ABOTT Company on the basis of fluorescent polarisation measuring. The lipids and creatinine were determined by means of DIMENSION LxR (DADE BEHRING Company).
Results: We got higher concentrations of homocysteine in patients after ischemic stroke (24-48 h) and in patients with vascular dementia then in healthy subjects. Our results show that the concentration of HDL cholesterol was significantly lower in the group with ischemic stroke and vascular dementia than in the control group. In the group with ischemic stroke, the concentration of LDL cholesterol was higher than 4.3 mmol / L in about 56% of patients, whereas in the group with vascular dementia in 60 % of patients. The Pearson correlation coefficient have show significant negative difference between serum homocysteine with HDL cholesterol concentrations at patients after ischemic stroke (r= -0.299; P =0.035) and patients with vascular dementia (r= -0.227; P=0.049) for P <0.05. Using Pearson correlation coefficient we have got no significant difference between serum homocysteine and concentrations of cholesterol and triglycerides in patients with ischemic stroke and vascular dementia for P <0.05. Comparison of homocysteine with LDL cholesterol (r=0.282; P=0.048) concentration in patients' serum with vascular dementia we found significant positive difference for P <0.05.
Conclusion: Our results showed that homocysteine concentration is inversely proportionate to HDL cholesterol concentration, i.e. as homocysteine concentration in serum increases, HDL concentration falls. Homocysteine concentration increases with LDL cholesterol concentration in serum of the patients with vascular dementia, which points to homocysteine impact in further atherosclerosis development.

T168

THE SERUM CONCENTRATION OF URIC ACID AND CORRELATION WITH LIPIDS IN PATIENTS WITH ISCHEMIC STROKE AND VASCULAR DEMENTIA

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Background: The epidemiological studies proved that concentration increase in uric acid can be a risk factor for cardiovascular diseases independently of other disorders.
Methods: The study covered 300 patients, diagnosed with the first ischemic stroke (100), 100 patients diagnosed with vascular dementia and 100 healthy subjects. Uric acid and lipids were determined by Dimension LxR automatic analyser of Dade Behring Company.
Results: The patients with ischemic stroke had hyperuricemia in 35 % and vascular dementia in 10 %. The uric acid increased 7 days after ischemic stroke for 5.3%, after 14 days for 9.5%. Using the Wilcoxon signed ranks test (Z = -2.736, P=0.006) was found statistically significant difference between the average concentration of uric acid after 24-48 hours and 14 days of ischemic stroke. According Mann-Whitney test (Z= -2.837, P=0.005; Z= -2.734, P=0.006) it was a significant difference between concentrations of uric acid after 7 and 14 days of ischemic stroke and control groups. Using the same tests, no significant difference between the average concentrations of uric acid in acute (Z= -0.458, P=0.647; Z= -0.614; P=0.539) and post-acute phase (Z= -0.700, P=0.484) of ischemic stroke and vascular dementia groups with the significance level of P <0.05. Using the Mann-Whitney test (Z=-2.241, P=0.025) was significant difference between the average concentrations of uric acid in vascular dementia and control group for P <0.05. Using Pearson's correlation coefficient, we found it was no significant correlation between serum uric acid concentration and concentration of cholesterol, triglycerides, and atherogenic index, HDL and LDL cholesterol in patients with ischemic stroke and patients with vascular dementia (P <0.05).
Conclusion: Uric acid concentration is higher in the group with ischemic brain stroke during the acute and post-acute phases and in the group with vascular dementia than in the control group. With the first symptoms of brain stroke, uric acid increases in serum of the people who suffered ischemic stroke and it remains increased if vascular dementia develops after ischemic brain stroke. Therefore monitoring of uric acid at patients with ischemic stroke is important because uric acid is more harmful than protective.

T169

FACTOR XIII-A SUBUNIT VAL34LEU POLYMORPHISM IN FATAL ATHEROTHROMBOTIC ISCHEMIC STROKE

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Background: Factor XIII (FXIII) is a key regulator of fibrinolysis and clot firmness. Val34Leu polymorphism of its potentially active A subunit (FXIII-A) leads to faster activation of FXIII, influences clot structure and provides a moderate protection against coronary artery disease. The effect of FXIII-A subunit (FXIII-A) Val34Leu polymorphism on the risk of atherothrombotic ischemic stroke (AIS) has been investigated in a few studies with contradictory results. In all previous studies only patients surviving AIS were enrolled and gender-specific effects were not explored.
Methods and patients: In this retrospective multicenter cohort, we investigated the effect of FXIII-A Val34Leu polymorphism on the risk of fatal AIS in women and men. DNA isolation and genetic determinations in case of 316 patients died of AIS were carried out on paraffin embedded tissue specimens. Genetic analyses for population controls and sex-matched controls performed on extracted genomic DNA from peripheral blood leukocytes.
Results: The prevalence of homozygous wild-type, and heterozygous genotypes, Leu34 carriers and Leu34 allele was not different significantly between the patient with fatal AIS and their respective sex-matched controls. Logistic regression analysis with age as covariant demonstrated that in females homozygous presentation of Leu34 allele represented a more than 3.0-fold increased risk of AIS with fatal outcome.
Conclusion: The results demonstrate that FXIII-A Val34Leu polymorphism does not influence the occurrence of AIS, but has an effect on the severity of its outcome. This effect is gender-specific and in homozygous females, the prothrombotic/antifibrinolytic effects of FXIII-A Val34Leu polymorphism seem to prevail.

T170

ENDOTHELIAL NO-SYNTHASE AND ENDOTHELIN-1 GENES POLYMORPHISMS AND THE LEVEL OF CIRCULATING ENDOTHELIAL CELL: THE NEW LABORATORY APPROACH TO ASSESSMENT OF ENDOTHELIAL DYSFUNCTION AND STROKE RISK

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Background: The vascular endothelium plays a pivotal role in blood flow regulation, vascular permeability and thrombogenesis. The number of circulating endothelial cells (CEC) may be the marker of vascular endothelium damage. From the other hand, the polymorphisms of endothelial NO-synthase (eNOS) and endothelin-1 (EDN-1) genes may influence upon endothelial dysfunction and development of cardio- or cerebrovascular disease. The aim of our study was to investigate the eNOS and EDN-1 genes polymorphisms, the quantity of CEC and their relationship in patients with acute lacunar stroke (LS).
Materials and methods: The T786S and G894T eNOS, Lys198Asn and 3A/4A EDN-1 were detected in 87 LS patients and 79 healthy controls by RT-PCR using the amplifier DT-96 and reagent kit “DNA-Technology” (Moscow, Russia). Level of CEC was determined in venous blood by flow cytometry using a fluorescently-labeled antibodies specific to CD45 and CD146 as number of nucleate cells with specified size and positive binding with anti-CD146, but negative binding with anti-CD45.
Results: The Lys198Asn EDN-1, T786S and G894T eNOS genotypes’ frequencies didn’t differ in patients and controls. But the tendency to prevalence of 3A/4A EDN-1 in healthy group was found – 58%, 37%, 5% and 68%, 29%,3% for 3A3A, 3A4A, 4A4A genotypes in controls and LS patients, respectively (P=0.1). At the same time, the level of CEC was lower in carriers of 3A4A or 4A4A genotypes compared to subjects with 3A3A genotype – 4.2±0.9 cell/mL and 8.8±1.9 cell/mL (P=0.1).
Conclusion: the results of our study may indicate protective role of 3A/4A EDN-1 gene polymorphism in vascular damage and cerebrovascular disease development and need further investigations.

T171

DEVELOPMENT OF PRELIMINARY CUT-POINTS FOR PROGRESSION FROM MILD COGNITIVE IMPAIRMENT TO ALZHEIMER'S DEMENTIA FOR THE VITROS® Aβ-42 AND VITROS TAU IMMUNOASSAYS

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Background: Cerebrospinal fluid (CSF) Beta amyloid1-42 (Aβ42) and tau protein assays were developed for the VITROS® 3600 Immunodiagnostic System. CSF AB42 and Tau have previously been demonstrated to correlate with risk of progression from cognitively normal (CN) to Alzheimer's disease (AD). Identification of at-risk patients earlier in the development and progression of disease is critical for therapeutic development and disease management. Appropriate assay cut-points are vital for the accurate determination of risk of disease progression. This study reports the performance of the VITROS AB-42 and Tau assays using preliminary cut-points.
Methods: Banked samples were obtained from an expert center with a defined protocol for collection and MCI classification. Diagnostic status was defined by clinical assessment for CN (n=30), AD (n=30), MCI-who remained dementia free (MCI-s; n=30) or MCI-who progressed to AD dementia (MCI-p; n=30). In parallel to the VITROS analyses a commercial RUO ELISA was performed. Method comparisons were performed for each analyte. Cut-point analysis was performed by receiver operator curve (ROC) analysis and sensitivity and specificity were calculated.
Results: Method comparisons between the VITROS and RUO assays gave correlation coefficients of >0.94 for AB42, tau and the tau/AB42 ratio. In the ROC analysis, area under the curve of >0.91 was observed with the AD&CN population for each of the assays. For the VITROS assays the maximum efficiency point for tau/Abeta ratio was 95.0 for AD&CN, 83.1 for MCI-p&MCI-s and 91.7 for MCI-p&CN. Cut-points determined from the MCI-p&MCI-s gave more consistent specificity and sensitivity for both populations than did a cut-point derived from the AD&CN groups. For the tau/AB42 ratio the observed specificity and sensitivity for MCI-p&MCI-s was 77% and 90% and for AD&CN was 97% and 93% respectively. A cut-point of 1.7 for the tau/AB42 ratio demonstrated sensitivity and specificity of 87% and 97% for MCI-p&MCI-s and 97% and 93% for AD&CN respectively.
Conclusions: The VITROS Tau and AB-42 assays using preliminary assay cut-points discriminated between MCI-p&MCI-s and AD&CN populations with high specificity and sensitivity as both stand alone assays and as a tau/ AB42 ratio.

T172

PROTECTIVE EFFECT OF AGMATINE – CLINICAL SCORE AND GSH CONCENTRATION IN EAE MICE

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Background: Experimental autoimmune encephalomyelitis (EAE) is CNS inflammatory and demyelinating disease, used as an animal model of multiple sclerosis (MS). EAE is characterized by infiltration of the CNS by lymphocytes and mononuclear cells, microglial and astrocytic hypertrophy, and demyelination which, cumulatively, contribute to clinical expression of the disease. The aim of the present study was to investigate the hypothesis that agmatine (AGM) provides protection against oxidative stress induced by EAE in mice.
Methods: EAE was induced in knockout CBA/H iNOS-/- mice, 3 months old (15±5 g), by immunization with myelin basic protein (MBP) in combination with Complete Freund's adjuvant (CFA). The animals were divided into five groups: control, EAE, CFA, EAE+AGM and AGM. The animals were sacrificed 24 days after EAE induction, and the levels of glutathione (GSH) were determined in 10% homogenate of the whole encephalitic mass (WEM), cerebellum and medulla oblongata.
Results: The clinical expression of EAE was significantly decreased ($P < 0.05$) in the EAE groups treated with AGM, compared to EAE mice, during disease development in both wild-type and knockout mice. Also, we have demonstrated a significant reduction of GSH level ($P < 0.001$) in EAE mice WEM, cerebellum and medulla oblongata tissue compared to the control groups, whereas AGM treatment increased GSH level in EAE mice compared to EAE group ($P < 0.001$).
Conclusions: The obtained results prove an important role of oxidative stress in the pathogenesis of EAE, whereas AGM protective effects offer new possibilities for a modified combined approach in MS therapy.

T173
DISCOVERY OF NEUROSARCOÏDOSIS BIOMARKERS BY PROTEOMIC ANALYSIS OF CEREBROSPINAL FLUID

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Sarcoidosis is a systemic granulomatous disease, which mostly affects lung, and many other organs such as central nervous system: this affection is called neurosarcoidosis, which affects 5 to 15% of all patients with sarcoidosis. The definite diagnosis is established on histological examination of brain granulomas. Angiotensin Converting Enzyme (ACE) is currently the most relevant biomarker of the disease, and is helpful to confirm a probable diagnosis; however, this test lacks sensitivity and specificity. We aim to find novel biomarkers of neurosarcoidosis in cerebrospinal fluid (CSF) by proteomic analysis, combining two-dimension electrophoresis (2-DE), fluorescence image analysis and mass spectrometry. We performed CSF proteomic profile of both patients (group S) and control subjects (group N). The statistical analysis of 2-D gels highlighted 42 spots significantly different between the two groups. 25 spots were picked and were subjected to tryptic digestion; the peptides were analyzed by MALDI-TOF and MALDI-TOF-TOF, giving rise to 10 identifications. Among the identified proteins, low-molecular-mass-kininogen and Vitamin-D-binding-Protein were increased, while transthyretin was decreased. These proteins have probably an intrathecal source and could be interesting candidates. Thereby, this study led to the identification of several proteins which can be used for the improvement of diagnosis and monitoring of neurosarcoidosis. These putative biomarkers have to be verified on the experimental set, confirmed on a validation set, and compared to the ACE measurement in CSF.

T174
DETERMINATION OF INHIBITION EFFICIENCY OF CARBAMATES INHIBITORS OF ACETYLCHOLINESTERASE

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Background: Alzheimer´s disease is an age dependent neurodegenerative disorder that destroys brain cells, causes problems with memory, thinking and behaviour. There are many hypotheses of the etiology of this disease. The cholinergic hypothesis relates with increased activity of acetylcholinesterase (ACHE) and the neurodegeneration with the loss of cholinergic neurotransmission. ACHE is an enzyme primarily responsible for the rapid elimination of acetylcholine (ACH), within one millisecond after its release at cholinergic synapses. Nowadays, for treatment of AD inhibitors of ACHE are used.
Methods: The first one, Ellman´s method (ELM) is based on measuring of yellow product which is formed in reaction between the thiol reagent 5,5′-dithiobis-2-nitrobenzoic acid and thiocholine (TCH), a product of substrate (acetylthiocholine, ATCH) hydrolysis by ACHE. Final concentration of ATCH in reaction mixture was 0.4 μM. The second one, electrochemical method is based on measuring of anodic oxidation current of TCH using square-wave voltammetry. Final concentration of ATCH in reaction mixture was 1 mM. The third one, pH-stat method is based on determination of actual concentration of acetic acid (HA) by continuous titration of HA with analytical solution of 0.1 M potassium hydroxide. Final concentration of ACH in reaction mixture was 1 mM. All measurements were carrying out with the final activity of ACHE in reaction mixture 0.2 U. For each measurement, the concentration of inhibitor was chosen experimentally so as to be visible inhibitory effect (slow hydrolysis).
Results: Inhibiting efficiency characterized by IC50 (concentration of inhibitor that causes a reduction of rate of inhibited reaction to one half compared to uninhibited reaction) was calculated. Inhibitor (4-chlorobenzyl phenylcarbamate) has the lowest IC50 (5.75 μmol/L) unlike inhibitor (3-phenoxybenzyl phenylcarbamate) (IC50 =12.1 μmol/L) using ELM. For each method, 4-chlorobenzyl phenylcarbamate has the lowest value of IC50.
Conclusions: To the according data, the most effective inhibitor is 4-chlorobenzyl phenylcarbamate. This compound was recommended to other testing (determination of cytotoxicity and partition coefficient).

T175

THE COMPARISON OF PCT, CRP AND WHITE BLOOD CELLS AT DIFFERENT STAGE OF SEPSIS IN NEWBORN

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Background: Neonatal sepsis is a major cause of neonatal mortality and morbidity. The early and rapid diagnosis of systemic infections is very important to start the right therapy on time. Procalcitonin (PCT) is an inflammatory marker used as indicator of severe bacterial infection. Although that C reactive protein (CRP) is a sensitive marker of inflammation, it is a disadvantage that increases after PCT. The aim of our study was to analyse the role of PCT and CRP as a markers for systemic infection in comparison with granulocytes in early diagnosis of sepsis in new born, in different stages.
Methods - PCT, CRP and granulocytes have been determined in 45 neonates of 1-21 days of age with different stages of sepsis, admitted at Prematurely Newborn Center, and in 10 healthy neonates as a control group. Patients have been classified into: patients with systemic inflammatory response syndrome (SIRS) (10 patients); with neonatal sepsis (23 patients); with neonatal sepsis and purulent meningitis (7 patients); and patients with septic shock (5 patients). The determination of PCT has been established with ELFA method, CRP with turbidimetric method and granulocytes with hematologic analyzer.
Results - PCT was significantly increased in neonates with septic shock (92,5 ng/mL) compared to the systemic inflammatory response syndrome- SIRS (41 ng/mL), neonatal sepsis (10.26 ng/mL), neonatal sepsis and purulent meningitis (9.8 ng/mL). The mean of controls were < 0.5 ng/mL. CRP is increased without statistical differences in all stages of sepsis in newborns with septic shock (93.2 mg/L) in cases with SIRS (45.64 mg/L), neonatal sepsis (70.02 mg/L), neonatal sepsis and purulent meningitis (61.98 mg/L) compared with controls (4.7 mg/L).Granulocytes were increased in all cases (100%) with SIRS, neonatal sepsis with purulent meningitis and septic shock, while in neonatal sepsis only in 78.3% .8.7% resulted within normal range and 13,5% lower than normal range.
Conclusion - We concluded that PCT and CRP in comparison with granulocytes are increased in all stages of sepsis, with higher values in septic shock. In patients with elevated serum CRP level, PCT may be used as a measure to support further the diagnosis of different stages of sepsis in newborn.

T176

EXPERIENCE OF SWEAT CHLORIDE ANALYSIS USING A LOW COST INDIGENOUS METHOD FOR THE DIAGNOSIS OF CYSTIC FIBROSIS IN INDIAN PEDIATRIC SUBJECTS

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Background: Cystic fibrosis (CF) is one of the most common inherited disorder of childhood. The condition was once thought extremely rare in India. Reports indicate that CF is far more common in people of Indian origin and is most likely under-diagnosed and often missed clinically. We report our experience in the diagnosis of CF using a simple low cost indigenous and validated method of sweat chloride analysis that can be used in resource poor settings.
Methods: A pre-validated indigenous low cost method (less than € 1.00 per test) is employed for the collection of sweat by pilocarpine iontophoresis and estimation of chloride in the eluted sweat using mercuric nitrate (Schales and Schales method).
Results: Thirty pediatric patients (16 male patients) with a median age of 3 years (IQR: 1yr 5 mo to 5 yrs 9 mo) were referred to the sweat testing facility of the clinical chemistry laboratory of a tertiary care pediatric teaching trust hospital of Kolkata, India; since its inception in June 2012. The average sweat collection was 224 mg. Requisite sweat of > 100 mg could not be collected in the first attempt for 6/ 30 patients. Most patients tolerated the procedure well and only three had minor burn injury. The most common cause for referral was recurrent cold and cough with respiratory distress, followed by recurrent pneumonia, failure to thrive, pancreatitis and history of meconium ileus in the neonatal period. Two children were born of consanguineous marriage. Out of 30, 2 patients had positive sweat chloride levels (> 60 mEq/L) for CF which was confirmed with a repeat procedure. These children are now under the care of an experienced pulmonologist.
Conclusions: The complexities of gene mutation analysis for the diagnosis of CF have led to a renewed interest in the sweat chloride analysis. A simple and low cost method based on quantitative pilocarpine iontophoresis can serve as an alternative to expensive methods to reliably diagnose CF in many resource poor settings. This may eventually help to treat and manage CF and avoid severe malnutrition and early mortality in children due to delayed diagnosis.

T177

DETERMINATION OF REFERENCE VALUES FOR CYSTATIN C IN NEWBORNS AT THE HOSPITAL VIRGEN MACARENA IN SEVILLE

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Background: Cystatin C is a single chain unglycosylated protein of low molecular weight. It´s 13.360 kD, 120 amino acids and two disulfide bridges. This protein is a good marker of renal function in newborn infants because of its independence from the weight, muscle mass and sex. Until today we have no knowledge of the existence of reference values for cystatin C in these children.
Methods: Blood samples of 90 children were collected at birth (from umbilical cord), at 72 h and seven days of life. The period of study was two years. Cystatin C was measured by nephelometry (BNII Siemens). Children were divided into three groups according to gestational age: group A (24-27 weeks), group B (28-33 weeks) and group C (34-37 weeks). The Kruskal-Wallis Test was used to relate cystatin C values according to gestational age. The statistical analysis was performed with the IBM SPSS Statistics 19 (New York, USA) for Windows with a statistical significance of $P < 0.05$.
Results: The range for Cystatin C in blood from umbilical cord at birth was from 1.45 to 1.64 mg /L; at 72 h was from 1.27 to 1.48 mg /L and at seven days of live was from 1.42 to 1.63 mg/L. By gestational age groups; the ranges of Cystatin C in blood from umbilical cord were: group A from 1.24 to 1.65 mg /L, group B from 1.42 to 1.56 mg/L and group C from 1.52 to 1.91 mg/L with significant differences between groups B and C ($P=0.034$). The ranges at 72 h were: group A from 1.00 to 1.26 mg/L, group B from 1.29 to 1.45 mg/L and group C from 1.22 to 1.67 mg/L with significant difference between groups B and C again ($P=0.030$). The ranges at seven days were: group A: from 1.20 to 54 mg/L, group B from 1.43 to 1.62 mg /L and group C from 1.34 to 1.81 mg /L without significant difference between groups ($P=0.329$).
Conclusions: We found that the reference values of Cystatin C in newborns are higher than in children over one year. The values at 72 h are lower than in the other two measurements for all groups. There are significant differences between the values in blood from umbilical cord at birth and blood at 72 h between term infants and post-term infants with a higher value of Cystatin C in the last group.

T178

EVALUATION OF CHANGES IN ACID-BASE METABOLISM IN PATIENTS WITH NEONATAL DEPRESSION INTERNED AT THE DEPARTMENT OF NEONATOLOGY OF THE HOSPITAL DE CLINICAS DE SAN LORENZO, FACULTY OF MEDICAL SCIENCES (FCM), NATIONAL UNIVERSITY OF ASUNCIÓN (UNA), AUGUST 2011 TO JULY 2012

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Background: Perinatal asphyxia is the damage to the newborn by an abnormality of the fetal gas exchange, generating a series of irreversible changes such as hypoxia, hypercapnia, metabolic acidosis. The purpose of the study was to assess changes in acid-base metabolism in patients with neonatal depression and identify the various perinatal and neonatal factors associated with the development of neonatal depression.
Methods: A retrospective, observational, descriptive trasverse cutting. We analyzed the medical records of newborns (NB) assisted in the Neonatal Intensive Care Unit (NICU) with a clinical diagnosis of neonatal depression.
Results: From August 1st, 2011 to July 31st, 2012, 56 newborn were assisted: 23% had a diagnosis of asphyxia (neonatal severe depression), 32% moderate neonatal depression and 45% mild neonatal depression. All newborn had blood gases with hypoxemia, hypercapnia and acidosis. The morbidity and mortality of newborns with neonatal depression diagnosis was 14%, with the highest percentage (46%) of newborns with perinatal asphyxia.
Conclusions: The newborn population mostly affected by mild and moderate neonatal depression weighted 2500 g, all male and gestational age greater than 37 weeks. Those with perinatal asphyxia weighted more than 2500 g, all male and with gestational age less than 37 weeks. Risk factors during the perinatal period such as: type of vaginal delivery, premature rupture of membranes and nuchal cord, were associated with the development of neonatal depression mild, moderate and severe. On the other hand: meconium aspiration syndrome, was associated with the development of neonatal mild depression, moderate and severe, commonly found in infants with perinatal asphyxia. There were 46 newborn with neonatal asphyxia also diagnosed.

T179

EARLY-ONSET NEONATAL SEPSIS: CORD BLOOD LEVELS BIOMARKERS

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Background: Different biochemical biomarkers have been used to identify patients with early-onset neonatal sepsis (EONS); such as C-reactive protein (CRP), Procalcitonin (PCT) and Interleukin-6 (IL6) in neonatal blood 24 hours after birth. The aim of this study was to investigate whether cord blood levels of CRP, PCT and IL6 were useful markers in the diagnosis of EONS, compared to neonatal blood levels.
Methods: Umbilical cord blood samples were obtained from 561 newborns and we established five study groups; group 1 (G1): without infectious risk factors not admitted to Neonatal Unit (Neo) (N=184), group 2 (G2): with suspected infection and risk factors, admitted in Neo (N=53), group 3 (G3): admitted in Neo for others causes (N=76), group 4 (G4): preterm newborns with a gestational age less than 37 weeks (N=236), and group 5 (G5): with EONS confirmed with blood culture or clinical sepsis (N=10). We studied biomarkers relationship, both in neonatal blood (neo) and cord blood (cb). We determined biomarker levels by automated methods (Roche Diagnostics®). We used U Mann-Whitney test comparing and we assessed Spearman correlations to compare different biomarkers. We used ROC curve analysis to calculate AUC.
Results: In G5, were found 7 patients with positive blood cultures and 3 with negative blood cultures but clinical sepsis. We only found significant differences comparing biochemical biomarkers in neonatal cord blood levels between G1 and G2 for PCT (P=0,002) and IL-6 (P <0,001). A positive and significant correlation between PCT (R=0,234, P <0,001) and IL6 (R=0,214, P <0,001) levels in cord blood and neonatal blood at 24hours after birth was found. IL6cb levels were greater predictors of an admission in Neo because of suspected infection than PCT and CRP (ROC for IL6=0,754 and PCT=0,641), and statistically significant in PCTcb and IL6cb in G2 compared to G1.
Conclusions: Biochemical markers of sepsis (CRP, PCT and IL6) in cord blood have not been useful; clinical data are the most decisive in the diagnosis of EONS. Biomarkers in cord blood are already predictors in neonatal blood in first hours after birth, and cut-off values for biochemical markers in cord blood could have clinical utility to decide a hospitalization with one or more infectious risk factors.

T180

ESTIMATING PEDIATRIC REFERENCE INTERVALS(RI) OF CALCIUM(CA), PHOSPHORUS(P) AND ALKALINE PHOSPHATASE(ALP). ALP WITH THE PROCEDURE RECOMMENDED BY IFCC (4-NITROPHENYL PHOSPHATE(NNP), BUFFER 2-AMINO-2-METHYL-1-PROPANOL(AMP) AT 37 °C) USING HOSPITAL-PATIENT DATA WITH THE MODIFIED HOFFMAN METHOD APPROACH

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Background: Serum Ca and P are extensively evaluated in pediatrics.ALP is present in a number of tissues including liver, bone, intestine, and placenta. The application of the procedure recommended by IFCC to assess ALP in analytical platform Cobas 501, Roche, generated a significant difference in the results obtained showing the need for reassessment of RI. The Hoffmann method for indirect estimation of the reference intervals from existing laboratory databases provides a mechanism for deriving RIs for difficult-to-study populations such as children.
Objective: To establish an RI for Ca, P and ALP in the pediatric population. ALP using the procedure recommended by IFCC.
Methods: One-year pediatric-hospital laboratory database for patients 0 to 18 years was used. Initially all subjects with any clinical diagnosis were excluded. RI of serum Ca and P were assessed using the modified Hoffmann method. For ALP, all subjects with Ca or P outside RI and elevated GGT, AST, or ALT serum levels were excluded, finally calculating the RI by the modified Hoffmann method. IQR was used as an outlier test. Serum determinations were done with the analytical platform Cobas501, Roche.
Results: We found for serum Ca mg/dl: 1-30 days (n: 30): 8.7-11.0, 30-365 days (n: 914): 8.9-10.9, 1-3 years (y) (n: 2351): 8.9-10.5, 4-6y (n:2096): 8.9-10.5, 7-9y (n:2114): 8.9-10.2, 10-12y (n: 2253): 8.9-10.2, 13-15y (n:2590): 8.8-10.1 and 16-18y (1558): 8.7-10.1. Serum P mg/dL: 1-30 days (n: 18): 4.0-7.3, 30-365 days (n: 948): 4.2-6.7, 1-3 years(y) (n: 2490): 3.9-6.0, 4-6y (n:2165): 3.7-5.7, 7-9y (n:2281): 3.8-5.6, 10-12y (n: 2435): 3.6-5.5, 13-15y (n:2766): 3.2-5.4 and 16-18y (1687): 2.9-5.0. Serum ALP U/L IR was 30-365 days Male (n: 181): 107-366, Female (n:180) 133-335; 1-3 years(y)Male (n: 583): 116-293, Female (n:499): 125-294; 4-6y Male (n:456): 110-286, Female (n: 438): 109-290; 7-9y: Male (n:512): 110-302, Female (n: 531): 128-318*; 10-12y Male (n:404): 91-290, Female (603): 98-348*; 13-15y Male (n:566): 106-343, Female (n: 672): 45-216* and 16-18y Male (334): 54-221, Female (n: 374): 36-149* (*significant difference P <0.0001).
Conclusions: RIs were calculated by age for the procedure recommended. ALP dimorphism was found for 7 years of age. Hoffmann's method proved to be useful in difficult-to-study population.

T181
ROLE OF THYROID HORMONE DYSFUNCTION AND CRP LEVELS IN NEONATAL SEPSIS

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Background: Thyroid hormone abnormalities are frequently encountered in patients with critical illness. Sepsis is an important cause of death of neonates in developing countries. The aim of the study was to evaluate the prognostic role of thyroid hormones (FT3, FT4, TSH) in neonates specifically with sepsis and septic shock and to correlate with levels of C-reactive protein (CRP). Materials: 40 neonates with sepsis were included in the study. Neonates with gestational age less than 37 weeks, body weight less than 2500 grams or with congenital abnormalities were excluded from the study. Septic neonates were further divided on the basis of their disease severity into non-survivors (n=12), shock-survivors (n=9) and sepsis survivors (n=19). 40 full term neonates without sepsis served as controls. Thyroid hormones and CRP were estimated by chemiluminescent immunometric assay and immunoturbidimetric assay respectively.
Result: FT3 and FT4 levels were significantly decreased ($p < 0.001$) in neonates with sepsis as compared to controls. No significant difference was observed in TSH levels. Non survivors had lower FT3 and FT4 levels ($P < 0.05$) compared to sepsis-survivor group. There was also a significant negative correlation between CRP and FT3 level in non-survivor group ($r = -0.60$; $P=0.02$) and septic shock survivor group ($r= -0.78$; $P=0.006$)
Conclusion: Low levels of FT3 and elevation in CRP correlate closely with decreased survival in septic neonates.

T182
A CLINICALLY RELEVANT MONOCLONAL GAMMOPATHY IN PEDIATRIC AGE

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Background: Monoclonal gammopathy (MG) is a uncommon finding in pediatrics. Only viral infections, transplantation and immunosuppressive therapy may induce B-cell proliferation leading to monoclonal component (MC) appearance in serum. Here we describe a case of clinically relevant MG in pediatric age. Methods: After receiving a request for a serum protein electrophoresis test (SPE) in a 12 years-old boy, information on the patient was collected. He was affected by ileocolonic Crohn's disease from April 2010, treated with nutritional therapy, prednisone and azathioprine. In February 2011, after a disease relapse, therapy with antibodies against tumour necrosis factor-α (infliximab and, later, adalimumab) replaced azathioprine. In November 2011 the boy was hospitalized for a further severe relapse. He was treated with corticosteroids in combination with adalimumab at increased dosage, achieving disease remission. A biochemical evaluation on the patient in remission was lastly performed in May 2012. SPE and MC immunotyping by immunosubtraction technique were carried out using capillary zone electrophoresis (Sebia Capillarys), whereas agarose gel immunofixation (IFE) of serum and urine was performed on Hydrasys (Sebia). Results: We retrieved in our LIS a SPE first performed on April 2010 showing a 4.7 g/L MC immunotyped as IgGλ. On November 2011 at SPE 3 MCs (concentrations: 3.7, 2.8 and 3.8 g/L, respectively) were detected and typed as IgAλ, IgMκ and IgGλ by IFE. A κ-type Bence Jones protein (BJP) was also detected in urine. Bone marrow examination was carried out to exclude lymphoid/myeloid malignancies (LMM) resulting in a negative pattern. At the evaluation performed on May 2012 only an IgGλ MC (4.5 g/L) was found in patient's serum, the κ-type BJP still persisting.
Conclusions: Crohn's disease in adults, particularly when treated by immunosuppressive drugs, can be associated with MG and an increased risk of developing LMM. At our best knowledge, no report of such association has been previously reported in pediatrics. This case illustrates a specific situation in which SPE execution in the young is recommended to prevent the risk of silent LMM development. It also shows that highly sensitive techniques are needed to detect small, but clinically relevant MCs.

T183

OPTIMUM CUT-OFF VALUE OF SALIVARY MELATONIN TO DISCRIMINATE BETWEEN ADHD CHILDREN AND A CONTROL GROUP

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Background: Altered patterns of melatonin circadian rhythm have been described in sleep disturbances, neurological, psychiatric and immunological conditions. Attention Deficit Hyperactivity Disorder (ADHD) is the most commonly psychiatric disorder in children, affecting about 3 to 5 per cent of children globally.The aim of this study is to determine if there is an alteration of the melatonin secretion pattern in ADHD children and therefore if it can be helpful for its diagnostic.
Methods: Salivary samples, timed at 180 minutes before and 60 minutes after going to sleep, were collected from 46 healthy (age range: 6-11, 50% males and 50% females) and 91 ADHD children (age range: 6-12, 76% males and 24% females). Suitable samples were centrifuged 10 minutes to remove particulate material and frozen at -70ºC until analysis. Quantitative determination of melatonin was performed by a direct non-extraction ELISA assay using DSX analyzer. Dim Light Melatonin Onset increase (ΔDMLO) was calculated between timed samples in patients as well as in healthy children. Data were analyzed by ROC curves using SPSS 15.0 statistical analysis package.
Results: Melatonin mean values found at -180 and +60 for the healthy and ADHD groups were: 1.8, 41.0; and 4.3, 27.3 pg/mL, respectively. In the healthy group, ΔDMLO was 39.2± 24.4 (SD) pg/mL, 1.7 times higher than in the diagnosed group ,23.0 ±15.9 (SD) pg/mL. The area under the ROC curve found was 0.71 (95%:0.612-0.806).Taking into account several cut-offs, we have considered 28 pg/mL as the optimum value, with 70% specificity and 63% sensitivity.
Conclusions: ΔDLMO values lesser or equal to the cut-off value may be suggestive of ADHD since this condition is related with a deficit in the secretion of melatonin. Therefore, ΔDLMO could be helpful in the diagnosis of ADHD and would be considered in the decision-making process.

T184

DIM LIGHT MELATONIN ONSET ASSESSED WITH ONLY TWO TIMED SALIVARY SAMPLES

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Background: Dim Light Melatonin Onset (DLMO) is the single most accurate marker for assessing the circadian pacemaker. The salivary melatonin measurement is a reliable alternative to blood samplings, being particularly suitable for children because it offers a non-invasive and stress-free collection. Altered patterns of melatonin circadian rhythm have been described not only in sleep disturbances but also in neurological conditions. We assessed if taking only two timed samples is enough to show an alteration in the DMLO pattern. For this purpose, we have determined DLMO in healthy and affected by Attention Deficit Hyperactivity Disorder (ADHD) children.
Methods: Salivary samples, timed at: 3 (-3), 2 (-2) h before, during (0), and 1 h after (+1) going to sleep, were collected from 46 healthy children and from 91 ADHD children diagnosed. Specific instructions for specimen collection were given. Suitable samples were centrifuged 10 min to remove particulate material and frozen at -70 ºC until analysis. Quantitative determination of melatonin in saliva was performed by a direct non-extraction ELISA assay on a DSX analyzer. Statistical analysis was performed by SPPS 15.0. Sex differences were tested by Kruska-Wallis' non-parametric test.
Results: Mean values at different times expressed as pg/mL were, in healthy children: 1.9, 3.0, 9.0, 27.2 and 41.0; in ADHD children were: 4.3, 5.1, 11.7, 20.8, 27.1; both in 3,2,1,0, and +1 h, respectively. The greatest difference among different timed samples was found between -3 and +1 h. We found no sex differences in any group.
Conclusion: DLMO pattern assessment could be simplified by considering only two timed samples at -3 and +1 h, making easier in this way the sampling taking process.

T185
VITAMIN D DEFICIENCY IN CHILDREN

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Aim :The vitamin D is very important in the bone metabolism , and plays a role in various organs , like hormone with systemic activity :paracrine and autocrine The lack of vitamin D is observed frequently not only in adult but also young boy and children.
Methods: We studied 225 children , 117 male and 108 female (average 10 years old). The Vitamin D is detected by instrument Architect (Abbott) . We have used the same cut-off for the normal value in adult people (>30 ng) (guideline SIMMONS).
Results: Ours data showed that in the 19% of young boy , the value were very low (Vit-D: 10-20 ng/mL), the 66% is low (Vit-D: 20-30 ng/mL), only the 15% were normal (Vit D:>30 ng/mL). The Vit D was decrease in the female , fat boy , and in seven boy with positive anamnesis of allergic asthma.
Conclusion: The decrease of vitamin D is a big problem in all world, and in adolescent is very marked . Another the related with other phatologie, like described in literature , stimulate the research, for the pathology correlated with low level of vitamin D. The public health committee should be proposed new model of life-style especially in the children.

T186
METABOLIC SYNDROME IN CHILDREN AND ADOLESCENT

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Introduction: Metabolic syndrome connected with obesity is a prominent problem, particulary in south Italy in pediatric under age. Such syndrome seems correlated to insulin -resistence that has been increasing the cardiovascular risk since that age.
Materials and methods: 225 children have been examined 117 male and 108 female (11-14 years old). The HOMA index (Homeostatic Assessment:insulin resistence) has been used to value the insulin resistence, fundamental venipuncture was performed after 12 h not have eaten. Glycemia is exspressed with mmol/L, insulinemia is expressed with uU/l , value HOMA index >2.5 shows a insulin resistence condition (O.Si.M.E.). To patients with HOMA >2.5 we have correlated parameters like B.M.I., abdominal girth (AG), total cholesterol, LDL, trygliceridemia and altered glycemia (not to have eaten) in conformity with study O.Si.Me (2006).
Results: Ours study has pointed out a significative percent 46% (101 patients) with HOMA index >2.5, one with diabetes mellitus type I, two (1.7%), with altered glycemia not have eaten (IFG), 20% had a BMI >75°<95° Pc, 29% a BMI >95°Pc, 14.8% of patients had an AG >75°<90° and 38.4% AG >90 Pc , 27% had a cholesterol with borderline values (v.n. >200 mg/dL) and 16% with high values >240 mg/dL. 8% had a LDL >130 (v.n.<130), and a 12% with trygliceridi > 200.
Conclusions: Ours data confirm that in pediatric under age the obesity is one of the most problem of health ,therefore only life styl's models can reduce the excess, for example reducing carboydrates consumption, saturated fat, inducing to increase the physic activity. Sanitary organizations should divulge to schools and families these conduct.

T187

CORRELATIONS OF SERUM 25-HYDROXYVITAMIN D WITH BONE TURNOVER MARKERS IN RICKETS

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Background: Serum markers of bone formation, as bone alkaline phosphatase (BAP) are useful indices of disease activity in rickets, being elevated in vitamin D [25(OH)D] deficiency. 25(OH)D, BAP, calcium [Ca(2+)] and magnesium [Mg(2+)] are important physiological regulators of bone homeostasis. The aim of the study was to assess the correlations between these markers involved in the process of bone remodeling in rickets.
Methods: The study enrolled 54 children and adolescents with rickets (aged 4-16 years) and 40 aged-matched healthy children. The vitamin D deficiency rickets was confirmed by clinical signs and low levels of Ca(2+), Mg(2+), and 25(OH)D. The serum levels of bone markers were measured by ELISA. Serum concentrations of Ca(2+) and Mg(2+) were determined by VitrosSlides quantitative technique.
Results: The majority of the children included the study group (74%) presented vitamin D deficiency (<37.5 nmol/L), the rest showed insufficiency (37.5-50 nmol/L). Serum levels of 25(OH)D were significantly decreased in study group than in controls (38.6±14.5 nmol/L, respectively 78.9±7.5 nmol/L, P <0.0001). BAP concentrations were significantly increased in rickets group (142.7±47.3 μg/L) than in controls (109.8±22.8 μg/L, P <0.003). In all subjects, 25(OH) D correlated negatively with BAP (r = -0.469, r^2=0.22, P <0.0001). Serum concentrations of ions were significantly lower in study group [Ca(2+): 3.89±0.20 mg/dL, Mg(2+): 1.75±0.06 mg/dL] than in controls [Ca(2+): 4.89±0.27 mg/dL, P <0.0001, respectively Mg(2+): 2.12±0.18 mg/dL, P <0.0001]. BAP levels did not correlate with Ca(2+) or Mg(2+) concentrations. Furthermore, BAP and 25(OH)D levels did not correlate with age or sex of the subjects.
Conclusions: In rickets, decreased vitamin D concentrations induce osteoclast stimulation. Serum levels of BAP are significantly increased, attesting osteoblastic activation. The present findings confirm that children with vitamin D deficiency rickets show increased bone turnover.

T188

ASSESSMENT OF LCT -13910CT AND LCT-22018AG SINGLE NUCLEOTIDE POLYMORPHISMS AMONG CHILDREN PRESENTING FOR GASTROINTESTINAL CONDITIONS CONSISTENT WITH LACTOSE INTOLERANCE: A HOSPITAL-BASED STUDY OF LEBANESE PATIENTS

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Background: Adult-type hypolactasia affects most of world's human population and limits the use of milk due to lactose intolerance. The diagnosis of adult-type hypolactasia is challenging: direct lactase assay using biopsy specimens of intestinal mucosa is an expensive and invasive method; many of the other methods remain imprecise. The -13910CT and -22018AG polymorphisms of the lactase gene are reliable predictors of lactase persistence in Caucasians-derived populations, with genotypes -13910CC and -20018GG being associated with hypolactasia. The Lebanese population, for which no such data have been reported, is known to be genetically close to the Caucasian population. The aim of this study was to assess the frequency of LCT-13910CT and -22018AG variants among Lebanese children presenting for suspicion of lactose intolerance in order to appreciate the applicability of genotyping those variants as a diagnostic test for hypolactasia during childhood.
Methods: The study included 69 patients presenting to the pediatrics department for gastrointestinal conditions consistent with lactose intolerance. 200ml of blood was used for DNA extraction. Two PCRs, based on the techniques described by Mulcare et al. (2004) and Mendoza et al. (2010) were performed for the respective genotyping of LCT-13910 and LCT-22018.
Results: Age ranges from 1 month to 21 years old; females were 36 (52%), males 33 (48%). For locus -13910, 58 patients (84.1%) were CC, 10 (14.5%) were TC and 1 (1.4%) was TT. For locus -22018, 21 patients (30.4%) were GG, 44 (63.8%) were AG and 4 (5.8%) were AA. Twenty one patients (30.4%) were homozygous at both loci (CC-13910/GG-20018), 34 (49.3%) homozygous CC for -13910 and heterozygous for -22018, 3 (4.3%) homozygous CC for -13910 and AA for -22018, 9 (13.0 %) heterozygous for both loci.
Conclusions: In the present study, we found a strong association between hypolactasia and LCT-13910 since 58 (84.1%) patients were genotype CC. If we also consider the double heterozygous (-13910CG/-22018AG) (9 patients), combined genotypes supporting hypolactasia would be present in 67 patients (97%). Genotyping those variants could therefore be considered as a potential beneficial diagnostic test for adult-type hypolactasia during childhood in Lebanese patient.

T189

EVALUATION OF A NOVEL, COMMERCIALLY AVAILABLE MASS SPECTROMETRY KIT FOR NEWBORN SCREENING INCLUDING SUCCINYLACETONE WITHOUT HYDRAZINE

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Background: Newborn screening for hereditary tyrosinemia type I (HT 1) is mandatory to identify infants at risk before life-threatening symptoms occur. The analysis of tyrosine alone is limited, and might lead to false-negative results. Consequently, the analysis of succinylacetone (SUAC) is needed. Current protocols are time-consuming, and above all, include hazardous reagents such hydrazine.
Methods: We evaluated a novel, commercial kit to analyze amino acids, acylcarnitines and SUAC with a significantly less harmful hydrazine derivative in a newborn screening laboratory. Dried blood spot specimens from 4,683 newborns and samples from known patients with inborn errors of metabolism (IEM) were analyzed by a novel protocol and compared to an in-house screening assay. All samples were derivatized with butanol-HCl after extraction from 1/8-inch DBS punches. For the novel protocol, the residual blood spots were extracted separately for SUAC, converted into hydrazone, combined with amino acids and acylcarnitines, and subsequently analyzed by mass spectrometry using internal isotope-labeled standards.
Results: All newborns were successfully tested, and 74 patients with IEMs including three with HT 1 (SUAC 1.50, 4.80 and 6.49; tyrosine levels 93.10, 172.40 and 317.73, respectively) were detected accurately. The mean SUAC level in non-affected newborns was 0.68 μmol/l (cut-off 1.29 μmol/l).
Conclusions: The novel assay was demonstrated to be accurate in the detection of newborns with IEM, robust, and above all, without the risk of the exposure to highly toxic reagents and requirement of additional equipment for toxic fume evacuation.

T190

RET-HE AS A MARKER OF SEVERE BACTERIAL INFECTION IN CHILDREN PRESENTING WITH FEVER

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Background. Clinical evaluation of a child with fever does not always give accurate results having a tendency to underestimate the likelihood of serious infection, and additional diagnostic data may increase accuracy of the final diagnosis. Reticulocyte hemoglobin equivalent (Ret-He) is used as an indicator of iron availability for erythropoiesis. In acute infection and inflammation with fever, functional iron deficit may result in Ret-He reduction. Hereby, we assess Ret-He characteristics as a diagnostic marker in acute infection in children in comparison to traditional markers: white blood cell count (WBC) and absolute neutrophil count (ANC).
Methods. Venous blood was collected from 173 children (median age, 1.8 years; range, 0.1-5.3 years) presented at emergency room with fever >38 °C and suspected acute infection. Diagnosis of infection was verified by routine clinical and laboratory evaluation, and the children were separated into 2 groups: having severe bacterial infection (the SBI group, n=50) or having no SBI (n=123). Blood counts were performed by means of a Sysmex XT-2000i analyzer. The measurement of the Ret-He was done using the XT-2000i RET MASTER software in a separate laser channel of the analyzer. Laser scatter (reflection) detects the fluorimetric signal after staining of the RNA, and then the system can ascertain the Ret-He through fluorimetric reading of the contents of reticulocytes. All statistical analysis was performed by the SPSS Statistics 20.0. Data were presented as median (P25-P75).
Results. Median Ret-He level was higher in the SBI group (24.8 pg; 21.6-27.9) when compared to the no SBI group (27.4 pg; 24.9-29.3), P <0.001. The lower reference limit for Ret-He in healthy children established at our institution is 28.4 pg. Area under the ROC curve (AUC) for Ret-He discriminating SBI vs no SBI patients was 0.68 (95%CI; 0.57-0.79). At cut-off 26.1, Ret-He had sensitivity 59% and specificity 70% for detecting SBI. AUC for WBC was 0.82 (95%CI; 0.73-0.90), AUC for ANC was 0.78 (95%CI; 0.68-0.87).
Conclusions. Ret-He has good diagnostic performance in children with fever. Thus, Ret-He may be used as an additional marker in laboratory work up for differentiation of SBI in children with fever.

T191

PEDIATRIC DYNAMICS AND REFERENCE INTERVALS FOR WHITE BLOOD CELLS PARAMETERS IN HEALTHY NEWBORNS AND CHILDREN IN RUSSIA

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Background. Complete blood count with leukocyte differentiation (CBC+DIFF) is one of the most relevant and frequently used laboratory tests in pediatric practice including neonatology. Children healthcare is critically dependent on the availability of reference intervals (RI). RI are crucial decision-making tools aiding clinicians in differentiating between healthy and diseased populations. However, for children such values often are lacking or incomplete. Hereby, we report RI for automated blood analysis by means of a Sysmex XT-2000i analyzer.
Methods. The CBC+DIFF analysis was performed in 364 samples from 190 newborns (cord blood [CB], n = 103; early neonatal period: ≤ 2 days, n = 53; 3 days, n = 82; 4 days, n = 52; ≥ 5 days, n = 74) and in 286 healthy children (age range, 3 months–17 years) at prophylactic medical examination. All manipulations with blood was made with informed parental consent. RI were generated according to the CLSI C28-A3 statistical guideline by means of SPSS Statistics 20.0 package.
Results. The CB samples were analyzed separately from other blood samples. Leukocyte, neutrophil, immature granulocyte and platelets levels were higher in CB than in neonatal samples up to age ≥ 5 days. During early neonatal period, white blood cells parameters had statistically significant negative correlation with age. Platelet counts displayed a statistically significant increase with age. In healthy children (3 month–17 years), the number of leukocytes, lymphocytes, eosinophils and platelets had statistically significant negative correlation with age, but the neutrophil number had statistically significant positive correlation with age. Age had no statistically significant influence on monocyte and basophil number. RI for indicated CBC+DIFF parameters were established for various age groups: early neonatal period, <1 year, 1-5 years, 5-10 years, 10-17 years.
Conclusions. Our data will help to implement the up-to-date RI for hematological parameters in pediatric clinical practice.

T192

LYMPHOCYTE SUBSETS AND ACTIVATION MARKERS IN CHILDREN WITH IMMUNODEFICIENCIES AND RECURRENT INFECTIONS

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Background: lymphocyte subsets are commonly determined if primary immunodeficiencies (PID) are suspected, but their interpretation in pediatric population is not straightforward. The objective is to identify peculiar distributions of lymphocyte subsets and activation markers in children with PID and recurrent infections.
Methods: We analyzed lymphocyte subsets (CD3, CD4, CD8, CD19, CD56/16) and activation markers (CD45RA, CD45RO, CD25, CD38, HLA-DR, TCR$\gamma\delta$) in 17 children with recurrent infections and 42 PIDs (11 antibody deficiencies, 2 SCIDs, 12 DiGeorge syndromes, 9 phagocytes defects, 8 autoimmunity syndromes), by flow-cytometry on peripheral blood. Reference ranges for normal children of same age were analyzed and expressed in percentiles. Wilcoxon test and cluster analysis were used for comparison.
Results: Younger children expressed absolute lymphocytosis, therefore 1000/mm3 cut-off was not effective in recognizing cellular defects in children younger than 6 (10° percentile: 1900/mmc). Among all children with recurrent infections, Tcell expression of TCR$\gamma\delta$ and CD25 was significantly different in subjects with antibody deficiency (XLA, Hyper-IgM, CVID: elevated TCR$\gamma\delta$, absent CD25) compared to children without PID (opposite pattern). This finding helps to define PID diagnosis when serum levels of Ig are quite normal. The markers' distribution in DiGeorge syndromes (low CD3, elevated CD19, NK, HLA-DR, TCR$\gamma\delta$ and CD45RO, absent CD25 and CD45RA) was statistically significant and cluster analysis confirmed this distinction, allowing identification of an undiagnosed girl with recurrent infections but without malformations.
Conclusions: Age-related analysis of immunophenotype is a useful tool to distinguish normal children with recurrent infections from PIDs. Extension of the study to higher numbers is required.

T193

ALKALINE PHOSPHATASE IN HEALTHY CHILDREN: PREVALENCE OF ELEVATED LEVELS AND REFERENCE INTERVALS

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Background: Transient hyperphosphatasemia (TH) is an often unnoticed entity. Many case reports have been published, and it is thought that this benign condition primarily affects children below 5 years of age. However, the prevalence among healthy children is unknown. Data from the Falun pediatric reference interval project was used to estimate the prevalence of high alkaline phosphatase (ALP) among healthy children, as well as calculating pediatric reference intervals.
Methods: Blood samples were collected from 694 healthy Caucasian children, aged 6 months to 18 years, recruited in child care centers and schools in the Falun region of central Sweden. All subjects were subjectively healthy and a questionnaire on diseases and medications was filled out by parents and by the older children. Serum samples were analysed on an Abbott Architect 8200ci. After exclusion of the high ALP results (>1000 U/L or >16,7 μkat/L) reference intervals were calculated using non-parametric statistics to define the 2.5 and 97.5 percentiles. Partitioning by age and gender was evaluated by the Lahti model (Clin Chem 2002;48:338).
Results: Six children had ALP results >16,7μkat/L (25, 41, 54, 58, 76 and 76μkat/L). These subjects included four females and two males, aged 7-22 months. The prevalence in the age group 6.0mo to 4.99y was 2.4% (6/250). None of the older children had ALP >16,7μkat/L. The present study did not include any follow up of these apparently healthy children, thus we cannot exclude others conditions than TH as an explanation for the elevated ALP. However, other general chemistry analyses, such as liver enzymes and calcium were in general normal in these children. After exclusion of these six children reference intervals were calculated for the remaining 688 children: 0.5-1y (FM) 1.73-7.59μkat/L. 2-8y (FM) 1.85-4.62. 9-14y (FM) 1.26-7.98. 15-18y (F) 0.61-3.71. 15-18y (M) 1.06-5.32.
Conclusions: The prevalence of high ALP (>1000 U/L or >16,7 μkat/L) among subjectively healthy children in our population is approximately 2.4% under the age of five years. Reference intervals vary with age and gender.

T194

TUMOR NECROSIS FACTOR ALPHA (TNFα) PROMOTER HAPLOTYPE CONCURS WITH HLA-DQA1 –DQB1 IN DETERMINING CELIAC DISEASE RISK

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Background: The rare A alleles at -308 and -238 positions of the TNFα gene have been associated with CD. Although they may be disease susceptibility factors, it is likely that they act as part of TNFα haplotypes resulting from the combination of more SNPs of the promoter. The aims of this study were to ascertain whether five SNPs in the TNFα promoter(-1031T>C,-857C>T,-376G>A,-308G>A,-238G>A) are associated singly or as haplotypes with CD and whether their effect is HLA-DQA1/DQB1 dependent.
Methods: 527 children (250 CD, 277 controls) were studied. TNFα promoter SNPs and HLA-DQA1 and –DQB1 alleles were analysed by RT-PCR.
Results: The TNFa-1031C (OR=2.540, 95%CI: 1.157-5.575),-857T (OR=0.421,95%CI: 0.282-0.627),-376A (OR=2.037, 95%CI: 1.109-3.741) and -308A (OR=4.486, 95%CI: 3.076-6.541), but not -238 alleles, were significantly CD-associated, this finding being confirmed at multivariate logistic regression analysis. Six possible haplotypes derived from the combination of these four SNPs were estimated using the Arlequin statistical software. CCGG and TTGG were more frequent in controls; CCAG,TCGA and CCGA were more frequent in CD; TCGG was equally found in controls and CD. TNFa promoter genotypes inferred from haplotypes were classified in six categories: 1) CD-associated/ CD-associated; 2) CD-associated/TCGG; 3) CD-associated/control-associated; 4) control-associated/TCGG; 5) TCGG/TTG; 6) control-associated/control-associated. A strong correlation was found between the first two categories and CD and between the last category and controls not only considering all cases, but also considering only those bearing HLA-DQA1/DQB1 risk alleles. Binary logistic regression analysis performed by including among predictors TNFα promoter genotypes and HLA-DQ haplotypes, documented a strong association between CD and homozygous HLA-DQ2 (OR=481; 95%CI:112-2068), heterozygous HLA-DQ2 (OR=73; 95%CI:26-208) or HLA-DQ8 (OR=14; 95%CI:5-45), but also an HLA-independent correlation with CD-associated TNFα promoter homozygous (OR=7; 95%CI:1,48-34,31) or heterozygous (OR=5; 95%CI:1,56-16,59) genotypes.
Conclusions: In conclusion the TNFα promoter haplotypes CCAG,TCGA and CCGA was shown to increase CD risk independently from HLA-DQ alleles suggesting the pivotal role of TNFα in the disease pathogenesis.

T195

SHORT- AND LONG-TERM EFFECTS OF INHALED CORTICOSTEROIDS ON THE BONE METABOLISM OF CHILDREN WITH AIRWAY DISEASES

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Background: Inhaled corticosteroids (ICS) are very effective in the treatment of bronchial asthma, but the effects of ICS therapy on the bone metabolism of children are poorly understood and the results remain controversial compared to those on controls. The aim of the study was to examine the biochemical markers of bone metabolism in children treated with ICS.
Methods: The authors examined the effects of high, low and cumulative doses of ICS in children with different airway diseases. Group I: 10 children (mean age 5.6 y) with subglottic laryngitis received an average daily dose of 825 μg fluticasone. Urine samples were collected before, and 24 and 48 h after the beginning of ICS treatment. Group II: 11 children (mean age 8.8 y) with bronchial asthma received a daily dose of 200 μg fluticasone. NTx levels were measured in urine samples before, and 2, 4 and 6 weeks after ICS treatment. Group III: 42 asthmatic children received ICS (budesonide, cumulative dose 452 mg) therapy for an average of 53 months. The serum levels of N-MID-Osteocalcin (Ocn) and betaCrossLaps (CTx) were measured with Roche tests (Roche Diagnostics, Germany) and from urine cross-linked N-telopeptides (NTx) with a VITROS 250 analyser (Ortho Clinical Diagnostics, USA). The studies in Groups I and II were self-controlled; in Group III the results were compared with healthy controls.
Results: In Group I there was a significant increase ($P < 0.01$) in the NTx values on days 1 and 2 of ICS therapy. In Group II there was no significant change in NTx. In Group III the urine and serum markers of the ICS group did not differ significantly from those of the healthy controls at any time.
Conclusions: 1/ ICS has systemic effects. Bone resorption is already influenced after 24 h of ICS treatment. 2/ However, the systemic effects of ICS are neither progressive nor cumulative. 3/ Despite these facts, the smallest effective dose of ICS should be administered as initial treatment.

T196

IMPACT CLINICAL PARAMETERS ON EVOLUTION OF RESIDUAL INSULIN SECRETION IN TYPE 1 DIABETIC PATIENTS BY RADIOIMMUNOASSAY OF C-PEPTIDE

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Introduction: Type 1 diabetes results from a β-cell destruction due to an autoimmune process leading to insulin deficency. This insulin deficiency may be assessed by measuring the C-peptide which is a marker of residual endogenous insulin secretion in insulin-dependent diabetics.
Objectives: To assess the C peptidemia in young diabetics in relation to their glycemic control and secondly to study the impact of clinical parameters on the evolution of residual insulin secretion (RIS).
Material and methods: 38 diabetics (18 boys, 20 girls), with mean age 13 +/- 6 years, age of onset (AO) 8 +/- 4 years and mean diabetes duration (DD) 6 +/-5 years. All patients receive a 2 daily injections regimen. The C-peptide levels were assayed by radioimmunoassay and the levels of glycated hemoglobin were measured by the method DCA2000. (Mean annual Value: 8.3+/-1.5% (2010), last value 7.8+/- 1.5%) (09/2012). Three groups have been considered depending on metabolic control (G1 HbA1c ≤ 7.5% DD: 3.6 years, G2 HbA1c >7.5% DD: 6.5 years, G3 HbA1c >7.5% DD: 6.6 years)
Results and discussion: Our results show higher C-peptide levels in G1 patients compared to G2 and G3 (Mean+/-SD: 1.03+/-0.1 ng vs 0.35 +/- 0.20 ng/mL and 0.35+/- 0.09 ng/mL). In G2 and G3 the C-peptide levels are increased insignificantly ($P = 0.058$) from childhood to adolescence. Furthermore, C peptidemia is negatively correlated with AO (maximum values when AO is 5-11 y and decreasing in all groups with later onset). In G2 C-peptide levels evolved from 0.44 ng/ml to 0 ng /mL after 10 years of DD respectively. RIS is correlated with insulin requirements (Insulin 1.5 UI/kg vs value of C-peptide: 0.28 ng/mL) and BMI.
Conclusion: C-peptidemia is correlated with metabolic control, insulin requirements, DD and also with AO and BMI. RIS could be promoted and maintained by some clinical parameters.

T197
PLASMA GLYCOSAMINOGLYCANS OF CHILDREN WITH JUVENILE IDIOPATHIC ARTHRITIS

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Background: Related with juvenile idiopathic arthritis (JIA), increased levels of proinflammatory cytokines as well as hyperactivity of proteolytic enzymes, may affect metabolism of the extracellular matrix components. However, the influence of aforementioned factors on tissue proteoglycans and glycosaminoglycans (GAGs) metabolism in children with JIA, is not fully elucidated. Tissue PGs/GAGs metabolism is reflected by the blood GAGs pattern. Hence, quantitative analyses of GAGs and matrix metalloproteinases (MMP-3 and MMP-10) and their tissue inhibitors (TIMP-1 and TIMP-2) in plasma of JIA patients were carried out to provide a clearer understanding of the role these macromolecules in the disease pathogenesis.
Methods: GAGs were isolated by the multistage extraction and purification using papaine hydrolysis, alkali elimination, as well as cetylpyridium chloride binding, from blood samples derived from healthy subjects and JIA patients before treatment and after achievement of remission state. Total amount of GAGs was quantified by the hexuronic acids assay. Plasma levels of MMP-3, MMP-10, TIMP-1 and TIMP-2 were quantified by ELISA kits from R&D Systems.
Results: We found statistically decreased level of plasma GAGs in the JIA patients before and after treatment (119.72±22.34 mg/L, 125.53±19.60 mg/L) compared to controls (156.06±19.84 mg/L). Plasma level MMP-10 and TIMP-2 were significantly lowered in untreated patients (736.01±296.02 pg/mL, 86.95±18.50 ng/mL) versus controls (905.34±446.22 pg/mL, 107.85±17.45 ng/mL). The disease remission led to normalization of these parameters. We revealed statistically elevated of MMP-3 and TIMP-1 levels in JIA patients plasma before treatment (22.47±2.28 ng/mL, 96.50±48.21 ng/mL) versus after treatment situation (5.49±0.57 ng/mL, 61.39±18.97 ng/mL) and controls (2.58±1.01 ng/mL, 74.23±21.75 ng/mL). Total plasma GAGs positively correlated with MMP-10 (r=0.382, P=0.001) in untreated JIA patients. A negative relationship was found between plasma GAGs and both MMP-3 (r=-0.425, P=0.002) and TIMP-1 (r=-0.266, P=0.025) levels in this group of patients.
Conclusions: Although the mechanisms leading to the development of disturbances of ECM components metabolism in the course of JIA still have to by elucidated, we postulate that disturbed proteolytic potential play a significant role.

T198
NEWBORN HAEMOGLOBINOPATHY SCREENING USING TANDEM MASS SPECTROMETRY IN SOUTHERN SPAIN

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Background. Some countries have included early detection of haemoglobinopathies in their newborn screening programs during the past decades, especially conditions for which there is greater evidence that early intervention is likely to be beneficial: Hb SS, Hb SC, Hb S-thalassemia. Our aim was to prove that tandem mass spectrometry (MS/MS) offers an efficient alternative methodology with respect to the currently used techniques (HPLC and IEF).
Methods. We analyzed 7712 samples (dried capillary blood spots on filter paper) obtained from newborn children during the first week of birth in our region (Andalucía) during 2012, as part of the neonatal screening program. For analysis, blood spots (3.2 mm) were punched into separate 96 well plates. Sample preparation was carried out using an adaptation of a method previously described (Boemer et al. Clin Chem 2008) based on the digestion of haemoglobin using trypsin treated with TPCK (Sigma-Aldrich®), resulting in a working solution ready for injection, and reducing the preparation time from 12 to 2 h. We loaded 20 μL aliquots of the working solution via a 10 μL loop injector into the electrospray source of a triple quadrupole mass spectrometer (API 3200, ABSciex). Samples were injected into the mobile phase stream [ACN:H2O (50:50)] using a HPLC pump (Perkin-Elmer). Analysis was performed in the multiple-reaction-monitoring (MRM) mode with an acquisition time of 120s (HbA: MRM 476.8>502.2, HbS: MRM 461.8>472.3, HbC: pseudo-MRM 694.4>694.4). All the cases detected were confirmed using HPLC (Variant, Bio-Rad).
Results. In total, 42 cases were detected, resulting in a global frequency of 0.54% (28 cases were AS phenotype, 13 AC phenotype and 1 sickle-cell anemia SC). Specificity with respect to HPLC was 100%. Maximum sensitivity obtained in the confirmed cases was sufficient for a screening program (1.3% HbS and 2.1 % HbC).
Conclusions. The method developed based on MS/MS can be applied to newborn screening of haemoglobinopathies, with adequate sensitivity and specificity. Its advantage with respect to HPLC is the lower reagent cost and the unequivocal confirmation of the haemoglobin variant. The disadvantages however, are the lack of standardization and the more complex sample preparation.

T199
QF-PCR AS A RAPID TECHNIQUE FOR ROUTINE PRENATAL DIAGNOSIS OF FETAL ANEUPLOIDIES

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Background: The most common chromosomal abnormalities identified at birth are aneuploidies of chromosome 21, 18, 13, X and Y. Prenatal diagnosis of fetal aneuploidies is routinely done by traditional cytogenetic culture, a major drawback of this technique is the long period of time required to reach a diagnosis. In this study we evaluated the QF-PCR as a rapid technique for prenatal diagnosis of common aneuploidies Method:This work was carried out on Sixty amniotic fluid samples taken from patients with one or more of the following indications: Advanced maternal age (3 case), abnormal biochemical markers (6 cases), abnormal ultrasound (12 cases) or previous history of abnormal child (39 cases). Each sample was tested by QF-PCR and traditional cytogenetic. Aneuploidy screenings were performed amplifying four STRs on chromosomes 21, 18, 13, two pseudoautosomal,one X linked, as well as the AMXY and SRY; markers were distributed in two multiplex QFPCR assays (S1 and S2) in order to reduce the risk of sample mishandling .
Results : All the QF-PCR results were successful, while there was two culture failures, only one of them was repeated. No discrepancy was seen between the results of both techniques. Fifty six samples showed normal patterns, three sample showed trisomy 21, successfully detected by both techniques and one sample showed normal pattern by QF-PCR but could not be compared to the cytogenetics due to culture failure, the pregnancy outcome of this case was a normal baby. Conclusion: Our study concluded that QF-PCR is a reliable technique for prenatal diagnosis of the common chromosomal aneuploidies. It has the advantages over the cytogenetic culture of being faster with the results appearing within 24-48 h, simpler, doesn't need a highly qualified staff, less prone to failure and more cost effective.

T200
HUMORAL IMMUNITY IN THE HEALTHY POPULATION OF THE CENTRAL REGION OF CUBA

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Background: In order to study the humoral immune response in healthy population of the central region of Cuba we establish reference values of immunoglobulins IgG, IgM and IgA and C3 and C4 complement proteins in three different and interesting moments in life from the immunological point of view (birth, childhood and adulthood) in apparently healthy population of the province of Villa Clara, Cuba.
Methods: With serum samples from 200 individuals were formed 3 study groups comprised 80 new born, 60 children and 60 adults. In each case were 50% female and 50% male. Using nonparametric tests found no significant differences between gender at each group was taken as a single sample. The values of reference were established for the immunological parameters according to age, taking the central interval (95%) delimited by the 2.5th and 97.5.
Results: It was observed that new born had lower serum complement proteins, while children showed slightly higher serum concentrations than adults. The immunoglobulins were significantly different in each study group.
Conclusions: Until the end of this work do not exist similar studies in the region that also characterize the humoral immunity, provide reference values to facilitate further studies to make decisions about possible prognoses and treatments of patients exhibiting inmunological symptoms.

T201
ASSOCIATIONS BETWEEN THE URINARY METABOLOME AND BREASTFEEDING

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Background: The identification of biomarkers for evaluation of lactation performance is of interest for clinical practice and lactation research. The presence of lactose in urine has previously been demonstrated at week 22 in pregnancy. Post partum lactose/creatinine has in some studies been associated with increased infant intake. The aim of this study was to investigate how the maternal urinary metabolite profile during and after pregnancy correlated with breastfeeding status.
Methods: The STORK Groruddalen study is a population-based cohort study of 823 pregnant women with diverse ethnic background. Morning urine samples were collected at three consultations (V1: gestational week (GW) 8-20, V2: GW 28, and V3: 10-16 weeks post partum) and analyzed using 1H-NMR metabolomics. NMR spectra were acquired at 300.0 K on a Bruker AV 600 spectrometer. Processing and analysis of spectra was carried out with the statistics package R. Metabolites were identified using published literature and the Human Metabolome Database. Urine profiles were individually normalized to the absolute creatinine concentrations. Breastfeeding status was recorded at V3.
Results: Urine profiles at V3 could distinguish between full (n=326), partial (n=156) and no (n=67) breastfeeding (no information or lack of sample; n=274). Lactose/creatinine concentrations differed significantly between the three groups of breastfeeding (μM/mM; ANOVA p-value = 9e-58; mean ± 95% CI = 300±20, 200±30 and 40±8, respectively), but glycine, creatine and two unidentified substances were also correlated with breastfeeding status. Earlier urine profiles at V1 and V2 were generally not correlated with successive breastfeeding, but increased glycine/creatinine ratios during pregnancy at GW 28 was associated with breastfeeding 10-16 week post partum (p = 2e-5; 340±30, 310±30 and 240±20, respectively).
Conclusions: Increased lactose excretion in urine post partum was associated with self-reported breastfeeding. Low level of urinary lactose during pregnancy was not related to inferior lactation performance. The observed association between breastfeeding and glycine excretion both mid-gestational and post partum should be interpreted with caution.

T202
N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE IN NEONATES WITH CONGENITAL HEART DISEASE

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Background: Clinical relevance of N-terminal pro-B-type natriuretic peptide (NT-proBNP) in pediatric patients with cardiac disease, particularly in neonates, is still argued. There are scarce data in the literature about the importance and possible use of NT-proBNP measurement in neonates with congenital heart disease (CHD).
Methods: Our goals were to determine the levels of NT-proBNP and their course during the neonatal period in a group of neonates without CHD (control group n=28, mean age 6,82±5,61 days), and to compare them with the results of a group of neonates with CHD (pathological group n=38, mean age 4,79±5,37 days), as well as to determine if there is a possible correlation between the clinical parameters (like fractional shortening of the left ventricle, presence of a cardiac shunt, cardiac shunt direction) and concentration of NT-proBNP in the pathological group. NT-proBNP concentrations were determined by the Roche electrochemiluminescent immunoassay.
Results: The concentrations of NT-proBNP varied greatly in the control group: they were highest in the first days of life, then decreased until the end of the neonatal period. A negative correlation between age and concentration of NT-proBNP was evident (r=-0,65, P <0,001). A significant difference was also found between NT-proBNP values in the first week and the following days of the neonatal period (P=0,012). The neonates with CHD showed significantly higher mean concentrations of NT-proBNP (31242,9±36822 ng/L) compared to the control group (6851±8095,9 ng/L, P <0,001). The neonates with CHD and low fractional shortening of the left ventricle had higher levels of NT-proBNP then those with CHD and normal fractional shortening (P=0,007). No differences have been found in the levels of NT-proBNP between the neonates with CHD with the shunt (n=33) compared to those without the shunt (n=5, P=0,276), or related to the shunt direction.
Conclusions: High concentration of NT-proBNP may be used in diagnosis of congenital heart disease in neonates additionaly to the other clinical findings. An accurate diagnosis of congenital heart disease depends strongly on the reference and decision values for NT-proBNP in the neonatal period.

T203

MEASUREMENT OF BASAL ANTI-MULLERIAN HORMONE LEVEL OF HEALTHY FERTILE WOMEN IN MACEDONIA

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Backgroud: With aging in women the number of primordial follicles is reduces, which is accompanied by a decrease in serum concentrations of anti Mulerian hormone. Several studies have shown that serum concentrations of anti mulerian hormone strongly correlate with the number of antral follicles and much more specific marker for predicting ovarial reserve and induction induction than age or other conventional serum tumor markers (follicle stimulating hormone, estradiol, or inhibin B).
Methods: The method of work is a classic sandwich ELISA (Anti Mullerian Hormone (Mullerian Inhibitoring substance), Immunotech, Beckam Coulter, Webster, TX). Experimental group consisted of randomly selected 126 healthy girls and women aged between 22 to 50 years from Skopje, Republic of Macedonia. Were divided into five groups according to age to see the distribution of AMH by age.
Results: Analyzed mean values of serum concentrations of AMH, median age and the standard deviation as a measure of individual deviation. The mean decrease in relation to increasing age is 1, 824±SD 0,7999 ng/mL. And that decline serum concentration is harder to 35^{th} year (approximately 2 ng/mL), after the 40^{th} year decreasing intensity decreases and is approximately 1 ng/mL. Median age and median concentrations are strongly positioned in linear digression R> 0,5167, which confirms that there is a positive correlation between age and serum concentrations of AMH.
Discussion: We confirmed that AMH strongly correlates with oocyte number. The results from our study are highly correlated with other similar studies of AMH. The serum level of AMH, was decreasing over aging, which is also correlated with decreasing of the number of antral follicles over aging. Thous makes AMH serum concentration a better marker marker for prediction the ovarial reseve in women then other markers.

T204

ASSOCIATION OF VITAMIN D AND SOLUBLE FMS-LIKE TYROSINE KINASE 1/PLACENTAL GROWTH FACTOR RATIO WITH PREECLAMPSIA IN THE SECOND TRIMESTER

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Background: Preeclampsia (PE) is a disorder emerging from placental dysfunction in the latter half of pregnancy. The condition is diagnosed following new-onset hypertension and proteinuria after 20 weeks of gestation and affects up to 2-5% of pregnancies worldwide. PE is one of the foremost complications of pregnancy and a major cause of maternal and fetal mortality. The angiogenic factors (Placental Growth Factor (PlGF), Soluble fms-like tyrosine kinase 1 (sFLT-1)) and 25-hydroxivitamin D(25-OH-D) may play an important role in the pathogenesis of angiogenesis. 25-OH-D is a risk factor for preeclampsia, its active form, 1,25-dihydroxyvitamin D3 stimulates VEGF vascular endothelial growth factor) expression in smooth muscle cells. PlGF is a pro-angiogenic factor that may play a role in placental development during pregnancy. sFLT-1 binds PlGF and prevents its interaction with its endogenous receptors. We sought to determine whether there is an association between midgestation serum 25-OH-D levels and angiogenic factor activity for development of preeclampsia.
Methods: Healthy women with term deliveries were used as controls (n=24). We identified 25 patients with risk of PE who met one or more of the following criteria (IMC >35, arterial pressure >140/80 mmHg without proteinuria, age >40 years, multiparious, pregestational hypertension or diabetes mellitus. From 25 women with risk of PE, we identified 5 cases with PE and 20 women without PE. We measured midgestation maternal serum of 25-OH-D and the angiogenic factors using chemiluminescent immunoassay.The ratio of sFLT-1/PlGF was determined to have more predictive value than either variable alone. Results were analyzed by SPSS15.
Results: There were significant differences in sFLT-1/PlGF ratio between controls, patients with risk and patients with preeclampsia (means ±SD:4.4±2.3, 7.1±3.7, 11.1±5.6 respectively, P:0.001). None of the angiogenic factors were significantly correlated with 25-OH-D, just found correlation between PlGF and sFLT-1 (correlation coefficient:0.446 P=0.001). The sFLT-1/PlGF ratio was the most predictive marker for preeclampsia (area under curve:0.820).
Conclusions: Our findings confirm that sFlt-1/PlGF ratio is better marker of development of preeclampsia than a single angiogenic factor.

T205

LOCAL INTERLEUKIN-8 AS BIOMARKER FOR ADVERSE PREGNANCY OUTCOMES IN PREGNANT WOMEN WITH BACTERIAL VAGINOSIS

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Background: Preterm birth (PTB) is an increasingly prevalent, complex condition associated with a high risk of infant mortality and morbidity, including cerebral palsy, blindness, hearing loss, and also hidden disabilities such as school difficulties and behavioural problems that become apparent and persist into adolescence. PTB has a recognized complex multifactorial etiology. Numerous clinical studies have shown a direct relationship between reproductive tract infection/inflammation and PTB. Among microbial alterations, bacterial vaginosis (BV) has received particular attention, because it is a highly prevalent condition among childbearing and pregnant women potentially treatable by antibiotics. Non-invasive biomarkers to assess which pregnant women with BV are more likely to have an adverse pregnancy outcome are highly warranted. Our aim was to assess if vaginal interleukin-(IL)-8 measured in early pregnancy is associated with adverse outcome among BV-positive women.
Methods: A total of 1,806 women were enrolled at <20 weeks' gestation, in Philadelphia, Pennsylvania, USA. 800 women were BV-positive (Nugent 7-10), 707 of them had birth outcome data. Vaginal IL-8 concentrations was measured in 105 BV-positive women who had an adverse preterm outcome, including 66 preterm births (20-<37 weeks, of which 52 were spontaneous) and 14 late miscarriages (12-<20 weeks), and in 295 BV controls (term normal birthweight infants). To asses whether IL-8 associated risk had a U-profile, the upper (>66th percentile) and lower (<33rd percentile) tertiles of IL-8 were compared with the middle tertile (33rd to 66th percentile).
Results: The 33rd percentile (first tertile) of vaginal IL-8 concentrations corresponded to 1140 pg/mL, and the 66rd percentile (second tertile) corresponded to 4830 pg/mL. None of the IL-8 tertiles was associated with increased risk for any adverse preterm outcome, nor preterm birth and miscarriage with or without exclusion of women with concurrent STDs.
Conclusions: IL-8 is not a risk biomarker for preterm birth among BV-positive women in early gestation at average 12 weeks' gestation.

T206

LEVELS OF PAPP-A IN THE 1ST TRIMESTER OF PREGNANCY IN RUSSIAN POPULATION WITH TWO-SITE MONOCLONAL ELISA

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Background. Pregnancy-associated plasma protein-A (PAPP-A) is an established biochemical marker that is using in the prenatal screening for chromosomal abnormalities in the first trimester of pregnancy.
Methods. Serum concentration of PAPP-A was measured using a monoclonal two-site ELISA (DS-EIA-PAPP-A).
Results. Maternal serum PAPP-A values from 3579 singleton white pregnant women with no Down in pregnancy outcome and 16 women with Down syndrome fetuses (Central Russia, mean age 27 years, range from 16 to 43 years) have been measured. Results of specimens testing were used to calculate median (normal range, 5th and 95th percentile) for each gestational week: 8 week of gestation – median 0.60 mIU/ml (from 0.19 to 1.82 mIU/m); 9 week of gestation – median 0.88 mIU/ml (from 0.3 to 2.03 mIU/m); 10 week of gestation – median 1.31 mIU/ml (from 0.28 to 2.84 mIU/m); 11 week of gestation – median 2.02 mIU/ml (from 0.25 to 4.65 mIU/m); 12 week of gestation – median 3.39 mIU/ml (from 0.22 to 6.46 mIU/m); 13 week of gestation – median 4.27 mIU/ml (from 1.49 to 9.11 mIU/m). A statistically significant correlation between PAPP-A medians and maternal body weight was found (p <0,05). Medians were ranged according forth body weights (45 kg, 60 kg, 75 kg, 90 kg) for each gestational week: 8 week of gestation – 0.73; 0.64; 0.51; 0.44 mIU/ml respectively, 9 week of gestation – 1.26; 0.97; 0.84; 0.73 mIU/ml, 10 week of gestation – 1.52; 1.34; 1.25; 1.13 mIU/ml, 11 week of gestation – 2.32; 2.13; 1.84; 1.48 mIU/ml, 12 week of gestation – 3.92; 3.35; 3.19; 2.41 mIU/ml, 13 week of gestation – 5.57; 4.86; 3.68; 3.32 mIU/ml. A downward trend in the medians PAPP-A with an increase age of pregnant women (P >0,05) was observed. For risk calculation in prenatal screening PAPP-A concentrations are indicated as MOM (multiple of medians). The median of MOMs for women with no Down in pregnancy for our population was observed as 1.07 and for Down syndrome pregnancies the medians of MOMs for PAPP-A are increasing during the first trimester: 11 week of gestation – 0.45; 12 week of gestation – 0.47; 13 week of gestation – 0.5.
Conclusions. We defined normal limits of serum PAPP-A in Central Russia population with test DS-EIA-PAPP-A. It is necessary for estimation of abnormal pregnancy risk.

T207

CORRELATION BETWEEN LIPID AND HORMONAL PROFILE AND INTIMA MEDIA THICKNESS AT DELIVERY IN PREGNANCIES COMPLICATED BY INTRAUTERINE GROWTH RESTRICTION

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Background: this study has examined the associations aIMT measured in utero during the early third trimester of pregnancy and neonatal and maternal lipid and hormone profile at delivery.
Methods:AGA=97, SGA=57 IUGR=11 were enrolled. The lipid profile (LP), Adiponectin (A), and insulin (I) and leptin (L) were measured in mothers (M) and fetuses (F) at delivery. Data were analyzed by Analyset.it,considering significant p <0.05.
Results: aIMT was significantly higher in IUGR than SGA and AGA (P <0.05).The LP maternal was significant higher compared to neonatal. The fetal concentration of L is lower than maternal but this difference is only significantly in IUGR (P=0.0343).Fetal L was positively correlated with birthweight (p <0.05).The fetal concentration of LDLox was significantly higher in IUGR than SGA and AGA (P <0.05).There was a hypertrigliceridaemia in SGA and IUGR groups than AGA (P= 0.05) and among SGA fetuses there is a significant positive correlation between fetal aIMT and tryglicerids concentration (P <0.05).There is a negative correlation between oxidized LDL and aIMT that among IUGR and SGA was not significantly positive.A and I have a significant positive correlation between maternal and fetal values.The concentration of A was significantly lower in the M than in the F (P <0.05) while NEFA were higher. Maternal A and NEFA in SGA group were significantly higher than in AGA ones (P <0.05). in SGA we had a significant negative correlation of increased fetal I with low aIMT, while in AGA we had no correlation and in IUGR a non significant positive correlation.There was in a non-significant way a correlation between high aIMT and maternal high I (SGA and IUGR). In AGA there is a significant negative correlation between mother A and fetal aIMT, while in the SGA group the correlation was significantly positive (P <0.05).
Conclusion: metabolic syndrome of the mother might reflect on the fetal metabolism and probably influence the health of fetal vessels. LP of the mother is correlated with LP of the F and newborns with low birthweight had higher chances to present higher colesterol and LDL. Low birthweight was associated with dislipidemic state as well as high tryglicerids were associated with increase aIMT in IUGR.

T208

MATERNAL SERUM SCREENING – BESPOKE CALIBRATION, DOES IT MAKE A DIFFERENCE?

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Background: Four biochemical markers, alpha-fetoprotein (AFP), freeβ or total human chorionic gonadotrophin (hCG), unconjugated estriol (uE3) and Inhibin A are used in the risk calculation for Trisomy 21 in the second trimester of pregnancy (14+2 – 20 weeks). All 4 markers were not originally designed for this purpose, consequently the standard concentrations supplied by the manufacturers are inappropriate for screening. It was considered if a bespoke set of standards was used this would improve the specificity and sensitivity of each assay.
Methods: Beckman Coulter Access2 AFP, Total βhCG, uE3 and Inhibin A kit methodology was used in all assays. Appropriate in house calibrator levels were calculated by determining the 5th and 95th centiles of each of the four markers from samples previously run in the laboratory. The manufacturer's standards were run to establish their calibration curve. This was followed by the in house standards and a set of six low, medium and high quality controls (QC's).
Results: A linear curve fit was the most appropriate for AFP, total βhCG and Inhibin A in house calibration. A log 10, log 10 linear transformation model was needed for uE3. Each QC level was read against both calibrations for each marker. Standard deviations (SD) were calculated for each of the controls for all four markers. AFP showed a mean SD for the low medium and high controls of 0.32525, 0.89245, 2.0532, total βhCG 3.7071, 5.1379, 9.8352, uE3 0.01259, 0.01763, 0.04234 and Inhibin A 2.7796, 4.0602, 9.3653 for the manufacturer's calibration curve. For the in house calibration curve the SD's were AFP 0.28366, 0.7454, 1.58916, total βhCG 3.13719, 4.3398, 8.0061, uE3 0.01032, 0.01783, 0.0521 and Inhibin A 2.7357, 3.8884, 8.8638.
Conclusion: It appears that the in house calibration curves provide a more then adequate fit over the range of standards used. The standard deviations associated with the back calculated QC values are, in the majority of cases smaller using the local in house calibration curve than those provided using the manufacturers software thus improving specificity and sensitivity.

T209

RESULTS SCREENING TEST FIRST TRIMESTER OF PREGNANCY AND PRENATAL DIAGNOSTICS KARYOTYPE IN BANJA LUKA CLINICAL CENTER

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Introduction: Down's syndrome (DS) is the most common chromosomal disorder in live births with a prevalence of 1: 650 – 800. Combined screening in the first trimester of pregnancy (10 to 14 weeks gestation) is used for the statistical evaluation of risk for Down's (DS), Edward¢s (ES) and Patau's syndrome (PS). Trisomy 21 is associated with biochemical (pregnancy-associated plasma protein-A, PAPP-A and free-beta hCG, β-hCG) and ultrasound markers (nuchal translucency, NT and crown rump length, CRL).
Objective: Our aim is to present the positive results of the screening test of the first trimester and results of karyotyping amniotic fluid samples obtained by amniocentesis in the second trimester of pregnancy.
Materials and Methods: The study population includes 1437 pregnant women between 10 and 14 gestational weeks. Biochemical markers, free β-hCG and PAPP-A were performed by electrochemiluminescence imunoassay on Roche COBAS E601 analyzer. Risk calculations were performed using antenatal screening software SsdwLab v5.0 (Roche). Karyotype analysis of fetus was performed on 16 metaphases. At 8 metaphases were done using GTG bands, while the other 8 metaphases determination was performed using classical colored slides.
Results: From 1437 pregnant women tested, high risk (cut-off >1: 250) was found in 171 (12.00%). Of the 171 high risk pregnancies, 69 (40.35%) had an increased risk for DS based on biochemical and ultrasound parameters, while ES and PS increased risk was found in 5.20% of patients. In 171 pregnant women with an increased risk for DS, amniocentesis was performed on 111, of which 108 (97.29%) had normal findings. Pathological karyotype was found in 3 cases (2.71%).
Conclusion: Screening plays an important role in the early detection of risk for DS and it is recommended for pregnant women, regardless of age. Early diagnosis can reduce the incidence of late stage termination, which would otherwise be routinely applied at the request of the patient. Screening tests do not give a definitive prenatal diagnosis. The final diagnosis should be made only by applying invasive methods and karyotyping.

T210

A NON-INVASIVE PRENATAL DIAGNOSIS EXPERIENCE IN SOUTHERN PART OF TURKEY: HIGH RESOLUTION MELTING ANALYSIS TO DETECT THE PATERNAL MUTATIONS OF CELL-FREE FETAL DNA IN MATERNAL PLASMA

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Backround: We examined paternal mutations in cell-free fetal DNA (cff-DNA) by high-resolution melting analysis (HRM) and investigated differences in the levels of fetal and total cff-DNA in mothers with β-thalassemia or sickle-cell traits.
Methods: We examined cff-DNA from 32 pregnant women with β-thalassemia, 57 pregnant women with sickle cell anemia (HbS), and 15 healthy pregnant women as control subjects. The plasma was separated from maternal peripheral blood within 1 h of sample collection, and the DNA was extracted from 1-mL plasma. DYS14 and β-globin genes were analyzed to detect fetal and total DNA by quantitative real time PCR. Paternal mutations were examined in cff-DNA by HRM analysis.
Results: We observed increased multiples of the median (MoM) levels of DYS14 in fetuses affected with HbS and decreased DYS14 MoM levels in β-thalassemia-affected fetuses. We successfully detected 22 paternal β-thalassemia mutations using HRM analysis in cff-DNA when the fetus had different mutation as the mother in β-thalassemias.. However, we could not distinguish cff-DNA from HbS-affected individuals.
Conclusion: Fetal DNA quantification and MoM levels were not informative for detecting HbS and β-thalassemias in early pregnancy. HRM is a useful method for non-invasive prenatal diagnosis for detecting paternally inherited mutations in the maternal plasma.

T211
NONINVASIVE PRENATAL DIAGNOSIS OF CYSTIC FIBROSIS

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Background: The possibility to retrieve fetal DNA from maternal plasma has made available a new source of fetal genetic material for noninvasive analysis of numerous fetal pathological conditions. Due to the scarcity of fetal DNA in maternal plasma and the difficulty in detecting fetal mutated alleles, noninvasive prenatal diagnosis (NIPD) has not yet attained a widespread clinical application. Our goal is to develop and validate accurate tests for NIPD which combine high sensitivity and ease of use during the first trimester of pregnancy. The project is focused on the set up of methodologies for the identification of fetal paternally inherited mutations/polymorphisms in CFTR gene.
Methods: Two research lines are been investigated: the development of amplification protocols based on CO-amplification at Lower Denaturation temperature-PCR (COLD-PCR) combined with Sanger sequencing and highly sensitive microarray substrates which could allow the detection of fetal minority sequences without any enrichment strategy.
Results: Full COLD-PCR: We have developed assays for the identification of fetal paternally inherited F508del and G542X mutations in maternal plasma. In total, 5 diagnoses were performed including 4 cases where the father carried the F508del mutation (in 2 of these the fetus inherited the paternal mutation) and 1 case where the father carried the G542X mutation and the fetus inherited the wild-type allele. In all cases the fetal paternal mutated allele was not detectable with conventional PCR while it became evident at different proportions after enrichment with full COLD-PCR. The results obtained were in complete concordance with those obtained on fetal DNA extracted from chorionic villi. Microarray: We have developed assays for the identification of fetal paternally inherited F508del and G542X mutations in maternal plasma in all the couples previously tested. The results obtained were in complete concordance with those obtained with COLD-PCR.
Conclusions: The application of the full COLD-PCR protocol and the microarray might be extended to any other CF gene mutation for noninvasive prenatal diagnosis of cystic fibrosis and other common genetic diseases and has the potential to be easily transferable to clinical diagnostic laboratories.

T212
RELATIONSHIP BETWEEN INSULIN SENSITIVITY AND LEPTIN IN PREGNANT WOMEN WITH GESTATIONAL DIABETES MELLITUS

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Background: Gestational diabetes mellitus (GDM) individuals have higher body fat than healthy. The obesity in GDM patients results in elevation of leptin concentrations. The aim of this study was to determine leptin influence on insulin sensitivity during the late pregnancy. The index QUICKI-IS is used to assess the insulin sensitivity.
Methods: Pregnant women (n=102, gestational weeks 24 vs 25±4) were included in the study . Based on 75 g 2-h OGTT, the participants were stratified into the following groups (IADPSG criteria): I-st group: healthy pregnant women with normal glucose tolerance (NGT), n=49; II-nd group: pregnant women with GDM, n=53. All women were tested for venous plasma glucose (by measuring of oxygen consumption), serum insulin (by ECLIA) and serum leptin (sandvich ELISA). The index QUICKI is defined as follows: QUICKI=1/[(log(Ins0)+log(Glu0)], where Ins0 is the fasting plasma insulin level (μU/mL) and Glu0 is the fasting blood glucose level (mmol/L).
Results: Both groups were compared for gestational age (non significant difference) and BMI (significal difference, P=0.011). Women with GDM had significantly higher levels of serum insulin (13.84±8.43 vs.11.35±7.38 μU/mL, P=0.02), higher levels of venous plasma glucose (5.93±1.04 vs. 4.63±0.28, P <0.0001) and serum leptin levels (16.96±11.89 vs.8.49±5.38 ng/mL, P=0.002) in comparision to NGT group. In contrast, insulin sensitivity calculated from QUICKI-IS was significantly lower for GDM group (0.56±0.11 vs. 0.65±0.1, P=0.001). Spearman correlation analysis revealed significant correlation between leptin and QUICKI index (r= -0.740 for NTG and r= -0.728 for women with GDM).
Conclusions: The women with GDM had significantly higher levels of serum insulin and leptin concentrations in comparision to the NGT group. A signicant negative correlation between leptin levels and insulin sensitivity is established by us. Our findings suggest that leptin might contribute to development of GDM by decreasing insulin sensitivity.

T213

BIOCHEMICAL CHARACTERIZATION OF HEALTHY NEWBORNS IN THE PROVINCE OF VILLA CLARA, CUBA

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Background: Laboratory studies in newborns constitute an important tool in the diagnosis and treatment of neonatal conditions. In our region are used as the reference limits reported in the literature, which generally come from abroad, which complicates the evaluation and interpretation of normal ranges in the neonate and, undoubtedly, play an important role in behavior and neonatal clinical management. In order to characterize biochemically healthy newborns Villa Clara was quantified various indicators in cord blood.
Methods: We studied 80 infants (40 females and 40 males) who met the inclusion criteria, and 22 biochemical parameters were measured in umbilical cord blood. Were established reference values for each parameter, taking the interval corresponding to the central interval interpencentile (95%) delimited by the 2.5 and 97.5 percentiles.
Results: The reference intervals determined for blood chemistry parameters were: urea: 2.01-5.45 mmol/L, creatinine: 24.65-95.75 μmol/L, SGPT: 0.47-28.70 U/L, SGOT: 3.35-89.20 U/L, total bilirubin: 20.86-148.37 μmol/L, alkaline phosphatase: 97.37-421.87 U/L, GGT: 12.13-176.12 U/L, cholesterol: 0.59-2.96 mmol/L, triglycerides: 0.07-0.97 mmol/L and VLDL: 0.31-0.45 mmol/L. Regarding immunological parameters the results were: IgM: 0.04-0.34 g/L, IgG: 7.26-13.43 g/L, IgA: 0.00-0.08 g/L, C3: 0.49-1.50 g/L and C4: 0.03-0.22 g/L. About micro minerals the results were: iron: 10.21-36.13 μmol/L, copper: 6.18-15.82 μmol/L and zinc: 11.15-24.09 μmol/L; and macro minerals: sodium: 102.60-150.40 mmol/L, potassium: 3.24-9.69 mmol/L, calcium: 1.08-2.72 mmol/L and magnesium: 0.45-1.15 mmol/L.
Conclusions: Knowledge of reference intervals presented in this study is an important social, scientific and economic, offering mothers and their children the opportunity to receive a quality service that ensures future welfare, and gives professionals health a new tool for the early diagnosis and successful of different complications in this age group, thus avoiding unnecessary medication and decreased hospital stay.

T214

HORMONE LEVELS IN EXTREME PREMATURITY: ESTABLISHMENT OF GESTATIONAL APPROPRIATE REFERENCE INTERVALS FOR FSH, LH AND PROLACTIN

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Background: Premature neonates, in particular extremely premature neonates, suffer from a number of common morbidities and prophylactic maternal glucocorticoid administration prior to delivery is now almost universal. Immaturity of the endocrine system and its potential impact on morbidity is the subject of numerous studies. Reports suggest significant differences in serum levels of pituitary hormones in extremely preterm neonates compared to older preterm and full term neonates. However, whilst serum hormones are often measured there is little normative data available for this cohort. The aim of this study is to develop reference intervals for three pituitary hormones measured in babies born between 25 and 32 weeks gestation.
Methods: Blood was collected from 249 (129 male and 120 female) extremely premature infants on successive occasions whilst inpatients in neonatal wards. All infants in this cohort did not have any evidence of ambiguous genitalia or other endocrine related abnormalities Samples (median of three per neonate) were collected in conjunction with routine capillary blood collection. The serum was analysed for prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) by automated electrochemiluminescence immunoassay (Roche Cobas 8000 - E602 module). Infants that did not survive beyond the equivalent of term were excluded from the statistical analysis.
Results: Reference intervals were established with samples collected from 230 of the 249 extremely preterm infants; representing 521 (267 male and 254 female) samples analysed throughout the first six weeks of life. The distribution was non-Gaussian and initial assessment established the 95% central range for each pituitary hormone. For male extremely preterm infants the ranges are: prolactin 605 - 4798 mIU/L; FSH 0.2 – 4.7 IU/L; and LH 0.3 – 8.3 IU/L. The female extremely preterm infant ranges are: prolactin 666 – 5854 mIU/L; FSH 5.7 – >174 IU/L; and LH 0.4 – 167 IU/L.
Conclusions: We describe gestation appropriate reference intervals for three pituitary hormones measured in the first six weeks of life for babies born <32 weeks gestation. Utilisation of these reference intervals will permit the correct and timely interpretation of results for this preterm population.

T215
ANTIOXIDANT DEFENSE IN PREGNANT WOMEN

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Background: Oxidative stress is described as an imbalance between the production of reactive oxygen species (ROS) and the level of antioxidant activity. Pregnancy is a state of oxidative stress arising from increased placental mitochondrial activity. Aim of the study was to assess differences in the levels of antioxidants in healthy pregnant women compared to non-pregnant women.
Methods: Antioxidants measurements were performed in 29 healthy pregnant (HP) and 22 healthy non-pregnant (HNP) women. Activity of glutathione reductase (GR), glutathione peroxidase (GPx) and FRAP (Ferric Reducing Ability of Plasma) were measured in plasma, CRP and uric acid (UA) levels were measured in serum.
Results: In plasma GPx activity a trend towards lower values in pregnant women (560,69 U/L for HP, 651,12 U/L for HNP; P=0,62), and towards higher values in FRAP in pregnant group (1,115 mmol/L for HP, 1,067 mmol/L for HNP, P=0,36) was found. No difference in the GR activity and in uric acid levels (58,04 U/L for HP and 59,44 U/L for HNP; 2,02 µmol/L for HP and 2,07 µmol/L for HNP) was observed. Significant increase in serum CRP was observed in pregnant compared to non-pregnant group (1,78 mg/L for HP, 0,44 mg/L for HNP, P < 0,01). Significant correlations between GPx activity and uric acid levels (P=0,026 r= 0,33) in both groups, between uric acid and FRAP in the HNP group (r=0,74, P <0,001) and between FRAP and CRP in the HP group (r=0,4340, P=0,039) were found. Conclusions: Our results indicate that the antioxidant defense assessed based on GR, GPx, FRAP and uric acid measurements does not differ significantly in pregnant compared to non-pregnant women. Uric acid appears to be an important low molecular weight antioxidant. Our findings suggest that oxidative stress in pregnancy is the result of increased production of ROS, which is not balanced by unchanged antioxidant defense. Significantly higher serum CRP levels in pregnant women indicate mild inflammatory response in this group.

T216
COPEPTIN: A NOVEL EARLY BIOMARKER FOR FOETAL GROWTH RESTRICTION?

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Background: Pregnancies complicated by foetal growth restriction (FGR) is a stressful maternal state with increased neonatal mortality and morbidity. FGR is usually diagnosed by ultrasound in the late second trimester. Early biochemical prediction of FGR is of great interest since early treatment has been shown to reduce risk of developing FGR in high risk pregnancies. An increased level of copeptin is found associated to various stress conditions, such as ischaemic stroke and sepsis. Copeptin remains stable ex vivo and is easy to measure in contrast to vasopressin and cortisol. The aims our study were 1) To establish first and second trimester reference ranges for copeptin, and 2) To investigate if first and second trimester levels of copeptin are elevated in pregnancies later complicated by FGR.
Methods: In this case-control study, we measured copeptin levels in maternal serum at gestational week 12 and 19. The blood samples were collected at Aarhus University Hospital, Denmark from 2002 to 2004. Pregnant women who subsequently developed FGR were defined as cases (N=39) and each case was matched on maternal age with 3 pregnancies not complicated by FGR (controls, N=119). Levels of Copeptin were analysed using a commercial kit from BRAHMS (Copeptin ultrasensitive Kryptor); an automatic immune fluorescent analysis. Reference ranges were calculated as 95% prediction intervals and presented as anti logarithms with 90% confidence intervals. Paired and unpaired t-test was performed testing the null-hypothesis of no difference within and between groups.
Results: No significant changes were found in Copeptin levels from week 12 to 19 in controls (P=0.61). Copeptin levels declined significantly from week 12 to 19 cases (P=0.02). No significant difference in copeptin levels was found between cases and controls in week 12 (P=0.10) and week 19 (P=0.81).
Conclusions: The decline in copeptin in FGR pregnancies was not clinically important, since it does not add any predictive information on FGR. An unspecific marker as copeptin is not a suitable single biomarker for prediction of FGR.

T217

sFlt-1/PlGF RATIO AS BIOMARKER IN THE DIAGNOSIS OF PREECLAMPSIA IN SINGLETON AND MULTIPLE PREGNANCIES.

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Background: Preeclampsia (PE) is a disorder characterized by the onset of hypertension and proteinuria after 20 weeks' gestation. Angiogenic/anti-angiogenic factors, as placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1), have been purposed as biomarkers in the monitoring of women at high PE risk. The aim of the study is to assess the utility of sFlt-1/PlGF ratio at the obstetric triage area to distinguish those women who will develop PE from those who will not. It has been described that sFlt-1 and PlGF show different concentrations in singleton and twin pregnancies, so we want to demonstrate if these differences are clinically relevant to diagnose PE when sFlt-1/PlGF ratio is used.
Methods: Serum samples from 214 pregnant women between 20 and 40 weeks' gestation were collected; 192 were singleton and 22 multiple pregnancies. sFlt-1 and PlGF concentrations were determined by electrochemoluminescence immunoassay (Cobas 6000, Roche Diagnostics). Performance of sFlt-1/PlGF ratio was analyzed using receiver operating characteristic (ROC) curve. Differences between groups were compared using Mann-Whitney U test. SPSS software was used for data analysis.
Results: Subjects were classified in two groups according to gestational age at sampling time: ≤34 (n=58) and >34 weeks' gestation (n=156). Women who developed PE (31 and 36 in each group, respectively) showed higher values of sFlt-1/PlGF ratio than normal pregnancies. Areas under ROC curves for the ratio were 0,857 (95% confidence interval: 0,751-0,962) and 0,797 (95% confidence interval: 0,711-0,884), for ≤34 and >34 weeks' gestation, respectively. Cutoff values for the ratio with best performance were 22 for ≤34 and 54 for >34 weeks' gestation. Ratios were significantly different when singleton vs multiple non-PE pregnancies were compared at ≤34 weeks' gestation, but similar diagnostic power was obtained either including or not multiple pregnancies.
Conclusions: Increased values of sFlt-1/PlGF ratio may be useful to identify women who will develop PE at the obstetric triage area in the third trimester. In this study so far, differences observed in sFlt-1/PlGF ratio between singleton and multiple gestations do not seem to have clinical relevance to diagnose PE in the third trimester.

T218

TRIMESTER-SPECIFIC REFERENCE LIMITS FOR THYROID HORMONES IN RUSSIAN POPULATION WITH THE MONOCLONAL ELISA

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Background: Maternal thyroid dysfunction may be associated with increased risks of adverse pregnancy and perinatal outcomes. Therefore, a local reference range for thyroid hormones in pregnant women is required.
Methods: Samples were tested for thyroid stimulating hormone (TSH), free thyroxine (FT4), total thyroxine (TT4), free triiodothyronine (FT3), total triiodothyronine (TT3), antibodies to thyroid peroxidise (TPO-Ab), human chorionic gonadotropin (HCG). All testing was performed using ELISA kits (RPC Diagnostic Systems, Russia).
Results: 360 serum samples from white women (Central Russia and Volgo-Viatsky Region, mean age 29) with normal pregnancy were examined. Reference limits (2.5th and 97.5th percentile) were calculated using TPO-Ab negative specimens with normal levels of HCG. Trimester specific median, and 2.5th – 97.5th percentile ranges for each analyte are reported below: TSH 1st trimester - median 1.26 μIU/mL, normal interval from 0.10 to 2.84 μIU/mL, 2nd trimester - median 1.39 μIU/mL, normal interval from 0.22 to 3.25 μIU/mL, 3rd trimester - median 1.61 μIU/mL, normal interval from 0.47 to 3.32 μIU/mL; FT4 1st trimester - median 13.7 pmol/L, normal interval from 10.9 to 22.9 pmol/L, 2nd trimester - median 13.0 pmol/l, normal interval from 9.9 to 16.9 pmol/L, 3rd trimester - median 10.7 pmol/L, normal interval from 8.3 to 14.9 pmol/L; TT4 1st trimester - median 123 nmol/L, normal interval from 84 to 172 nmol/L, 2nd trimester - median 133 nmol/l, normal interval from 107 to 165 nmol/L, 3rd trimester – median 127 nmol/L, normal interval from 97 to 158 nmol/L; FT3 1st trimester - median 2.97 pg/mL, normal interval from 1.96 to 5.0 pg/mL, 2nd trimester - median 2.99 pg/mL, normal interval from 2.25 to 4.54 pg/mL, 3rd trimester - median 2.72 pg/mL, normal interval from 2.11 to 3.97 pg/mL; TT3 1st trimester - median 1.4 ng/mL, normal interval from 0.86 to 2.15 ng/mL, 2nd trimester - median 1.61ng/mL, normal interval from 1.12 to 2.33 ng/mL, 3rd trimester - median 1.73 ng/mL, normal interval from 0.94 to 2.42 ng/mL.
Conclusions: We determined trimester-specific normal limits for serum thyroid hormones in Central Russia population with the ELISA (RPC Diagnostic Systems, Russia). These intervals may reduce the risk of mis-interpretation of thyroid function during pregnancy.

T219

THYROID DISORDERS IN WOMEN OF REPRODUCTIVE AGE IN KOSOVO

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Background: Thyroid diseases are very common in women of reproductive age. Normal thyroid function is essential for maintaining normal reproductive capacity. Any type of thyroid dysfunction undiagnosed and untreated may be the cause of infertility. Goal of the investigation was to evaluate thyroid disorders in women of reproductive age in Kosovo. In this study were included women of reproductive age 18 to 40 years, frequented in our clinic, from January 2006 up to December 2010, who are trying to conceive and women that have undergone methods of assisted reproduction technologies. Testing of Thyroid-stimulating Hormone (TSH), triiodothyronine (T3), thyroxine(T4), Thyroid antibodies (aTPO-Ab, aTg-Ab) and prolactin was performed in all women. A total of 860 infertile women underwent thyroid screening test and 176 females in a control group. Of these, 16.1% (106 of 860) were with hypothyroidism (subclinical and overt Hypothyroidism). Hypothyroxinemia was tested in women with elevated TSH and women with TSH within range. Hypothyroxinemia was seen in 0.39% (4 of 754) of the tested women with TSH within range and was seen in 3% (4of 106) of the tested women having elevated TSH. Women with elevated TSH received aTPO Ab test and of these, 14.9% (16 of 106) tested positive. Females with thyroid disorders and ovarian dysfunction have increased period of infertility in comparison with control group. Thyroid disorders are one of the causes of infertility. Women that have undergone methods of assisted reproduction technologies and have thyroid disorders with positive TPO-Ab have increased risk of miscarriage. Based on many scientific facts we therefore propose that a systematic screening of TSH, freeT4 and TPO-Ab could be considered in all women with infertility

T220

SELECTIVE TRANSFERT OF A SINGLE BLASTOCYST STAGE EMBRYO: A COMPARISON VITRIFICATION / SLOW FREEZING

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Introduction: Data from the literature tend to show the benefit of the blastocyst stage embryo vitrification compared to slow freezing. However, no study has examined the contribution of vitrification under a policy of selective transfer of a single embryo. The aim of our study was to compare the outcome of the first and second attempts at IVF/ICSI couples who benefited from a transfer of a single embryo at the blastocyst stage with slow freezing or vitrification of embryos.
Materials and methods: This retrospective study compares two groups of pairs of balanced numbers (n=35) for whom cryopreservation of surplus blastocysts was performed by slow freezing (n=128) or vitrification (n=130). Recruitment periods protocols for slow freezing and vitrification respectively extend from January 2007 to December 2011 and from November 2010 to March 2012. Couples enrolled in their first or second attempt at IVF/ICSI, including the woman's age is less than or equal to 36 years with at least one supernumerary blastocyst quality for cryopreservation (Gardner classification: B3/or B4 AA, AB, BA or BB) are included. Methods of embryo culture (G® series), slow freezing/thawing (G-Blast® Kit Freeze/Thaw Kit G-Blast®) and vitrification/warming (Rapid Lives Blast®, Rapid Warm Blast®) are performed according to the recommendations provider (Vitrolife, Sweden).
Results: The two groups of couples have considered similar demographic parameters. After freeze/thaw slowly, the survival rate of blastocysts was lower (68%, n=63 blastocysts thawed) compared with blastocysts vitrified/warmed (86%, n=35 blastocysts warmed). The number of embryos thawed/warmed and transferred is similar for both groups (1.09±0.06 vs. 1.11±0.07). After thawed embryo transfers or reheated, a significant difference was observed concerning the implantation rate (slow freezing: 9% vitrification: 36%) and pregnancy rate (slow freezing: 4%; vitrification: 32%) between the two groups ($P < 0.05$).
Conclusion: Culture until the fifth day of embryonic development allows the application of an effective policy of selective transfer of a single blastocyst. However, slow freezing (or) blastocyst (s) supernumerary (s) appear to lose this benefit. The preliminary results obtained by vitrification/warming are very encouraging

T221
PLACENTAL GROWTH FACTOR (PLGF) AS MARKER FOR PREDICTING PRE-ECLAMPSIA.

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Background. Placenta growth factor (PlGF) is a homodimeric glycoprotein, 46-50 kDa in size, belonging to the vascular endothelial growth factor (VEGF) sub-family. We perform this study to evaluate the ability of placental growth factor (PlGF) measurements in gestational weeks 24-28 to predict pre-eclampsia.
Methods. In this study we include 30 pre-eclamptic and 40 healthy pregnant women. Levels of PlGF were measured in the stored serum. Pre-eclamptic subjects were also divided into women with early-onset (<37 weeks) and women with late-onset pre-eclampsia (> or =37 weeks).
Results. Levels of PlGF were found to be higher in the pre-eclamptic group in both trimesters. No differences were found between early- and late-onset pre-eclamptic. This finding was confirmed by high specificity (97.06%) and sensitivity (88.89%), a positive predictive value (94.12%) and a negative predictive value of (94.29%) for PlGF.
Conclusions. PlGF might be used as markers for predicting pre-eclampsia.

T222
ANGIOTENSIN-CONVERTING ENZYME (ACE) AS A PREDICTIVE MARKER OF PREECLAMPSIA AT 19-21 WEEKS OF GESTATION

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Background: Preeclampsia (PE) is a syndrome of unknown etiopathogenesis. Previous studies about preeclampsia have focused on the increase in free radicals in the feto-placental unit with poor perfusion. It is believed that the renin-angiotensin-aldosterone system (RAAS) has a role in the poor perfusion of the placenta. It is uncertain whether there is a preexisting impairment in RAS in preeclamptic pregnant women or not. The aim of this study was to investigate the role of angiotensin-converting enzyme (ACE) serum levels as a useful marker in the prediction of PE at 19-21 weeks of gestation.
Methods: We included 101 pregnant women with a priori risk of developing PE (inclusion criteria: preexisting renal disease, chronic hypertension without proteinuria, history of PE in a previous pregnancy, etc) in a prospective study of one year and 80 controls. PE was diagnosed if normotensive woman had two systolic blood pressure measurements of ≥140 mmHg repeated in a 6 hour interval and/or a diastolic blood pressure measurements of ≥90 mmHg after the 20th gestation week; together with proteinuria of more than 300 mg/24 hours specimen (6 h interval). ACE levels were measured by spectrophotometry assay (Biosysten, ATOM). Student's t test was used to compare ACE serum levels in both groups: Group 1 (pregnant with PE) (G1), Group 2 (control group of normotensive pregnant women) (G2). We used ROC curve analysis to calculate the area under the curve (AUC).
Results: Of the 101 pregnant women included, 9 developed PE. When we compared G1 and 2, we obtained significant differences for ACE serum levels (53.7±19.7 vs 30.4±12 U/L) (P <0.001). AUC for ACE was 0.882 and the cutoff point with better sensitivity and lower false positive rate was 40.4 U/L (sensitivity=66.6%, specificity=85%, positive predictive value=33.3%, negative predictive value=95.7% and false positive rate=15%).
Conclusions: ACE is useful marker to predict the subsequent development of PE at 19-21 weeks of gestation.

T223

SOLUBLE FMS-LIKE TYROSINE KINASE 1: A PROMISING DIAGNOSTIC BIOMARKER FOR ECTOPIC PREGNANCY

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Background: Ectopic pregnancy (EP) remains a considerable cause of maternal morbidity and mortality worldwide. It is diagnosed using a combination of transvaginal ultrasound and serial b-human chorionic gonadotrophin (bhCG) serum levels. Therefore is important to develop a biomarker that can differentiate between an EP or an intrauterine implantation. The aim of this study was to investigate if placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt1) serum levels were useful markers to differentiate an EP of a missed abortion (MA).
Methods: We included 70 pregnant women (35 EP and 35 MA) at 6-9 weeks of gestation with βhCG serum levels between 800-3500 UI/L. PlGF and sFlt-1 levels were measured by electrochemiluminescence assay (Roche Diagnostics®, Manheim, Alemania). U Mann-Whitney test was used to compare the two markers in both groups and we used ROC curve analysis to calculate the area under the curve (AUC).
Results: When we compared EP group with MA group, we didn't find significant differences for bhCG (1854±1070/2089±1859 UI/L) (P= 0.780) because selected pregnant women presented bhCG levels between 800-3500 IU/L as an inclusion criteria. PlGF levels were similar in both groups (16.1±3.6/15.2±3 pg/mL) (P =0.277). We only obtained significant differences for sFlt-1 (80±20/170±125 pg/mL) (P <0.001). The AUC for sFlt-1 was 0.829 and the cutoff point with better sensitivity and lower false positive rate was 85 pg/mL (sensitivity=77%, specificity=94%, positive predictive value=93%, negative predictive value=80% and false positive rate=5%).
Conclusions: sFlt-1 is a useful marker to differentiate between an EP or a MA when bhCG levels are similar in both groups.

T224

PLACENTAL GROWTH FACTOR AND SOLUBLE FMS-LIKE TYROSINE KINASE 1 AS MAIN BIOMARKERS OF PREECLAMPSIA AND INTRAUTERINE GROWTH RESTRICTION

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Background: Placental growth factor (PlGF) and soluble fms-like tyrosine kinase-1 (sFlt-1) are altered in preeclampsia (PE) and intrauterine growth restriction (IUGR). The aim of this study was to investigate if PlGF and sFlt1 serum levels are useful markers in the prediction of PE and/or IUGR at 11-13 weeks' gestation.
Methods: We included 101 pregnant women with a priori risk of developing PE (inclusion criteria: preexisting renal disease, chronic hypertension without proteinuria, history of PE in a previous pregnancy, etc) in a prospective study of one year. PE was diagnosed if normotensive woman had two systolic blood pressure measurements of ≥ 140 mmHg repeated in a 6 hour interval and/or a diastolic blood pressure measurements of ≥ 90 mmHg after the 20th gestation week; together with proteinuria of more than 300 mg/24 hours specimen (6 hour interval). IUGR was defined as a birth weight below the 10th percentile for gestational age. PlGF and sFlt-1 levels were measured throught electrochemiluminescence (Roche®, Manheim, Alemania). U Mann-Whitney test and student's t test were used to compare the two markers in different groups: Group1 (pregnant with PE) (G1), Group2 (pregnant women with IUGR) (G2) and Group3 (pregnant women at risk for PE but ultimately not develop PE or IUGR) (G3).
Results: Of the pregnant women included, 9 developed PE and 16 developed IUGR. When we compared G1 and 3, obtained significant differences for PlGF levels (35.4±11.1/60.7±26.3 pg/mL) (P=0.004) and the sFlt-1/PlGF ratio (43 [26-52] / 24 [17-35] (P =0.033). When we compared the IUGR cases, G2 with G3, no significant differences were found for any marker studied. Finally we compared G1 and G2 and we observed significant differences only for PlGF levels (35.4±11.1/69.4± 24.4 pg/mL) (P=0.009) Conclusions: PlGF and SFlt-1/PlGF ratio are useful markers to predict the subsequent development of PE but not IUGR, in the first trimester of pregnancy.
Conclusions: PlGF and SFlt-1/PlGF ratio are useful markers to predict the subsequent development of PE but not IUGR, in the first trimester of pregnancy. Of the two markers studied PlGF has a higher AUC for the diagnosis of PE.

T225
EVALUATION OF THE DYNAMICS OF THYROID HORMONES LEVELS DURING NORMAL PREGNANCY BY ELISA

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Background: Pregnancy is associated with changes in thyroid function, which are result of a normal physiologic state.
Methods. Levels of thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (TT4), total triiodothyronine (TT3) during normal pregnancy were evaluated using ELISA kits (RPC Diagnostic Systems, Russia).
Results: Two groups of samples were investigated. The first group consisted of 360 serum samples from white women with normal pregnancy (Central Russia and Volgo-Viatsky Region, from 4 to 39 gestational weeks). The second group included 103 serum samples from white non-pregnant women (the same region). The Mann-Whitney U test (in case of non-normal distribution) and t-statistic (in case of normal distribution) were used to compare differences between two independent groups. TSH level was lower in pregnancy state (1 trimester - median 1.26, P <0.001; 2 trimester - median 1.39, P=0.003; 3 trimester - median 1.61, P >0.05) than in normal state (median 1.83 μIU/mL). FT4 level was lower in pregnancy state (1 trimester - median 13.7, P >0.05; 2 trimester - median 13.0, P <0.001; 3 trimester - median 10.7, P <0.001) than in normal state (median 14.1 pmol/L). FT3 level was the same in pregnancy state (1 trimester - median 2.97, P >0.05; 2 trimester - median 2.99, p >0.05; 3 trimester - median 2.72, P >0.05) as in normal state (median 2.91 pg/mL). TT4 level was greater in pregnancy state (1 trimester - median 123, P <0.0001; 2 trimester - median 133, p <0.0001; 3 trimester - median 127, P <0.0001) than in normal state (median 96 nmol/L). TT3 level was greater in pregnancy state (1 trimester - median 1.40, P <0.0001; 2 trimester - median 1.61, P <0.0001; 3 trimester - median 1.73, P <0.0001) than in normal state (median 1.19 ng/mL).
Conclusions. We observed a statistically significant suppression serum TSH in the 1 trimester of pregnancy when the human chorionic gonadotropin levels reach their peak. Concentrations of FT4 decrease during pregnancy in contrast to FT3 due to the high affinity of thyroxine-binding globulin for T4. Total T4 and T3 levels increase from 1 to 2 trimesters and they reach a plateau from 2 trimester to term. We defined normal trimester specific limits for these hormones in Central Russia population.

T226
CORRELATION BETWEEN SERUM HOMOCYSTEINE AND CERULOPLASMIN LEVEL IN THE LAST PART OF PHYSIOLOGICAL PREGNANCY

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Background: Pregnancy in humans is a physiological state in which there are majors anatomic and metabolic changes in order to support and provide the needs of the growing conceptus. Homocysteine is an amino acid which contains sulfur and derivates from demethylation of methionine. Hyperhomocysteinemia in pregnancy it has been associated preeclampsia, spontaneous abortion, and premature delivery. The objective of our study was to investigate the correlation between homocysteine concentration and ceruloplasmin in the last part of physiological pregnancy.
Methods: We studied 45 women enrolled in the following groups: (1) (n =15) non-pregnant women (control group), (2) (n=15) second trimester of pregnancy (group 1), (3) (n=15) third trimester of pregnancy (group 2). We measured serum homocysteine concentrations using an enzymatic method, C Reactive Protein (CRP) were determined by immunoturbidimetry. As the most important plasmatic antioxidant factor, the level of ceruloplasmin was determined using the Ravin method.
Results: Compared with the non-pregnant group (7.83±0.98 μmol/L), serum concentrations of homocysteine were significantly low in pregnant women groups (P <0.001). No significant difference was found between pregnant women in the second trimester (4.25±0.69 μmol/L) and pregnant women in the third trimester (4.08±0.89 μmol/L) (P >0.05). The serum levels of ceruloplasmin in pregnant women in the second (43.75±2.50 mg/dL) and the last trimester of pregnancy (46.60±6.20 mg/dL) were higher than in the reference group (32.11±1.62 mg/dL) (P <0.001). There was no significant modification of the serum ceruloplasmin concentration between group 1 and group 2 (P >0.05). No significant correlation was observed between homocysteine and ceruloplasmin.
Conclusions: The concentrations of serum homocysteine were significantly lower in pregnant women groups compared with non-pregnant controls, but between the pregnant women groups no difference was found. The main plasma antioxidant factor, ceruloplasmin was increased in pregnant women groups in comparison with the reference group. No correlation was found between homocysteine and ceruloplasmin.

T227
OXIDATIVE STRESS MARKERS IN PREGNANCY WITH PATHOLOGICAL CARYOTIPE

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Fetal trisomy 21 is the most frequent form of aberrant karyotype in pregnancy and screening methods for detection of fetal trisomy 21 are urgently needed. Cu, Zn- superoxide dismutase is an enzyme with important roles in oxidative stress equilibrium and the physical location of the gene encoding Zn, Cu-superoxide dismutase on chromosome 21 makes it a likely candidate for the Down syndrome screening. The aim of this study was to investigate the role of the fetal trisomy 21 on the redox system status in mothers and to investigate the possibility of using anti- oxidative enzymatic activities in the prenatal screening for the Down syndrome fetuses in the first trimester of pregnancy. Erythrocyte hemolysates from the study group (30 pregnancies with the fetal trisomy 21) were obtained and compared with the control group erythrocyte hemolysates (50 healthy pregnancies with the normal karyotype) for the following pro-oxidative (lipid peroxidation) and anti-oxidative parameters (SOD, catalase, gluthatione peroxidase activities and acidum uricum). The standard biochemical markers of the aberrant karyotype in the first trimester (feta-HCG, PAPP-A) were also compared between the control and the study group. Our data indicate that there is a significant increase in the lipid peroxidation and superoxide dismutase activity in the study group compared to the control group and no change in the catalase activity between the two groups. Additionally, we found a significant decrease in the gluthatione peroxidase activity in the study group compared to the control group, and an increased level of acidum uricum and of the index of oxidative stress in the study group. The analysis of our data suggests that there may be a change in the oxidative stress balance in the study group which may lead to the excessive hydrogen peroxide production. The analysis of the oxidative stress parameters in the control group suggests that there is a balance between the pro- and anti-oxidant parameters. The test for the biochemical markers of the aberrant karyotype (free beta HCG and PAP-AP) was positive in 93.75% of the pregnancies with the fetal trisomy 21. Of all parameters investigated, the values for the SOD activity in the 13th and 14th gestation week showed the best prognostic value.

T228
ANALYSIS OF SERUM PROTEIN FRACTIONS FROM WOMEN WITH RECURRENT PREGNANCY LOSS

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Recurrent pregnancy loss occurs in 1 5% of women at reproductive age. Miscarriage incidence correlates with, among other factors, gestational age, women's age and the number of previous abortions. In most women, no cause of recurrent pregnancy loss is usually found. Therefore it seems important to study all factors possibly inducing pregnancy disorders.
Objective: We assessed the fractions of serum proteins from those women with recurrent pregnancy loss, who gave vaginal birth once prior to having at least 3 miscarriages, and those who had never given birth.
Methods: The study group consisted of 52 women (aged 36.0±4.9) with recurrent pregnancy loss. Nine of them (17%) reported one earlier regular pregnancy ending with childbirth without complications. Control group comprised 30 non-pregnant women (aged 36.1±3.6), who had given vaginal birth to healthy children at least twice. Serum protein fractions were separated by electrophoresis in the SDS PAGE buffer system using a Mini PROTEAN 3 cell device. BioRad SDS PAGE Molecular Weight Standards covering mass range of 6.5-200 kDa were used as a reference. Gels were stained with Coomassie Blue R 250 solution. BioRad QuantityOne software was used for the assessment of molecular weight of each protein fraction.
Results: Electrophoretic separation revealed 39 protein fractions of 10 243 kDa. Particularly interesting was a 38 kDa fraction present exclusively in serum of women with recurrent pregnancy, who had never given birth. Another fraction (74 kDa), not detected in the control group, was found in all women with recurrent pregnancy loss. Protein fractions of 76 and 151 kDa were present only in the control group.
Conclusions: The presence of atypical, low-or mid-weight proteins, including a 38 kDa fraction, in women with recurrent pregnancy loss may potentially play a role in the pathomechanism of this disorder.

T229
LIPID PEROXIDATION IN OVERWEIGHT AND OBESE PREGNANCY

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Background: Overweight and obesity is a growing problem all over the world also for women of reproductive age. The study was undertaken to evaluate the role of oxidative stress (OS) in overweight and obese pregnant women. 8-isoprostanes (8-IP) were estimated as a marker of lipid peroxidation in maternal urine and amniotic fluid.
Methods: We prospectively recruited Slovenian pregnant women undergoing amniocentesis at at Department of Obstetrics and Gynecology at University Medical Center Ljubljana between 15 and 27 weeks of gestation. Eighty non-smoking women with healthy, singletone pregnancy and normal pregnancy outcome were included. The groups were formed according to WHO body mass index (BMI) classification, as “lean and normal weight pregnant women” (BMI <25) and “overweight and obese women” (BMI ≥25). Urine and amniotic fluid samples were collected at recruitment. 8-isoprostanes were assayed by 8-isoprostane ACETM Competitive Enzyme Immunoassay Kit (Cayman Chemical, Ann Arbor, MI, USA) following manufacturer instructions. Normally distribution of data was checked with Kolmogorov-Smirnov test. Non-parametric Mann Whitney U-test and Spearman`s correlation were used for statistical analysis. A p value <0.05 was considered to indicate statistical significance.
Results: We found significant difference in amniotic fluid 8-IP concentration between groups (P <0,05). The 8-IP level in amniotic fluid was lower in the group with BMI ≥25 (19.87 ng/L) than those in the group with BMI <25 (24.66 ng/L). There was no difference in urine 8-IP between groups. The 8-IP in amniotic fluid and urine did not correlate with each other.
Conclusions: We successfully detected 8-IP in urine and amniotic fluid. Absence of correlation between maternal and fetal compartment indicate that there might be different level of oxidation products excreted to maternal and fetal side. Our results of comparison between defined groups were not consistent with previous findings and expectations.

T230
BIOCHEMICAL PROFILING STUDY IN UMBILICAL CORD BLOOD AS PREDICTORS OF NEONATAL DAMAGE.

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Background: During pregnancy inflammatory,metabolic and immunologic disorders affecting fetus,are known,such as abortion,intrauterine growth retardation (IGR), low birth weight and neonatal death. The objective was to analyze different biochemical parameters (BP) in maternal venous blood (MVB) and umbilical cord blood (UCB) of newborn from healthy mothers and mothers with basal pathologies and associated to gestation that allow the early detection of perinatal complications.
Methods: Samples from MVB (283) and UCB (283) were analyzed. Delivery was by cesarean. Mothers and newborn were classified controls (C-n=99), pathological (P-n=184). Maternal pathologies: diabetes, hypertension, anti-phospholipid syndrome, hyper/hypotiroidism, intrahepatic-cholestasis and genital infections. Pathological newborn: IGR and/or fetal distress. BP: Glucose, urea, creatinine, uric acid (UA), bilirrubin (B), proteins (P), albumin (A), transaminases (ALT/AST), alkaline-phosphatase, gammaglutamyltranspeptidase (GGT), creatinkinase (CK), lactatedehydrogenase, iron, calcium, phosphorus, magnesium, sodium, potassium (K), cholesterol (CHO), triglycerides (TG), hsCRP were determined by recommended methods-Roche autoanalyzer. Student/Mann Witney tests were applied, P <0.05.
Results: -P newborn from P mothers showed significant decrease:in gestation weeks (GW) and newborn weight (NW) with respect to C newborn from C mothers (P <0.05); significant increases in CHO, TG, UA, K, B, AST, GGT (P <0.05) and significant decreases in CK, P, A (P <0.05). -P mothers related to C mothers showed significant increase in UA, ALT, AST, GGT (P <0,05).
Conclusions: In P newborn from P mothers with respect to C, the decrease in GW/NW would be related to IGR that accompany these pathologies; increases in CHO, TG, UA, K, B, AST, GGT to cellular destruction associated to maternal pathologies and to deficit in pulmonary development by IGR; decreases in CK, P, A to IGR. The increase of UA, ALT, AST, GGT from P mothers with respect to C mothers would be associated to inflammatory process. A future study with greater number of samples by maternal pathology, is proposed.

T231
BLOOD LIPID PROFILE IN SECOND AND THIRD TRIMESTER OF PREGNANCY

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Pregnancy induces significant metabolic changes. The concentration of plasma lipids increase appreciably during pregnancy. The lipid levels are affected during maternal hormonal changes(rise in insulin, progesterone, 17 beta-estradiol, Human placental Lactogen). Other maternal factors such as BMI (Body Mass Index), maternal weight gain, nutrition, pre-pregnancy lipid levels and various medical complications of pregnancy may also have significant effects on lipid metabolism and plasma levels. To test any changes in lipid profile, we determinated cholesterol, tryglycerides, HDL- and LDL-cholesterol in 33 pregnant women three times. I - second trimester (24 +/- 3 gestation weeks), II - third trimester (36 +/- 1Gestation week) and III - after delivery (30 +/- 10 days). Method: Enzimatic colour tests for quntitative determination of cholesterol, truglycerides, HDL- and LDL-cholesterol in human plasma. The measurement were classified into the following categories: second trimester (24 +/- 3 GW), third trimester (36 +/- 1 GW) and after delivery (30 +/- 10 days).
Results: I group - cholesterol mean 6,77 +/- 1,097 mmol/L; tryglycerides mean 2,476 +/- 0,719 mmol/L; HDL- mean 1,87 +/- 0,496 mmol/L; LDL- mean 3,763 +/- 0,894 mmol/L; II group – cholesterol mean 7,15 +/- 1,279 mmol/L; tryglycerides mean 3,366 +/- 1,495 mmol/L; HDL- mean 1,728 +/- 0,395 mmol/L; LDL- mean 3,94 +/- 0,912 mmol/L; III group – cholesterol mean 5,72 +/- 1,032 mmol/L; tryglycerides mean 1,48 +/- 1,073 mmol/L; HDL- mean 1,624 +/- 0,504 mmol/L; LDL- mean 3,23 +/- 0,758 mmol/L. Conclusion: cholesterol, trygycerides, HDL- and LDl-cholesterol increase to third trimester and decrease after delivery.

T232
DETERMINATION OF MEDIAN LEVELS OF THE FREE BETA SUBUNIT OF HUMAN CHORIONIC GONADOTROPIN IN WOMEN FROM RUSSIAN FEDERATION USING A TWO–SITE MONOCLONAL ELISA

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Background. The level of free β subunit of the human chorionic gonadotropin (free β-HCG) is an important serum marker for biochemical screening in the first trimester of pregnancy.
Methods. Serum concentration of free β-HCG was measured using a two-site monoclonal ELISA (DS-EIA-GONADOTROPIN-free βHCG).
Results. We evaluated serum samples from pregnant white woman from Central Russia and Volgo-Viatsky Region, Russian Federation, mean age 27 years, range from 16 to 40 years. The study population consisted of 3345 singleton, nondiabetic pregnancies at 8-13 weeks of gestation resulting in the delivery of phenotypically normal neonates and 16 pregnancies with trisomy 21. Median values and 95 % confidence intervals were calculated for 8-13 gestational weeks. 8 weeks of gestation: median 46.5 ng/mL (12.1 to 169 ng/mL), 9 weeks of gestation: median 44.7 ng/mL (12.7 to 134.3 ng/mL), 10 weeks of gestation: median 43.8 ng/mL (6.2 to 135.8 ng/mL), 11 weeks of gestation: median 38.9 ng/mL (10.6 to 133.0 ng/mL), 12 weeks of gestation: median 35.9 ng/mL (8.2 to 135.6 ng/mL), 13 weeks of gestation: median 17.3 ng/mL (4.75 to 115.6 ng/mL). MoM values were calculated by dividing an individual's marker level by the median level. In cases of Down syndrome (trisomy 21) the median MoM values of free β-HCG were significantly higher than in control groups: 11 weeks of gestation - 2.4 MoM, 12 weeks of gestation – 1.8 MoM, 13 weeks of gestation – 7.8 MoM. In control groups Median Mom consisted 1.0±0,004. The study population was divided according to maternal weight. Medians for groups with different weight (45, 60, 75, 90 kg) were calculated for every week of gestation. 8 weeks of gestation (54.1, 46.5, 44.3, 32.8 ng/mL), 9 weeks of gestation (56.8, 52.5, 46.1, 30 ng/mL), 10 weeks of gestation (55.0, 43.9, 39.4, 37.3 ng/mL), 11 weeks of gestation (48.4, 38.9, 34.5, 32.8 ng/mL), 12 weeks of gestation (42.5, 35.9, 34.8, 27.4 ng/mL), 13 weeks of gestation (22.0, 18.2, 14.3, 5.5 ng/mL).
Conclusions. We defined normal limits of maternal serum free β-HCG in Central Russia population with "DS-EIA-GONADOTROPIN-free βHCG". It is necessary for estimation of the risk of abnormal pregnancy. A significant relationship between free β-HCG values and maternal weight was obtained.

T233
EVALUATION OF 2ND TRIMESTER BIOCHEMICAL AND ULTRASONOGRAPHIC MARKERS FOR THE PREDICTION OF PREECLAMPSIA

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Background: Recent studies have examined the combination of biochemical and ultrasonographic markers for the prediction of preeclampsia and found that the addition of uterine artery Doppler data to the biochemical markers data improved the predictive performance of biochemical markers in both 1st and 2nd trimester. We aimed to evaluate the performance of the biochemical markers:Placental growth factor (PlGF), soluble fms-like tyrosine kinase-1 (sFlt-1), Asymmetric dimethylarginine (ADMA) and human Neutrophil Gelatinase-Associated Lipocalin (NGAL) in the 2nd trimester of pregnancy for the prediction of preeclampsia.
Methods:The data used in this study are part of a wider ongoing investigation project on biochemical and ultrasound markers for the prognosis of adverse pregnancy outcomes.In this nested case-control study we prospectively measured PlGF, sFlt-1,ADMA,NGAL and Doppler uterine artery pulsatility index (PI) in 12 pregnancies that developed preeclampsia and 41 uncomplicated pregnancies,in the 2nd trimester (20th – 26th week) of pregnancy.
Results: ADMA (123±27.9 μmol/L) and NGAL (51.8±28.2 ng/mL) concentrations were significantly higher while PlGF (225±152 pg/mL) was significantly lower in preeclamptic pregnancies compared to normal pregnancies (104±25.3; 30.4±20.2; 330±157 respectively) in the 2nd trimester. Also uterine artery PI was significantly higher in preeclamptic pregnancies (1.24±0.42) compare to normal pregnancies (0.95±0.30). The concentrations of sFlt-1 didn't differ significantly between the two groups. We evaluated a logistic regression model to predict the probability of preeclampsia using as predictors the concentrations of biochemical markers, the PI and the maternal body mass index (BMI). Using the forward stepwise selection method we found that BMI, sFlt-1 to PlGF ratio and NGAL were significantly independent predictors. The model with these predictors had sensitivity 67% and specificity 96% for the prediction of preeclampsia. The addition of PI in the model didn't improve the sensitivity and specificity.
Conclusions: During 2nd trimester of pregnancy, ADMA and NGAL were significantly increased while PlGF significantly decreased in preeclamptic pregnancies. sFlt-1 to PlGF ratio and NGAL together with maternal BMI are effective predictors.

T234
FETAL URINE ANALYSIS TO PREDICT POOR POSTNATAL FUNCTION IN TWO CASES OF CONGENITAL URINARY OBSTRUCTION

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Background: Untreated fetal lower urinary tract obstructions have a mortality rate of up to 45%. We evaluated the clinical usefulness of fetal urine analysis for the prediction of poor postnatal renal function in two cases.
Methods: Patient 1: 26th week, diagnosis of left fetal abdominal mass (suspected multicystic-dysplastic kidney). Patient 2: 14th week, diagnosis of fetal megabladder. In both cases, 7 echoguided cystocentesis were performed. Urine fetal samples were assayed for electrolytes, creatinine, albumin, osmolality, β2-Microglobulin and CystatinC on Cobas311-Roche, BNII nephelometer Siemens,Osmometer AI.
Results: In patient 1, the parameters of renal function were elevated, supporting an impaired renal function (Na:130.1±3.1 mEq/L; K: 4.14±0.38 mEq/L; Cl:109.3±2.1 mEq/L; Osmolality: 258.1±7.4 mOsm/Kg; Creatinine: 8.8±1.4 mg/dl; β2-Microglobulin: 0.47±0.11 mg/dL; CystatinC: 0.07±0.01 mg/dl). At day 4 after birth, the child underwent left nephroureterectomy, with a definitive diagnosis of segmental form of renal dysplasia. The cystourethrography, performed at 3 months of age, showed a right healthy renal parenchyma, with a normal renal function. In patient 2, the fetal urine biochemical tests were normal (Na:61±16.6 mEq/L; K:2.57±0.5 mEq/L; Cl:50±18.4 mEq/L; Osmolality: 134.3±30.14 mOsm/Kg; Creatinine: 13.14±4.4 mg/dL; β2 Microglobulin: 0.40±0.23 mg/dL; CystatinC: 0.03±0.02 mg/dL) suggesting a normal renal function,even in the presence of megabladder, suggesting Prune-Belly syndrome. At birth, general conditions were fair with normal biochemical parameters and urinary tract ultrasound. The statistical analysis of biochemical tests of the two patients showed significant differences for Na, K, Cl, Osmolality and CystatinC ($P < 0.05$), but not for creatinine and for β2-Microglobulin (Student's test).
Discussion: The use of echo guided invasive techniques has allowed to consider the fetus as a little patient and invasive approaches can be made with a very low risk-benefit ratio.The biochemical evaluations on fetal urines, have made possible the monitoring of the disease,leading to pregnancies up to 37th and 38th weeks,respectively, with appropriate approach and without aggressive treatment. In conclusion, the biochemical tests have been of great support to the formulation of the diagnosis and to reduce the intra-bladder pressure.

T235
COST/EFFECTIVENESS OF RH NEGATIVE PREGNANT WOMEN MANAGEMENT

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Background: Currently, to prevent alloimmunization to Rhesus D antigen (Rh) during pregnancy, all Rh-negative women receive prophylaxis (anti-D antibodies). Measurement is therefore not targeted to women who have a specific need. Knowing fetal RhD would allow identifying women who are actually at risk of alloimmunization. There are several options to identify these women, but their cost/effectiveness (C/E) is still unknown.
Methods: A virtual population of 10000 Rh negative pregnant women was built to simulate the C/E of preventing alloimmunization. The model considers four options: 1) the systematic use of anti-D immunoglobulin, 2) fetal RhD genotyping, 3) immunological determination of the father Rh factor, 4) mixed screening: immunological determination of the father Rh factor, followed, if the result is positive, by fetal RhD genotyping. The outcomes were measured for the first and an eventual second pregnancy (in 54.93%). Outcomes considered were: 1) the total direct costs under the perspective of the public health care system; 2) the number of fetus with hemolytic disease (HDF), 3) infant mortality.
Results: Regarding the first pregnancy, two options emerged as the most C/E options: systematic prophylaxis and immunological Rh typing of the father with overlapping confidence intervals between both of them. Regarding the second pregnancy, immunological typing of the father emerged as the most C/E option when the outcome considered was the number of fetus without HDF. When, as an outcome baby's survival at 28 days was considered, the results were similar to those of a first pregnancy. In all cases (first or second pregnancies and the combination of the two) fetal genotyping option doesn't appear to be C/E. In sensitivity analysis, fetal RhD genotyping appears as the most C/E option if the cost of fetal RhD genotyping drops below $CAD 140.
Conclusion: Immunological Rh typing of the father and routine prophylaxis are the most C/E options. Whereas the immunological typing of the father option probably could not be adopted by the majority of clinicians, routine prophylaxis remains the preferred option. However, this could change if the cost of RhD fetal genotyping goes below $CAD 140.

T236
VOLATILE ORGANIC COMPOUNDS IN AMNIOTIC FLUID DURING NORMAL HUMAN PREGNANCY

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Background: Amniotic fluid is essential for fetal development and maturation. Biochemical composition changes during pregnancy and reflects the metabolic status of the fetus and the mother. Volatile organic compounds (VOC) are present in various biologic fluids of the organism (urine, blood, saliva) and many are in the expired air. The endogenous or exogenous biochemical and physiological functions are not yet clearly understood. However, their presence may indicate a pathological process. The purpose of this study was to identify the VOCs present in amniotic fluid during physiological pregnancy and to analyze the relationship between these compounds and the lifestyle of the mother.
Methods: The amniotic fluids were collected during the second trimester of pregnancy in 84 women. VOC composition was analyzed with a mass spectrometer (MS5973, Agilent®) coupled with gas chromatography (GC6890, Agilent®).
Results: One hundred and twenty-three VOCs were detected in the amniotic fluid with a relative abundance between 0.001 and 63%. These compounds belong to 13 chemical families (alkanes, Maillard compounds, halogens, terpenes...) and the majority of them (90/123) had an exogenous origin. Acetone was the most abundant compound (relative abundance 63%) and most common (present in 100% of amniotic fluid). A statistical association was found between tobacco consumption by the mother and some VOC presence [benzene (P=0.0098), styrene (P=0.0007), 2.5 dimethylfurane (P=4.91x10-8), but-3-enenitrile (P= 6.234x10-6)].
Conclusion: During normal pregnancy, the fetus is exposed to many VOC with mainly exogenous origins and with some carcinogenic properties. This compounds presence results in part of lifestyle of the mother and in particular tobacco addiction. Consequences on the child's long term become are to be determined.

T237

FIRST TRIMESTER COMBINED PRE-NATAL SCREENING: EVALUATION OF EIGHT YEARS AS FMF CERTIFIED LABORATORY

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After eight years as Fetal Medicine Foundation (FMF) Certified Laboratory, the authors want to demonstrate the importance of using correct technology and correct procedures that allow detecting the highest number of Positive Cases of Trissomy 21, 18 and 13, and lowering the unnecessary amniocentesis.
Methods: Screening analysis was performed between June 2005-2012. The FMF-CL uses a immunoassay analyser with Time-Resolved-Amplified-Cryptate-Emission (TRACE technology) – KRYPTOR (BRAHMS). The biochemical markers are Free βhCG and PAPP-A for First Trimester Screening, from BRAHMS. The three level Internal Quality Control are analysed on a daily basis, with a Coeficcient Variation less than 3%. The Certified Laboratory participates in UK-NEQAS for First Trimester Downs Syndrome Screening. For statistics evaluations the laboratory uses a ANOVA with 95% of Confidence Interval, and descriptive statistics permormed on Minitab Software.
Results: All the procedures recommended by FMF, including quality control, technology used for biochemistry markers and the ultrasonographic data that the laboratory is working with (an essential component of biochemical screening is accurate dating of the pregnancy by ultrasound, otherwise the detection rate is reduced by about 10%), allow us to give very accurate risks, and decrease the number of amniocentesis in women aged 35 or more. We evaluate in our population the correlations between Free Beta-HCG/ PAPP-A MoMs and several maternal characteristics, including racial origin, weight, smoking and method of conception, being evident an increase in PAPP-A MoMs in smokers, and decrease in diabetic patients. We analyze the cross-effect of the smoke and diabetes in both biochemical variables. In both we verify the effect potentiating of factors that are not as evident when only one is present. We interpreting the results between PAPP-A-MoMs and birth weight, and compare with other bibliography.
Summary of effects of various factors on the MoMs studied,
Discussion: An alternative strategy for first-trimester combined screening is for biochemical testing and ultrasound scanning to be carried out in two separate visits, with the first done at 9 to 10 weeks and the second at 12 weeks, allowing earlier assessment. Based on bibliography, this method would improve the detection rate from 90% to 93%. In this period 20486 patients were examined. The median maternal age at term was 33 years, and there were 23% aged 35 or more. The risk estimated was 1:300 or higher in 3,35% fetuses. Techinal variations with more than 4% of Coeficient Variation in the determination of Free βHCG and PAPP-A, can give variations in the calculation of the risk over than 25% [Spencer, 2003, DS News]. Laboratories that do no follow the FMF criteria can present False Positive Rates (FPR) of 30% and over. Our FRP is 3,14%. Regarding FMF the FPR maximun expected is 5%. The smoker variable and diabetes are effectively important parameters when combined risk is performed, and the decision action is taken based on FMF criteria and there accurated risks.

T238

USING SOFTWARE SSDWLAB 5 IN FIRST AND SECOND TRIMESTER FOR SCREENING DOWN'S, EDWARD'S AND PATAU'S SYN

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Background: First and second trimester screening are indispensable in early identification of Downs, Edwards and Pataus syndrom in fetuses. We performed combined protocol which included maternal biochemistry(free beta-hCG, PAPP-A ,AFP and beta hCG) and sonografic determination(CRL, NT,BPD). In first trimester screening was performed between 11-14 weeks SsdwLab version 5.0.This software makes use of an algorithm described by Palomaki and is based on mathematical calculation using Gaussian multivariate distribution. Risk analysis is based on maternal age, NT,CRL as well as on the results of biochemical parameters free beta hCG and PAPA-A, corrected by different factors like e.g. maternal weight, smoking and ethnic background of the pregnant woman.The risk for a trisomy 21affected pregnancy in second trimester,14-18 weeks , can be calculated using the algorithm as described by Wald and the respective assay-specific prametars.
Methods: Free beta-hCG, PAPP-A, AFP, beta hCG were performed by electrochemiluminescence immunoassay on COBAS E 411 immunoassay analyzer.
Results: Total of 1806 samples from clinical routine with known outcome were examined for one year. 42 were from pregnancies with confirmed Down's syndrome in first and 38 in second trimester. 8 out of the 1806 samples were positive for Edward's or Patau's syndrome in first trimester. Cut-offs for Trisomy 21 and 18-13 were 1:250.
Conclusion: Using software SsdwLab version 5.0 we were screening 2,7% pregnancies with risik in first and 2,1% in second trimester.

T239

THE USE OF NEW LABORATORY MARKERS IN THE DIAGNOSIS OF ENDOMETRIAL CARCINOMAS - THE PILOT STUDY

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Background: Abnormal or pathological findings on the endometrium is a frequent finding in women of childbearing age, as well as in postmenopausal women. The most important finding is endometrial cancer, which is currently one of the most common gynecological malignant tumors. Diagnosis is usually set up on the basis of histopathological examination of biopsy material. There is currently no reliable biomarker that could diagnose suspected or established on the basis of biochemical tests. Calgizzarin (S 100A-11) and TFF are proteins, which in addition to physiological functions in the body and interfere significantly in carcinogenesis. Their abnormal values in serum could therefore may indicate pathological changes in the endometrium. Interesting for the diagnosis of endometrial carcinoma is the AIF-1. Their concentrations were till now used as a diagnostic indicator of Ca endometrium.
Aim: to verify the effectiveness of diagnostic determination of AIF-1 TFF, TFF-2, TFF-3 and S100A-11 in the diagnosis of endometrial carcinoma.
Methods: 44 women were middle age tested. S100A-11 (BioVendor, DSX) TFF-1 (BioVendor, DSX), TFF-2 (BioVendor, DSX), TFF-3 (BioVendor, DSX), AIF-1 (BioVendor, DSX) and Ca-125 (Siemenes, Centaur XP) were analysed in sera.
Results: 30 women without endometrial carcinoma and 14 women with carcinoma were tested. Individuals with carcinoma had significantly higher values S100 A-11 and TFF-3 in sera (S100 A-11 7.2 vs. 4.2 ng / ml, P <0.01; TFF-3 1.0 vs. 2.7 ng / ml, P <0.05). Subgroup with with carcinomas did not significantly differ in values of Ca 125, TFF-1, TFF-2 and AIF with the healthy subgroup. Linear regression model with stepwise regression included only as a diagnostic marker of choice S-100 A11.
Conclusions: S100 A-11 appears as a potential indicator of the presence of endometrial cancer. Similarly interesting appears TFF-3. In later stages of the project, we will investigate these parameters in subjects repeatedly endometriosis and monitor their prognostic significance. In Conclusion, firts results reveal a real possibility to use these biomarkers in diseases with endometrial cancer.

T240

SPERM FATTY ACID COMPOSITION AND THE MALONDIALDEHYDE LEVELS IN HUMAN SEMINAL PLASMA

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Background: Infertility appears to be very common problem of reproductive-age couples nowadays. The semen quality decreases for example because of lifestyle, infections, autoimmune or chronic diseases. The aetiology and pathogenesis are still not exactly understood, accordingly majority is considered idiopathic. Sperm lipid membrane, mainly consisted of polyunsaturated fatty acids (PUFAs), is particularly susceptible to oxidative damage. Good sperm qualities are important to suitable sperm motility and successful fertilization. Lipid peroxidation occurs when the production of potentially destructive reactive oxygen species (ROS) exceeds natural antioxidant capacity of the cell. PUFAs in the phospholipid membrane are the most attacked substrate. Many markers of oxidative stress are described; malondialdehyde (MDA) is one of the most determined. It is mutagenic, carcinogenic and atherogenic.
Methods: MDA levels were measured using HPLC with fluorescence detection as a MDA(TBA)2 adduct. To seminal plasma sample EDTA solution and 2-thiobarbituric acid were added. The mixture was stirred and incubated. After derivatization, cold n-buthanol was added and the mixture was vortexed and centrifuged. The buthanol layer was filtered through nylon filter and transferred into cramped vial. Phospholipid PUFAs were determined by GC-FID. The raw semen specimens were centrifuged, supernatant was carefully removed and the pellet was used further. After protein precipitation, lipids were extracted and separated by preparative TLC into 5 classes. Phospholipid fraction was isolated and after the addition of an internal standard it was hydrolyzed and converted to methyl esters of fatty acids. This way prepared samples were analyzed.
Results: MDA levels in a group of all patients ranged between 1.12-1.84 μmol/L, in smokers (S) between 1.03-1.91 μmol/L and in nonsmokers (NS) between 1.15-1.81 μmol/L. Total sperm membrane phospholipid fatty acids were profiled into 4 groups - saturated acids (S 60.59%, NS 61.07%), PUFA ω3 (S 14.52%, NS 14.93%), PUFA ω6 (S 10.06%, NS 10.07%) and other acids (S 14.84%, NS 13.94%).
Conclusions: High levels of PUFAs are proper for sperm membrane motility and flexibility. Despite this, PUFAs are susceptible to lipoperoxidation.

T241
FREE BETA HUMAN CHORIONIC GONADOTROPIN AND PREGNANCY-ASSOCIATED PLASMA PROTEIN A IN THE FIRST TRIMESTER SCREENING

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Background: The aim of this research is to study the influence of the lapsed time between the extraction and the determination procedure in the case of free beta human Chorionic Gonadotropin (free beta hCG) and Pregnancy-associated plasma protein A (PAPP-A), and its effect on the calculation of risk of chromosomal anormalities. Free beta hCG and PAPP-A increases over time, but it is to be determined how long it needs to cause a significant difference on the values and on the associated risk for the prenatal screening.
Materials and methods: A group of 38 women in the first trimester of pregnancy were included in the study, blood samples were taken during the routine check-up. Blood samples were stored and transported to Virgen del Rocío Laboratories in the same conditions (uncentrifuged and in isothermal iceboxes). Once in the laboratory the samples were centrifuged and analyzed using the electrochemiluminescence immunoassay "ECLIA" on cobas 8000 (Roche Diagnostics).
Results: Both beta hCG and PAPPA increase over time. There were significant changes among the determinations corresponding to the samples extracted four, seven, and nine hours before the analysis (P=0.007). Both beta hCG and PAPPA have a linear ascending trend as the statistics and the profile graphs indicate (P=0.000). Comparing two to two (the different levels of the factor -time lapsed since the extraction-), the critical levels associated to every comparison show that there are relevant differences among all the combinations of the different levels for both variables (P=0.000). The associated risk has notable difference among the determinations carried out at different times from the extraction (P=0.000). Such a difference, however, has no influence on the screening results.
Conclusions: Normal handling of samples is likely to increase the results of the determination of free beta hCG y PAPP-A as time till their processing increases. However, in most cases, these variations do not affect the screening results (positive or negative screening).

T242
THE IMPACT OF BILIRUBIN PHOTOTHERAPY ON NEUROBLASTOMA CELL VIABILITY

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Background: Pathological newborn jaundice is a severe condition which may lead to irreversible damage of the neonatal brain. To prevent this potentially fatal disease, endangered neonates are routinely treated by phototherapy, in which lipid-soluble bilirubin is transformed onto more polar bilirubin photoisomers (PIs). However, only scarce data exists on the potential biological effects of these PIs, which might account for certain side effects accompanying neonatal phototherapy. Thus, the aim of our study was to assess whether PIs may affect viability of neuronal cells.
Methods: The effect of bilirubin PIs was studied on neuroblastoma cell line SH-SY5Y incubated with bilirubin (10-100 umol/L) for 24 hrs and exposed to photo-irradiation using clinical phototherapeutic lamp (Dräger Photo-Therapy 4000). Bilirubin PIs were determined by HPLC (C18 column, isocratic elution with 92% 0.1 mol/L di-n-octylamine acetate/8% water). Cell viability was tested by staining with crystalline violet, cell apoptosis was measured by MTT test.
Results: Phototherapy of the neuroblastoma cells exposed to bilirubin lead to significant decrease of the bilirubin concentration (by 15-25%) due to its photoisomerization. Surprisingly, significant decrease in viability and increased apoptosis rate were observed in neuroblastoma cells exposed to various levels of bilirubin PIs ($P < 0.05$ for both parameters, and most of bilirubin concentrations). Possibility, that our observations on cell viability might be due to the effect of photo-irradiation per se, was excluded in separate experiments, in which no effect of phototherapy on neuronal cell proliferation was observed.
Conclusion: Here we report that bilirubin PIs may be even more harmful for neuroblastoma cells compared to non-irradiated unconjugated bilirubin, and this observation might account for some known side effects of bilirubin phototherapy, especially under conditions where bilirubin PIs clearance from the body is impaired.

T243

SERUM NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN AND PLASMA NITRIC OXIDE LEVELS IN PREECLAMPTIC AND HEALTHY PREGNANTS

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Background: We aimed to evaluate serum neutrophil gelatinase associated lipocalin (NGAL) and plasma nitric oxide (NO) levels in preeclamptic and healthy pregnant women above twentyfour gestation weeks.
Materials and Methods: 49 healthy and 21 preeclamptic, totally 70, pregnant women participated voluntarily in the study. Presence of 140 mmHg and above systolic and 90 mmHg and above diastolic blood presure which emerges after 20th gestation week, proteinuria more than 300 mg/24 hour and edema has been used as diagnostic criterion for preeclamptic pregnant women. Measurements of serum NGAL and plasma NO were performed with ELISA and photometric method, respectively.
Results: Serum NGAL (ng/mL) and plasma NO (μM) levels of healthy and preeclamptic groups did not show a statistical difference [124.68 (72.42-218.82) (medyan (min-max), (39,83±15,84); 120.44±50.88, 37,07±14,48 (Mean±SD), respectively]. In preeclamptic group, a statistically meaningful correlation was found between level of NGAL and body mass index of sampling time, creatinin and NGAL, total protein and NO and albumin and NO.
Conclusion Serum NGAL levels, correlated with serum creatinin levels in our study, may be the early marker of renal damage which may develope mainly due to inflammation and endothel damage. We could not find a statistical difference for serum NGAL and plasma NO levels between healthy pregnant and preeclamptic groups. Varieties peculiar to humans in preeclampsia, impossibility of obtaining first trimester tissue material as an evidence of inadequate trophoblast invasion, different appearance of maternal reaction to underlying main pathology in every case may restrict clarification of etiopathogenesis.

T244

INTERPRETATION OF APTT PROLONGATION – A POSTANALYTICAL EXTERNAL QUALITY ASSESSMENT SURVEY OF 16 COUNTRIES

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EFLM/EQALM Working group for Postanalytical External Quality Assessment

Background: Activated partial thromboplastin time (APTT) is the second most common coagulation test performed in clinical laboratories worldwide. It is sensitive to quantitative and qualitative defects of intrinsic coagulation factors and it also might detect presence of factors' specific and nonspecific inhibitors. The aim of our study was to assess the extent of laboratory practice variation in Europe when dealing with an unexpected prolonged APTT result.
Methods:The survey was designed as a case report with multiple-choice questions. It was distributed electronically through EQALM and ECAT representatives to laboratories in 35 countries, with an invitation letter aimed at persons responsible for coagulation.
Results:16 countries provided 95% out of 902 answers received, with an average response rate of 20% (range 2-77%). The results show considerable variation in handling an unexpected prolonged APTT, in terms of preanalytical errors exclusion approach and techniques, subsequent mixing studies and results reporting. When considering preanalytical factors, only 37% of laboratories use a laboratory method for excluding heparin presence in the sample. In spite of the fact that mixing studies represent a straightforward way to gather initial orientation on possible causes of truly prolonged APTT (presence or lack of inhibitors), 27% of participating laboratories do not perform them at all, while further 12% do it only upon physician's specific request. Among those who perform mixing studies, there is great variation in the sources of normal plasma, which is also non-buffered in at least half of the laboratories, while the possible effect of mixture preincubation is not known in 45% of cases. The interpretation of three possible mixing studies scenarios has also shown substantial variation. It is worth mentioning that almost half of the responders were not sure about the characteristics of their APTT reagent, ie. its relative sensitivity to factor deficiencies or LA presence.
Conclusion:There is considerable variation in laboratory practice concerning evaluation and interpretation of prolonged APTT results. This might be an indication towards a possibility of false or mis-interpretations during investigations of the reason behind a prolonged APTT result.

T245
COMPARISON OF KRAS GENOTYPING METHODOLOGIES: DOKUZ EYLUL UNIVERSITY MOLECULAR ONCOLOGY LABORATORY EXPERIENCE

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Background: KRAS is a predictive marker for the response to EGFR based targeted therapies in metastatic colorectal patients. KRAS mutation testing for codons 12 and 13 is recommended by the American Society for Clinical Oncology (ASCO) and National Comprehensive Cancer Network (NCCN) guidelines for planning CRC chemotherapy. DNA sequencing, PCR or microchip based methods for determine the KRAS gene mutations are used in many routine laboratories. No specific methodology is recommended for this test. The aim of this study was to compare 5 methods of KRAS mutation analysis tests and provide practical and affordable method.
Methods: Five different methods for the analysis of KRAS codon 12, 13 from formalin fixed paraffin embedded tissues were evaluated. ARMS-PCR (DxS, Therascreen KRAS Mutation Kit, Manchester, UK), PCR/reverse-hybridization test strips (ViennaLab K-Ras StripAssay, Austria), classical DNA sequencing (Beckmancoulter, GenomeLabTM DTCS-Quick Start Kit, USA), pyrosequencing (Qiagen, PyroMark Kras Kit), microarray based gebotyping technology (Autogenomics Inc., Infinity Biofilm Chip Microarray, KRAS Assay, USA). Agreement was assessed by κ (Kappa) statistics using SPSS (Version 19.0, SPSS Inc., Chicago, USA).
Results: In this study, each methods were compared with each other. The results of DNA sequencing by ARMS-PCR are κ1=0.529 (95% CI, 0.18–0.87), by pyrosequencing κ2=1; (95% CI, 1.00–1.00) and by microarray κ3=0.89 (95% CI, 0.68–1.09). The results of ARMS-PCR by by pyrosequencing are κ4=0.78 (95% CI, 0.38–1.17), microarray κ5=0.65 (95% CI, 0.36–0.94) and by PCR/reverse-hybridization test strips κ6=0.61 (95% CI, -0.04–1.237). The results of pyrosequencing by microarray κ7=0.76 (95% CI, 0.35–1.18). The results of microarray by PCR/reverse-hybridization test strips are κ8=0.60 (95% CI, -0.05–1.27).
Discussion: This method comparison study provides data on KRAS mutation analyses. The results suggest that commercial services may provide different results. Pyrosequencing reliability is outstanding according to DNA sequencing. The lack of concordance among results of some methods, suggest that quality assurance programs will be necessary to ensure consistent and accurate results.

T246
PREANALYTICAL RATING PHASE OF BLOOD SAMPLING: SURVEY

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Introduction: Preanalytical errors are counting up to 70% of the total number of errors in laboratory diagnostics. According to the ISO 15189:2007 preanalytical phase can be defined in chronological order, which is based on the requirements of clinicians, preparation of patients, taking the primary samples, transport to the laboratory, and it ends when the analytical process begins. The study was conducted at the CCBL clinics and it was based on the process of blood sampling among nurses with secondary education and nurses with college degree, in order to access the actual assessment.
Materials and methods:The anonymous survey which included 7 questions of closed type (Likert scale; never=1, rarely=2, often=3 and always=4) had a purpose to examine the blood extraction procedure as preanalytical phase of laboratory practice among nurses with secondary education and nurses with college degree at Clinical Center Banja Luka (N=882).
Results: Of the total number of participants in the survey, 53% gave responds. Of that percentage (53% answered question), 85% were nurses with secondary education, and 15% of nurses with college degree.The mean value and standard deviation of all procedures was determined to be 3,27 ±0,60. The rating time record of blood sampling was 2.42, and this value represents the lowest score of all the survey questions. The best score obtained in the survey was based on the question refereeing to the mixing test tubes, and it was determined to be mixing blue and purple caps immediately after extracting blood and its value was 3.95.
Conclusion: Our results highlight the need to educate the majority of staff responsible for blood sampling , in order to prevent the source of errors and quality assurance of clinical laboratory testing. For the achievement of high quality standards, it is necessary to continuously educate the personnel directly involved in the blood sampling.

T247
BIOLOGICAL VARIATION REFERENCE DATA: TIME FOR DEVELOPMENT OF STANDARDS?

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Background: Characterization and understanding of the biological components of variation in laboratory data is a fundamental requirement for laboratory medicine specialists. The data are used in the setting of quality standards and in the determination of significance of change in two consecutive results (reference change values (RCV). The characteristics of the data define their utility to providers and users of services, and so methods of data production and other data attributes are of great importance. There are parallels with production and use of reference values as identified by the IFCC and more recently in CLSI guidance. That approach identifies need for characterization of populations studied, methods for production of data, and the statistical treatment of data. The need for this degree of definition is accepted in the context of reference values, but not so in the context of biological variation data (BV). Indiscriminate application of poorly characterized, or produced, BV data leads to delivery of erroneous quality standards and RCVs, and compromises other applications of BV data. It follows that there is a need for standards for production of BV data, standards for reporting of them, and standards for their transmission, since these all impact on their utility and commutability.
Methods: A working group has been constituted by the EFCCLM to consider the issues. As a first step the group has undertaken work to deliver a checklist to help users and publishers of biological variation data to appraise existing and future studies of biological variation data in a structured way.
Results: An outline critical appraisal checklist has been developed to be further validated, and is available for wider consideration, discussion and testing (www.biologicalvariation.com/Tools.html). The checklist identifies critical factors to be considered, ranging from the need to adequately describe populations studied and the analytical methods used, to the importance of detecting outlying data and appropriate use of statistics.
Conclusions: There is a need for standardization in the context of BV. An outline critical appraisal checklist has been developed to enable assessment of existing data sets and promote good practice in delivery of new data.

T248
EXTERNAL QUALITY ASSURANCE FOR 25-OH VITAMIN D (RESULTS OF 2010-2012 CYCLES)

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We reported the results of the “Immunocheck” External Quality Assessment (EQA) scheme for 25-OH vitamin D (25OHD)-2010-2012 cycles- conducted by the QualiMedLab-CNR (Pisa, Italy) in co-operation with ProBioQual (Lyon, France), including 250 among Italian (25%) and French (75%) laboratories (2012).
Methods: Each participant, identified by a code number, indicated on a datasheet the method of analysis used, and the results obtained assaying the control samples. An on-line access permits to visualize analyzed and archived data, including distribution of all results and those of the participant’s own method group. All control materials (12 samples/year) are delivered at ambient temperature as lyophilized samples at the beginning of the annual cycle. Six rounds are expected, each one including two different test samples. The 2010-11 cycle included four samples with deficient (≤10 ng/mL), six and five samples with insufficient (11-29 ng/mL), and two and three with sufficient concentration (≥30 ng/mL). The 2012 cycle, actually not completed, included three with deficient and seven samples with insufficient concentration.
Results: Number of EQA participants increase during the 2010-12 period (from 110 to 169 and 259). In parallel, there has been a significantly reduction in inter-laboratory imprecision, from 29% in 2010 to 26% and 23% in 2011 and 2012, respectively. Majority of laboratories utilized automated immunoassay (90%), while 10% of the overall participants had manual assays. Liaison DiaSorin was the method most utilized (40.3%), then Roche (23.5%), IDS (10.9%), ABBOTT Architect (10.9%) DiaSorin RIA (4.1%), others (10.5%). The within-method variability between laboratories (CV %) resulted 12.5, 18.5, 12, 14, and 17.6% for Liaison, Roche, IDS, Abbott, Diasorin-RIA, respectively. The majority of methods are positively biased with respect to the Consensus Mean (21.9, 11.5, 15.2 and 0.6% for total Liaison, IDS, Sorin-RIA and Abbott, respectively), the exception being the Roche which had a mean bias of −23.5%.
Conclusions: Results of the present study indicate that running EQA schemes are needed to allow the comparison of results from different laboratories and to reliably evaluate their performance.

T249
THE CDC HORMONE STANDARDIZATION PROGRAM

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Steroid hormone measurements are increasingly used in patient care and public health. However, the accuracy and reliability of hormone tests prevent appropriate detection, treatment and prevention of diseases. The aim of this effort is to standardize steroid hormone measurements such that accurate and comparable measurements are obtained regardless of the measurement procedure. The CDC has established higher order reference measurement procedures for total testosterone and total estradiol in serum using LC-MS/MS to assign target values to matrix-based materials. These measurement procedures are traceable to primary reference materials and to JCTLM certified reference measurement procedures. As part of the CDC Reference Laboratory Services non-pooled sera from single donors obtained following the protocol from CLSI C37-A with target values assigned are being provided for calibration. As part of the Standardization Services the CDC is operating a standardization certification program, HoSt. In this program, 4 blinded challenges are sent over the period a year. Values obtained are used for bias assessment as described in CLSI EP9-A2 and final assessment criterion is based on biological variability data. At present, 22 participants are enrolled in the HoSt Testosterone Program. Participants include clinical, academic, and pharmaceutical laboratories as well as immunoassay manufactures. 82% of participants have been able to meet the established criterion and laboratories that are successful are published on the CDC website (http://www.cdc.gov/labstandards/hs.html). Estradiol has been added to the HoSt program and is executed in the same manner. The CDC is also working with external quality assessment providers on accuracy-based surveys as well as monitoring services for large clinical trials. In addition the CDC is working to establish reference ranges using the NHANES data set that can serve as common reference ranges for laboratories that are standardized to the CDC. The CDC Hormone Standardization Program collaborates closely with its partners such as the Partnership for Accuracy in Hormone Testing (PATH) to assure clinical and public health needs are met. Initial data show improvements in testosterone measurements performed in patient care.

T250
THE ROLE OF THE SERBIAN CHAMBER OF BIOCHEMISTS IN THE QUALITY IMPROVING OF WORK AT THE CLINICAL BIOCHEMICAL LABORATORIES

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Background: Serbian Chamber of Biochemists was founded as an independent, professional organization whose purpose is to promote the conditions necessary for performing duties required by the professions of medical/clinical biochemists. The aim of the work is the implementation of testing in the process of human resource evaluation, laboratory evaluation and implementation of internal and external control of work with recommendations for their improvement.
Method: represents an independent research which is conducted using of Questionnaire on the organization of clinical biochemical laboratories (the study sample included 10% of all registered laboratories). The data were analyzed by the descriptive statistics method.
Results: represent the present structure of employees with university degrees. The results show that in primary and secondary activity are working most of physicians-specialists in biochemistry, while pharmacy graduates – medical biochemistry specialists are most represented at the tertiary level. By the form of ownership, 84,4% laboratories belonged to the public and 15,6% to the private sector. Pharmacy graduates – M. Sc. in medical biochemistry are most represented in the private sector. By the educational level of lab managers, specialist in biochemistry was present in 90% of private laboratories and 85,2% of public laboratories. Biochemists participation on the work of professional collegiums at the primary health care level, is 68,8%, at the secondary level 77% and at the tertiary level 85,7%. Implementation of internal work control, in 90% of laboratories, is carried out daily with commercial control material, with commercial control material periodically in 18,8%, frozen pool serum daily in 15,6%, and with frozen pool serum periodically in 3,1%. System control “yesterday-today” is done in 28% of laboratories. 88% of laboratories are involved in external monitoring. Here is a significant statistical difference between the private sector-20%, compared to public sector-100% of laboratories. Research has shown that only one laboratory is accredited according to ISO 17025, while the ISO 9001 certification was performed in 4 laboratories. There is a significant difference between private and public sectors, by the opportunities for professional training (20% of the private sector and 78% of public sector institutions).
Conclusion: condition of the human resources does not satisfy the standards. The relation, between basic profile and specialists in medical and clinical biochemistry, is uneven. Status of health workers is not regulated within the health system. Accreditation is at the very beginning. It is necessary to legislate the implementation of external and internal work control.

T251
QUALITY SPECIFICATIONS BASED ON BIOLOGICAL VARIATION – WHERE ARE WE?

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Background: Consensus recommendations on the quality targets in laboratory medicine supports hierarchy quality model. In this concept, a model higher in the hierarchy should be preferred over lower one. The analytical performance in the majority of laboratories in our country is evaluated according to manufacturer recommendations, which is the lowest model in the hierarchy. Thus, we aimed to compare analytical coefficients of variation (CVa) against criteria for imprecision based on biological variation from five different laboratories.
Materials and methods: Imprecision of 17 biochemistry parameters (amylase, AST, ALT, bilirubin – total and conjugated, Ca, Cl, CK, CK-MB, creatinine, CRP, glucose, K, LD, Na, urea and total protein) was monitored by analyzing two levels of commercial quality control material, three times per day, over one year. CVa was monitored on four different platforms (AU2700, AU480, Architect ci 4100 and Cobas c501). Obtained CVa for each platform was evaluated against criteria for imprecision based on biological variation.
Results: Comparison against biological variation was as follows: all monitored parameters met the optimum quality specifications only on AU480, while CVa monitored on the rest of the platforms (AU2700, Architect ci 4100 and Cobas c501) did not meet quality specifications based on biological variation for four parameters (sodium, chloride, calcium and total protein).
Conclusions: According to our results sodium, chloride, calcium and total protein did not meet quality requirements on three different platforms. One of the possible reasons is low within-subject biological variation which is difficult to achieve with available analytical methods.

T252
EVALUATION OF THE VERIGENE RESPIRATORY VIRUS PLUS NUCLEIC ACID TEST (RV+) FOR RAPID DETECTION OF INFLUENZA VIRUSES

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Background: The Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) (Nanosphere, USA) is a fully automated sample-to result platform capable of detection of respiratory viruses-specific nucleic acids directly from clinical specimens. This qualitative nucleic acid multiplex test based on nucleic acid amplification (NAT) followed by hybridization to capture gold nanoparticle-conjugated probes which are utilized to detect the presence of captured target DNA. This system differentiates influenza A and its subtypes (H1, H3, and 2009 H1N1), influenza B, and RSV subtypes (A and B). We evaluated the ability of the RV+ assay to detect influenza A and B virus from clinical specimens.
Methods: Nasopharyngeal swabs, taken from 210 patients-influenza A (n=80), B (n=80), A and B mixed (n=1), and influenza-negative(n=102) were analyzed using Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) assay, the Seeplex® RV15 ACE Detection (Seegene, Seoul, South Korea), and the Anyplex FluA/B Typing Real-Time Detection (Seegene, Seoul, South Korea).
Results: Compared to the Seeplex® RV15 ACE Detection, Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) assay had sensitivities and specificities of 100% and 100% for influenza A, and 100% and 99.4% for influenza B. Compared to the Anyplex FluA/B Typing Real-Time Detection, Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) assay had sensitivities and specificities of 95.8% and 100% with RNA samples, and 87.5% and 100% with unprocessed specimens.
Conclusions: The RV+ assay for detection of influenza A and B was simple and relatively rapid with performances comparable to that of the conventional molecular methods- approximately 2.5 hour with 15-minute hands-on time. However, a low throughput is a drawback of this assay as only one sample can be tested at a time.

T253

USING BD LABORATORY CONSULTING SERVICES™ TO UNDERSTANDING THE IMPACT OF THE PREANALYTICAL PHASE ON SAMPLE QUALITY AND SAFETY, A MULTI COUNTRY PERSPECTIVE

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Background: The complexity of the preanalytical (PA) phase has precluded standardisation of PA processes, despite its impact on sample quality, laboratory efficiency, or patient & healthcare worker safety. The BD Laboratory Consulting Service™ Preanalytical Review provides a consistent method, based on a standard data collection form, to audit PA procedures and practices in hospitals in different countries. Blood collection processes were assessed from storage of blood collection materials through specimen collection, transportation, processing of the samples and the resulting sample quality. By following the samples through the complete process, it was possible to link specific PA attributes to sample quality deficiencies.
Methods: A consistent method and data collection form were used for audits (N=48) of all blood collection systems. Data were collected by observation of PA phase procedures and practices and resultant sample quality.
Results: The PA phase was observed for 3597 blood collection tubes over 1350 collections. Sample quality was assessed for 8016 chemistry and 3532 coagulation tubes. For collections that resulted in hemolysed samples, 48% had prolonged use of tourniquet, 31% used catheters and for 38% the disinfectant was not allowed to dry. For serum samples with fibrin where the PA process had been observed, 26% had less than 30 minutes between collection and centrifugation and 81% had not been mixed. The following list gives the percentage of collections where a particular behaviour was observed, incorrect patient identification procedure, 56%; tubes labelled prior to collection 61%; coagulation tubes filled to less than 90% of tube volume 7%; gloves not worn 37%; incorrect activation of needle safety device 19%.
Conclusions: The BD Preanalytical Review standardised methodology allows comparison of results between departments and between institutions and countries. The prospective nature of the reviews permits identification of issues within an institution based on more data than that from rejected samples alone. It makes the link between collection procedures and the consequences for the sample quality & efficiency of the institution, providing evidence for all those involved that improved compliance has an impact.

T254

VALUATION OF NON CONFORMITIES IN LABORATORY: HOW TO IMPROVE THE QUALITY PROCESS FOR PATIENT SAFETY

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Objective: Evaluate the incidents that negatively influence in the safety of the patient, registered during the period January -September 2012, which were action derived necessary improvement.
Method: We have a record system of Non-Conformities (NC) which the whole personnel has access by login and password. The detected NC and the corrective actions are selected. Statistical methods be used for describing our collection of data: a) failures identification; b) frequency; c) realized action; d) actions done: corrective immediate action (CI), preventive action (PA), critical incident; e) percentage of not solved/solved NC.The quality manager compiles every term these entries and proceeds to its evaluation and reporting that is sent to the Direction. The report includes: a) percentage of registered NC; b) causes; c) effects; d) corrective/preventive measures; e) graphics. This report is object of Annual Review for the Direction.
Results: There are 9.929 (3.16%) NC from 314.219 related to errors in the patients' identification, 23,9% are not repairable mistakes. Effect: the samples cannot be processed. Corrective immediate action: rejection of requests. Preventive action: information from the Unit of Communication of the Laboratory to the Center/Service petitioner. 38.9% are errors of codification in the steering wheels of request (repeated and/or incorrect codes), CI: recoding by the Laboratory, research and communication causes to the Center/Service petitioner. 23,1% incorrect requests (no origin, no destination, no age, no sex). 14,1% are errors detected in samples. Cause: urgent tests in non-urgent requests that overcome the maximum time among extraction/receipt and the use of inadequate containers, Effect: rejection of samples.
Conclusions: The NC valuation provides a starting for his management and control. Is proposed to increase the formation/information in the whole implied personnel actions, in extraction points in order to adapt them to the quality procedures pertinent.

T255
ONE YEAR EXPERIENCE OF A QUALITATIVE SCORING SCHEME FOR EQA BLOOD SMEAR INTERPRETATION

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Aim: In 2012 the regional EQAS organizer has set up a peripheral blood smear qualitative scoring scheme, following the EQALM guidelines.
Methods: Registered laboratories in the smear scheme were 179. Samples were selected by the scientific committee of the haematology unit of the AOUC laboratory. One sample of 4 different blood smears stained with MGG was sent to the participants for morphological evaluation: a chronic lymphatic leukaemia (LLC), an haemolytic anemia (HA), an acute lymphoblastic leukaemia (LLA).and a primary myelofibrosis (PMF) Relevant patients details and complete blood count were included. The participants were asked to select up to three significant morphological abnormalities using a coding list provided by the EQAS organizer. A diagnostic suggestion (free text) was well accepted. For each survey, individual results were assessed against the consensus answers given by the referees. The assessment of individual results was calculated using a score system. At the end of each exercise each laboratory received a report with the morphological alterations identified by the participants and by the referees, the reached score, a clinical background of the patient including relevant immunophenotype or cytogenetic data, the diagnosis.
Results: Overall participation in this morphology scheme was high (70%). Different performance levels were registered relative to pathological cells: the mean score of exercise n°1 (LLC) was 0.40, of exercise n° 2 (HA) was 1.70, of exercise n° 3 (LLA) was 0.70 and of exercise n° 4 (PMF) was 1.3, indicating that pathological lymphoid cells were the most difficult to identify by the participants. Different performance levels were detected relative to the laboratory category: higher score were shown by great laboratories, specialized laboratories and hospital laboratories. No significant differences were found between private and public laboratories.
Conclusions: The results revealed substantial concordance with data of literature. We are going to improve the EQA morphology scheme by the introduction of a list of suggested diagnosis and the delivery of a report, at the end of each survey, with a short discussion of the case, some comments and bibliography.

T256
INTERLABORATORY COMPARISON OF METHODS IN LABORATORY MEDICINE IN PREPARATION FOR ACCREDIATION OF THE LABORORATORY

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Background: The College of American Pathologists (CAP) has been involved in interlaboratory comparison surveys since 1946. The European Community Confederation of Clinical Chemistry (EC4) has established a working group on laboratory accreditation. The aim of this group is to explore the possibilities for harmonisation of accreditation and quality systems in clinical laboratories in the European Community (EC) (2). Part 1 of ISO/IEC Guide 43 provides guidance on the development and operation of laboratory comparisons for use in proficiency testing schemes [see also DIN EN ISO 15819:2007; points 5.6.6. and 5.6.7.].
Methods: The Department of Laboratory Medicine of the hospital consists of two laboratories in different towns. The distance between the laboratories is 18 kilometres. The Department of Laboratory Medicine will be accreditated by Deutsche Akkreditierungsstelle GmbH [DAkkS] according to the Guidelines 93/42/EWG; 90/385/EWG and DIN EN ISO 15189 in the future.
Results: Passing-Bablok-Regression and Pearsons Correlation Coefficient (R) were calculated for the following metohods: Creatinine (Y= 0,949x+4,21; R=0,9992), Bilirubine total (y=1,02x-0,39, R=0,9947), sodium (y=x-1, R=0,839), potassium (Y=x, R=0,987), chloride (Y=x, R=0,947); aspartate aminotransferase (Y=x-0,01, R=0,985), alanine aminotransferase (Y=0,923x-0,0004, R=0,988), gamma-glutamyltransferase (y=0,980+0,059, R=0,999), C reactive protein (Y=1,019x-0,48, R=0,999), TSH (Y=1,004x, R=0,985), BUN (Y=1,006x-0,02, R=0,998), uric acid (Y=0,984x-1,3, R=0,999), cholesterol total (Y=0,967x+0.07, R=0,995), HDL cholesterol (Y=0,993x-0,031, R=0,994), LDL cholesterol (Y=0,938x+0,109, R=0,995), triglyceride (Y=1,017x-0,044, R=0,999), total protein (Y=0,977x+0,608, R=0,986), albumine (Y=x, R=0,994), phosphorus (Y=0,941x+0,049, R=0,992), creatin kinase (Y=1,008x+0,0042, R=0,999), alkalic phosphatase (Y=1,017x, R=0,985), alpha-amylase (Y=1,014x-0,002, R=0,999), lipase (Y=1,013x-0,07, R=0,988), lactate dehydrogenase (Y=0,938x+0,109, R=0,995), calcium (Y=1,09x-0,21, R=0,95); cholinesterase (Y=1,025x+5,18, R=0,989), magnesium (Y=x, R=0,954) and iron (Y=0,987x-0,205, R=1,00).
Conclusions: Since two years an interlaboratory competition system of the most tests in clinical chemistry, haematology and haemostaseology has been installed. The competition system works IT-based. The competition system shows that the routine measurement procedures have an acceptable traceability to the reference systems.

T257
COMMUTABILITY OF TWO REFERENCE MATERIALS FOR TWO COMMERCIAL LITHIUM ASSAYS

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Background: The traceability of lithium measurements in serum is ensured by the availability in the JCTLM database of two secondary reference materials (RM), i.e. the NIST SRM 956c (electrolytes in frozen human serum) and the IRMM BCR-304 (lyophilised human serum). As these RM are intended for use in the calibration and traceability validation of commercial systems, information on their commutability is central. Here we tested the commutability of these RM using two commercial methods measuring serum lithium, based on direct potentiometry (Ion-Selective Electrodes Direct, Roche Cobas Integra 400) and on a colorimetric approach (Multigent Lithium, Abbott Architect c16000), respectively. Methods: A total of 27 leftover human serum samples were collected, aliquoted and stored at -80 °C until their use. We measured lithium concentrations with the two systems in each biological sample, in SRM 956c (3 levels) and in BCR-304 in duplicate in two different runs on the same day. Manufacturer's control materials were used to validate analytical runs. The commutability of RM was estimated from Deming regression analysis of the measured results in native samples using the 95% prediction interval (95PI) and multiples of the standard error of regression (Sy-x), in accordance with the CLSI C53-A standard. Results: The SRM 956c results did not fall inside the 95PI based on the results for the native clinical samples. In addition, using an acceptance criterion for commutability of ±3 times the experimental Sy-x (±0.066), the relative residuals (rr) for SRM 956c (−2.956 level 1, −4.044 level 2, −3.209 level 3) were all outside the acceptable range. On the other hand, BCR-304 results fall inside the 95PI, but its rr (−0.197) was not within the acceptable range. Conclusions: Our results show that SRM 956c was not commutable between the methods evaluated. BCR-304 showed better, although not perfect, commutability and should be preferred to align lithium assays to higher-order references. The uncertainty of BCR-304 (2.9%) is however relatively high and this may become an issue for fulfilling the goal of acceptable uncertainty of lithium measurement for clinical laboratories (±4.3%), as calculated assuming a time interval between doses of 12h and a drug average half-life of 24h.

T258
INTEGRATION OF CONTINUOUS QUALITY IMPROVEMENT FOR BIOSAFETY WITH THE CONTINUOUS QUALITY IMPROVEMENT FOR THE ENTIRE LABORATORY: OUR EXPERIENCE

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Background: Biosafety laboratories must ensure adequate safety conditions to avoid potential hazards associated with the handling of biologic materials, the manipulation of genomes, the creation of synthetic organisms, and the spread of multidrug-resistant bacteria. The protection of laboratory workers from disease agents transmitted by aerosols, droplets, blood and body fluids is a must in different sections of a laboratory such as chemistry, hematology, virology, etc. CDC and NIH addressed the topic in their publication BMBL. Recently, CDC published the Guidelines for Biosafety Laboratory Competencies. This guideline outline the importance of the competencies of the laboratory workers in terms of skills, knowledge, and abilities required for working with biologic agents at the three highest biosafety levels (BSLs 2-4). More recently, the publication of the Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories outline the culture of safety, the biological risk assessment and the fundamental safety practices in diagnostic laboratories. Moreover, it is crucial the integration of continuous quality improvement for biosafety with the continuous quality improvement for the entire laboratory.
Methods: Of the four domains that BLC follows, we started to introduce the Domain 1 Potential hazards which is comprehensive of the following sub-domains: Biologic materials and Chemical materials. We classified our laboratory personnel (technicians, laboratory specialists and manager) in three different level (entry-, mid-, and senior level) on the basis of the skills and competencies. Results: on a total of 16 and 51 specialists and technicians respectively we trained 8 and 37 at midlevel, 6 and 10 at senior level, 2 and 4 were entry level. Conclusions: the implementation of these guidelines among laboratory workers increased the awareness of biorisk, including willingness to report concerns, response to incidents, and communication of risk. Moreover, it developed an enhanced culture of safety that is open and nonpunitive, encourage questions, and is willing to be self-critical.

T259
CALCULATION REFERENCE VALUE CHANGE IN BIOCHEMISTRY LABORATORY

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Background: As one aspect of quality control in the laboratory, we have to provide objective meanings for changes in consecutive results. If we want to establish with confidence a change between successive results, the difference between both has to be higher than the variation of three factors: pre-analytical variation (CVp), analytical variation (CVa) and intra-individual biological variation (CVi). If we standardize pre-analytical procedures, the CVp is considered minimal and the total variation is reduced to CVi and CVa components. Two consecutive results are significantly different if numerical variation between both is greater than the combined variation inherent to the two results. This value is called Reference Value Change (RVC).
Methods: Analytical variation coefficients were obtained from internal quality control measured by an Advia 2400 Autoanalyzer (Siemens Healthcare Diagnostics) during 2011. To calculate the RCV the following equation was used: RCV = 21/2 *Z*(CVa2 +CVi2)1/2.
Results: We show the results of the calculation of CVa and RCV for a 95% confidence for each analyte, respectively: Glucose: 1.90, 16.65; urea: 2.65, 34.87; uric acid: 1.48, 25.28; cholesterol: 3.12, 17.28; creatinine: 3.88, 18.20; triglycerides: 5.28, 59.74, total bilirubin: 2.43, 66.30; direct bilirubin: 9.06, 105.03 , gamma-glutamyltransferase: 4.01, 39.83; alanine aminotransferase: 3.63, 68.09; aspartate aminotransferase: 3.16, 34.12; creatine kinase: 3.12, 63.78; amylase: 3.56, 5.26; alkaline phosphatase: 3.35, 20.02; iron: 4.60, 74.54; albumin: 2.73 , 11.45; calcium: 1.94, 7.53, total protein: 2.24, 9.72; Sodium: 1.42, 4.38, potassium 0.98, 13.58; chlorine: 1.78, 5.96.
Conclusions: The RCV is easy to calculate and is the most appropriate tool to evaluate changes in successive results. Biological quantities with both high imprecision and an elevated CVi, results in high RCV. The incorporation of the VRC to lab reports is very useful to interpret changes in two consecutive measurements of the same analyte.

T260
EVALUATION OF THE IMPACT OF STANDARDIZATION PROCESS ON THE QUALITY OF SERUM CREATININE DETERMINATION IN ITALIAN LABORATORIES

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Background: Creatinine determination in serum is the key indicator of kidney glomerular function. A reference measurement system for standardization of creatinine measurements is available and virtually all IVD manufacturers have recently aligned their creatinine assays to this system. The aim of this work was to verify if and how these standardization efforts have improved the state of the art of creatinine determination in Italy.
Methods: An analysis of Prolarit EQAS results using control materials with target values assigned by a traceable method (enzymatic assay calibrated against the NIST SRM 967) was carried out.
Results: Results obtained during 2006, 2010, and 2011 schemes by participating laboratories showed a general good alignment at creatinine concentrations ~2.00 mg/dL, with 2011 results – except for one method group – well inside the desirable bias (±4%). At higher concentrations, whereas the overall bias was small in 2010, for some groups using alkaline picrate (AP) methods it became significantly negative in 2011. The performance markedly worsens at creatinine physiologic concentrations, where a significant positive bias (up to ~20%) is still present for most of the AP-based analytical systems. Unexpectedly, with few exceptions, no evident improvement in individual assay bias was noted from pre- (2006) to post-standardization (2011) periods. The enzymatic method groups were the only always presenting an acceptable bias for all concentration levels, in addition to showing the lowest between-laboratory variability. The number of laboratories using enzymatic methods, however, still remains only 7% of the total.
Conclusions: Our EQAS performance data indicate that most of the current “standardized” creatinine methods based on AP reaction do not perform well, mainly at the lower creatinine concentrations. This inaccuracy of creatinine measurements can adversely impact the estimation of glomerular filtration rate by equations and the evaluation of kidney function in pediatrics.

T261

QUALITY AND PATIENT SAFETY IN HEALTHCARE: ASSESSMENT OF PERFORMANCE OF PREANALYTIC PHASE IN CLINICAL LABORATORIES BY SIX SIGMA OR QUALITY INDICATORS

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Background: The preanalytic (Pre-A) phase is responsible 46%-77% of total errors in the total testing process. Every laboratory should have a policy for detection and prevention of errors. The frequency of errors should be determined systematically in a standardized manner. In this study, we aimed to assess the performance of Pre-A phase by two methods: the Six Sigma approach and Quality Indicators (QIs) Model developed by the IFCC Working Group on ''Laboratory Errors and Patient Safety'' (IFCC WG-LEPS). We compared them for effectiveness.
Methods: We selected the most possible Pre-A errors (sample-improperly labeled; sample-hemolyzed; sample-improper transport; sample-insufficient; sample-damaged; sample-clotted; information (clinical)-insufficient; order-incorrect; order-duplicate; container-inappropriate). We first standardized the terminology and structured the reporting system in our Hospital Information System (HIS) and Laboratory Information System (LIS). Data were collected monthly for the period of January 2012–June 2012; analyzed, and the QIs and sigma metrics were calculated. QIs were assessed according to the quality specifications (QSs) proposed by the IFCC WG-LEPS. We have chosen the quality performance as 4.6 sigma which shows 10% waste with 1 000 DPM. The QI value and sigma metrics for each error was evaluated.
Results: The QIs for "sample-hemolyzed" and "sample-clotted" didn't match the QS for desirable performance. The sigma metrics which are found below 4.6 are as follows: 3.2 (sample-hemolyzed), 4.2 (sample-clotted), 4.4 (sample-insufficient) and 4.3 (sample-improperly labeled). The results obtained with both methods are comparable.
Conclusions: Our results showed that both QIs Model and Six Sigma approach may be useful for assessment of performance of preanalytic phase. But, the Six Sigma approach may be more promising because the QSs for QIs are not defined and standardized yet. Every laboratory can choose the most applicable assessment method in its own situation, but the HIS and LIS should be structured for error collection and data management, and the skills of laboratory staff also should be improved on data management and the usage of quality tools in order to identify and eliminate the error sources.

T262

COMPARING, BY USING SIX SIGMA LEVEL OF THE ANALYTICAL PERFORMANCES OF DIFFERENT LABORATORIES WHICH USE VARIOUS ANALYZERS OF BIOCHEMISTRY

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Background: The analytical performances of medical laboratories, in terms of patient safety, should be satisfactory. Six sigma level is the most expressive measurement of quality in the evaluation and comparing of the performances of laboratories. In this work, it is purposed to compare the six sigma levels of biochemistry parameters in different laboratories using various analyzing systems.Methods: In the work, it is defined the six sigma levels belonging to the total biochemistry parameters of the Central Biochemisty Laboratory at Gazi University Hospital that have the Beckman Olympus AU2700 devices and the different three laboratories. The six sigma levels are calculated by using internal QC data.Results: While the parameters having sigma levels ≥6 in our Biochemisty Laboratory using the Beckman Coulter systems are glucose, creatinine, uric acid, total bilirubin, AST, ALT, ALP, HDL, Ca, direct bilirubin for level 1; those having sigma levels <3 are urea, Na, Cl for level 1. While the parameters having sigma levels ≥6 in the laboratories using the Abbott systems are glucose, creatinine, cholesterol, TG, HDL, total protein, Ca, K, GGT, direct bilirubin for level 1; those having sigma levels <3 are urea, total bilirubin, AST, ALT, LDH, Cl for level 1. While the parameters having sigma levels ≥6 in the laboratories using the Siemens systems are glucose, TG, HDL, LDH, K for level 1; those having sigma levels <3 are urea, ALT, Ca, Na, Cl, GGT, direct bilirubin for level 1. While the parameters having sigma levels ≥6 in the laboratories using the Roche systems are glucose, uric acid, AST, ALT, ALP, TG, HDL, total protein, LDH, Ca, K, GGT for level 1; those having sigma levels <3 are Na for level 1. Conclusions: It is examined that the six sigma levels as an indicator of analytical performances in different laboratories are similar in some parameters but different in others. These differences can result from not only the performances of analyzers but also the factors such as the criteria belonging to the QC procedures that are used by respected specialist. As a result, in terms of patient safety, the performances of laboratories can be evaluated by this easy and valid method and if they are under expected performance, it could be made improvement works.

T263
EXTERNAL QUALITY ASSESSMENT (EQA) FOR QUANTITATIVE FECAL BLOOD IN STOOL (FIT)

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Background. Colorectal cancer (CRCA) is the second most frequent malignant disease in Europe. CRCA screening we began in the Czech Republic in 1994, and population-based national screening with FOBT was started in 2002, since 2009 with immunochemical test (FIT). External quality assessment (EQA) of haemoglobin determination in the stool has been started in January 2012 as a part of the national EQA. The aim was to improve the analytical quality of new, quantitative FIT for colorectal cancer screening. This EQA programme is provided by SEKK (member of EQALM) which is accredited according to ISO/IEC 17043:2010 and provides EQA programmes for Czech and Slovak republics. This study will compare our new Czech FOB EQA with 15 years running ECQS annual Eiken program for users of OC-Sensors. Methods. In the Czech Republic the Faecal Occult Blood (FOB) EQA programme is organised 2-times per year, in Japan EQCS once per year, with 2 liquid samples produced by Eiken. The results are collected using web application and evaluated according to the ISO13528. We use robust means of all results as the assigned value for each sample. Also group based statistics (based on manufacturers of kits) are evaluated.
Results: There ran 2 rounds of the new FOB EQA programme up to now. Participants in two rounds - May (n=34) and October (n=32) - analysed samples with OC-Sensor analyser (mean CV% were 10 and 8.7), Sentinel-Gold methods (mean CV% were 16 and 18) and Smart Eurolyser POCT (mean CV% were 52 and 76). Eiken ECQS data from 880 facilities indicating smaller CV% with OC-Sensor Diana (3.5 and 3.2), OC-Sensor Micro (5.2 and 3.7), others analyzers (5.8 and 12). Conclusions. The new FOB EQA programme that started in January 2012 was joined by 33 clinical laboratories analysing quantitative FIT samples. Our results confirm the importance of the newly implemented FOB EQA for the quantitative haemoglobin in the stool samples and support further activities aimed to improve quality and optimization of the colorectal cancer screening programme. The results obtained in the Czech EQA rounds show higher CV in comparison to the Eiken EQAS. It is probable that this behaviour is also influenced by the number of participants which is uncomparably larger in the Eiken's EQAS.

T264
BIOLOGICAL VARIATION AND REFERENCE CHANGE VALUE OF PROCALCITONIN IN HEALTHY INDIVIDUALS

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Background: The measurement of procalcitonin (PCT) in patients with systemic inflammation, infection and sepsis has considerable utility, from both a diagnostic and a prognostic point of view. PCT has demonstrated excellent correlation with the severity of infection, and its monitorization has been employed for antibiotic management of patients with systemic bacterial infections. Our objetive was to establish the biological variation (BV), index of individuality (II) and reference change values (RCV) of PCT in healthy individuals.
Methods: Samples were collected from 45 subjects (PCT <0.5 ug/L) at 24 hours intervals over 5 days (PCT half-life=24-30 h). PCT was assayed using a Cobas e411 (Roche Diagnostics). The analytical coefficient of variation (CVA) was calculated from the between-run data quality control (N=111). BV data were estimated using nested analysis of variance, according to the method published by Fraser and Harris. The II was calculated as the ratio CVw/CVg (within- and between-subject biological coefficients of variation respectively). The RCV was estimated using the formula RCV=Z(2)1/2 (CVA2+CVw2)1/2 , where Z denotes the level of statistical significance (Z=1.96 for P <0.05).
Results: The PCT values ranged from 0.02 to 0.46 ug/L (225 points). The analytical variability estimated by CVA was 7.11%. The BV coefficients, CVw and CVg, were 29.94% and 60.75%, respectively. The index of individuality was 0.49 (II <0.6 suggest marked individuality). The clinically significant difference between two consecutive results estimated by RCV was 85.23% (P >0.05).
Conclusions: The low index of individuality shows that the use of population-based reference limits is inadequate for interpretation. In this case, the use of the RCV has been proposed to be the best reading strategy. The inclusion of this data in diagnostic algorithms for sepsis may increases the usefulness of PCT, mainly for monitoring the effectiveness of therapy.

T265

A TWO-YEAR EXPERIENCE OF IMPLEMENTING GENETICAL EXTERNAL QUALITY CONTROL FOR RHD FETAL GENOTYPING BY CNRHP

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Background: A positive RHD fetal genotyping diagnoses the RH1 fetomaternal incompatibility for the anti-RH1 alloimmunized pregnant women. For the non-immunized ones, a negative test will avoid injection of IgRH. Since the RHD fetal genotyping became a key to the monitoring of RH1 negative pregnant women, an increasing number of laboratories performed such test. It appeared essential for the CNRHP and part of its missions, to offer a quality assessment program based on an external quality control (EQC). The CNRHP can rely on more than ten year experience in the fetal RHD genotyping by PCR from maternal blood and its EN ISO 15189 acreditation to establish such control. The aim of this presentation is to review the EQC program two years after its launch.
Methods: Positive control specimen were prepared from RH1 negative plasma donors spiked with various concentrations of RH1 positive plasma in order to reflect RH1 positive fetuses of different gestational ages. Negative control specimen, made from RH1 negative plasma donors, remained unspiked. Once tested, the samples were conveyed to the laboratories with a feedback form where they had to state the material and methods used, the results and the clinical biological interpretation. The control samples were sent twice a year.
Results: Over these two years, 9 series of samples were prepared and sent to 6 or 7 laboratories (3 in 2010, 4 in 2011, 2 in 2012) reaching each year a 100% response rate. In 2010, the EQC results were consistent with those expected although the laboratories use different extraction and amplification protocols. In 2011, two laboratories made erroneous clinical interpretations despite right analytical results. In 2012, the EQC concluded to both analytical or/and interpretation errors. Only a single laboratory returned the right analysis with the good clinical interpretation.
Conclusion: The presented EQC responds to the criteria required to evaluate the practices of laboratories performing fetal RHD genotyping. The ideal EQC should be prepared from maternal plasma from a single pregnant woman containing a predetermined quantity of fetal DNA but the collection is impossible in practice. The next step is the transfer of EQC program conducted by the CNRHP to an EN ISO/IEC 17043 certified entity.

T266

IMPLEMENTATION OF A PROFICIENCY TESTING FOR THE ASSESSMENT OF THE PREANALYTICAL PHASE OF BLOOD RNA

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Background: Molecular in vitro diagnostics will play an important role in future health care practice and gene expression profiling promises to provide insight into normal biological and pathological processes with the hope of predicting disease outcome and indicating individualized courses of therapy. In this field, significant improvements of downstream assays and data analysis (analytical process) have been made during the last years. In contrast, the influence of the pre-analytical steps, such as sample collection and stabilization, have been highly underestimated. SPIDIA (Standardisation and improvement of pre-analytical procedures for in vitro diagnostics, www.spidia.eu) is a four-year large-scale integrated project funded by the European Commission that works on the standardization and improvement of pre-analytical procedures for in vitro diagnostics in order to close the gap between the more elaborated analytical procedures and the less standardized pre-analytical processes.
Methods: We performed a multicenter study within the EU-granted SPIDIA project to investigate blood collection and shipping influence on some RNA quality parameters: yield, purity, integrity, RT-qPCR interference and IL1B, IL8, c-Fos and GAPDH gene expression. Two models were designed: ExpA - Ten laboratories collected blood from an own donor into two different tubes (with or without stabilizer) and extracted RNA at two different times; Exp B - Blood was drawn from a single donor and shipped to ten laboratories in two different tubes (with or without stabilizer) for RNA extraction.
Results: In both models and collection tubes, reliable results were obtained for purity, yield, GAPDH expression, and interferences. For blood collected in unstabilized tubes we observed a substantial variation in RIN (ExpA) and in transcription levels of IL1B, IL8 and c-Fos (ExpB). Overall the variability was higher among data obtained from unstabilized blood samples.
Conclusions: We defined the experimental setup for a larger ring trial throughout Europe. The chosen downstream analyses verified their potential, serving as adequate markers to test quality of blood RNA.

T267
HARMONIZATION OF AN LC-MS/MS ASSAY FOR THERAPEUTIC DRUG MONITORING OF THE IMMUNOSUPPRESSANT DRUG TACROLIMUS

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Background: Monitoring blood levels of the immunosuppressant drug tacrolimus in solid organ transplant recipients is considered the standard of care. Trough concentrations are commonly monitored and are generally regarded as a good surrogate for tacrolimus exposure. Thus, dose adjustments that are critical to regulating the appropriate level of immunosuppression are made in part based on laboratory results. While liquid chromatography-tandem mass spectrometry (LC-MS/MS) is often considered the "gold-standard", tacrolimus test results are not yet harmonized across laboratories because of the wide variety of calibrators, sample pre-treatment protocols and instrumentation used today. Here we demonstrate the successful harmonization of tacrolimus measurements using a commercially available test kit, through a proficiency testing survey. Further, we compare these results to a reference measurement procedure for tacrolimus.
Methods: A 40 member whole blood panel (spanning the range of approximately 2.0 – 25 ng/mL tacrolimus) consisting of twenty pooled patient samples and twenty tacrolimus-supplemented samples was prepared and distributed to seven laboratories in the United States and Europe. All testing was performed on a common LC-MS/MS system and as specified in the kit's directions for use. Four patient pools were prepared in sufficient volume to allow for value assignment by an exact-match isotope dilution mass spectrometry method (EM-IDMS), i.e., a reference measurement procedure (RMP).
Results: Good agreement between all laboratories was observed for the proficiency testing panel (2.0 – 5.4% CV for patient pools and 3.7 – 12.2% CV for supplemented samples). Further, for the four sample pools having value assignment by EM-IDMS (4.58, 7.66, 11.90 and 19.82 ng/mL) the difference between this RMP and the mean measurements from the participating laboratories ranged from 0.6 – 4.4%, demonstrating excellent accuracy of this routine assay across the currently accepted therapeutic range for tacrolimus.
Conclusions: The authors believe this is the first study to demonstrate harmonization of an LC-MS/MS assay for tacrolimus across multiple laboratories and should lay the foundation for similar surveys involving other measurands.

T268
ACCREDITATION OF CLINICAL BIOCHEMISTRY LABORATORY DZ "SAVSKI VENAC" BY THE STANDARDS OF THE AGENCY FOR ACCREDITATION OF HEALTH CARE INSTITUTION OF SERBIA (AZUS)

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Background: Agency for Accreditation of Health Care Institutions in Serbia was established on the basis of the Law on Health Care to perform professional, regulatory and development activities. This law bestows her setting standards for the accreditation of health institutions. Accreditation is a process for evaluating the quality of health care institutions by applying the optimal level of acceptable standards. Standards have been developed and recommended by health experts and used the guidelines of the International Association for quality in health care.
Methods: Qualitative research methods: a retrospective analysis and database searching.
Results: Accreditation Standards for Primary Care Centres may be divided into three categories: 1. Patient care standards: These standards are structured to follow the process of care for the patient, from the time of intake into a primary care service, through planning and delivery of car to completion of care or referral to another form of care. 2. Support service standards: Support service sections have been developed: environment, human resources and information management. 3. Leadership: These standards cover the leadership in process accreditation goes through the following phases: Application for accreditation: Primary Care Centre institutions obtain the necessary documents from the AZUS. Self-assessment: performed by the staff. They assess the quality of work and evaluate compliance with the standards. In this way they are looking at areas that could be improved. Assessment by surveyors: is an independent evaluation by a trained assessor hired by AZUS. Continuous audit: contribute to maintaining the level of quality that is achieved. Laboratory accreditation in Primary Care Centres is based on a comparison of laboratory performance with the given standards. AZUS has developed 8 standards.
Conclusions: Accredited facility has proved to have: detailed procedures for all activities that are conducted, comprehensive quality system that actively searches for problems in providing services and tries to solve them. The advantages gained through accreditation are as follows: improving the quality of health services, reducing risk and increasing safety for patients and employees and reducing costs.

T269
ESTABLISHING QUALITY ON PROCESS IMPROVEMENT AT PRE-EXAMINATION PHASE - THE WAY FORWARD

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Introduction: Our laboratory that serves 300-bed General Hospital started operation on August 2010 and we achieved College of American Pathologists (CAP) accreditation 8 months later. Quality indicators on specimen unacceptability are measured, collected and data-transformed knowledge served as continuous process improvement. We report our laboratory's journey into operational delivery performance at the pre-examination bench.
Material and methods: At first contact with specimens on pre-examination bench, staff at Core Laboratory use predefined quality indicators to accept specimen. Quantitative analysis data on rejected specimens are tracked weekly during our Quality Management Meeting. Specific actions are acted upon and data are being interpreted. Interventions taken and process monitored. Data on unacceptability (August 2010-December 2012) were calculated for trends and drifts.
Results: Longitudinal data showed weekly unacceptability decreased from 2% to 0.4% (lowest) after several Plan-Do-Check-Action cycles. Interventions such as introduction of laboratory phlebotomist service, lectures on specimen collection are conducted during the Resident and Nursing Orientation Days, reporting of unacceptable specimens to Incident Hospital Reporting System managed by Medical Affair Department and returning of memo received from doctors/nurses for minor amendment on forms/tubes back to wards, out-patient clinics and Emergency Department(ED). ED had the highest % of specimens being rejected because of anti-coagulated tubes clotted (50%), under-filled citrated tubes (20%), labeling errors (15%) and insufficiency (10%).
Conclusion: Specimen rejection has remained at mean 0.7% (1.1% to 0.4%) since March 2011. Similar data from Microbiology Division shows mean rejection at 9% with poor specimen collection as the main specimen rejection criterion. Plan to improve specimen quality at ED is being discussed. In summary, monitoring of quality indicators during regular meetings, follow-up actions and interdepartmental cooperation ensured continuous process improvement in attaining stable quality service and patient safety.

T270
"MEASUREMENT UNCERTAINTY IN THE MEDICAL LABORATORY - IMPLEMENTATION AND EVALUATION OF TWO DIFFERENT FORMULAS IN CLINICAL CHEMISTRY PARAMETERS: TOTAL CHOLESTEROL, CREATININE AND GLUCOSE MEASUREMENTS."

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Background: Laboratory Accreditation by ISO:15189 requires the application of uncertainty calculation in medical laboratories. Specifically it requires laboratories to calculate this data, and to provide patient results along with their associated uncertainty values. The Guide to the expression of uncertainty in measurement describes in general terms the calculation of measurement uncertainties (MU); however, the specific aspects of clinical laboratory testing are not addressed. Several models of uncertainty have been studied, but there is no consensus on implementation.
Methods: The aim of the study was to investigate two potential MU formulas (MU-A and MU-B) within a Portuguese ISO:15189 Accredited Clinical Laboratory. Results were compared with Total Error, currently accepted by the Portuguese Institute for Accreditation as MU value. Three accredited analyte measurements were considered: Total Cholesterol, Creatinine and Glucose, measured in human serum samples using two Cobas® 6000–c501(Roche®) analysers.
Results: Cholesterol - Normal: TE=4.7; 5.4%; MU-A=4.2; 4.8%; MU-B=4.6; 5.1%; Pathological: TE=4.2; 4.5%; MU-A=3.7; 4.1%; MU-B=4.1; 4.4%; Creatinine - Normal: TE=12.5; 10.9%; MU-A=11.6; 10.4%; MU-B=9.9; 8.5%; Pathological: TE=10.5; 9.9%; MU-A=10.2; 9.8%; MU-B=8.2; 7.7%; Glucose - Normal: TE=3.9; 4.4%; MU-A=3.6; 4.0%; MU-B=7.0; 7.2%; Pathological: TE=3.9; 4.6%; MU-A=3.6; 4.0%; MU-B=7.0; 7.3%
Conclusions: MU-B formula was capable to provide reliable values, allowing definition of procedure's variability, well representing the dispersion of values reasonably attributable to the measurand final result. Became clear the necessary Investment from manufacturers, Reference Laboratories and International Organisations to promote and produce certified reference material with high metrological traceability, focusing values at levels of critical decision, with uncertainties associated to the assigned values. General laboratory investment is also needed to improve practice in the pre-analytical phase, but also to assess and evaluate their own specific pre-analytical uncertainty. In addition, guidelines and tables with new goals/limits, defined according to the evaluation methodologies and tools being introduced in the clinical laboratory, must be developed.

T271
EVALUATION OF FLUORIDE-CITRATE MIXTURE IN PRESERVING GLYCEMIA AFTER COLLECTION.

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Introduction: The preanalytical loss of glucose during the first 1-2 h after collection constitutes a large source of error that interferes with both diabetes diagnosis and the process of clinical decision-making regarding management. The standardized pre-analytical processing (i.e.: centrifuging samples immediately in a refrigerated centrifuge and placing the removed plasma promptly into an ice slurry) is not a practical solution in our outpatient blood collection area composed of 51 blood collection facilities. As it has been reported that acidification of blood drawn into tubes containing sodium fluoride inhibits glycolysis quickly, our aim is to study in our setting whether glucose level in blood collected into tubes containing fluoride-citrate mixture is effectively preserved within two hours after collection.
Material and methods: The study was carried out from 25 selected outpatients coming to our blood collection unit. Venous blood was drawn into both two tubes prepared with lithium heparin and two tubes containing fluoride-citrate mixture. Pairs of tubes were stored under transport conditions for one or two hours and then centrifuged and analyzed for glucose. Paired Student's t-test was used for glucose levels randomized comparison.
Results: Significance paired Student's t-test revealed that glucose levels in tubes prepared with lithium heparin and stored one hour after collection were higher than those stored two hours after collection by approximately 8 mg/dL on average ($P < 0.05$). There was not statistically significant difference of glucose levels between both times as far as tubes prepared with fluoride-citrate mixture were concerned. The closeness between both DIF1FLU and DIF2FLU curves shows that there is no change in blood glucose level at any patient over two hours as far as citrate–fluoride mixture is concerned. On the contrary, the gap between DIF1HEP and DIF2HEP curves reveals that glucose level falls in the course of two hours at every patient as far as tubes prepared with lithium heparin are concerned.
Conclusions: We conclude that drawing blood into tubes containing fluoride-citrate mixture to preserve glucose within two hours after collection is an excellent alternative for the standardized preanalytical processing.

T272
SELECTION OF REFERENCE GENES FOR EXPRESSION ANALYSES IN LIVER OF RATS WITH IMPAIRED GLUCOSE METABOLISM

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Background: Hepatic gene expression studies are vital for identification of molecular factors involved in insulin resistance. However, the need of normalized gene expression data leads to the search of stable genes useful as reference in specific experimental conditions. To evaluate expression stability of potential reference genes for real-time PCR gene expression studies, in rats with insulin resistance, early programmed in intrauterine environment for maternal insulin resistance and triggered by exposure to a high sucrose and fat diet in adult life.
Methods: Male rats coming from insulin resistant (F1IR) or normal (F1N) mothers were fed a standard rodent diet from postnatal day 21 to 56, and then divided in two groups each. One group from normal and one from insulin resistant mothers were fed a high sucrose and fat diet (groups F1IR + HSFD and F1N + HSFD respectively) and the rest were fed a control diet (groups F1IR + CD and F1N + CD) for 14 days. Afterwards, glucose metabolism related tests were performed. After liver extraction, RNA was isolated and gene expression analyses of seven potential reference genes (Actb, Gapdh, Gusb, Hprt1, Ldha, Rpl13a and Rplp1) were performed. LinRegPCR software was used to analyze raw data and determinate baseline corrections, threshold lines, efficiency of PCR reactions and corrected Cq values. Evaluations of gene expression stabilities and of the number of necessary genes for normalization were assessed with geNorm tool.
Results: All samples from all groups showed acceptable PCR amplification efficiencies. The most stable genes were Rplp1, Ldha, Hprt1 and Rpl13a and the less stable was Gapdh. For all groups, just 2 to 3 of the most stable genes were necessary to optimal gene expression data normalization in rat liver.
Conclusion: Genes encoding ribosomal proteins are the most appropriated for normalization of expression data in the presented animal model. By contrast, Gapdh, one of the more used genes in normalization, is not recommendable due to its high intergroup variation.

T273

EXTERNAL QUALITY ASSESSMENT SCHEME FOR FAECAL OCCULT BLOOD TESTING (FOBT): AN ESSENTIAL TOOL TO ASSURE THE RELIABILITY OF RESULTS

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Introduction: The colorectal cancer (CRC) is the most common newly-diagnosed cancer and the second most common cause of cancer deaths in Europe. The objective of a CRC screening programme is to discover disease in its early latent stages and, consequently, reduce mortality. To achieve the potential benefit of CRC screening, quality must therefore be optimal at each step in the process. The reliability of FOBT results and their decisional levels are fundamental to assure the correct identification of clinical condition. International guidelines recommend the adoption and use of an Internal Quality Control procedure and the satisfactory participation in External Quality Assessment Scheme (EQAS) to ensure standardized and reliable FOBT results. In this context the Centre of Biomedical Research implemented a specific EQAS to monitor and assess the analytical performances of laboratories. The aim of this work is to describe the results obtained during these years.
Methods: The results of participants to EQAS, from 2006 to 3rd survey of 2012 (46 samples, about 90 participants), have been analysed and grouped on the basis of concentrations. In particular the inter-laboratory variability (CV%) of diagnostic systems used by participants have been evaluated and results have been compared with each decisional level used.
Results: The concentrations range, the percentage of agreement among laboratories about results interpretation and the CV% average related to quantitative methods are reported. <15ug/L: negative = 98.3%, CV% = 67.5%; 30-50ug/L: negative = 74.7%, positive = 24,7%, doubtful = 0.6%, CV%=30.6%; 55-79ug/L: negative = 64.9%, positive = 33,3%, doubtful = 1,8.0%, CV% = 14.6%; 80-96 ug/L: negative = 48.4%, positive = 45.8%; doubtful = 5.8%, CV% = 12.8%; 100-182 ug/L: positive = 89.7 %, negative = 9.5%, doubtful = 0.8%, CV% = 9.3%; 220 -1381ug/L: positive = 98.1%, negative = 1.9%, CV% = 10.1%. Moreover, data demonstrate that only the 77% of laboratories use the cut-off advised by international recommendations (100 ug/L).
Conclusion: The participation in EQAS is a useful tool for clinical laboratories to monitor their performance, to know the improvement needs and analytical performance of diagnostic system commercially available.

T274

EXTERNAL QUALITY ASSESSMENT SCHEME FOR BLOOD GAS ANALYSIS: AN ESSENTIAL TOOL TO CONTROL THE RELIABILITY OF RESULTS OF POCT

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Background: Many regulatory bodies have developed standards for the performance and governance of Point-of-Care Testing (POCT). In accordance with the risk management perspective, all users of POCT are required to undergo specific training managed and coordinated by the laboratory which must assume the responsibility of POCT. Moreover, the adoption of an Internal Quality Control procedure and the satisfactory participation in External Quality Assessment Scheme (EQAS) to ensure standardized and reliable POCT results, are recommended. The Department of Laboratory Medicine of Padova consists of 3 laboratories and a specific laboratory staff has the role of supporting all POCT and blood gas analyzers in the 3 hospital sites. Since 2011, more than the 4 blood gas analyzers in the laboratories and 20 POCT decentralized in care units participated in the EQAS of the Centre of Biomedical Research. The aim was to investigate the performances of the 24 blood gas analyzers over 20 months for ten parameters of the EQA scheme: pH, pO2, pCO2, tCO2, Na+, K, Cl-, Ca++, Glucose and Lactate.
Methods: Data relating the performances of the 24 blood gas analyzers (10 Siemens RapidLab 1265, 13 Siemens RapidPoint 405 and 1 IL Gem Premier 3500) were extracted from the overall data of EQAS participants (145), for a total of 2234 results. In the EQAS the analytical performance is calculated as Index Score (IS) with the following acceptability limits (Total error, %), based on biological variation: pH = 0.5, pO2, pCO2, tCO2 = 8.6, Na+ = 2.0, K = 4.4, Cl- = 3.3, Ca++ = 6.1, Glucose = 5.2, Lactate = 15.2.
Results: Total number of unacceptable performances (PNA), for parameter: pH=0, pO2=29, pCO2 =8, tCO2=9, Na+= 2, K = 2, Cl- = 1 Ca++=15, Glucose=16, Lactate=0. For blood gas and glucose the higher number of PNA was associated to samples with lower concentrations. For pO2 and Ca++ the higher number of PNA was observed for the 3 POCT of a single hospital site. These results are also affected by pre-analytical problems.
Conclusions: EQA Program for blood gas analysis appears an essential tool to monitor the analytical quality of POCT. It allows to identify the POCT with unacceptable performances that need corrective actions coordinated by the laboratory.

T275

BLOOD GAS ANALYSIS IN ITALY: STATE OF THE ART RESULTING FROM THE EXTERNAL QUALITY ASSESSMENT PROGRAM.

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Background: External Quality Assessment (EQA) Program for blood gas in Italy was implemented for the first time in 2009 by the Centre of Biomedical Research. At present 80 laboratories and 65 POCT (Point of Care Testing) for a total of 145 are participating. The scheme consists of four surveys of two liquid control samples each.
Aim: the evaluation of the state-of-the-art of the parameters analyzing the inter-laboratory variability and the degree of harmonization among diagnostic systems.
Methods: Data from 20 control samples distributed from 2010 to 2012 EQA cycles, grouped for diagnostics systems (IL GP3000 = 27, IL GP4000 = 18, Radiometer ABL = 20, Roche Omins/Cobas = 7, Siemens RapidLab = 16, Siemens RapidPoint = 57) were analyzed.
Results: Inter-laboratory variability (in the concentration range studied), calculated as mean CV% and (range): pH (7.10-7.64) = 0.11 (0.07-0.14), pO2 (67.5-149.3 mmHg) = 4.98 (3.55-6.58), pCO2 (17.0-79.7 mmHg) = 3.74 (2.48-5.46), tCO2 (17.6-26.6 mmol/L) = 3.10 (2.45-3.95), Na+ (112.6-165.2 mmol/L) = 0.67 (0.46-0.95), K+ (2.74-6.61 mmol/L) = 1.62 (0.81-2.69), Cl- (74.0-119.0 mmol/L) = 1.25 (0.87-1.88), Ca++ (0.54-1.89 mmol/L) = 2.20 (1.42-3.72), Glucose (1.90-20.1 mmol/L) = 2.95 (2.38-4.06), Lactate (0.88-7.90 mmol/L) = 4.33 (3.75-5.26). The analytical variability results sufficiently low for all parameters with the exception of pO2 and lactate whose CVs% mean are between 4 and 5. RapidLab shows the higher variability for pO2, Ca++ and Glucose, RapidPoint for pCO2 and tCO2, GP3000 for Na+ and Lactate, GP 4000 for K+ and ABL for Cl-.Harmonization among diagnostic systems is very high for pH and Na+, while some critical situations appear for pO2, tCO2, Ca++, Cl- and Lactate. E.g.: for pO2, Omnis/CobasB221 and RapidLab show a bias (positive and negative, respectively) depending on concentration; for Ca++ Radiometer ABL shows a positive bias depending on concentration; for Cl- GP4000 shows a mean bias of +3.6%.
Conclusions: EQA Program for blood gas analysis is useful to define the state of the art of analytical performance of diagnostics system commercially available and appears an essential tool for clinical laboratories to monitor the analytical quality of POCT.

T276

ACCURACY ASSESSMENT OF SERUM CREATININE MEASUREMENT ON ROCHE MODULAR DP AND VITROS ANALYZERS

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Background: Serum creatinine concentration is critical for the assessment of renal function. We aimed to develop an isotope dilution liquid chromatography tandem mass spectrometry (LC-IDMS/MS) method for measuring creatinine, and to assess the accuracy of serum creatinine measurement on Modular DP (Roche) and Vitros analyzers.
Methods: Serum samples were deproteinized by methanol containing the internal standard (IS) D3-creatinine. Supernatants were analyzed using a LC-MS/MS (API 5000) system in positive ionization mode. The mobile phase consisted of 95% of 0.025% formic acid and 5% of methanol. Creatinine and IS were quantified using ion transitions of m/z 114 to 44 and 117 to 47, respectively. The method was calibrated with the purified standard reference material SRM 914a from NIST and assessed the accuracy by analyzing SRM 967. Serum creatinine concentrations in patient serum samples (n=40) determined by kinetic Jaffe method (Roche), enzymatic method (Vitros), and LC-MS/MS were compared.
Results: Intra-assay imprecision of the LC-MS/MS method was 1.5% and 0.4% at 73.0 and 536 μmol/L creatinine, respectively, while inter-assay imprecision was 4.3% and 1.0%, respectively. The method was accurate indicated by the recovery in SRM 967 (99.8±1.0% and 99.6±0.6% of target value for levels 1 and 2, respectively). A good correlation was obtained between the LC-MS/MS method and Jaffe method (Y=0.984X-0.006, r=0.999), and LC-MS/MS and enzymatic method (Y=1.028X-0.125, r=0.999). Hemolysis (up to 20 g/L) caused falsely high results in Jaffe method, but tended to have lower creatinine results in enzymatic method.
Conclusions: We have developed a fast and simple method for the quantification of serum creatinine by isotope dilution LC-MS/MS and used this method as a reference method to validate serum creatinine automated assays.

T277
COMMON URINALYSIS –CURRENT PRACTICES AND WAYS TO IMPROVE THEIR RELIABILITY

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Introduction: Urinalysis is one of the first tests used in medicine from the uroscopy to qualitative and quantitative automated analyses today. It makes up to 12% of the workload in the routine Bulgarian laboratory. It consists of 10 – 13 dry chemistry parameters and microscopic investigation, performed by qualified medical personnel. Urinalysis is used for screening, diagnostic and monitoring purposes. The laboratory is responsible for reliable test results. The goal is to present our experience in applying automated analyzers (DIRUI H800/FUS100) in the routine urine lab, the dry chemistry and microscopy quality control, and explain a surprising source of interlaboratory variation in the microscopy.
Material and methods: Two years long we are using DIRUI H800/FUS100, >35000 urines are tested, all with dry chemistry and microscopic investigations. Productivity is presented as time/sample for 1, 10 and 230 samples. The quality is controlled with materials, purchased by the producer and through manual microscopic confirmation of selected samples - with pathology. Control cards can be plotted manually or with DIRUI software. Regression comparisons are calculated between manual (x) and automated (y) counts of RBC and WBC in 292 urines, analyzed by the workstation and manual microscopic.
Results: The productivity of the workstation is 1 min/sample (230 sample series), 1.5 min/sample for 10. Single sample takes 2.5 minutes (all with 10 dry chemistries + microscopy). Comparison of WBC counts n = 162; y=1.189.x+1.3 r =+0.913; RBC counts n = 130 y=0.847.x + 7.3; r = +0.870. We were surprised to see, that the “high power field (HPF)” of objective 40 is not a reliable measuring unit to present the microscopic findings. We measured the diameters of HPF 40x of 12 microscopes and calculated a CV of 31%; when the HPF surface is calculated the CV rises up to 73%!
Discussion: Optimal productivity and quality of results is achieved through automation and standardization of the urinalyses. The technician is responsible for the quality and is an expert evaluates the findings on the PC screen, verifies them and selects samples for manual microscopic confirmation

T278
ENZIMATIC CREATININE: LIMIT OF BLANK (LOB) AND VERIFICATION OF DETECTION LIMIT (LOD). USE OF ACTIVATED CHARCOAL AS BLANK SAMPLE

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Background: LoD is the lowest amount of analyte in a sample that can be detected regardless of error. LoB is the highest measurement result that is likely to be observed for a blank sample (without analyte). In a Clinical Chemistry lab it is necessary to know analytical performance at some analytes ´ low concentrations; for example, enzymatic creatinine, used in pediatrics replacing Jaffe reagent, whose Limit of Quantification is high. The difficulty lies in getting a sample whose creatinine concentration is zero. So, we verified Creatinine Enzimatic reagent`s LoD manufacturer´s claim.
Methods: reagent: Creatinine Plus, Roche Diagnostics. Analyzer: Cobas 6000. To set the blank limit, we use an activated charcoal adsorbed serum, whose protein concentration was 6,7 g/dL and albumin 4,6 g/dL. This ensures a suitable matrix, similar to patients’ samples. In this serum, the creatinine concentration reported by the instrument was 0,00 mg/dL. The adsorbed sample was processed to get 20 replicates, in order to calculate mean and standard deviation (SD) of absorbance measurements. To calculate LoB we used LoB = meanblk + 1.65 SDblk. In order to express LoB in mg/dL, we used a serum of known concentration. We plotted a straight line with these two mg/dL points, to interpolate LoB absorbance. To verify LoD, we measured 35 replicates (at different days) baby’s serum dilutions (initial concentration: 0.14 mg/dL) and calculated SD. LoD=LoB+1.645 SD; LoD manufactures’ claims = 0.056 mg/dL . Results: LoB = 0,048118 (expressed as absorbance); LoB = 0,009 mg/dL (< 0,01 mg/dL); LoD=0,046 mg/dL.
Conclusions: We obtained LoD=0.046 mg/dL, lower than manufacturer’ claims (0.056 mg/dL). So, LoD’s Creatinine Plus Reagent is therefore verified and may be used to measure creatinine in pediatrics and in adults patients with decreased muscle mass, such as, burned, mutilated patients and pregnant women.

T279
EMPOWER YOUR LAB - NEW INTEGRATED EQA DESIGN

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Background: Labquality has experience in performing external quality assessment (EQA) services for more than 40 years. Recently it has become clear that there is a need for new kinds of modern and even integrated EQA-services in particularly with the aim to empower medical laboratories for their future tasks, such as contribution to the development and implementation, as well as participation in global health-care policies.
Methods: Labquality recently developed the Empower product line comprising the following 4 pillars: (i) master comparisons with panels of single donation sera (ii) virtual EQA-1 and (iii) virtual EQA-2 based on monitoring of patient percentiles and internal quality control (IQC) data across laboratories, and (iv) conceptual/statistical education to share a common vision on analytical quality.
Results: Master comparisons give laboratories a calibration fix-point and information on basic quality of their assays and own performance; patient percentile monitoring serves as a real-time quality indicator for their daily performance; patient- and IQC-monitoring establish evidence about mid- to long-term variation of the instrument-calibrator-reagent combination, backed-up by information from other laboratories of the peer group; in case of unacceptable lot-to-lot variation, laboratories have a basis for factorizing; the link patient/IQC data strengthens the quality management/assurance system of laboratories.
Conclusions: Labquality's new Empower product line adds on to the knowledge of the reasons for assay variation, strengthens the laboratories' position in claims versus manufacturers and creates a tool for developing realistic quality goals and for strengthening the physician/laboratory interface by transparent communication on performance. It delivers data on the performance of assays from other manufacturers that may help laboratories in decisions on the acquisition of new instruments. Last but not least, the education by EQA-organizers on analytical quality, backed-up by evidence created from the empower project, will allow a common understanding between manufacturers and laboratories about realistic performance specifications and the needed quality management/assurance activities.

T280
IMPROVING THE COMPARABILITY OF PARATHYROID HORMONE MEASUREMENTS – A REPORT FROM THE IFCC WORKING GROUP FOR PTH

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Background: Renal physicians strive to maintain PTH concentrations for patients with chronic kidney disease (CKD) within guideline limits, but poor method comparability means there is currently serious risk of clinical misinterpretation. The potential for under-or over-treatment is significant, representing a major challenge to patient safety.
Method: At a meeting convened in September 2010 and attended by representatives of relevant clinical and scientific professional organisations and manufacturers of most PTH methods, the current status of PTH measurement was reviewed and priorities for improvement identified. The IFCC Scientific Division subsequently established a Working Group for PTH with the aim of undertaking this work.
Results: The Working Group is actively raising awareness of clinical implications of method-related differences in PTH among both clinicians and laboratorians. Establishing pre-analytical requirements for PTH is also a priority with a systematic review of stability and specimen requirements currently in preparation. In the longer term, re-standardization of PTH methods in terms of an appropriate International Standard (IS) is required. Provided its commutability can be assured, the recently established IS 95/646 for PTH1-84 is a suitable candidate. Establishment of a well-characterized panel of samples of defined clinical provenance to enable manufacturers to determine appropriate reference intervals and clinical decision points is also being undertaken by the Working Group and will provide an invaluable clinical resource. Recent developments in mass spectrometry mean that a candidate reference measurement procedure for PTH is now achievable and will represent major progress. Concurrently, evidence-based recommendations on clinical requirements and performance goals for measurement of PTH are being developed.
Conclusions: Improving the comparability of PTH results requires support from many stakeholders but is achievable.

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UK NEQAS PILOT SCHEME FOR ANTI-MÜLLERIAN HORMONE – EARLY EXPERIENCE

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Background: Anti-Müllerian hormone (AMH) is increasingly used to assess ovarian reserve in women undergoing investigation for infertility and/or assisted conception. A Pilot United Kingdom National External Quality Assessment Service (UK NEQAS) scheme for AMH has been operating since July 2010. Participants receive monthly specimens to assess analytical performance. Occasional surveys of practice are also undertaken. There are currently 93 participants, a substantial proportion of whom are outside the UK.
Methods: Participants receive five liquid serum specimens monthly and are requested to return their results to the EQA centre within three weeks. A personalised report summarising their results is returned with the next set of specimens a week later. In 2011 surveys were sent to 75 laboratories participating at the time; 70 % of these laboratories are located outside the UK. The survey focussed on clinical applications, reference intervals and interpretation.
Results: Most participants (63%) report results in pmol/L but the remainder use mass units. Five methods are currently represented in the scheme. Between-laboratory agreement is poor, both overall (the coefficient of variation was 24% in 2011) and within-method, particularly at low concentrations. The absence of an International Standard probably contributes to the differences observed. Surveys of practice show wide variation in reference intervals quoted, even by users of the same method; relatively few centres use age-related reference intervals, despite good evidence that AMH concentrations change with age. Decision limits for assessing ovarian reserve also vary widely, so that the same AMH concentration might be interpreted differently depending on the centre in which it was obtained. For example decision levels used to assess risk of ovarian hyperstimulation syndrome ranged from >28.6 to >70.0 pmol/L. Most laboratories use reference intervals provided by the manufacturer, 31% have determined their own reference intervals and 24% use values from the literature.
Conclusions: There is a need to improve between-method agreement of AMH assays and to achieve consensus about units for reporting, appropriate reference intervals and reliable decision levels for assessing ovarian reserve.

T282
SUITABILITY OF THE MEASUREMENT IMPRECISION OF COMMON URINARY BIOCHEMICAL ANALYTES

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Background: Imprecision monitoring is central in the performance evaluation of an analytical system. To be suitable in clinical setting, the measurement imprecision should fulfil goals derived from biological variation data of the corresponding analyte, when available. Here we aimed to evaluate the long-term imprecision in our laboratory of measurements of 11 urinary analytes measured on the Roche Cobas 6000 system.
Methods: Data were obtained for evaluated analytes by drawing up an internal quality control (QC) programme based on daily measurements of a lyophilized control material (Assayed Urine Control – Level 3, Randox Laboratories, lot no. 580UC). CVs were calculated along the whole examined period (February 2012 to September 2012) (n=165) and compared with corresponding goals for imprecision derived from biological variability data (analyte concentration in 24 h urine sample), with the exception of urinary chloride and glucose for which biological variability data are missing.
Results: Analyte concentration means and CVs (optimum goals in parentheses) were as follows: albumin, 182 mg/L, 3.0% (15.3%); calcium, 194 mg/L, 2.9% (6.9%); chloride, 258 mmol/L, 2.2% (not available); creatinine, 1.91 g/L, 2.1% (6.0%); glucose, 2.78 g/L, 1.9% (not available); magnesium, 323 mg/L, 3.5% (11.4%); phosphate, 852 mg/L, 2.0% (6.6%); potassium, 105 mmol/L, 2.1% (6.8%); sodium, 193 mmol/L, 1.9% (6.0%); urea, 14.7 g/L, 2.5% (5.7%); and urate, 214 mg/L, 2.7% (6.2%).
Conclusions: Our study shows that in routine laboratory practice and over a clinically and analytically relevant time-span, the imprecision of the common urinary analyte measurements on the Cobas 6000 system fulfils optimum goals of analytical performance. Although intra-individual biological variability of urinary analytes is usually relatively high, resulting in less stringent analytical goals when compared to those of serum measurements, the excellent CVs of urinary analyte measurements qualify the suitability for clinical application of these tests.

T283
REGULATORY SCIENCE FOR LABORATORIES IN GLOBAL AND ETHIOPIAN SITUATIONS

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Objective: To explore the regulatory science and describe its application for medical Laboratories comparing Global and African orientation with Ethiopian harmoniztion.
Method: Seminar Review was conducted on electronically available documents on Regulatory Science, Health and Laboratories regulations in the Global and Sub-Sahara African perspectives.
Result: A total of about 480 documents were searched based on relevance of their topics. 60 documents including 22 web pages have been upraised for this seminar. Documents from regulatory review meetings reports, books, scientific reviews, policy documents and guidelines have been given priority during appraisal of the documents. The outcome of the study has been organized in to four categories: the regulatory science1, the FDA2 and CLIA'882 experience, International Health Regulation3, harmonization in Sub-Sahara Africa3 and Experience of Ethiopia4.
Discussion: Regulatory science is the science of developing new tools, standards, and protocols to assess the safety, efficacy, and quality of medical devices. The regulations of medical devices in developed countries are guided by national agencies and overseen for standardization and collaboration by ICH. Having committed to share responsibility in regulation and maintenance of global health, ICH is working to develop and standardize global prospects to ensure safety, effectiveness and quality of human use of medical devices. The World Health Assembly passed IHR-2005 enabled the WHO and member states to improve local and global capacity for detection, intervention, and response to diseases of international and public concern. Both the ICH and WHO are working in collaboration to enable countries to manage their population health and communicate their findings for global concern. Ethiopia has established Food, Medicine and Health care Administration and Control Agency by proclamation 661/2009 17 years after National Health Policy had been documented. Tools, standards and protocols to measure substantial equivalence of devices and practices is not yet developed.

T284
ANALYSIS OF INTESTINAL PARASITES IDENTIFYING CLINICAL LABORATORIES PARTICIPATING IN A PROGRAM OF EXTERNAL QUALITY ASSESSMENT

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Background: External Quality Control (EQC) is a procedure in which an organization outside clinical laboratories for assessment methods of analysis, including testing coproparasitologic useful in the diagnosis and treatment of intestinal parasites infections, however, there are problems diagnosis, mainly of overdiagnosis and also the lack of experience of laboratory personnel in identifying parasites, which promotes the indiscriminate use of antiparasitic drugs. The objective is to evaluate the ability of clinical laboratories linked to a program of EQC in the correct identification of the parasites during the period of 2007-2010.
Methods: We developed an analytical study of the results obtained by clinical laboratories linked to the EQC program of the Facultad de Ciencias Químicas, Universidad Autónoma de San Luis Potosí, México (PEEC), in the period of 2007-2010, where on average per year involved 71 laboratories. The identification was assessed by sending photomicrographs of the following parasites: Blastocystis hominis, Giardia lamblia, Entamoeba histolytica/dispar, Entamoeba coli, Trichuris trichiura, Enterobius vermicularis and Ascaris lumbricoides. For statistical analysis were used Microsoft® Office Excel and Epi InfoTM programs.
Results: 867 results were analyzed, 579 of protozoa (479 successful) and 288 of helminths (287 successful). T. trichiura and A. lumbricoides scored 100% acceptable results (142 and 78) responsible for helminth infections more common in Mexico. E. vermicularis scored 98,5% correct results. For protozoa, E. coli was the most successful identified 92% (70), G. lamblia followed by 83% (123) and B. hominis with 81% (175) and E. histolytica/dispar with 79% (111). The ability to identify protozoa against helminths, was 59,9 times to correctly identify helminths ($P < 0,001$). The identification analysis protozoa, is 2.5 times the ability to identify E. coli to B. hominis ($P < 0,05$) and 4,4 times the ability to identify E. coli vs. E. histolytica/dispar ($P < 0,001$). No differences between helminths.
Conclusions: Clearly there are problems in identifying protozoa; laboratories with unsatisfactory results should implement training programs to improve identification skills.

T285

APPLICATION OF GUIDELINES AS A REQUIREMENT FOR ACCREDITATION OF CLINICAL CHEMISTRY LABORATORIES: THE DUTCH EXPERIENCE.

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Background: Quality and quality management are concepts within the clinical chemistry laboratory that always have received considerable attention. An important tool to demonstrate the quality of the products and services of the laboratory is accreditation. In the Netherlands, medical laboratories are accredited according to the CCKL-mark, based on the ISO 15189 standard. It is acknowledged in this standard that a country can have its own specific regulations or requirements. Within the Netherlands Society for Clinical Chemistry and Laboratory Medicine (NVKC) there is a growing need for guidelines with recommendations on laboratory procedures.
Methods and results: According to a newly adopted procedure within the NVKC, three guidelines were recently developed with financial support from the Dutch Quality Fund for Medical Specialists (SKMS): 'Anemia protocol in primary care', 'Consultation by laboratory specialists' and 'Point-of-care testing in primary care'. All members of the NVKC could comment on the concept guidelines. Comments and suggestions for improvement were processed in a final version. After approval by the Quality Committee of the NVKC, the guidelines were put to vote during a plenary membership meeting and accepted. Each of the guidelines contains minimum standards as well as target standards. Minimum standards provide a lower limit of appropriate care that laboratories must comply to. In individual cases one can by exception deviate from this, if substantiated. Target standards provide optimal care. Ideally, these standards should be pursued by laboratory protocols.
Conclusions: 1) The main goal for the development of NVKC guidelines was to harmonize processes between clinical chemistry laboratories, thereby improving quality. 2) The Dutch procedure of authorizing guidelines implies an active demand for input of the NVKC members and a transparent feedback regarding the processing of this input. A formal status of the guidelines was obtained by approval during a plenary membership meeting. 3) Application of the guidelines is greatly improved by subjecting them to audits. Specifically for the Netherlands, implementation of these professional guidelines is a requirement for accreditation of clinical chemistry laboratories.

T286

A TRACEABILITY CHAIN FOR THE STANDARDIZATION OF DIRECT BILIRUBIN TESTS

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Background: Beyond the determination of Total Bilirubin, differential diagnosis of an icterus requires discrimination between the water-soluble post-hepatic Direct Bilirubin fraction (consisting of glucuronidated β- and γ-bilirubin, covalently protein-bound δ-bilirubin and photo-bilirubin, a water-soluble cis-trans-isomer of α-bilirubin) and the Indirect unconjugated α-bilirubin. The Doumas reference method for the measurement of Total Bilirubin is in place, while for Direct Bilirubin neither a specific method nor a standard material is available yet. In the following we suggest a traceability chain for the standardization of Direct Bilirubin.
Methods: A fast HPLC method (less than 15 minutes) was able to resolve the α-, β-, γ-, and δ- species and photo-bilirubin into clearly separated fractions and to quantify those using NIST reference material Unconjugated Bilirubin as a standard. The Doumas reference method for Total Bilirubin and the Roche methods BILTX and BILD2 were used for bilirubin determinations.
Results: Comparison of the HPLC with the routine and reference methods results indicated that β-, γ-, and δ-bilirubin were correctly recovered by the routine methods BILTX and BILD2. This was also true for photo-bilirubin, a very polar fraction that formed upon exposure of α-bilirubin samples to blue light, and for ditaurobilirubin, a non-physiological conjugated bilirubin that gave a separate HPLC peak when dissolved in water, but was transformed rapidly and quantitatively into δ-bilirubin when added to serum. Therefore, human sera with low total bilirubin ($\leq 5\,\mu M$) were supplemented with different concentrations of ditaurobilirubin (up to 170 μM) and used as physiological direct bilirubin standards. They gave an excellent correlation when measured with the Doumas Total Bilirubin reference method compared to the routine method BILD2, showing their suitability for the standardization of Direct Bilirubin routine tests.
Conclusion: A traceability chain for the standardization of Direct Bilirubin assays has been established from the Doumas Total Bilirubin reference method via a commercially available bilirubin derivative to the Direct Bilirubin concentration in human sera.

T287
PERFORMANCES OF THIRD PART QUALITY CONTROL IN A COAGULATION SYSTEM

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The guidelines for the running of the Internal Quality Assessment (IQA), conceived by the working group SIBIoC in 2008, report both the characteristics the materials must have and the formalities of execution and validation of IQA. Among the characteristics, internal control materials must have, the guidelines report they have better to be independent (of a third part) in case of validation of the analytical series, that is to say different in comparison to those either manufactured or supplied together with the reagents. The use of third part materials allows to highlight problems of degradation and/or shift of the calibrator that could be hidden if calibrators and control materials with the same matrix are used. The aim of this work was to evaluate the performances of third part control materials on a coagulation system. We tested two Biorad control levels and, for each one, we had 30 determinations for PT, APTT, Antitrombin (AT) and Fibrinogen (FGB). The Biorad controls were tested on ACL TOP 700 instrument using dedicated reagents (RecombiplstaTin, SynthAsil, Liquid AT, QFA). The coefficients of variation (CV%) relating to the values obtained from each test, were for the normal and pathological level as follows: PT=4.0% and 4.6%, APTT=3.5% and 3.0%, AT=3.0% and 4.0%, FBG= 6.0% and 6.0%. All the CV% resulted to be lower than the target of total error defined on the basis of biological variability (PT=5.3, aPTT=4.5, AT=8.3, FBG=13.6). They also resulted to be lower than the optimal acceptable limits proposed by the accredited EQA Program managed by the Centre of Biomedical Research of Padua and defined on the basis of the biological variability but modified on those of the state-of-the-art (PT=5.3, aPTT=6.7, AT=6.3, FBG=6.8).
The results of this work demonstrate that the tested third part materials have characteristics of precision corresponding to the quality objectives defined both on the basis of the biological variability and of the state-of-the-art. Moreover, having the tests been performed up to 48 hours from their reconstitution, they show a good stability and it is obvious to think that processing them within 24 hours from reconstitution, as happens in a routine process, can further ameliorate the performance above all for PT and APTT.

T288
CELIAC DISEASE DIAGNOSIS AND MONITORING: COMPARISON BETWEEN CHEMILUMINESCENCE AND ELISA ANTITRANSGLUTAMINASE AND ANTI-DEAMIDATED GLIADIN PEPTIDES MEASUREMENTS

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Background: Celiac disease (CD) diagnosis is based on anti-tissue transglutaminase (tTG) and anti-deamidated gliadin peptides(AGA) determination. The majority of commercially available assays for tTG and AGA are ELISAs with sensitivity and specificity above 95%, never reaching however 100%. The aim of this study was to compare the analytical performancesand the clinical utility for CD diagnosis and monitoring of tTG and AGA of the IgA and IgG classes measured by new chemiluminescent(QUANTA Flash®, Inova) and established ELISA (QUANTA Lite®, Inova) methods. The one run measurementsof IgA and IgG tTG and AGA (h-tTG/DGP screen) by QUANTA Flash® and QUANTA Lite® were also evaluated.
Methods: We studied 321 children (155 CD; 166 controls). tTG IgA and IgG, DGP IgA and IgG, h-tTG/DGP screen were measured by QUANTA Flash® and QUANTA Lite®.
Results: Intra- and inter-assay coefficients of variations of QUANTA Flash® were: 1.6% and 3.0% for tTG IgA, 3.7% and 4.0% for tTG IgG, 8.6% and 6.8% for DGP IgA, 8.6% and 3.2% for DGP IgG, 5.1% and 2.4% for h-tTG/DGP screen. The most sensitive (96.1%) and specific (97.0%) test for CD diagnosis was QUANTA Flash® tTG IgA (cut-off:16 U), while the less sensitive (82.1%) and specific (78.6%) was QUANTA Lite® tTG IgG (cut-off:4 U). We performed binary logistic regression analyses considering CD diagnosis as dependent, and including as predictors QUANTA Flash® or QUANTA Lite® tTG IgA and IgG, DGP IgA and IgG, and h-tTG/DGP screen. QUANTA Flash® allowed to obtain a better correct overall classification of patients (96.60%) with respect to QUANTA Lite® (95.14%). Among QUANTA Flash® predictors, tTG IgA only was selected as significantly correlated with CD (r=0.263, P <0.0001, Exp(B)=1.0506, 95% CI=1,0286-1,0731). 18 CD children on GFD had a complete follow-up with measurements at 4, 12 and 24 months. QUANTA Flash® tTG IgA was the most reliable index (Repeated measures analysis of variance, between subject effect F=5.03, P <0.05) in monitoring GFD.
Conclusion: QUANTA Flash® tTG IgA measurement is an extremely sensitive and specific index not only for CD diagnosis, but also for the monitoring of CD patient on GFD.

T289

EVALUATION OF A PARTICLE-ENHANCED TURBIDIMETRIC CYSTATIN C ASSAY ON THE ROCHE COBAS 8000 ANALYZER AND ASSESSMENT OF CYSTATIN C-BASED EQUATIONS FOR ESTIMATION OF THE GLOMERULAR FILTRATION RATE

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Backgrounds: Estimation of the glomerular filtration rate (GFR) is essential for the evaluation of patients with chronic kidney disease (CKD). Cystatin C is a low molecular protein that freely filtered through the glomerulus and reabsorbed and catabolized by tubular cells. Recently, serum cystatin C-based equations were proposed as markers for estimation of GFR. The aim of this study was to evaluate the analytical performance of new cystatin C particle-enhanced turbidimetric assay (PETIA) on the COBAS 8000 analyzer and compared it particle-enhanced nephelometric assay (PENIA). Estimated GFR, which was generated from cystatin C-based equations, was compared by using two cystatin C assays.
Methods: PETIA cystatin C measurements (n=185) were performed on the COBAS 8000 analyzer (Roche Diagnostics,Switzerland). PENIA cystatin C measurements (n=185) were done by using BN II nephelometer (Siemens N-Latex Cystatin C, Germany). . Serum creatinine levels were analysed by rate-blanked and compensated Jaffe method in Cobas 8000 analyser. Estimated GFR (eGFR) for these cystatin C assays was calculated using the equations recommended by the manufacturers: Grubb et al. and Hoeks et al. Imprecision, limit of detection, quantification and linearity data were determined. Bland-Altman analysis was used to examine the differences between the assays.
Results: The measurement range of cystatin C PETIA was between 0.4 mg/L and 8.0 mg/L. Within-run and between-run CVs were less than 5% at two quality control levels. PETIA cystatin C values were higher than PENIA results (mean of differences: -0.14, SD: 0.16, %95 confidence limits: -0.45-0.17). Both assays are linear with a good correlation coefficient (r=0.989). The Bland–Altman analysis for the eGFR calculated with the two assays showed a systematic bias.
Conclusion: PETIA cystatin C method is a reliable alternative to determinate of the cystatin C with good linearity, precision and accuracy on the Cobas 8000 analyzer. It provides a valid quantitative measurement of cystatin C with comparable % CVs in quality-control as well as patient samples. Additionally, PETIA provides a rapid analysis of a large number of samples with a low turnaround time and is well suited to detect renal dysfunction with a 24 h availability.

T290

SIMULTANEOUS DETERMINATION OF SIROLIMUS AND EVEROLIMUS BY LC-MS/MS AUTOMATED PROCEDURE: A FOUR-YEAR EXPERIENCE IN KIDNEY TRANSPLANTATION RECIPIENTS

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Background: Therapeutic drug monitoring (TDM) of immunosuppressive drugs as well as sirolimus and everolimus is frequently used in organ transplantation for dosage adjustment. High-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS) is a reliable technique for TDM with many advantages. The main objective of this study was to evaluate on-line solid-phase extraction method in combination with LC-MS/MS for the simultaneous determination of sirolimus and everolimus in whole blood.
Methods: The whole blood sirolimus and everolimus levels (n=7720) were determined in kidney transplantation recipients between December 2008 and November 2012. The current method uses protein precipitation for sample preparation. Analyses were performed using a triple quadrupole LC-MS/MS with a C18 column (Tandem Gold, Zivak Company, Turkey). Blood samples (100 μL) were prepared by protein precipitation. Ascomycine was used as the internal standard (ZinMass Immunosuppressants LC-MS/MS Analysis Set (Whole Blood). Mass spectrometric analysis was performed by selective ion monitoring with an electrospray ionization in positive mode. Four-level blood calibrators and three internal quality control materials at different concentrations were used for assay. External quality control assessments were done by using the international proficiency testing scheme (www.bioanalytics.co.uk, England).
Results: The analytical time was 4 min and the assay was linear from 1.5 to 50 μg/L for everolimus and sirolimus. Calibration curves were linear between the ranges. Within-run and between-run CVs were less than 10% for three quality control levels. The limits of detection and quantification were determined (sirolimus: 0.66 μg/L, 1.98 μg/L ; everolimus: 0.48 μg/L, 1.60 μg/L, respectively). The external quality control results showed acceptable agreement with other methods.
Conclusions: This method allows for the simultaneous determination of sirolimus and everolimus in whole blood in a short time with high linearity, precision and accuracy. LC-MS/MS method provides a reliable and rapid automated procedure that can be preferable for therapeutic drug monitoring of immunosuppressive drugs in clinical laboratories.

T291
RAPID AND SIMPLE SANDWICH IMMUNOASSAY FOR DETECTION OF TOTAL CYANOBACTERIAL HEPATOTOXINS (MICROCYSTINS AND NODULARINS)

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Background: Cyanobacterial blooms cause local and global problems with their potent toxins and pose serious health risks for human and animals. Microcystins (MCs) and nodularins (Nods) are structurally related cyclic peptide cyanobacterial toxins having more than 100 variants. Most MCs and Nods are potent hepatotoxins, tumor promoters and possible carcinogens. The WHO guideline value for cyanobacterial toxin MC-LR in drinking water is 1 μg/L. Commercially available immunoassays are based on indirect competitive method and require hours to perform. Simple and rapid, yet sensitive methods for first line screening of total MCs and Nods are in high demand for assessment of water quality and safety.
Methods: Antibody fragment (scFv) capable of recognizing immunocomplexes (IC) consisting of a capture antibody (Ab) bound to MCs or Nods were selected from a synthetic phage displayed Ab library. The scFv was expressed in E. coli as fusion with Alkaline Phosphatase (AP), purified and used in sandwich assay for detection of total MCs and Nods. In a one-step assay reagents and toxin standards in water were added together, incubated for 10 min followed by one washing step and signal development. Signal of Eu labelled anti-AP Ab was detected by time-resolved immunofluorometry. Total assay time was 15 min.
Results: It is very rare and difficult to establish generic sandwich format assay for small molecules (~1000 dal) where both capture and secondary Ab must have generic binding capabilities. Using synthetic phage display library, we have successfully isolated high affinity anti IC scFv with generic specificities towards MCs and Nods and developed a 15 min rapid and simple single-step sandwich format assay. The sensitivity (blank+2SD) of the assay for major hepatotoxins (MC-LR, -dmLR, -RR, -dmRR, -YR, -LY, -LF -LW, and Nod-R) varies from 0.02 to 0.1 μg/L (4-20 pg/well).
Conclusions: The assay was capable of detecting all the tested nine major hepatotoxins at levels ten times below the WHO guideline limit (1 μg/L). The assay is very rapid and simple having only one washing step. It offers robustness for cost-effective automation and high throughput possibilities for fast screening of large number of samples for the detection of total MCs and Nods.

T292
NEW INSTRUMENT FOR EASY DETERMINATION OF RHEOLOGICAL PARAMETERS OF ERYTHROCYTES

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The evaluation of the rheological parameters of human red blood cells allows diagnose possible alterations that can induce serious complications in microcirculatory disorders and diseases such as diabetes, hypertension, anemia, etc. The red blood cell (RBC) must be able to deform without breaking or obstruct microcapillaries but there are certain diseases in which the membrane is altered and cannot normally be distort or break.
In this context, we developed a new computerized rheometer to determine the stationary and dynamic rheological parameters of RBCs for economical and easy use in conventional and specialized biochemical laboratories, which is in patenting step. This new instrument is based on laser diffractometric technique and has a GUI (Graphic User Interface) for easy use to an unskilled operator. As in the Erythrodeformeter developed previously by Rasia et al, this instrument permits to evaluate the viscoelasticity of RBC by applying the laser diffractometry technique. In order to carry out these measures, a thin layer of RBC is suspended in a viscose media and placed between two parallel concentric disks. In the stationary mode, the lower disk rotates at constant speed and in the dynamic mode, it moves at sinusoidal oscillating speed at frequencies in physiological range. Photometric signals from diffraction pattern are used to determine all RBC rheological parameters, which are averaged over several millions of cells. The parameters obtained with the new rheometer correlate with the erythrocyte rheological parameters obtained with other similar instruments but they are more complete and accurately. The new erythrocyte rheometer can determine all the rheological parameters of erythrocytes in a simple and easy form to help in the diagnosis of vascular and haematological diseases and allow analyze their evolution in terms of treatment and medication that the patient receives. Also, the easy determination of the dynamic viscoelasticity of RBCs permit to know the part of the membrane that is altered in order to design a medication according to the disease.

T293

COMPARISON BETWEEN ANALYTIC RESULTS OBTAINED BY TWO VERY COMMON HPLC SYSTEMS IN HAEMOGLOBIN DIAGNOSTICS: EVALUATION ISSUE AT CSMR

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Background: The CSMR (Centro Studi Microcitemie di Roma) deals from more than 50 years with prevention and diagnosis of Thalassaemia syndromes and haemoglobinopathies. The determination of the haemoglobin fractions HbA2 and HbF, very important parameters to reach diagnostic results, is currently performed using the cation exchange HPLC method. In this study we compared the results obtained for the haemoglobin fractions HbA2 and HbF by the Variant II system (BIO-RAD Laboratories, Hercules, CA, USA) and the G8 system (TOSOH HLC-723 G8). Furthermore, when possible, we compared the analytic results of abnormal haemoglobin fractions according to identifying and quantitative capacity.
Methods: 156 samples coming from our ambulatory were studied. These samples were analyzed as routine, by the HPLC Variant II (BIO-RAD Laboratories, Hercules, CA, USA) and successively, they were examined by the G8 system (TOSOH HLC-723 G8). The analyzed samples were divided into three subgroups: a. Hb A2 <2.0% (n=15) b. 2.1%< Hb A2 <3.2% (n=111) c. Hb A2> 3.3%. (n=30). In all subgroups the median calculation was carried out for the HbA2 and HbF. We used the Pearson correlation coefficient to compare the HbA2 and HbF obtained values. Some samples already selected by the Variant II for the presence of rare haemoglobin variants, were tested by the G8 as well.
Results: The analysis of the results obtained by the two methods pointed out a correlation coefficient of 0.99 for both HbA2 and HbF. This result indicates a very strong correlation. As for the HPLC separation of the examined haemoglobin variants, we obtained excellent results by the G8 system even if the retention times are different respect with Variant II'S. We highlighted the following haemoglobin variants: Hb G Copenhagen, Hb O Arab, Hb San Diego, Hb Koln, Hb Toulon and one more Delta variant still under identification.
Conclusions: Both systems provided equivalent results that can be interpreted in the same way in diagnostic terms. The excellent overlap obtained by two different systems, leads us to consider also the G8 HPLC as a reliable support for first diagnosis level in thalassaemia and haemoglobinopathies.

T294

STUDY OF REPRODUCIBILITY OF A NEW CAPILLARY ELECTROPHORESIS INSTRUMENT (MINICAP FLEX PIERCING) AT CENTRO STUDI MICROCITEMIE OF ROME

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Background: Centro Studi Microcitemie of Rome has been working for years in prevention and diagnosis of hemoglobinopathies. For the detection of these pathologies the identification and the precision in the quantification of the hemoglobin fractions HbA2 and HbF are crucial.
Aim of the study: In this study we tested a new Capillary electrophoresis instrument, the MINICAP Flex Piercing (SEBIA, Parc Tecnologique Leonard de Vinci, Evry Cedex, France) to evaluate the precision performance of quantitative measurements. For this purpose we performed intra and inter assay study on HbA, HbA2 and HbF. Assessment was also extended to hemoglobin variant HbS as it is widely present in our laboratory.
Materials and methods: For intra- and inter-assay reproducibility, 5 samples were selected as follow: normal whole blood from patient of our ambulatory; whole blood with elevated HbA2 from patient of our ambulatory; heterozygous A/S from patient of our ambulatory; SEBIA Pathological HbA2 Control; SEBIA Normal HbA2 Control. All assay were performed on samples stored at -80 °C. Reproducibility within-run: each sample was run 5 times on both capillaries, using the Minicap Hemoglobin procedure. The same protocol was then repeated the same day using a different reagent lot number. The mean, Standard Deviation (SD) and Coefficient of Variation (CV; n=20) were calculated for each sample and each hemoglobin component. Reproducibility between-runs: the 5 samples were run within the same series for 10 consecutive days, using Minicap Hemoglobin procedure. The same protocol was repeated the same day using a different reagent lot number. The mean, SD and CV (n=20) were calculated for each sample and each hemoglobin component.
Results: In the within run study we have evaluated a good reproducibility for HbA2 (1.26<CV%<1.70), HbS (CV= 0.49) and HbA0 (0.05<CV<0.31). As expected, since there is evidence in literature, the CV of HbF is major (CV=5.66). The assay between runs shows a good reproducibility too. Infact for HbA2 we have calculated 1.12<CV%<1.77, for HbS the CV is 0.75 and HbA0 shows a CV from 0.05 to 0.47. As expected the CV of HbF is major (CV=8.1).
Conclusions: The results show a good performance of the Minicap flex pearcing about its precision in the reproducibility of the results.

T295
ANALYTICAL PERFORMANCE OF COBAS C311 IN THE PEDIATRIC LABORATORY

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Background: The Cobas c311 analyzer (Roche, Mainheim; Germany) is routine clinical chemistry analyzer suitable for laboratory with workloads of 50-200 samples per day. The aim of our study was to evaluate analytical performance of Cobas c311 in the pediatric laboratory.
Methods: The validation was performed for 30 analytes: glucose, urea, creatinine, uric acid, total bilirubin, direct bilirubin, ALP, AST, ALT, GGT, LDH, CK, AMY, total protein, albumin, CRP, IgA, IgG, IgM, ferritin, triglyceride, cholesterol, HDL, iron, calcium, magnesium, inorganic phosphorous, sodium, chloride and potassium. The imprecision study consisted of within-run imprecision (N=15) and between-run imprecision (N=15). Method comparison study was done with routine analyzer AU400 (Beckman Coulter, USA; N=20). Results were judged according to quality specification given in Biological variation database by Ricos and colleagues.
Results: For all tested analytes coefficients of variation (CV) were below 5% for within-run imprecision. Of 30 tested analytes, 26 of them fulfilled quality specification for total imprecision except: creatinine, sodium, chloride and ALP. The Passing and Bablok regression analysis was used for method comparison study. Linear equations, as well as 95% confidence intervals for intercept and slope were calculated. The proportional error was found for: total bilirubin, direct bilirubin, CK, AST, LDH, CRP and magnesium. The constant error was revealed for: GGT, IgG and chloride. Both constant and proportional errors were detected for urea and IgM. Other tested analytes were in agreement with routine method.
Conclusions: Overall analytical performance of Cobas c311 was satisfactory. Imprecision study for majority of tested analytes was acceptable. However, method comparison study showed a need for regression line modifications in almost half of tested methods. Both imprecision and inaccuracy requirements were not satisfied only in case of chloride measurement. Despite of imperfect analytical performance Cobas c311 analyzer can be implemented in routine pediatric laboratory after certain adjustments.

T296
THE CONVENIENT PURIFICATION OF BIOCONJUGATED UPCONVERTING NANOPARTICLES BY HIGH GRADIENT MAGNETIC SEPARATION

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Background: Upconverting nanoparticles (UCNPs) have the unique luminescent property of converting low-energy infrared light into visible emission which can be utilized in reporter and imaging technologies. However, as the particle size of the UCNPs approaches the size of biomolecules, the handling of reporters becomes cumbersome with traditional purification methods such as centrifugation. While the lanthanide-doped crystal structure of UCNPs is responsible for the upconversion luminescence, the lanthanide dopants also bring paramagnetic properties to the UCNPs. This enables the use of high gradient magnetic separation (HGMS) as a method to purify and separate them and their conjugates. HGMS produces high magnetic gradients which can capture even weakly paramagnetic materials.
Methods: UCNPs were bioconjugated with an excess amount of streptavidin or monoclonal antibodies and thereafter purified from unbound biomolecules by HGMS or by conventional method (ultrafiltration). The purification efficiency of the two methods was compared by analyzing e.g. the yield and by using the bioconjugated UCNPs as reporters in a heterogeneous bioaffinity assay.
Results: The HGMS-purification of the bioconjugated UCNPs resulted in higher yield compared to ultrafiltration, as it is difficult to detach the UCNPs from the filter membrane and redisperse them into solution. Ultrafiltration also tends to aggregate the UCNPs which was observed as higher non-specific binding and standard deviation when they were used as reporters in a heterogeneous assay. A more sensitive assay was performed by using the HGMS-purified UCNPs.
Conclusions: HGMS is a fast, convenient and highly selective purification method for UCNPs and it can be used to separate bioconjugated UCNPs from unbound biomolecules or other reagents used for their surface modification process producing highly sensitive reporters with high yield and purity. It can also be used for buffer exchange and for concentrating the UCNPs which are difficult with other purification methods. The separation is solely based on the intrinsic paramagnetism of luminescent lanthanide dopants without the need to embed separate, optically inactive magnetic materials within the UCNPs.

T297
HIGH-THROUGHPUT ANALYSIS OF AN IMMUNOSUPPRESSANT DRUG PANEL IN WHOLE BLOOD USING ULTRAFAST SPE/MS/MS

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In many clinical research laboratories, liquid chromatography-mass spectrometry (LC/MS) methods of analysis of immunosuppressant drugs have proven superior because of their increased sensitivity and selectivity. We evaluated the ability of an ultrafast SPE/MS/MS system to simultaneously analyze tacrolimus, everolimus, sirolimus, and cyclosporin A in whole blood. MS methods for tacrolimus, everolimus, sirolimus, and cyclosporin A and their corresponding internal standards were optimized for analysis by QQQ MS. Calibration standards for each analyte were prepared in bovine whole blood. The whole blood samples were mixed with water and precipitated using a zinc sulfate and methanol solution containing the internal standards. Precipitated samples were gently mixed and then centrifuged. Following centrifugation, supernatants were transferred to a 96-well plate for analysis. Samples were analyzed using an Agilent RapidFire high-throughput mass spectrometry system coupled to a QQQ mass spectrometer. A C18 column was used for online SPE. Prepared calibration standards were run in triplicate over a series of days to establish both intra- and inter-day precision and accuracy. Cyclosporin A had both intra- and inter-day accuracies within 15% and CV values less than 6% for all concentrations within the linear range (7.8-1000 ng/mL). The method for all four analytes had excellent linearity within their respective measured ranges with an R2 value greater than 0.995. Signal-to-noise ratios were calculated by looking at peak to peak height and found to be greater than 40:1 at the limit of quantitation for all four analytes. To further evaluate this method, identical human samples were analyzed by RapidFire and a traditional LC/MS/MS method. Excellent correlation was found for the two methods. Based on these results: tacrolimus, everolimus, sirolimus, and cyclosporin A can be accurately and precisely measured in whole blood. All four immunosuppressant drugs were simultaneously analyzed in a 12 MRM panel in less than 13 seconds per sample using ultrafast SPE/MS/MS. While the analytical results were comparable to LC/MS/MS, the analysis time was approximately 10 times faster. This methodology is capable of throughputs >270 samples per hour.

T298
QUANTITATIVE ANALYSIS OF FREE AND TOTAL THYROID HORMONES IN SERUM USING LC/MS/MS

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Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) has become an essential tool for small molecule quantitation due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis of multiple analytes. Thyroid hormones can be challenging compounds to analyze due to the low levels in biological matrices such as plasma relevant to clinical research. In order to address this challenge, a sensitive liquid chromatography-tandem mass spectrometry method for the simultaneous analysis of Thyroxine (T4), 3,3',5-Triiodothyronine (T3) and 3,3',5'-Triiodothyronine (rT3) in serum samples has been developed. Two separate sample preparation techniques were used for the determination of the thyroid hormones – protein precipitation for the total concentration and ultracentrifugation for the free concentration. A labeled internal standard was included for each of the analytes to ensure accurate quantitation. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard. Standard liquid chromatography (LC) was used with a reverse-phase C18 analytical column. Final concentrations were calculated by comparing the response of the analyte to a known concentration of internal standard and plotting the result on a calibration curve developed using stripped human serum spiked with standards. The LC-MS/MS parameters were optimized and calibrated over the range of 1 pg/mL to 1000 pg/mL for free T4, T3 and rT3 and 1 pg/mL to 1000 pg/mL for total T3 and rT3 and 1 ng/mL to 1000 ng/mL for total T4 hormone concentrations. The calibration curves show excellent linearity and reproducibility across the entire range of analysis. Accuracy of the methodology was verified using NIST Standard Reference Material (SRM 971 Hormones). A sensitive and specific LC/MS method has been developed the simultaneous analysis of Thyroxine (T4), 3,3',5-Triiodothyronine (T3) and 3,3',5'-Triiodothyronine (rT3) in serum. A simple filtration sample preparation for free thyroid hormones and a liquid-liquid extraction sample preparation for total thyroid hormones allows for determination down to low pg/ml levels.

T299

POLYADENYLATED SEQUENCING PRIMERS ENABLE COMPLETE READABILITY OF SHORT PCR AMPLICONS ANALYZED BY DIDEOXYNUCLEOTIDE SEQUENCING

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Background: Dideoxynucleotide DNA sequencing is one of the principal procedures in molecular biology. Loss of an initial part of nucleotides behind the 3 end of the sequencing primer limits the readability of sequenced amplicons. We present a method which extends the readability by using sequencing primers modified by polyadenylated tails attached to their 5 ends.
Methods: In the study, seventeen samples were tested. Performing PCR, we amplified eight amplicons of six human genes (AMELX, APOE, HFE, MBL2, SERPINA1 and TGFB1) ranging from 106 bp to 680 bp. For sequencing we used BigDye Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems. Standard or polyadenylated sequencing primers for each gene were used in parallel. The purified extension products (BigDye XTerminator Purification Kit, Applied Biosystems) were separated using an ABI 3130 Genetic Analyzer with POP7 polymer.
Results: When standard primers were present in the sequencing mixture, the loss of nucleotides fluctuated between 20 and 53 according to the amplicon. Polyadenylation of the sequencing primers minimized the loss of bases in all amplicons. Complete sequences of shorter products (AMELX 106 bp, SERPINA1 121 bp, HFE 208 bp, APOE 244 bp, MBL2 317 bp) were obtained. Also in the case of TGFB1 products (366 bp, 432 bp, and 680 bp, respectively), the lengths of sequencing readings were significantly longer if adenylated primers were used.
Conclusions: Single strand dideoxynucleotide sequencing with adenylated primers is a universal, easier, cheaper, and less time-consuming way to achieve complete or near complete readability of short PCR amplicons when compared to the standard double strand sequencing.
The study was supported by the research projects NT11334-4/2010 and NT11344-4/2010 from the Ministry of Health, Czech Republic.

T300

CELLULARITY CONTROL AND MICROBIOLOGICAL SAMPLE SUITABILITY: KEY-ROLE OF REAL TIME-PCR IN SEXUALLY TRANSMITTED DISEASES REPORTING

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Background. The use of Real-Time PCR (RT-PCR) to diagnose sexually transmitted diseases (STD) such as C. trachomatis (CT), Human Papillomavirus (HPV), U. urealyticum (UU), U. parvum (UP), M. hominis (MH) and M. genitalium (MG) infections, needs an adequate standardization during pre/post-analytical phases. Aim of this study was to standardize: 1) pre-analytical phase, with a correct DNA sample extraction modality from biological matrix like urine (UR), seminal fluids (SF), urethral swabs (US) and cervical swabs (CS) using internal control (IC) and endogenous control (EC); 2) post-analytical phase, using cellularity cut-off and range cycle threshold (Ct) to define bacterial/viral load.
Methods. DNA from 933 samples (135 UR, 110 SF, 135 US and 553 CS) was extracted with EZ1 DNA Tissue or Virus kit (Qiagen) and amplified by RT-PCR to detect CT (artus, Qiagen), MH, MG, UP and UU (Nuclear Laser); HPV detection and genotyping was performed by end-point PCR to amplify L1 gene (Innolipa HPV Genotyping Extra, Innogenetics) and by microarray technology targeting E1 gene (INFINITI HPV Genotyping, Autogenomics); sample cellularity was calculated by quantitative RT-PCR amplifying human HPRT1 gene (Cell Control, Argene).
Results. CT, MH, MG, UU, UP and HPV positivity respectively was: 8.6%, 8.8%, 2.25%, 9.1%, 27.8% (8% with Ct>30) and 17.33%. Average cellularity/PCR in UR, US and CS was respectively: 1,661±384, 5,629±1,174 and 8,068±990. Samples with less than 300 cells/PCR for UR and 750 cells/PCR for US and CS are classified unsuitable. L1 High Risk (HR)-HPV positivity was 12,1% vs 15,9% of E1 positivity.
Conclusions. Reported data demonstrate: 1) using IC and EC enables pre-analytical optimization with an analytical quality increase; 2) cellularity/PCR allows to evaluate sample suitability while a range of Ct is useful to evaluate bacterial/viral load; 3) results interpretation is fundamental to diagnose HR-HPV (with integrated DNA) and to give an appropriate clinical significance to low viral loads; 4) more data are required to standardize sample cellularity control.

T301

VIRTUAL CROSS-MATCH ON SINGLE ANTIGEN LUMINEX ASSAY IS PREDICTIVE OF COMPLEMENT-DEPENDENT CYTOTOXICITY CROSSMATCH IN KIDNEY TRANSPLANTATION.

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Background: Detection of antiHLA donor-specific antibodies (DSA) was performed using CDC (complement-dependent cytotoxicity) up to the introduction of solid phase based assays. ELISA and, more recently, Luminex technology based on polystyrene microspheres coated by HLA molecules, has highly increased DSA detection sensitivity. Virtual crossmatch (VXM), based on Single Antigen (SA) Luminex assay, is used to compare HLA donor specific antigens and recipient antiHLA antibodies specificities. Therefore it may significantly decrease time of pre-transplant immunogenetic tests. In addition the results are more reliable than CDCXM (complement-dependent cytotoxicity crossmatch). Aim of this study is to verify whether VXM result is predictive of CDCXM outcome.
Material and Methods: 403 CDCXM combinations performed between 2009 February and 2012 March (184 sera samples from patients in renal transplant waiting list tested against lymphocytes from 43 deceased donors) were analyzed to assess whether VXM is predictive of CDCXM outcome.VXM was carried out using antiHLA specificities assigned performing a Luminex SA assay.
Results: 275 sera samples were negative at Luminex screening for research of HLA antibodies, 256 were CDCXM negative, 19 were positive; 15 of 19 positive sera had negative CDC reaction with Dithiothreitol addition, bearing out hypothesis that CDCXM positivity is due to antiHLA IgM or autoantibodies presence. Both CDCXM and VXM were performed on 128 positive sera samples, 81 with concordant XM results(63,3%); 47 positive-positive: in 35 (74%) SA assay showed at least one DSA with MFI (Mean Fluorescence Intensity) value ≥5000, supporting that the higher is MFI Ab value, the more predictive is VXM on CDCXM outcome. 47 samples (36,7%) XM contrasting: 33 CDCXM- /VXM+ is probably due to either non fixing complement anti HLA Ab of clinical relevance, or Luminex higher sensitivity than CDCXM; 14 CDCXM+/VXM-, is maybe due to non HLA Ab : further analysis should therefore be carried on to consider eligible donor an otherwise excluded one.
Conclusions: VXM, especially with high DSA MFI, is predictive of CDCXM outcome.

T302

VITAMIN D LC-MS/MS: OPTIMIZATION OF CHROMATOGRAPHIC PARAMETERS TO INCREASED 60% OF COMMERCIAL KIT THROUGHPUT

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Background: In the last 5 years many companies developed In Vitro Diagnostic (IVD) Kits for the quantitation of Vitamin D3 and D2 (Vit-D) for Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS) instrumentation. This analysis is particular difficult for the presence of several metabolic Isomers and contaminants. The IVD kit ensures to the clinical laboratory a correct quantification validating the peaks separation between the Vit-D and their contaminants. All Vit-D commercial kits need 5 minute analysis time with a throughput of 96 samples/8 hours (h). In the present work we evaluate an implementation of IVD commercial Kit to obtain an increased throughput of up to 160 samples per days.
Methods: The LC-MS/MS are composed by 1290 chromatographic system and 6460 triple quadrupole (Agilent Technologies). All the analysis was performed with the Chromsystems MassChrom® Vitamin D3/D2 kit. In the present work we: 1-increase the flow rate of trapping/Washing samples 2- increase the flow rate of washing analytical column; 3-overlap the analysis.. To guarantee the chromatographic validation we don't change the elution and, the reduction in time of modified steps, are compensated by the increasing of flow. This compensation permit to maintain the same washing mobile phases volume without change in Vit-D peaks width, separation between isomers/contaminants, etc.
Results: The evaluation of chromatographic parameters was performed on deuterated Vitamin D3 (internal Standard - ISTD). The base width of ISTD is 0.20 min and the separation between ISTD and its contaminant peak is 0.20 min in both methods. Our experience highlight a strong stability of the Chromsystems modifies method. The calibration are stable up to 9 months with a CV% 100%±20% for both high and low level internal Quality Controls.
Conclusions: The Chromsystems MassChrom® Vitamin D3/D2 kit was optimized to obtain a 3 minutes method without any change in chromatographic peaks separation without loose the original kit validation. The LC-MS/MS modified Chromsystems assay is a very robust method with up to 9 months stable calibration. The increment of 60% in throughput of this new method allow a better use of LC-MS/MS instrumentation in clinical laboratory hospital

T303
EMIT ASSAY INCREASE RISK OF REJECTION IN PATIENTS TREATED WITH MICOPHENOLIC ACID?

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Background: Mycophenolic Acid (MPA) is the third most widely immunosuppressant in solid organ transplantation. Several studies highlight the necessity of an accurate and sensitive drug monitoring. The assay to monitor MPA are Tandem Mass spectrometry coupled with Liquid Chromatography (LC-MS/MS), High Performance Liquid Chromatography (HPLC) and Immunoassay like Enzyme Multiple Immunoassay Technology (EMIT). Several studies show an EMIT overestimation of MPA between 14% to 35%. These value are calculated as media of the Altman-Bland test of all samples. In the present study we want to evaluate the EMIT overestimation at different MPA concentration ranges and highlight the bias between the therapeutic range (1-3.5 mg/L), the extra-therapeutic range (>3.5 mg/L) and low level therapeutic range (1-2 mg/L). Methods In this study we evaluated 43 serum samples from patients who had different organ transplantation. The serum was analysed with MassTox® Series A MPA (Chromsystems GmbH) for MPA. The same samples were analysed with Syva EMIT kit (Siemens Healthcare Diagnostic GmbH). All statistical analysis were performed with Analyse-it v2.20; the data comparison was calculated with Deaming Fit, the bias was calculated with Altman-Bland Test. Results LC-MS/MS show an interassay CV=3.94% and an inaccuracy of 1.09% at 1.94 mg/L. The comparison with EMIT was LC-MS/MS=0.85*EMIT+0.32 with a positive bias for EMIT of 33.5%. If we divide the samples by EMIT concentration range, the EMIT positive bias are: 1-2 mg/L: 46.4% SD: 11.7; 2-3 mg/L: 33.6% SD: 16.8; 3-4 mg/L: 20.2% SD: 15.4; >4 mg/L: 21.4% SD: 7.0. The EMIT bias in the therapeutic range (1-3.5 mg/L), is 35.2% (SD: 16.9). The EMIT bias for samples with higher concentration (>3.5 mg/L) is 19.8% (SD: 7.8). Conclusions The overall correlation between EMIT and LC-MS/MS show an overall EMIT overestimation of 33.5% coherent with data published in literature; but, at low concentration range 1-2 mg/L show a bias of 46.4%. The adeguate MPA minimum level, with cyclosporin coadminstration, is fixed at 1.3 mg/L for Kidney and 1.4 mg/L for Hearth transplantation (HPLC assay). In both cases an EMIT concentration of 1.5 mg/L overestimate the real concentration of 1.0 mg/L (HPLC or LC-MS assay) with increased risk of rejection

T304
INTER-BATCH VARIATION OF THE BINDING SITE FREELITE LIGHT CHAIN ASSAYS

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Introduction: Analysis of serum free light chains utilising the Freelite assay is commonplace for identifying and monitoring patients with B cell disorders. This study compares inter-batch variation for 3 batches of the Freelite assay on 8 different platforms within a single laboratory (Beckman Coulter AU400, Siemens Advia 1650, Roche C6000, Roche Modular P, Beckman Coulter Immage, Roche Integra 400, The Binding Site SPAPLUS and the Siemens BNII).
Methods: 3 batches of reagent for each platform were used to test two sets of samples; 1) >30 serum samples from individuals with both normal and elevated light chain levels; 2) >45 serum samples from healthy adult individuals. Sample set 1 was compared between batches, on the same platform using results from a single batch as the predicate values and correlating the remaining two batches results. Sample set 2 was compared to the manufacturers 95th percentile reference normal ranges of 3.3-19.4 mg/L for kappa light chains and 5.71-26.3 mg/L for lambda light chains. Analysis was performed on SPSS and Analyse-it.
Results: For sample set 1, Passing and Bablok regression slopes for kappa were 0.97x-0.14 / 0.97x-0.08 (AU400), 1.11x-0.81 / 1.11x-0.04 (Advia), 1.01x-0.62/1.10x-1.85 (C6000), 1.11x+0.06 / 0.98x+0.84 (Modular P), 1.01x-0.03 / 0.94x+0.23 (Immage), 1.00x-0.38 / 1.00x-0.15 (Integra), 0.91x+0.3 / 0.89x+0.16 (SPAPLUS), 1.01x-0.49 and 0.96x-0.19 (BNII). Passing and Bablok slopes for lambda were 1.00x+0.16 / 1.14x+0 (AU400), 1.00x-0.16 / 0.94x-0.19 (Advia), 1.08x-0.53 / 1.14x-1.84 (C6000), 0.87x+0.26 / 0.94x-0.02 (Modular P), 1.15x-4.44 / 1.05x-2.27 (Immage), 0.93x+1.59 / 0.95x+0.93 (Integra), 0.99x-0.19 / 0.93x+0.03 (SPAPLUS), 0.96x-0.66 and 0.97x-1.26 (BNII). For sample set 2 the median values and distributions were not significantly different (kappa p=0.404 and p=0.461, lambda p=0.4 and 0.461 respectively) with an overall mean 95th percentile range of 4.40-15.33 mg/L for kappa and 7.05-20.02 mg/L for lambda.
Conclusion: Interbatch agreement for the last 3 batches produced was within acceptable limits. Furthermore, reference range comparison showed that there was no difference between the results returned. These results support the role of Freelite measurement in prolonged patient monitoring.

T305

ASSESSING ANALYTICAL QUALITY OF GLUCOSE MONITOR: A COMPARISON BETWEEN POCT SYSTEMS AND MULTIPARAMETER ANALYZER

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Background: The blood glucose test is one the most frequent in the hospital and it is a cornerstone in the diagnosis and monitoring of diabetes and management of hyperglycemia control in critical and non critical settings. Inside our hospital, on bedside glucose test takes account of about 50% of inpatient glucose measurements and different instruments for measuring glycaemia are used. The aim of the study was to demonstrate whether Roche Point of Care instruments for glucose measurements have the required accuracy compared with reference method (hexokinase) on multiparametric laboratory analyzer.
Methods: Following the protocol of evaluation of Mahoney (2007) of split sample design methodology, glucose from arterial blood gas analysis (left over) was measured at the same time in double on the following systems produced by Roche Diagnostics (Mannheim, Germany): multiparameter analyzer Cobas C8000 (hexokinase, reference method), blood gas analyzer Cobas b123 (glucose oxidase), glucometer Accu-Chek Inform II Professional (glucose dehydrogenase modified). The results were evaluated according to the CLSI EP9-A Method Comparison.
Results: We evaluated 90 samples with glucose values ranged between 1.06 and 30.61 mmol/L. (haematocrit range: 22.1-50.6%) The comparison between methods showed excellent correlations: in particular between Cobas b123 and Cobas C8000 (y =0.93+0.24, r =0.99, Standard Error Estimated = 0.715) and between Accu-Chek Inform II and Cobas C8000 (y = 0.98+0.28, r =0.998, Standard Error Estimated =0.281). The analysis with the Consensus Error Grid shows that 100% of the results fall within the acceptable range (Zone A. No effect on clinical action) in both of comparison.
Conclusions: Our study shows an excellent correlation between the three Roche system methods for blood glucose measurement. For an analytical point of view, the Roche POC glucose testing systems are suitable for use in ICUs and other medical and surgical areas, according to the range of measurement considered, in interchangeable way.

T306

SWITCHING FROM IMMUNOASSAY TO LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) FOR IMMUNOSUPPRESSANT DRUGS DETERMINATIONS

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Background: The Toxicology and Clinical Pharmacology Laboratory performs about 14.000 immunosuppressant drugs determinations every year, about 50 test in a day, concluded by 3 pm of the day of sample receiving.
Methods: Until 2011 the utilized methods were Immunoassay: CMIA (Chemiluminescent Microparticles Immuno Assay) on Architect (Abbott) for ciclosporin, tacrolimus and sirolimus; QMS (Quantitative Microsphere System) on CdX90 (Tema Ricerca) for everolimus; CEDIA (Chemiluminescent Enzyme Donor Immuno Assay) on Aries (IL-Instrumentation Laboratory) for Mycophenolic acid. From 2012 the utilized method is LC-MS/MS (Liquid Cromatography/Mass/Mass) on UPLC-TQD (Ultra Performance Liquid Cromatography-Triple Quadrupole Detector) Waters with Chromsystems assay kit (column, deutered internal standard, calibrators, quality control and mobile phase) Immunoassay sensivity is 25 ng/mL for ciclosporin, 1.5 ng/mL for tacrolimus, 2 ng/mL for sirolimus, everolimus and 0.3 ug/mL for mycophenolic acid. LC-MS/MS sensivity is 5ng/mL for ciclosporin, 0.5 ng/mL for tacrolimus, sirolimus, everolimus and 0.1 ug/mL for mycophenolic acid. Even if LC-MS/MS is the reference method, comparison between LC-MS/MS and immunoassay patient's data was necessary to support the Clinicians for patient therapeutic drug monitoring, during method switching.
Results: The linear regression LC-MS/MS versus immunoassay and correlation coefficient are: Ciclosporin y=0.7791x+16.725 / r =0.990991, Tacrolimus y=0.821x+0.5924 / r=0.929904, Sirolimus y=0.7041x-0.1783 / r=0.916859, Everolimus y=1.0727x+1.0434 / r=0.774135, Mycophenolic acid y=0.8059x-0.2485 / r=0.948176.
Conclusions: Our LC-MS/MS ciclosporin, tacrolimus, sirolimus and mycophenolic acid results are lower than the immunoassay results (ciclosporin -18%, tacrolimus -20%, sirolimus -25% and mycophenolic acid -25%. Only the LC-MS/MS everolimus results are higher than the immunoassay results (+13%), because of a known undervalue of QMS immunoassay. After the results discussion with the Clinicians, we switched from immunoassay to LC-MS/MS for all immunosuppressant determinations, with the warranty to make available all results every day, by 3 pm of the day of sample receiving.

T307
AUTOMATED BLOOD CELL COUNTS: PRELIMINARY EVALUATION

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Background: The today automation facilities applied to haematological laboratory systems have changed the diagnostic scenario and help the role of Clinical Pathologist in improving test and supporting clinical decision. These results are achieved also applying the sophisticated analytic philosophies coming from the today advanced scientific research to the routine activity. The aim of our investigation is to evaluate and compare, on the same samples, the analytical performances of six automated blood cell counters.
Methods: For this preliminary evaluation, a random 20 samples (out of 300 from those obtained in the routine) were selected. Each sample has been processed on the following hematologic instruments: 1) Abbott Cell-Dyn Sapphire, 2) Siemens Advia 2120, 3) ABX Pentra DX 120, 4) Beckman Coulter DXH 800, 5) Sismex Xe 5000, 6) Abbott Cell-Dyn Ruby, and the results obtained on leukocyte, lymphocyte, erythroblast and reticulocyte absolute count were used. The concordance Lin's Coefficient for each pairwise comparison and the graphical Bland and Altman analysis were applied.
Results: the range of measurements (from instrument 1) was 1.22-28.5 for leucocytes, 0.75-16.7 for lymphocyte, 0-56.4 for erythroblast, 0.002-0.27 for reticulocytes. The best/worse coefficient (for each comparison) was as follows: leucocyte 0.99 (5 vs 6)/0.21 (1 vs 6), lymphocyte 0.82 (4 vs 6)/0.11 (1 vs 4), erythroblasts 0.96 (2 vs 5)/0.01 (2 vs 3), reticulocytes 0.92 (2 vs 5)/0.59 (3 vs 4).
Conclusions: For the evaluated parameters, the comparisons reveal a relatively low concordance between the hematology analyzers, probably due to the different technologies employed, particularly for leukocytes and lymphocytes. On the contrary we have observed a good concordance for erythroblast and reticulocyte absolute count. Our results, though drawn from a very small sample, suggest that several issues must still be resolved, for instance the analytic quality of the count of certain populations (namely: leukocytes) probably due to technological limits in searching for morphologic abnormalities of immature or atypical cells.

T308
ABILITIES OF THE FACTOR FOR CORRELATING DIMENSION® JAFFE CREATININE METHOD TO THE IDMS REFERENCE MEASUREMENT PROCEDURE IN ASSESSMENT OF CHRONIC KIDNEY DISEASE

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Background: To improve the performance of serum creatinine (sCr) measurement for the reliable of estimated glomerular filtration rate (eGFR), all major sCr assays have been standardized to the isotope dilution mass spectrometry (IDMS). However, this was not claimed by the Dimension Jaffe method but the manufacturer provided a correction factor to achieve IDMS traceable results. We compared sCr values derived from the manufacturer's factor to IDMS traceable enzymatic method. Agreement of CKD classification based on the eGFR was also assessed.
Methods: sCr was measured in 2,660 patients on the Dimension RxL MAX analyzer using the non-IDMS modified Jaffe method (non-IDMS sCr), then a correction factor (-0.168 mg/dL) was used to convert the results to IDMS creatinine values (corrected sCr). The samples were also performed by IDMS traceable enzymatic method (IDMS sCr) on the Vitros 350 analyzer. The CKD-EPI equation was used to calculated eGFR.
Results: According to a factor derived from creatinine within the range of 0.30-2.5 mg/dL, 2,636 samples were used in data analysis. The median creatinine concentration with a range for corrected sCr (0.70 mg/dL, 0.27 to 2.31 mg/dL) was significantly lower than non-IDMS (0.87 mg/dL, 0.44 to 2.47 mg/dL) and IDMS sCr (0.78 mg/dL, 0.31 to 2.25 mg/dL), P <0.05. The bias was -0.08 mg/dL. The Passing-Bablok regression showed a slope of 1.0374 (95% confidence interval [CI], 1.021 to 1.053) and intercept of -0.124 mg/dL (95% CI, -0.136 to -0.111) with a high Spearman's rank correlation coefficient of 0.927 (95% CI, 0.922 to 0.932), P <0.0001. The limit of agreement was -0.23 to 0.06 mg/dL. Based on the eGFR corresponding to CKD stage 1 to 5 defined by Kidney Disease Outcome Quality Initiative (K/DOQI), the corrected sCr classified patients into CKD stages for 82.9%, 14.0%, 3.8%, 0.3%, 0% while the IDMS sCr were 73.6%, 21.3%, 4.7%, 0.4%, 0%, respectively. The overall consistency patients was 87.5% which demonstrated good agreement between both methods (kappa=0.657).
Conclusions: The corrected sCr derived from factor for correlating Dimension Jaffe creatinine method to the IDMS method correlated and agreed well with the IDMS traceable enzymatic method. No patient was discordantly classified by more than one group of any CKD stages.

T309

MEASUREMENT OF 1,25-DIHYDROXYVITAMIN D2 AND D3 IN BLOOD BY LC-MS/MS UTILIZING ION FUNNEL TECHNOLOGY

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Liquid chromatography triple quadrupole mass spectrometry (LC-MS/MS) has become an essential tool for small molecule quantitation due to its high sensitivity and specificity, excellent reproducibility and the ability to perform simultaneous analysis of multiple analytes. 1,25-dihydroxyvitamin D – a metabolite of vitamin D – has proven to be a challenging compound to analyze due to the low pg/ml range relevant to clinical research. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard. Final concentrations were calculated by comparing the response of the analyte to a known concentration of internal standard and plotting the result on a calibration curve developed using stripped human plasma spiked with standards. A UHPLC system configured for sample enrichment was also explored. This system consisted of two pumps, a 2 position/6 port valve, an enrichment column and a reverse-phase C18 analytical column. 1,25-dihydroxyvitamin D3 in extracted serum samples displayed a low pg/ml limit of quantitation (LOQ). The calibration curves shows excellent linearity and an R2 >0.998. The LOQ displayed good reproducibility and precision with a CV of <20%. A sensitive method has been developed for the quantitation of 1,25-dihydroxyvitamin D2 and D3 in blood using ultra high performance liquid chromatography (UHPLC) and triple quadruple (QQQ) mass spectrometry enhanced with dual ion funnel technology.

T310

SIMULTANEOUS MEASUREMENT OF THYROXINE (T4), TRIIODOTHYRONINE (T3) AND REVERSE TRIIODOTHYRONINE (RT3) BY TWO DIMENSIONAL LIQUID CHROMATOGRAPHY AND TANDEM MASS SPECTROMETRY IN NEGATIVE ION MODE

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Accurate and precise measurement of thyroid hormones in serum or plasma is important for clinical research. In this work, we present a reliable and sensitive method for the measurement of thyroid hormones. Liquid chromatography with online sample cleanup was employed to minimize manual sample preparation. This was coupled to a triple quadrupole mass spectrometer utilizing dual ion funnel technology to increase ion sampling and attain the highest sensitivity. The LC configuration comprising a loading and an analytical column allows cleaning hydrophilic unretained matrix components while trapping the target analytes at low organic mobile phase composition during the loading process. During the analysis process, the column valve is switched and the mobile phase strength is increased to transfer the target analytes onto a narrow bore sub-2 μm analytical column. The LC configuration also enables injecting a large sample volume. Thyroxine (T4), triiodothyronine (T3) and reverse triiodothyronine (r-T3) were spiked in charcoal stripped serum after removing thyroid binding globulin and other large proteins. In negative ion mode, r-T3 can be quantified using its two unique multiple reaction monitoring (MRM) transitions at 649.8>604.6 and 649.8> 462.8. T4 can be quantified using the MRM transition at 775.8 >126.9 and T3 can be quantified using the MRM transition at 649.8>126.9. Critical aspects of LC/MS/MS method development affecting sensitivity are identified for quantifying thyroid hormones at the low pg/mL level. Under optimal LC and MS/MS conditions, LODs of 1 pg/mL for T3 and 2 pg/mL for T4 and rT3 as well as LLOQs of 2 pg/mL for T3 and <5 pg/mL for T4 and rT3 were obtained. The linear correlation coefficients in the range from 2 pg/mL to 100 pg/mL were >0.99 for the three analytes. Free thyroid hormone concentrations can be reliably quantified at the low pg/mL level by LC/MS/MS. The advantage of using mass spectrometry consists not only better specificity, but also capability of quantifying multiple analytes within one experiment.

T311

DETERMINATION OF OPIATES IN DRIED BLOOD SPOTS USING NOVEL FLOW-THROUGH TECHNOLOGY COUPLED TO LC/MS/MS

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The use of dried blood spot (DBS) technology in clinical research and pharmacokinetic studies has significant advantages to conventional plasma sampling because it allows sampling of small blood volumes, easy sample shipping and storage, and alleviates many concerns related to the handling of biohazardous materials. DBS coupled to highly sensitive LC/MS/MS systems promises significant advantages in bioanalytics both in the toxicology and pharmaceutical industry. A novel, fully automated online card extraction system coupled to a triple quadrupole mass spectrometer has been demonstrated for the analysis of opiates. This enables efficient, highly sensitive and specific, automated flow-through analysis of DBS cards by LC/MS/MS. This integrated analytical system greatly reduces analysis time and manual experimental errors. This work describes the online analysis of DBS cards for the determination of opiates. Excellent sensitivity down to 1ng/mL, linearity with an R2 >0.99, and precision with CV <15% were demonstrated for the online extraction method. The quantitative performance capabilities of the online extraction method was evaluated and compared to that of a conventional offline extraction hole punching method and consistent results were observed. The flow-through analysis of DBS cards by LC/MS greatly reduces analysis time and manual experimental errors. Excellent sensitivity, linearity, dynamic range, precision, accuracy, and reproducibility, and the quantitative performance capabilities of the online extraction method were demonstrated. This DBS analysis approach can be readily applied to clinical research, forensic toxicology and pharmaceutical, research studies.

T312

EVALUATION OF IMMUNOGLOBULIN FREE LIGHT CHAIN (FREELITETM) ASSAYS ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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Introduction: Here we describe the development of serum immunoglobulin free light chain (FreeliteTM) assays for The Binding Site's Next Generation Protein Analyser (NGPA). The NGPA analyser is a random-access bench top turbidimetric analyser capable of a wide range of on board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour, and multiple methods of antigen excess detection. Precision is promoted by single use cuvettes which are automatically loaded and disposed of.

Method: NGPA kappa and lambda FLC assays were compared to existing Freelite (BNIITM) assays for 141 kappa (79 normal, 62 monoclonal, mean 535.20 mg/L, range 1.13-17,868.19 mg/L) and 129 lambda (79 normal, 50 monoclonal, 275.06 mg/L, range 0.52-3833.94 mg/L). Precision by repeat measurements was assessed at high (134.01 mg/L and 129.75 mg/L) and low levels (6.47 mg/L and 7.56 mg/L) (kappa and lambda respectively). Linearity was determined by serial dilutions from 30.99 mg/L to 3.10 mg/L (kappa) and 30.52 mg/L to 0.76 mg/L (lambda), measured in triplicate with results compared to expected values. 71 kappa and 79 lambda samples identified for the purpose of antigen excess testing were analysed.

Results: 149 kappa samples mean 535.20 mg/L (range 1.13-17,868.19 mg/L) compared to mean 540.77 mg/L (range 2.37-20,000.00 mg/L) on the predicate device (R2 =0.98). Similarly 129 lambda samples gave a mean value of 275.06 mg/L (range 0.52-3833.94 mg/L) compared to mean 288.83 mg/L (range 0.51-4480.00 mg/L) on the predicate device (R2=0.96). Kappa returned a precision CV at the high level of 2.2% (mean 134.01 mg/L, range 130.59-140.38 mg/L) and the low level of 4.9% (mean 6.47 mg/L, range 6.09-6.91 mg/L), whilst lambda returned 1.0% (mean 129.75 mg/L, range 126.61-131.32 mg/L) and 3.7% (mean 7.56 mg/L, range 7.14-7.96 mg/L). Both kappa and lambda gave acceptable linearity (R2=0.99 and 0.98 respectively) with a maximum deviation of 11.9% from the expected result. 9/9 kappa and 9/9 lambda samples identified for antigen excess were automatically re-diluted on the NGPA.

Conclusions: We conclude that these assays are rapid, accurate and precise and protected from false low results by the instruments automatic antigen excess check function.

T313
EVALUATION OF A C4 ASSAY FOR USE ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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Introduction Serum complement consists of around 30 proteins that have a fundamental role in immune system functionality. Inherited deficiencies in C4 are associated with an increased risk of developing systemic lupus erythematosus (SLE). Conversely, high levels of circulating immune complexes in SLE can reduce serum levels of complement components. C4 deficiency is also associated with glomerulonephritis and vasculitis. Here we describe the development of serum C4 assay for Binding Site's Next Generation Protein Analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems.The assay has a measuring range of 0.064 – 0.9 g/L at the standard 1/10 sample dilution, with sensitivity of 0.0064 g/L. High samples are remeasured at a dilution of 1/20 with an upper measuring range of 0.128-1.8 g/L.
Method: Correlation to the Binding Site C4 assay for the SPA PLUS was performed using 33 samples (mean 0.344; range 0.152 – 0.7160). Intra-run precision was assessed by measurement of twenty replicates of samples at 0.787 g/L and 0.105 g/L. Furthermore, precision was assessed at the clinical decision point of 0.154 g/L. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range and comparing expected versus observed results (0.001 – 0.882 g/L).
Results: Correlation with the C4 SPA PLUS assay demonstrated good agreement when analysed by Passing-Bablok regression; y=1.08x - 0.03. The assay was shown to be linear over the range of 0.001 – 0.882 g/L; y=1.013x + 0.0177 mg/L (R^2 = 0.998). Intra-run precision produced the following results: sample 1 (0.787 g/L) CV of 1.59%, sample 2 (0.154 g/L), CV of 2.17% and sample 3 (0.105 g/L), CV of 2.83%
Conclusions: We conclude that the C4 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

T314
DEVELOPMENT OF AUTOMATIC ANTIGEN EXCESS DETECTION PARAMETERS FOR IMMUNOGLOBULIN FREE LIGHT CHAIN (FREELITETM) ASSAYS ON THE ROCHE COBAS® C501

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Background: Studies have shown that immunoglobulin free light chain (FLC) measurements aid in the diagnosis and monitoring of patients with AL amyloidosis, non-secretory myeloma and light chain myeloma. The inherent nature of monoclonal FLC makes antigen excess a possibility even for polyclonal based assays. Here we describe the development of automatic antigen excess protection for use on existing FLC assays (FreeliteTM) on the Roche cobas® c501.
Method: Reaction kinetics of monoclonal patient sera prone to antigen excess (5 kappa, mean c501 result 5,345.12 mg/L, range 502.62-12,672.00 mg/L; and 4 lambda, mean c501 result 4,046.5 mg/L, range 1,590.00-6,213.00 mg/L) and 4 blood donor sera (mean kappa 10.51 mg/L, range 9.14-13.18 mg/L; mean lambda 11.41 mg/L, range 10.90-12.14; mean ratio 0.93, range 0.79-1.21) were analysed to set threshold limits for antigen excess protection. These thresholds were then validated using 67 blood donor serum, 68 kappa monoclonal and 32 lambda monoclonal patient sera.
Results: Samples in antigen excess were typified by a high initial rate of reaction, which rapidly slows as the detecting antibody becomes saturated (early delta OD 125.5, late delta OD 14.0). This compares to non-antigen excess samples which show a slower, more sustained rate of reaction throughout the assay time (early delta OD 28.0, late delta OD 38.7). 22/40 kappa samples (mean 314.64 mg/L, range 2.64-4,919.00 mg/L) gave a false low result when assayed without antigen excess protection whilst for lambda this was 7/32 samples (mean 879.01 mg/L, range 7.68-4,738.00 mg/L). When assayed with antigen excess thresholds active 0/40 kappa samples gave a false low result (mean 3,103.82 mg/L, range 20.06-40,930.00 mg/L) whilst 0/32 lambda samples gave a false low result (mean 3,548.30 mg/L, range 7.68-56, 949.00 mg/L).
Conclusions: All samples previously identified as being in antigen excess on the c501 analyser were automatically rediluted using these thresholds. Implementation of these parameters will improve throughput and prevent samples being mis-reported.

T315
VERIFICATION OF ANALYTICAL PERFORMANCE AND COMPARABILITY OF BLOOD GAS ANALYZERS

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Background: Verification is confirmation, with objective evidence, that specific requirements have been fulfilled, difficult in blood gas analyzers due to low sample's stability and aqueous control solutions. If two instruments are available, comparability must be ensured. Results´ difference from same patient may have clinical impact. So, we verified performance and assured comparability of two blood gas analyzers Cobas b 221, Roche.
Methods: Repetibility (CLSI EP-15): 3 control levels tested automatically in an AUTO QC MODULE. Accuracy: linear regression between own results and EQAS Riqas' consensus mean, bias was calculated at different concentrations. Total Error (TE) compared to Quality Requirement (TEa) RCPA: TE <TEa. Linearity (CLSI EP-6): 7 solutions (COOX/MSS) with known levels of O2, N2 and CO2, salts, buffers and metabolites. Uncertainty: applying Nordtest to Riqas'results. Comparability (EP-C54-A): with patients' samples. Acceptability criterion: RCPA.
Results: We verified precision manufacturer's claims for: pH, pCO2, pO2, Hematocrit (Hto), Ionized calcium (Cai), Sodium, Potassium, Chloride, Bilirubin (Bb), Glu, Lac, Total Hemoglobin (Hbt), Oxygen Saturation, Oxyhemoglobin (O2Hb), Carboxyhemoglobin (COHb), Reduced Hemoglobin (HbH) and Methemoglobin (METHb). Linearity range: pH 6,87-7,69; pCO2: 13-130 mmHg; Hto: 22-75%; Cai: 0,43-2,52 mmol/L; Na: 90-173 mEq/L; K: 2,0-8, 8 mE/L; Cl: 68-132 mEq/L; Hbt: 6,2-23g/dL; O2Hb: 35-93,8%; COHb:2,8-28,2%; HbH:2,0-22,3%; METHb: 1,6-14,6%; Bb: 4,0-23,8 mg/dL; Glu: 29-460 mg/dL; Lac: 1,4-13,3 mmol/L.. We verified accuracy and acceptable performance regarding TEa for: pH, pCO2, Lac, Cai, Na. K, Cl and Glu in the three levels. We couldn´t verify pO2 linearity and accuracy. Uncertainty %: pH=0,2; pCO2:6,21; pO2:13,05; Cai:2,51; Na:1,48; K:2,29; Cl:4,71; Glu:14,69; Lac:15,91. 3 level results comparability was obtained for pH, pCO2, pO2, Na, K, Cl y Cai.
Conclusions: both instruments meet quality requirements and comparability for all parameters except pO2, due to possible contamination with ambient air.

T316
AQT90 FLEX - PRACTICABILITY AND IMPACT ON TNI TAT IN AN EMERGENCY LABORATORY

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Background: The goals of this study were to evaluate the analytical performance of TnI (Troponin I) on whole blood AQT90FLEX analyzer (Radiometer ApS),random access POC (Point Of Care), and impact on TnI TAT (Turn Around Time) though its implementation in a emergency section of central laboratory. The practicability of AQT90FLEX has been analyzed with a Test Operator's Evaluation.
Methods: Correlation and concordance studies were performed collecting blood sample from ED (Emergency Department) and Cardiac Intensive Care Unit (CICU). Samples were drawn in duplicate: whole blood EDTA (K3EDTA) to be tested on AQT90FLEX and serum tube to be tested on ARCHITECT (Abbott-i2000SR). Total imprecision was determined using 3 levels of quality control materials and 2 plasma pools. The TnI TAT of the sample from ED analyzed with AQT90 FLEX was compared to the TnI TAT from ARCHITECT.TAT data were collected for several days in the slot time 8.30 AM–02.30 PM. Practicability of AQT was evaluated through a questionnaire of 22 parameters, data were collected from different professional role.
Results: Correlation of patient between the AQT and the ARCHITECT demonstrated the following regression: AQT cTnI = 0.1276 ARCHITECT+ 0.0073 r =0.989 (95% Confidence 0.9819 to 0.9938); concordance was 96%; day to day imprecision was CV% between 3.08 and 8.38 for concentration between 0.035 and 1.4 ng/mL. TAT analysis: AQT90 FLEX TnI <45'=32 samples, between 45' and 60'=15 , >60'=11; Abbott-i2000SR TnI <45'=15 samples, between 45' and 60'=14 , >60'=28, average TAT on AQT90 44', Abbott- i2000SR 67'. Test Operator's Evaluation: Device design 4/5, Infection prevention 5/5, Practicability 5/5, Measurement 4/5, Overall 5/5.
Conclusions: Troponin I is a very reliable marker that allows early diagnosis of ACS (Acute Coronary Syndrome), but requires short TAT.Both good practicability of the random access analyzer AQT90FLEX, with ability to work from capped primary whole blood sample tube, and the high degree of correlation with laboratory results allow to TnI TAT to be improved by an emergency section of a central laboratory. The safe and simple technology is suitable for measurements of cardiac troponin also by unskilled personnel in other settings such as emergency department and cardiac care unit.

T317
METHOD COMPARISON OF FOUR AUTOMATED CA 15-3 IMMUNOASSAY SYSTEMS

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Background: Measurements of serum cancer antigen (CA) 15-3 are used to monitor tumor recurrence and treatment of patients with advanced breast cancer. Most systems use two monoclonal antibodies against (MAbs) 115D8 and DF3, to detect two epitopes in the molecular structure of the polymorphic epithelial mucin MUC1, commonly referred as CA 15-3. In the present study, we compared the methods of 4 automated CA 15-3 immunoassay systems.
Methods: Results obtained in individual immunoassay systems, Centaur CA 15-3 (Siemens Healthcare Diagnostics), Cobas e411 CA15-3 (Roche Diagnostics) and Architect i1000SR CA15-3 (Abbott Laboratories), with the same set of 142 samples were compared with the Kryptor CA 15-3 (Thermo Scientific) system as our reference method using Passing-Bablok regression and differential diagrams according to Bland-Altman.
Results: Poor agreement [y=1.09x+0.62 (r = 0.98; n = 142; 95% confidence intervals for slope and intercept, 1.01-1.14 and -1.47-3.51)] was found between Cobas e411 CA 15-3 (y) and Kryptor CA 15-3 (x). On the other hand, the comparison between Centaur CA 15-3 (y) and Kryptor CA 15-3 (x) revealed the highest degree of agreement with a slope of 1.03 (95% confidence interval, 0.95-1.12), and a negligible intercept (-0.49 U/mL; 95% confidence interval, -3.94 to 2.21) with r = 0.97. Acceptable agreement was observed [y = 1.05x-2.26; r = 0.96; n= 142; 95% confidence intervals for slope and intercept, 0.98 to1.13 and -4.68 to 0.61)] between Architect i1000SR CA15-3 (y) and Kryptor CA 15-3 (x). When method comparison studies were analysed using difference plots with Kryptor CA 15-3 in the abscissa, the mean differences were: 11.38 (Cobas e411 CA 15-3), 1.35 (Centaur CA 15-3) and 15.64 U/mL (Architect i1000SR CA15-3).
Conclusions: In our study Cobas e411 CA 15-3 method had the highest slope with Passing-Bablok analysis. Data from Bland-Altman differential plots suggest significant individual differences among individual samples, mainly for high concentrations, which occur in patients with active breast cancer. Even for the method (Centaur) that showed the best agreement with Kryptor CA 15-3, substantial intermethod differences exists for some samples, indicating that redetermining the baseline is required when changing method.

T318
EVALUATION AND COMPARISON BETWEEN METHODS FOR SERUM FREE LIGHT CHAIN IN PATIENTS WITH MONOCLONAL GAMMOPATHY

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Background: The measurement of serum free light chains (FLC) is recommended for diagnosis, monitoring and prognosis of monoclonal gammopathies. In addition, specific assays on automated nephelometers and turbidimeters are available. Many factors need to be considered when deciding upon the most appropriate method for measuring FLC because the discovery of the monoclonal component can be unexpected and require further testing for confirmation.
Methods: The aim of this study was the evaluation and comparison of two commercial kits: the new N Latex FLC (Siemens) and the FreeliteTM (Binding Site) assay, applied respectively on a BNP ProSpec, Siemens and Cobas C 6000, Roche, in accordance with manufacturer's instructions. We included in this study 108 samples of patients with monoclonal gammopathies, admitted to the Istituto Nazionale Tumori, in order to compare methods and 60 serum samples from healthy donors to establish Reference interval with new N Latex FLC.
Results: The T test shows significant statistical difference between methods for FLC κ (p=0.01) and κ/λ ratio (p=0.02), non significant for FLC λ (p=0.69). A moderate correlation was observed in the method comparison (Pearson coefficient) for FLC κ (r=0.89), for FLC λ (r=0.60) and κ/λ ratio (r=0.78). Cohen Method shows the concordance for FLC κ= 89%, for FLC λ = 65% and for κ/λ ratio=84%. A relevant difference in the results obtained from the two methods was observed in a patient with λ chain multiple myeloma. The FLC λ concentration was highly abnormal with the FreelightTM , whereas in the N Latex FLC assay the levels were within the reference ranges. This difference can depend to the specificities and affinities of the antibodies. The precision data, obtained according to CLSI, demonstrated the high precision for both assays with CV lower to 5% for FLC κ and λ in the within- day and lower 2% in the between- run. The Reference interval which we calculated for N Latex FLC are similar to those reported by manufacturers.
Conclusions: Our study demonstrated that there is only a moderate concordance between the two FLC assay and further studies are needed in order to verify the performances of these methods in the diagnosis and monitoring of monoclonal gammopathies.

T319

IMPLEMENTING EXACTIVE ULTRA HIGH RESOLUTION MASS SPECTROMETER AND EXACTFINDER DATA PROCESSING SOFTWARE IN TARGETED AND UNKNOWN SCREENING METHOD FOR URINE ANALYSIS

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Introduction: Fast methods allowing identification of unlimited number of compounds with capability of retrospective data analysis are required in toxicology labs for quick and confident analysis. Using ExactiveTM ultra-high resolution mass spectrometer with ExactFinder TM data processing software meets these expectations.
Methods: Urine was spiked with internal standards, diluted 20 times with water and analyzed on PFP column with 15 min LC gradient. Mass spectrometer was equipped with ESI source. Full scan data in m/z range 100-1000 mu followed by Higher Energy Collisional Dissociation (HCD) was collected in positive and negative switching ionization modes. Resolution was 50K and 25K (FWHM) for full scans and fragmentation scans respectively. ExactFinder software identified target compounds base on exact mass, retention time, isotopic pattern, and presence of fragments. Unknown compounds were identified based on exact mass and isotopic pattern; proposed molecular formulas were searched against ChemSpider libraries.
Results: Data collected with screening application on Exactive Orbitrap mass spectrometer and processed with ExactFinder software correlates very well with GC-MS and immunoassay data. All compounds identified with GC-MS and immunoassay were identified with our method. Additional compounds and more metabolites confirming presence of parent drug were identified. Collected data allowed for high confidence identification using m/z, retention time, isotopic pattern and all ions fragmentation spectra. We also found that information about retention time is not necessary required.
Conclusion: Implementation of Exactive ultra high resolution mass spectrometer and ExactFinder software allowed us to develop efficient screening method which correlates well with traditional screening techniques, allows for unlimited number of target compounds, retrospective data analysis and unknown compounds identification.

T320

THERAPEUTIC DRUG MONITORING OF 8 NEW ANTICANCER AGENTS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Introduction: The treatment of some cancers has shifted from conventional chemotherapy drugs to chronic treatment with molecular targeted therapies. Targeted therapies include drugs such as Tyrosine kinase inhibitors (eg: Imatinib, Dasatinib, Nilotinib…). A highly sensitive and selective method has been developed for the analysis of eight drugs using LC/MSMS.
Methods: 50 µL of plasma samples were extracted with methanol and the organic layer was diluted into the mobile phase. The injection volume was 15 µL. Quantitation was performed using the imatinib-D8 as an internal standard. Chromatographic separation was achieved using a gradient on a C18 column. The mobile phases were the following: A was water containting 0.1% formic acid and 10 mM ammonium formate and B was acetonitrile containing 0.1% formic acid. Flow rate was 300 µl/mn. Analytes were quantified using electrospray ionization in positive mode.
Results: Calibration curves were established from 100 to 10000 ng/ml in human plasma, calculated and fitted by 1/x2 weighted linear regression. The square of the sample correlation coefficients, R2 were between 0.977 and 0.995 (n=10). Replicate analysis of quality control samples at the three concentrations (low, medium and high) was used for the intra and inter-assay precision and accuracy determination. Intra-day and inter-day imprecision values were below 15%. The percentages of deviation between experimental and theoretical concentration were also below 15%. The specificity of the method was examined by analyzing different blank human plasma extracts with or without internal standard. No interferences were observed. The limits of quantitation were 100 ng/mL for all compounds except for dasatinib that was evaluated at 50 ng/mL.
Conclusion: This method is the first broad range LC-MS/MS assay covering the major currently in-use TKIs. It has been validated according to international regulations and can be used to evaluate patient adherence to therapy and pharmacokinetic studies.

T321

COMPARISON OF A NEW HBA_{1C} ANALYZER (HLC-723 GX) WITH TWO ESTABLISHED SYSTEMS

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Background: Evaluation of glycated haemoglobin fraction (HbA1ac) levels is now widely accepted and performed in the monitoring of diabetic patients. High-performance liquid chromatography (HPLC) is considered the gold standard measurement procedure and this is the most frequently used technique in clinical practice. The objetive of this study was to compare three automated HbA1c tests using the same measurement principle, HPLC with cation-exchange columns. One of these is a new automated analyzer in its first evaluation study: HLC-723 GX Analyzer.
Methods: One hundred and thirty six whole blood samples were collected by ramdon process for this study. The three automated tests were perfomed by Bio-Rad Variant II Turbo with 1.5 minutes HbA_{1c} program, Bio-Rad Variant II with 3 minutes HbcA1c program and HLC-723 GX Tosoh Analyzer. 54 samples were analyzed by the three tests while 136 samples just by Variant II Turbo 1,5 min and Tosoh GX. A database of results was generated using Microsoft Excel and multiple regression analysis model was used for estimating the relationships among the results from the three tests. Passing-Bablok was used to obtain the correlation between Tosoh GX and Variant II Turbo 1,5 min; and Tosoh GX and Variant II 3 min.
Results: Multiple regression analysis model generated a coefficient of determination (R2) of 99,65% giving a strong concordance between the results from the three tests. Passing-Bablok expressed a good correlation in the different test combinations: GX and Variant II Turbo 1,5 min (Intercept 0.10, 95% CI [-0,1561 to 0,1000]; slope 1.00, 95% CI [1,0000 to 1,0303]) ; GX and Varian II 3 min (Intercept 0.25, 95% CI [-0,1929 to 0,2500]; slope 1.00, 95% CI [1,0000 to 1,0714]).
Conclusions: We found excellent correlation between the three HPLC methods. This comparison study showed that a change test would not affect the monitoring of diabetes patient. The conclusions are particularly relevant to HLC-723 GX Analyzer as this is its first evaluation.

T322

COMPARATIVE ANALYSIS OF HLA-B27 IMS-SANDWICH ELISA, FLOW CYTOMETRIC TEST AND PCR-SSP IN TYPING HLAB-27

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Background: HLA-B27 antigen displays a degree of association with rheumatic diseases like ankylosing spondylitis(AS),Reiter syndrome and acute anterior uveitis. HLA-B27 typing is one of the useful tools for confirming association of AS with HLA-B27 phenotype in the clinical diagnosis. Most commonly used three types of test for HLA-B27 typing are 1. serological-flow cytometric (FC), 2. cytological- conventional micro-lymphocytotoxicity test (MLTC) and recently 3.Genotyping -polymerase chain reaction (PCR) with sequence specific primers (PCR-SSP) and PCR with sequence specific oligonucleotides (PCRSSO) . Aim of our study was to rule out the efficacy of IMS-sandwich ELISA for HLA-B27 typing in our setup.
Methods: We compared results from three different methods Flow cytometric (FC), IMS-sandwich ELISA and PCR-SSP in 200 patients suspected of rheumatic diseases.Taiwan Advance Bio-Pharmaceutical HLA B-27 screening kit was used for IMS-sandwich ELISA. Flow cytometric was performed by using HLA-B27 BD Biosciences screening kit and tools. DNA was extracted from the samples showing positive and ambiguous screening test results. DNA extraction was done by standard salting out procedure from whole blood. PCR-SSP was performed by using GmbH,Germany.
Results: Out of 200 samples,IMS-sandwich ELISA showed 69 positive and 3 ambiguous test results while Flow Cytometric showed 70 positive and 2 ambiguous test results. Extracted DNA from these samples was further analyzed by PCR-SSP.All72 samples(including positive and ambiguous test results from screening test) were HLA-B27 positive by PCR-SSP by assigning cut off value CtB27 22.42 to discriminate HLA B-27 positive samples from negative. IMS-sandwich ELISA showed specificity of 100% and sensitivity of 95.8% while flow cytometric showed 100% specificity and 97.2% sensitivity with compared to PCR.
Conclusion: PCR is most reliable among these three techniques employed ,but it is not practical in our set up due to lack of infrastructure,and limited number of sample load . Post-PCR identification steps as gel electrophoresis, time -consuming procedure and prone to contamination are limitations of PCR, simple test procedure, cost effectiveness, 100% specificity and around 96% sensitivity makes IMS-sandwich ELISA best option for our set up.

T323

ACCURACY OF THREE AUTOMATED 25-HYDROXYVITAMIN D ASSAYS IN HAEMODIALYSIS PATIENTS

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Introduction: Patients on dialysis therapy form an important subgroup for vitamin D measurements with high needs for accurate monitoring. Suboptimal 25 (OH)D concentrations increase the risk for hypocalcaemia, secondary hyperparathyroidism and mortality. Despite standardization efforts, unresolved discrepancies and unacceptable bias persist among immunoassays compared to reference methods, particularly in samples from diseased patients with atypical matrix composition such as haemodialysis patients. We evaluated the accuracy of 3 automated assays on Architect i2000sr (Abbott), ModulaE170 (Roche) and iSYS (IDS) analyzers for 25(OH)D measurement in comparison to a higher reference isotope dilution/online solid-phase extraction liquid chromatography tandem mass spectrometry (ID-XLC-MS/MS) method, in serum from haemodialysis patients. Material and methods: All three routine assays were heterogeneous, competitive immunoassays (Architect, iSYS) or vitamin D binding protein assay (ModularE170), measuring 25(OH)D2 and 25(OH)D3. We studied 99 haemodialysis patients (47 women, 52 men, age 24-94y) and a healthy control group of 50 blood donors (34 women, 16 men, age 20-65y). Measurements were double blind performed in three different laboratories with a different operator and aliquot for each method.
Conclusion: Not all automated 25(OH)D assays equally accurate measure samples from haemodialysis patients compared to samples from healthy subjects. We suggest a possible role of matrix effects like elevated urea or other retained metabolites in haemodialysis sera, causing incomplete binding disruption between 25(OH)D and DBP. Architect shows the highest deviation from ID-XLC-MS/MS, almost consistently producing lower results, as well as for haemodialysis as healthy subjects. Architect falsely assigned 48.5% haemodialysis patients as having suboptimal (<30 ng/mL) and 12.2% patients as insufficient (<20 ng/mL) levels. Despite the lower degree of correlation with ID-XLC-MS/MS, iSYS showed a slightly better performance than ModularE170 in the clinically important concentration range 10-40 ng/mL. ModularE170 showed in overall the best fit crossed the line of identity and highest correlation coefficient, and is considered as the most reliable method to measure 25(OH)D in haemodialysis samples.

T324

DETERMINATION OF HYALURONIC ACID IN SERUM: ADAPTATION OF THE HA IMMUNOTURBIDIMETRIC KIT REAGENTS (CORGENIX, USA, DISTRIBUTED IN FRANCE BY ELITECH) ON UNICEL® DXC 880I (BECKMAN COULTER)

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Background. Large unbranched glycosaminoglycan, the hyaluronic acid (HA) is widely distributed in the extracellular matrix and a small quantity is found in blood, essentially degraded in liver sinusoïds. Excellent direct biomarker of liver cirrhosis, it is used in combination with other blood markers in many algorithm-based scores, which provide an assessment of degree of fibrosis in chronic liver diseases (alternative to the invasive liver biopsy). Latex immunoturbidimetric assays to measure serum HA has been developed, adapted on automated analyzers, but no adaptation exist on Beckman Synchron® Systems.
Materials and methods. Then, we adapted the dosage of serum HA on a UniCel® DxC 880i (method NIPIA at 940 nm) with the reagents of the HA Immunoturbidimetric Test Kit from Corgenix. Sera from hospitalised patients, harvested on dry tube with gel separator (Greiner Bio-One), were analyzed according to the protocol of validation of methods VALTEC (SFBC).
Results. Good imprecision: intra-assay CV's were 4.2% at 70 μg/L and 3% at 286 μg/L, inter-assay CV's 5.2% at 50.7 μg/L, 3.1% at 189 μg/L and 2% at 484 μg/L, with detection limit = 16.5 μg/L and linearity >785 mg/L; no hook-effect until 50,000 μg/L, no interference with bilirubin nor turbidity in the condition of protocol; the Bland Altman difference plot and the Passing-Bablock regression analysis (y = 0.97x + 5.44; r = 1.0; n = 42) show a good correlation with the ELISA method (HA test Kit from Corgenix). Stability of calibration was verified during 8 days with the reagents on-board and 30 days if stored stoppered at 2-8 °C.
Conclusion. The good performances of this method and its good correlation at every level with the ELISA method, already used in routine for the liver function tests, considered as reference method, thus allow us to use this turbidimetric adaptation on Synchron® Systems, and to completing the range of parameters needed for the calculation of the fibrosis scores on these analyzers.

T325
USE OF GEM PREMIER 3000 IN CARDIAC SURGERY UNDER EXTRACORPORAL CIRCULATION

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Background: Use of extracorporal circulation (ECC) during cardiac surgery results in hemodynamic and metabolic alterations, disbalance of blood cell components and electrolytes. These dynamic changes require quick and precise measurements of blood gas parameters (pH, pCO2 and pO2), electrolytes (Na+, K+, Cl–), glucose, lactate and oximetry (hemoglobin and hematocrit).
Aim of Study: Comparison of a cell analyzer GEM PREMIER 3000 (IL) with a conventional analyzer of blood-gas analysis and electrolytes OMNI C (Roche), as well as comparison of Hb and Hct measured with reference spectrophotometric and microfuge method, respectively.
Methods: Precision of analyses was compared in parallel series on both analyzers (n=20). Regression analysis – y-intercept, slope, bias, correlation coefficient, was used for statistics and assessment of difference. Blood arterial samples from patients cooled to 28 and 32oC, during aorta clamping, after rewarming to 36-37oC, and after ECC, were analyzed. Cardioplegic solution KIRKLIN (Laboratorium Dr. G. Bichsel AG) was used for cardiopulmonary bypass (CPB).
Results: Mean values and bias did not show significant differences between pH, pCO2, pO2, Na+, K+, Cl- and glucose when measured with OMNI C and GEM. Lower values of hematocrit and hemoglobin were measured with GEM in comparison to OMNIC (Hct: R2 0.90; bias–6.3; Hb: R2 0.89; bias–29.3), with microfuge method for Hct (R2 0.94; bias – 4.3) and spectrophotometric method for Hb, measured by Sysmex SF3000 (R2 0.73; bias–40.2) in the arterial samples obtained during CPB.
Conclusion: GEM measures the Hct by electric conductance technique, and the Hb is calculated from the measured Hct. Plasma impedance varies depending on alterations in ion strength, protein concentration, and colloids during the CPB, and the lower values of Hb and Hct might result in reluctant transfusions of erythrocyte concentrates.

T326
OPTIMIZATION OF AN INTACT PTH ASSAY FOR USE IN MONITORING THYROIDECTOMY PATIENTS.

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Background: Determination of parathyroid hormone (PTH) is performed as an aid in diagnosing and monitoring bone disease. It is also of clinical relevance in assessing calcium disorders, and in managing patients undergoing thyroidectomy or parathyroidectomy. There are many assays for PTH; but because of a lack of standardization, low PTH levels frequently seen after surgery and the heterogeneity of circulating PTH entities, a careful method evaluation is warranted. The aim of this study was to evaluate the technical performance and the clinical validity of a routine intact PTH immunoassay.
Methods: The evaluation was performed on two Beckman Coulter Access immunosystems, LXi 725 and DxI 800, (LXi/DXi). The imprecision study (within-run and between-day), was performed using quality control materials and patient plasma pools at two different concentrations. The correlation studies involved a series of samples selected to cover the analytical range, with an emphasis on samples at the lower end tested on the LXi/DXi and an Elecsys 2010 (Roche Diagnostics). Data was analyzed by Bland-Altman difference plot and Passing/Bablok regression analysis.
Results: Imprecision studies yielded within-run and between-day coefficients of variation of 3.2% -4.8% and 9.0% -16%, respectively at 1.9, 11 and 15 pmol/L. Using patient plasma pools, the total imprecision was respectively 15% and 10% for the around medical decision thresholds established at 1.8 and 3.0 pmol/L. LXi/DXi PTH results correlated variably with Elecsys PTH (r2 ~0.780) over the analytical range. The unweighted linear regression analysis yielded slopes of 0.793 – 1.026 and intercepts of -0.25 to 0.21 pmol/L. The mean difference for surgery patients was -0.25 to -0.18 pmol/L. Despite LXi/DXi and Elecsys “Intact PTH” assays are all one step sandwich chemiluminescence-immunoassays, correlation deviations were expected due to differences in immunoassay standardization.
Conclusions: Our data suggest LXi/DXi intact PTH gives information regarding medical decision thresholds that are similar to that of the Elecsys 2010 assay, supporting the applicability of the LXi/DXi assay to monitor thyroidectomy and parathyroidectomy patients.

T327
VITAMIN D3 A COMPLEX ANALYTE, SHIFTING FROM HPLC TO AN AUTOMATED METHOD

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Background: Vitamin D3 (VitD3) has become a household name in the clinical laboratory, but that doesnt mean the dust has setle on the debate as to which analite to use for patient management (D3, D2 or total) or which method to use to determine its value; analytical method choices ranges from the most complex in terms of time and lab equipment as RIA and HPLC, to the most straightforward molecular immunology methods where a broad range of automated solutions are comercialy available. As more and more determinations of VitD3 are requested from laboratories the need to shift from more time consuming methods like HPLC to automated ones with lateral processing capacities is difficult to evade.
Methods: We conducted a method comparisong between Alligen's Vitmaine D2/D3 HPLC vs. Siemens' Centaur Vitamine D, Roche's Elecsys Vitamine D and DiaSorin's Liaison Vitamin D. Following the guidelines outline by the Clinical and Laboratory Standards Institute (CLSI) in 2002, that is we selected 40 patient's serum samples with known prior values covering a broad range, but still within the values regularly observed in tested population, we then randomized the samples into four series, and processed them in two runs in parallel on every instrument. A conventional linear regresion plus the statisitcs recomended in the CLSI guideline was performed using MS Excel.
Results: Linnear regression: Centaur y = 0.3823x + 5.6624 R^2 = 0.4605 , Elecsys y =0.7595x - 4.9448 R^2 =0.7569, y = y = 0.7295x - 5.222 R^2 =0.7102, intra-series differences were assessed obtaining Centaur Tle 61,4, Elecsys Tle 50,06, and Liaison Tle 53,34, that is to say there were no signinificant intra-series differences, The acceptability limit for intermethod comparisons was for the Centaur DX' 0,18, Elecsys DX' 0,25 and for the Liaison 4,0, under those conditions no sample was rejected, and the linnear regresion data were accepted.
Conclusion: The goodness of fit of the automated assays evaluated were not optimal but a trend is easly decirnible where the Elecsys is superior to the Liaison which in term is superior to the Centaur; only three methods were assessed, more are commercially available and laboratories should carefully consider which to choose to incorporate to their instruments and methods

T328
DEVELOPMENT OF A RAPID AUTOMATED SPE PROCEDURE FOR THE MEASUREMENT OF PLASMA COTININE BY LC-MS/MS

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Background: Cotinine is the primary metabolite of nicotine and the preferred biomarker for assessing cigarette smoke exposure. Several liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been described for measuring cotinine in biological fluids. Such analyses often require lengthy sample preparation protocols involving protein precipitation and solvent evaporation. We describe a novel LC-MS/MS method for the measurement of cotinine using a simplified solid-phase extraction (SPE) procedure.
Methods: Extraction of cotinine from plasma samples was carried out by an HTS-PAL autosampler robot, using disposable SPE cartridges. Cotinine was quantified by LC-MS/MS with electrospray ionisation (ESI) using multiple reaction monitoring (MRM) and cotinine-d3 as internal standard.
Results: The assay was linear over the analytical range 0.0001 mg/L – 1 mg/L. Limits of detection and quantification were 0.14 ng/mL and 0.23 ng/mL, respectively. Intra- and inter-assay imprecision of cotinine in all samples was <5% relative standard deviation. The analytical recovery of cotinine spiked into plasma was >95%.
Conclusion: We have developed and validated a rapid, sensitive and specific LC-MS/MS method for the determination of cotinine in plasma, using a straightforward automated SPE protocol. This method can be applied to routine monitoring of smoking cessation in both the clinical and forensic environment (e.g. psychiatric patients within the secure forensic unit).

T329
STUDY COMPARING TWO CHEMISTRY ANALYZERS

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Background: When a measurement procedure replaces another, you have to prove it estimating the systematic error (SE). The aim of this study was to check the interchangeability of two procedures.
Methods: The study was performed for 5 days, and 15 duplicate samples were processed. The reference procedure was a PPE Modular (Roche®) and the evaluated analyzer was an Advia 1800 (Siemens®). The serum parameters evaluated were: urea, creatinine, total bilirubin (TB), direct bilirubin (DB), total cholesterol (TC), triglycerides, HDL-cholesterol (HDL), uric acid (UA), phosphorus, calcium, magnesium, albumin, total protein (TP), sodium, potassium, chloride, aspartate-aminotransferase (AST), alanine-aminotransferase (ALT), alkaline-phosphatase (ALP), gamma-glutamyltransferase (GGT), amylase, creatine-kinase (CK), lactate-dehydrogenase (LDH), cholinesterase, iron, glucose, transferrin, C-reactive protein (CRP), antistreptolysin O (ASO), rheumatoid factor (RF), LDL-cholesterol (LDL) and ferritin. The results were analyzed by: Analysis of differences. The differences (D) and relative percentage differences (RD) between procedures were calculated, describing themselves as the mean (Dm or RDm) and standard deviation (SD o SRD). The confidence interval (95% CI) of the mean were calculated. Linear regression. The results of both methods were represented, obtaining the slope (b), intercept (a) and 95% CI.
Results: Analysis of differences. The systematic difference was constant for chloride (95% CI (Dm) did not include 0), and systematic difference was proportional for chloride and cholinesterase (95% CI (RDm) did not include 0). Linear Regression. The systematic difference was constant for DB, TC, HDL, phosphate, albumin, TP, chloride, AST, ALT, CK and CRP (a >0), and for creatinine, TB, UA, ALP, amylase, LDH, cholinesterase, iron, transferrin and ASO (a <0). Showed a systematic proportional difference, TB, triglycerides, UA, potassium, ALT, ALP, GGT, CK, LDH, cholinesterase, iron, ASO and glucose (b >1), and creatinine, TC, albumin, TP, AST, transferrin and RF (b <1).
Conclusions: In order to correct the proportional SE it is necessary to review the calibration process and also the reference values, especially for chloride and cholinesterase.

T330
COMPARISON OF THE PT/INR ASSAY WITH USING TWO DIFFERENT REAGENT:QUIKCOAG PT AND TECHNOPLASTIN HIS ON CEVERON ALPHA COAGULATION ANALYZER

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Comparison methods, Behçet's disease, vitiligo disease

Objective: Prothrombin time (PT) is a coagulation parameter that reflects the activity of the extrinsic coagulation pathway (factors VII, X, V and II, and fibrinogen), starting from tissue factor-induced activation of factor VII and ending in fibrin formation. Our purpose to compare PT/INR values with using BioMedica QuikCoag PT and Technoclone Technoplastin HIS (heparininsensitive) commercially available kits on Ceveron alpha coagulation analyzer (Austria).
Materials and methods: The study was conducted in a large, multi-specialty general hospital (Usak State Hospital). We compared 78 patients plasma PT/INR values with using BioMedica QuikCoag PT Reagent (Canada) and Technoclone Technoplastin HIS PT Reagent (Austria) commercial kits on Ceveron alpha automated coagulation analyzer (Austria). 2,7mL venous blood samples were simultaneously collected from each subject with BD vacutainer tubes (0.109M sodium citrate) and centrifuged at 2,500xg for 15 min. The PT-INR was measured immediately in the laboratory with using both kits in consecutively on Ceveron alpha coagulation analyzer. Results from the BioMedica and Technoclone kits were compared using the two-sample t-test. p value <0.05 was considered to indicate statistical significance.
Results: A total of 78 patients mean age was 51±21 (32 male 46 female). The mean level of PT 14,77±5,80 with Technoplastin HIS and 20,66±12,19 with QuikCoag PT. The mean level of INR 1,42±0,65 with Technoplastin HIS and 1,67 ±1,16 with QuikCoag PT. We obtained higher level of PT/INR test results with QuikCoag PT than Technoplastin HIS PT. We found statistically significant difference between PT and INR results with using two-sample t-test (respectively P=0,000 and P=0,000).
Conclusion: The results obtained using BioMedica reagents are not equivalent to those obtained using Technoclone reagents.

T331

EVALUATION OF PANACLEAR MMP-3 "LATEX", A REAGENT FOR HITACHI LABOSPECT 008

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Background: MMP-3 (matrix metalloproteinase 3) is an enzyme produced in the synovial cells and chondrocytes of joints and is possibly most closely associated with collagen degradation and cartilage destruction. In recent years, reagents applicable to ELISA and automated analyzers have been developed and used frequently in the diagnosis of rheumatoid arthritis, prediction of responses of this disease for treatment and evaluation of the disease activity. An improved reagent with latex turbidimetric immunoassay method for LABOSPECT 008 has recently been launched and its basic performance was evaluated.
Method: The reagent PANACLEAR MMP-3 "Latex" (Sekisui Medical Co., Ltd.) and the clinical chemistry analyzer LABOSPECT 008 (Hitachi) were used for the following evaluations: (1) reproducibility (2) dilution linearity (2 level of MMP-3 samples were diluted with physiological saline) and limit of detection (2.6SD method), (3) interference (evaluated with Interference Check (Sysmex) and ascorbic acid), (4) correlation (with an existing reagent on JCA-BM1650 (JEOL Ltd.) in combination), and (5) probe contamination test (evaluated on 42 parameters of the same pre-installed module).
Results: (1) Within-run reproducibility of CV (n=20) :1.35% (Control L ; mean 110.9 ng/mL), 0.78% (Control H ; mean 435.4 ng/mL), 1.62% (pooled serum ; mean 99.2 ng/mL) (2) Linearity was up to 1500 ng/mL and limit of detection was 9.85 ng/mL. (3) No influences were observed by bilirubin <200 mg/L, hemoglobin <5 g/L, RF < 550 U/mL or ascorbic acid <500 mg/L in sample. (4) Good correlation with the existing reagent was noted, with the regression formula being y = 0.991x－13.9 and the correlation coefficient being 0.985 (N=92). (5) Probe contamination test: No influence from contamination was noted on any of the 42 parameters.
Conclusion: Improved PANACLEAR MMP-3 "Latex" with LABOSPECT 008 was shown in the present study as having favorable performance. The improved reagent will be useful for measuring of MMP-3 in clinical laboratories.

T332

EVALUATION OF A SINGLE LATEX-ENHANCED ASSAY FOR MEASURING COMBINED SERUM FREE LIGHT CHAINS IN CLINICALLY RELEVANT SAMPLES ON THE SPA PLUS TURBIDIMETRIC ANALYSER

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Background: Recent studies indicate a relationship between diseases associated with B cell activation, renal dysfunction and inflammation and elevated levels of combined κ and λ polyclonal serum free light chains (cFLC). Furthermore, cFLC concentrations have been linked to adverse outcomes in both normal, chronic kidney disease and systemic lupus erythematosus (SLE) populations. Here we describe Combylite™, a single, latex-enhanced, turbidimetric assay for the measurement of cFLC and its comparison to summation of results from individual κ and λ assays (Freelite®)
Methods: cFLC levels were measured in presentation and follow up sera from patients with a variety of disorders using Combylite on a SPA PLUS analyser (The Binding Site Group Ltd, TBS). The assay utilises polyclonal antibodies specific for κ and λ free light chains, and the results were compared with summated κ and λ. Monoclonal samples with abnormal κ/λ ratios (0.26-1.65) were excluded from the analysis.
Results: The measuring range was 6.25-200 mg/L with a sensitivity of 0.625mg/L. Inter-assay CVs for samples with high (169.66 mg/L), intermediate (53.98 mg/L) and low (12.23 mg/L) cFLC concentrations were 5.5%, 5.5% and 14.4%, respectively. The assay was linear over a range of 6.05-223 mg/L with a regression of measured against expected values of y=1.03x-3.4, R2=0.99. Comparison was made with summated FLC values by measuring samples from controls (n=193, range 6.7-93.9 mg/L) and patients with SLE (n=372, 7.1-164 mg/L), rheumatoid arthritis (n=332, 8.5-108.1 mg/L), lymphoma (n=37, 6.2-252.6 mg/L), liver disease (n=361, 5.4-337.4 mg/L), cardiovascular disease (n=406, 8.2-284 mg/L), and CKD (n=515, 20.8-259.1 mg/L). Passing-Bablok fit was y=1.02x-2.39 and the linear fit was y=0.98x-0.91 with an R2 of 0.94.
Conclusions: Measuring cFLC levels may provide a hitherto unavailable insight into B cell activation. Combylite is a single, rapid and reproducible immunoassay and further work is required to establish its utility in a variety of disorders.

T333
COMPARATIVE STUDIES OF THE ACCESS HCV AB PLUS ASSAY

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Background: The aim of this study was to assess the performance of the Access® HCV Ab PLUS (Bio-Rad) on the Beckman Coulter Immunoassay UniCel® DxI 800 system (Beckman Coulter Inc.) in terms of specificity and sensitivity using serum and plasma samples. All results were compared to Elecsys® Anti-HCV II assay (Roche Diagnostics) and ADVIA Centaur® HCV assay Siemens).
Methods: Comparison studies were performed on UniCel DxI 800 system, MODULAR® ANALYTICS E170 system (Roche Diagnostics) and ADVIA Centaur XP system (Siemens). 500 non-selected serum samples from a routine laboratory, 3 commercial HCV seroconversion panels and one anti-HCV low titer performance panel were used on each system for specificity and sensitivity testing.
Results: 488 of the 500 non selected samples were true negative. The specificity was 99.59% (95% CI: 98.53-99.95%) for all assays. The 2 false positive samples were different on UniCel DxI 800, MODULAR E 170 and AVDIA Centaur XP. The concordance with MODULAR and ADVIA Centaur was 99.20%. The clinical sensitivity from 12 positive samples was 100% for all assays. Using 3 commercial seroconversion panels plus one anti-HCV low titer performance panel, Access HCV Ab PLUS and Elecsys Anti-HCV II assays showed good performance and were more sensitive than the ADVIA Centaur HCV assay.
Conclusions: The performance of the Access HCV Ab PLUS assay on the UniCel DxI 800 immunoassay system was excellent in terms of specificity and sensitivity. The specificity and the clinical sensitivity were equivalent for the 3 assays. The seroconversion sensitivity was better on UniCel DxI and Modular than on ADVIA Centaur. Adapted for high throughput routine testing, the Access HCV Ab PLUS assay performed on UniCel DxI 800 immunoassay system is fully suited for the screening of HCV infection in diagnostic laboratories.

T334
LUMIPULSE® G1200, AN AUTOMATED CLEIA PLATFORM SHOWING GOOD PERFORMANCE FOR DIFFERENT TUMOUR MARKERS

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Background: Tumour markers are commonly used to detect a relapse of disease in oncologic patients during follow-up. It is important to evaluate new assay systems for a better and more precise assessment, as a standardized method is currently lacking. Aim of this study was to assess the concordance between an automated chemiluminescent enzyme immunoassay system (LUMIPULSE® G1200) and our reference methods using 6 tumour markers.
Materials: Serum samples from 821 oncologic patients, representing a variety of diagnoses, were analyzed using LUMIPULSE® G1200 and our reference methods. Serum values were measured for the following analytes: PSA, AFP, CEA, CA125, CA15-3, and CYFRA. For the determination of CEA, AFP and PSA, an automatic analyzer based on chemiluminescence (Access2) was applied as reference method. To assess CYFRA, CA125, and CA15-3, an immunoradiometric manual system was used (CisBio).
Results: The concordance between LUMIPULSE® G1200 and both reference methods was as follows: PSA 97%; AFP 96%; CEA 95%; CA15-3 91%; CYFRA 96%. A lower concordance (82%) was found when CA125 was assessed. In this group, 9 samples showed high disagreement between LUMIPULSE® G1200 (CLEIA) and the immunoradiometric manual system. After diluting the samples and retesting with the reference method, a higher concordance (98%) with undiluted LUMIPULSE® G1200 values was obtained. These data demonstrate the presence of the 'hook effect'. The precision of each assay was assessed by testing 6 serum samples. Each sample was analyzed for all tumour biomarkers in duplicate and in three different runs. The coefficients of variation (CVs) were less than 6.3% and 6.2% for within-run and between-run variation respectively.
Conclusions: Our data suggest an overall good agreement between all methods. However, some artifacts were obtained with immunoradiometric system and suggest the presence of an artifact secondary to the 'hook effect'. CLEIA automated assay showed a good reliability in all samples.

T335
"ISO LIKELIHOOD RATIO LINES": A TOOL FOR THE INTERPRETATION OF RECEIVER OPERATING CHARACTERISTIC CURVES

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Background: Receiver Operating Characteristic curves (ROC) are widely used as a measure of the accuracy of diagnostic tests. However, their interpretation in terms of post-test probability is not straightforward. To ease that interpretation we present a simple tool, based on Likelihood Ratios (LR).
Introduction: ROCs are functions of the Sensitivity (Se) and Specificity (Sp), calculated for all the possible cut-off points. LRs are the ratios of the probabilities of a test result in a specified diseased population versus a non-diseased population. Each LR value is determined by Se and Sp pairs linearly related through a straight line that we propose to call "Iso Likelihood Ratio line" (isoLR). In dichotomous tests, chosen cut-off points define the positive and negative results, whose LRs are respectively called Positive Likelihood Ratio (LR+) and Negative Likelihood Ratio (LR-), both functions of the Se and Sp at the chosen cut-offs. It follows that it is possible to plot isoLRs in ROC graphs.
Methods: Plot of isoLRs in ROC graphs.
Results: The isoLRs for the LR+ and LR- are represented as straight lines passing through points 0,0 and 1,1 respectively, with slopes equal to their values. They define areas for LR+s higher or equal and LR- lower or equal then their respective values. The intersections of the ROC with the isoLR+ and isoLR- define cut-off points for the positive and negative tests respectively. The part of the ROC that will eventually fall between these points corresponds to a "gray zone". For reference, we plot by default the IsoLR corresponding to LR values of 10, 4, 2 for LR+ and to their reciprocals (1/10, 1/4 and 1/2) for LR-. According to the specific clinical use of the tests, other LRs can be chosen and the corresponding isoLR are easily plotted.
Conclusions: In ROC graphs, isoLRs lines are very easy to plot and allow the definition of cutoffs and areas with specified LR values. These areas can be directly interpreted in terms of post-test probability, using the respective LRs and pre-test probabilities. IsoLRs can also be useful in method comparison studies, if common LRs are established to standardize the interpretation.

T336
RAPID DETERMINATION OF BILIRUBIN IN BIOLOGICAL SAMPLES BY COLORIMETRIC–SOLID-PHASE EXTRACTION-FIBER OPTIC REFLECTANCE SPECTROSCOPY

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Background: Bilirubin is a product of red blood cell breakdown. Normally, bilirubin is carried in the blood and passes into your liver, where it's removed and becomes part of bile. Bilirubin is not normally present in the urine. Only conjugated bilirubin is excreted into the urine and normally only trace amounts can be detected in urine. Normal bilirubin levels are less than 1.0 mg/dL. Bilirubin in your urine may indicate liver damage or disease. Analysis of urine is an important nursing procedure. It is relatively cheap and requires minimal amount of training. However, it gives vital objective information about the patients internal functioning.
Method: A new and selective colorimetric solid-phase extraction (C-SPE) procedure for the determination of Bilirubin in urine samples was proposed. The solid-state sensor is based on the reaction of bilirubin or mesobilirubin in presence of an oxidizing agent to convert the blue color and the analytes in samples were extracted onto a solid sorbent matrix and then quantified directly on the adsorbent surface by using a miniature reflectance spectrometer. An oxidation method is generally considered to be more accurate than a diazo method for the determination of bilirubin in urine. The measurements were carried out at a wavelength of 642 nm since it yielded the largest divergence different in reflectance spectra before and after reaction with the bilirubin.
Results: Under the optimum experimental conditions, a linear calibration curve for bilirubin was obtained over the 0.12–5.85 μg L-1 concentration range studied and the limit of detection was 0.1 μg mL-1. There were no significant differences between RSD (%) values for intra-day and inter-day precision, which indicates the method was reproducible. The proposed method was applied to the determination of BLB in urine samples with a recovery for the spiked samples in the range of 96-102%.
Conclusions: The proposed sensor was applied for the detection of bilirubin in the urine sample and satisfactory results were obtained. The above observations showed that the developed sensor might be promising in the detection of bilirubin in clinical samples. The sensor is small and of low-cost. Furthermore, it can be made into a portable instrument for in situ measurements.

T337
EVALUATION OF THE HLC-723GX ANALYSER (TOSOH BIOSCIENCE) FOR THE DETERMINATION OF HBA1C

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Background: HbA1c is the commonest marker for monitoring diabetes mellitus and treatment could be modified according to the results. Since a few years ADA and WHO proposed it as a test for the diagnosis of type 2 diabetes mellitus. For these 2 applications accuracy is necessary. When a new device is launched it is necessary to evaluate its features in order to be confident with the results.We evaluated the latest generation analyser of the Tosoh family: the HLC-723GX (GX). GX is a compact analyser which separates the haemoglobin fractions using the cations exchange HPLC method.
Methods: Intra and inter assay precision was evaluated with control specimens. Accuracy was evaluated with specimen from the European Reference Laboratory (ERL). Correlation was performed with patient specimens after they were tested for HbA1c. Interference of glucose, cyanate, acetate normally eluting in the fraction called labile A1c was assessed by overloading and/or by removal.The possibility to correctly separate the most common variants was evaluated.
Results: The intra assay and inter assay precision was in agreement with the specifications of the manufacturer: for example, coefficients of variation (CV) in the inter assay protocol, were respectively 0.89% and 0.28% for respective values 5.28% (34 mmol/mol) and 10.11% (87 mmol/mol). The reproducibility was also tested with patients samples with variant haemoglobins. The results were for HbS: CV= 0% for HbA1c at 5.6% (38 mmol/mol) and for HbC CV = 0% for an HbA1c at 4.8% (29 mmol/mol). The bias observed with specimens from ERL versus assigned values was 0.38%. We did not observe any interferences with stable HbA1c when labile A1c levels were below 5%, HbF below 45% or HbS below 35%. For the correlation to G8, 119 samples were tested. The regression line was GX = 0.99G8 +0.08. At least according to samples received every day, we observed that the most common haemoglobin variants were correctly separated.
Conclusion: GX is a small analyser for the determination of HbA1c which presents all the features of a big one. This analyser allows laboratories with low test volumes to be confident in the results. We appreciate particularly the close to equal consumption of each buffer.

T338
COMPARISON OF POLYCLONAL ANTIBODY-BASED ASSAYS FOR FREE LIGHT CHAIN KAPPA AND LAMBDA TO MONOCLONAL ANTIBODY-BASED ASSAYS

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Background: The measure of serum concentrations of free light chain (FLC) kappa and lambda is useful in diagnosis and follow up of plasma cell dyscrasia. Nowadays there are two different methods to determine them, both based on a nephelometric immunoassay, but they differ in the type of antibody used, one of them is a monoclonal antibody and the other is a polyclonal antibody.
Methods: The study was performed with 49 serum samples from patients with monoclonal gammopathy. Freelite™ FLC Kit was compared against N Latex FLC, both using the nephelometric system Siemens BNII.
Results: Correlations between both assays, using Spearman´s rho, were 0.928, 0.901 and 0.923 for kappa, lambda and kappa/lambda ratio, respectively. In order to evaluate the concordance, we used Bland and Altman´s graphic methods, this study showed a good concordance in low values (below 100), but this does not happen in the case of high values. Besides, we decide to categorize data in function of their reference intervals, so the estimated concordance indexes for kappa, lambda and kappa/lambda ratio were 0.829, 0.785 and 0.788 respectively. The distribution of conflicting cases for each parameter was symmetrical, using McNemar asymmetry test.
Conclusions: The correlation between the two methods was good, and also the concordance with categorized data. For this reason both methods are reliable for the determination of free light chains in monoclonal gammopathies.

T339

COMPARISON OF C REACTIVE PROTEIN ANALYSIS BY NEPHELOMETRY (BN II) AND TURBIDIMETRY (ADVIA 2400)

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Background: Ultrasensitive CRP kits that can quantify very low concentrations, are being increasingly used in the risk evaluation and prognostic value of heart diseases. Recently, a new CRP method, wide-range C-reactive protein (wrCRP), has been developed for ADVIA chemistry systems by Siemens to measure serum CRP levels in the low range of concentrations with good accuracy. The aim of this study is to compare the values of CRP concentration measured by two different methodology to assess the transferability of results: method 1-BNII nephelometer hsCRP(Siemens)and method 2-wrCRP Advia2400 (Siemens).
Material and methods: We analyzed 55 samples of serum by two methods: (y) hsCRP: immunonephelometry. With a detection limit of 2.97 mg/L. (x) wrCRP: enhanced immunoturbidimetric assay with particles of polyethylene glycol (PEG) coated with anti-CRP antibodies which agglutinate in the presence of CRP. Measure a wide concentration range, with a detection limit of 0.1 mg/L. Data were statistically analyzed using the MedCalc statistical package by Spearman correlation and Passing-Bablok regression. Significance was set at P <0.05.
Results: There is a good correlation between both methods, with a coefficient of correlation r=0.981 (P <0.01). Median CRP concentrations for method 1(y) was 7.20 mg/L (range: 2.97-285) and by method 2(x) was 9.21 mg/L (range:0.07-306). Passing-Bablok regression equation was y=0.721+2.06x (95%CIslope: 0.69-0.74; 95%CIintercept:2.03-2.45). There is constant and proportional error which generally yields higher concentrations with the wrCRP method and the results are not transferable. Is necessary to review the current reference values to match the new wrCRP method. Excluding values <3 mg/L (detection limit of hsCRP method), correlation improved significantly, finding a r=0.996 (P <0.0001). The new regression line was y=0.773x-0,148 (95%CIslope:0.753-0.839; 95%CIintercept:-1.01-0.245). The new relationship demonstrates that there is systematic proportional error but not constant.
Conclusion: There is a good correlation between both methods. The turbidimetric wrCRP assay is a reasonable alternative to hsCRP nephelometric assay besides being cheaper and faster.

T340

INFRARED ANALYSIS OF URINARY STONES: A VERY USEFUL TOOL FOR THE CLINICIANS.

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Background: Kidney stone disease is a common illness with multifactorial etiopathogenesis. Methods for urinary stone analysis are classified in two main categories: semi-quantitative and quantitative. In our laboratory we use the semi-quantitative methods that are the most widespread into the laboratory for routine analysis. These last methods can only identify the presence of individual ions without differentiate mixtures and the results are operator dependent. The aim of this paper is to compare semi-quantitative method (DiaSys) with a quantitative method (FT-IR spectroscopy technique) for urinary stone analysis, in order to introduce in our laboratory a more reliable technique.
Material and methods: We have analyzed 16 urinary stones arrived from Urology Department of our Hospital. The semi-quantitative analyses were performed using Urinary Calculi Analysis kit (DiaSys) according to the manufacturer's instructions. The same samples were analyzed by FT-IR using the "Perkin Elmer Spectrum One FT-IR Spectrometer" In this case each sample was prepared as follows: 3-5mg of the stone was powdered in a pestle, homogenized with 300 mg of potassium bromide (KBr) and converted into a pellet through a press. All FT-IR spectra of kidney stones were then computer matched against a library of spectra (NICODOM IR Library) so as to generate a precise report on the various stone components.
Results and conclusions: A comparison of results obtained using the two methods from these 16 stones shows good overlapping of the results, in fact the main substances identified with the semi-quantitative method are also identified with the FT-IR technique. Instead, there are substantial differences on identification of substances present in trace amounts. in fact FT-IR technique shows a high sensitivity and permits to accurately identify stone composition. Identification of gallstones composition is essential for clinicians to find out the underlying cause of kidney stones and to decide whether to treat patients therapeutically or surgically. For these reasons, according to these preliminary tests, the introduction of the FT-IR technique in clinical chemistry laboratory routine may be more responsive to clinicians expectations.

T341
AUTOMATION AND CONSOLIDATION OF CLINICAL CHEMISTRY AND IMMUNOCHEMISTRY ASSAYS: THE EXPERIENCE OF AN ITALIAN HOSPITAL-BASED CLINICAL LABORATORY

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Background: In this study we evaluated change in efficiency and efficacy in our Laboratory after implementation of a total Laboratory automation for Clinical Chemistry and immunochemistry.
Methods: We recently implemented a total laboratory automation for Clinical Chemistry and immunochemistry in our hospital-based clinical Laboratory: Thermo EnGen pre-analytical automation with a track system connecting two Vitros Fusion 5.1 and two Tosoh AIA-2000 analyzers.
Results: For Vitros Fusion 5.1 CV was <5% within-run and <6% inter-assay. For Tosoh AIA-2000 analyzers, within-run CVs were usually <7% and below <8% inter-assay. Functional sensitivity of ferritin was 5 mg/L with a CV of 18% and for glucose was 1.1 mmol/L with a CV of 12%. No drift effects were observed; neither there was any carry-over, due to samples or reagents. When evaluating the functionality of the whole automation system we observed a turn around time (TAT) 90% <10 min for tubes needing check-in and exit only. For tubes needing a check-in, centrifugation – exit cycle, TAT 90% was <30 min. Tests for clinical chemistry and/or immunochemistry on-line analyzers showed a TAT 90% <105 minutes.
Conclusions: We conclude that the adopted automation system effectively reduces the labor associated with specimen processing; decreases the number of laboratory errors that occur with specimen sorting, labeling, and aliquoting; and improves the integrity of specimen handling throughout the steps of specimen processing.

T342
COMPARISON BETWEEN METHODS OF DIRECT AND CALCULATED LDL CHOLESTEROL

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Background: LDL cholesterol (LDL-C) is a known risk factor for cardiovascular disease. The reference method for measurement is ultracentrifugation. For the daily routine, an estimate is made using the Friedewald formula or direct measurement using automated methods.
The aim of this study was to compare the direct measurement of LDL-C (dc-LDL) with the estimate LDL-C (cc-LDL) calculated.
Methods: 1742 patients were studied. Measurements of total cholesterol (TC), HDL cholesterol (HDL-C), triglycerides (TG) and dc-LDL were performed in the autoanalyzer Advia 1800 (Siemens®). The cc-LDL was calculated using the Friedewald's formula (=TC-HDL-(TG/5)). Patients were classified into five groups according to the TG values: ≤150, 151-200, 201-300, 301-400 and >400 mg/dL. The mean and standard deviation of biochemical parameters, and the percentage of patients with cardiovascular risk (LDL-C ≥130 mg/dL) were calculated. Student's-t-test was achieved for statistical data analysis.
Results: In relation to the concentration of TG we observed that dc-LDL and cc-LDL presented similar results. For TG ≤150 mg/dL was 110±31 (dc-LDL) and 114±33 (cc-LDL); for TG between 151-200 mg/dL was 118±35 (dc-LDL) and 118±37 (cc-LDL); for TG between 201-300 mg/dL was 122±34 (dc-LDL) and 116±38 (cc-LDL); and for TG between 301-400 mg/dL was 117±33 (dc-LDL) and 104±35 (cc-LDL). The percentage of individuals with risk of cardiovascular disease in the general group was 30% (dc-LDL) and 31% (cc-LDL). If TG ≤150 was 27% (dc-LDL) and 29% (cc-LDL); TG: 151-200, 36% (dc-LDL) and 35% (cc-LDL); TG: 201-300, 35 % (dc-LDL and cc-LDL); TG 301-400, 38% (dc-LDL) and 15% (cc-LDL); and TG >400, 32% (dc-LDL) and 10% (cc -LDL). The correlation between dc-LDL and cc-LDL in the general group was r^2=0.844. For TG ≤150, 151-200, 201-300, 301-400 and >400, was 0.866; 0.848; 0.837; 0.803 and 0.696, respectively.
Conclusions: For patients with TG <400 mg/dL the dc-LDL and cc-LDL methods presented similar results. In hypertriglyceric patients (TG >400 mg/dL) the correlation is weaker. The dc-LDL method doesn't appear to offer any advantages over the cc-LDL, so from a practical and economic point of view, we consider best to use cc-LDL in patients with TG <400 mg/dL.

T343
OPTIMIZED ROOM TEMPERATURE STABLE, READY-TO USE, ONE-STEP RT-PCR MIX

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Background: PCR Mixes, as well as enzymes and amplification mixes components, are usually stored at a -20° or +2/8°C. As a consequence, the reproducibility of an assay prepared from freezed and thawed material is critical. The aim of the present work is the achievement of a freeze-dried mix to perform, in a single step, an amplification starting from a RNA template in a ready-to-use, pre-dispensed, flexible and room-temperature storable format.
Methods: Each test tube of the one-step RT-PCR Mix contains: reaction buffer, dNTPs, MgCl2, Reverse Transcriptase, Hot Start DNA Polymerase, preservatives and stabilizers, in a freeze-dried form. Performances were evaluated on a 7500 Real-time PCR System (Applied Biosystems) by TaqMan Ribosomal RNA Control Reagents (Life Technologies). The thermocycling protocol was: 48 °C 30', 1 cycle; 95 °C 10', 1 cycle; 95 °C 15", 60 °C 1', 40 cycles. For the freeze-drying process an epsilon 2-12D freeze-dryer (Martin Christ) was used. Accelerated and real-time stability studied were conducted according to the internationally accepted guidelines.
Results: An optimized one-step RT-PCR mix, evaluating different compositions was developed. The formulation showed good performances; a Ct of about 20 with 10 pg of Human Raji RNA was obtained. The performances before and after freeze-drying were evaluated. The lyophilization procedure does not impact on mix functionality. Cts before and after freeze-drying were comparable, 19.87 and 19.88 respectively. Preliminary accelerated stability studies data allows to assign to this product at present a shelf-life of 10 months at room temperature. Real-time stability studies, are still ongoing.
Conclusions: The one-step RT-PCR Mix is a new ready-to-use and room-temperature storable mix useful for efficient one-step RT-PCR. The format (one vial/one test) and the room temperature storage allow the reduction of the time needed for preparation of the amplification mix, and the reduction of risk of contamination. Moreover the dried format permits large flexibility in the volume of template added to the mix. This one-step RT-PCR Mix is universal, applicable to different fields, gene expression, virology, etc. This mix is a valuable tool for the development of molecular diagnostic tests.

T344
INTERLABORATORY COMPARISON OF METHODS FOR 34 DIFFERENT ROUTINE BIOCHEMICAL PARAMETERS ON THE BECKMAN COULTER AU5800, ROCHE COBAS 8000, ROCHE COBAS 6000, ROCHE MODULAR PE, OR THE SIEMENS ADVIA 2400 ANALYTICAL SYSTEMS

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Background: A lack of reproducibility of results obtained in medical laboratories using different methods is still a major health-economic burden and not infrequently hinders the continuous and efficient treatment of the patient. We therefore performed an interlaboratory comparison of routine biochemical parameters between 4 different laboratories using different methods of analysis.
Methods: 998 anonymized residues of serum samples submitted to our laboratory were analyzed for the concentrations/ enzyme activities of 34 routine biochemical parameters. Serum samples were selected to obtain groups with 6 different ranges of concentrations per parameter, each group containing a maximum of 20 samples. Samples were split, stored in aliquots at -20 °C until measurement, and then analyzed in parallel in 5 different laboratories, using either the Beckman Coulter AU 5800, Roche Cobas 8000, Roche Cobas 6000, Roche Modular PE, or the Siemens ADVIA 2400 analytical systems. Statistical analysis (linear regression analysis) of the results was performed using SPSS Statistics.
Results: 23193 individual analyses in 998 samples were performed on each analyzer, actual sample throughput varied significantly between analyzers. Overall, the interlaboratory comparison of methods revealed an acceptable reproducibility, however, in some cases, coefficients of determination R2 varied significantly depending on the compared analyzers (e.g. for Creatine Kinase-MB, lipase). The best coefficient of determination were calculated for gamma-glutamyl transpeptidase.
Conclusions: Despite some outliers on the low side our data demonstrate an overall acceptable agreement in the measured values between the tested laboratories and analytical systems.

T345

GREAT IMPACT OF THE BRAND-NEW SYSMEX XN-SERIES AUTOMATED HEMATOLOGY ANALYZER ON WORKFLOW EFFICIENCY AND CLINICAL UTILITY

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Introduction: Recently, higher-quality testing environment has been increasingly required. To counteract these demands, Sysmex has introduced the XN-3000 automated hematology analyzer. Here we report our evaluation of usefulness of the XN-3000 and the integrated hematology testing system. Method: (1) Basic performance: We evaluated precision, linearity, correlation with the manual method. (2) Usefulness of new function: Low WBC mode, PLT-F (fluorescence) channel, WNR channel (real time NRBC count), Body Fluid mode, Automatic re-testing capability (3) Usability: We evaluated the usability of newly introduced functions of CNA-NET, which include automated smear preparation, selection of specific sample and sample requiring manual count. Results: (1) Basic performance: Precision was 0.42-5.21 (CV%), and linearity was confirmed up to WBC 378×109/L, RBC 7.81×1012/L, HGB 235 g/L, HCT 72.1%, and PLT 1,691×109/L. The correlation coefficient of WBC classification ratio with the manual method was 0.612-0.987. (2) In the evaluation using samples with WBC £ 0.5×109/L, WBC precision was 1.29-4.17(CV%) for Low WBC mode, and the correlation with the manual method was 0.999. In the evaluation using samples with PLT £ 50×109/L, the correlation coefficient between PLT-F and the manual method was 0.980. Usefulness of PLT-F can provide data with high accuracy and precision for in the case of giant and small platelets, and interference particles like fragment red cell and small RBC. Furthermore, PLT-F methods provide immature platelet fraction. WNR channel and Automatic re-testing capability reduced manual retesting and examine few manual procedure. Body Fluid mode was excellent. (3) Usability: In term of time for reporting was 50 – 94% reduction. The number of analyzer operator for 500 samples/ day decreased from 1.5 to 0.8 person.
Conclusion: Basic performance and new function was satisfactory. It is possible to return efficient report in a short time. Use of the automated re-testing function and the CNA-NET made reporting time and processing time reduced. The newly introduced integrated hematology testing system improved the quality of the clinical laboratory testing.

T346

MULTIPLEX DETERMINATION OF NINE SECOND-LINE ANTI-TUBERCULOSIS DRUGS BY UPLC-TANDEM MASS SPECTROMETRY

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Background: Therapeutic Drug monitoring (TDM) anti-tuberculosis (TB) drug concentrations is helpful for patients that show a slow response to treatment and especially for multidrug-resistant cases. We developed a rapid method that simultaneously measures the blood concentrations of nine major second-line anti-TB drugs, including streptomycin, kanamycin, clarithromycin, cycloserine, moxifloxacin, levofloxacin, para-aminosalicylic acid, prothionamide, and linezolid using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS).
Methods: Serum samples were extracted with acidified methanol followed by neutralization with NaOH. A HSS T3 column and gradients of ammonium formate and acetonitrile in 0.1% formic acid were used for UPLC separation. Drug concentrations were determined by multiple reaction monitoring in positive ion mode, and assay performance was evaluated. We applied the devised method to TDM of each drug by analyzing random serum samples from patients treated with second-line drugs (n=62).
Results: The preparation of samples including acidified methanol extraction followed by neutralization steps gave a relatively good recovery and ionization efficiency, and chromatographic separation was achieved within 3 min/sample. Within-run and between-run imprecisions were 2.8–11.1% and 3.7–11.6%, respectively at the concentrations of low and high levels for each nine drug. The lower limits of detection and quantitation were 0.05–0.5 μg/mL and 0.25–5.0 μg/mL, respectively. Linearity was acceptable at 5 level concentrations for each drug. Evaluation of ion suppression showed no effects, except streptomycin, kanamycin and cycloserine with close elution times to the void volume of the column. Pilot application of devised method on the limited number of samples from the patients showed dispersed distribution of serum concentration of each drug.
Conclusions: The performance of our devised techniques for MS/MS detection was generally acceptable. The devised method allows for rapid, sensitive, and reproducible quantification of nine second-line anti-TBs drugs and should be helpful for drug monitoring in TB treatment.

T347

QUATIFICATION OF HUMAN SERUM HEPCIDIN - 25: EVALUTION OF AN ENZYME-LINKED IMMUNOSORBENT ASSAY VS MASS SPECTROMETRY

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Background: Hepcidin-25 is a central regulator in the iron metabolism, holding potential in the diagnosis of iron-related disorders. Mass spectrometry methods for Hepcidin measurement are referred to as reference method; however, there is an increasing clinical need for specific immunoassays.
Methods: Serum samples from 68 healthy subjects (HS), 65 patients with liver-disorders (LD) and 225 hemodialysis patients (HD) were stored at -70 °C until analysis using a commercial enzyme-linked immunosorbent assay (ELISA), (Bachem, St.Helens, UK). The study aimed at evaluating ELISA-kit performance in terms of precision and accuracy. For precision, we applied constructed controls of low and normal levels while for accuracy, the results were compared to measurements using LC-MS/MS.
Results: Using ELISA, the results in HS showed ranges of 9-68 μg/L and 2-44 μg/L in males and females respectively; which are in accordance with published data. Intra- and inter-assay results showed CV of 18% and 13% for low and normal controls and 18% and 19% respectively. The overall correlation between ELISA results to those obtained by LC-MS/MS was good (r =0, 9). Correlation between Hepcidin-25 and ferritin in HS was also satisfactory (r =0, 87). Hepcidin-25 levels showed lower values in HS (male's median 18 μg/L) than LD (male's median 31 μg/L). HD had significantly higher results (male's median 150 μg/L). They were also more prone to processing errors with LC-MS/MS resulting in 29 samples failing to get a result. In addition, there was a higher variation of LC-MS/MS results at low levels. Females showed lower median values than males in all subgroups except hemochromatosis patients (male's median 26 μg/L, female's median 29 μg/L).
Conclusion: The overall correlation between both methods was good, showing that the studied ELISA-kit is a reliable method for quantification of Hepcidin-25. Close follow-up of precision should be considered. Results from ELISA had a tendency to show higher levels than results from LC-MS/MS, which became more prominent at high Hepcidin levels. This demonstrates the necessity of establishing local reference ranges. Considering ELISA's capacity to readily be set up in most laboratories it should become the routine method for quantification of Hepcidin.

T348

FAST AND EFFICIENT SAMPLE PREPARATION OF TOXIN-PRODUCING CYANOBACTERIA FOR qPCR ANALYSIS

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Background: Toxic cyanobacterial blooms are a serious risk to public health worldwide. Quantitative polymerase chain reaction (qPCR) is a practical tool for risk assessment and toxicological studies related to toxic cyanobacteria. Sample preparation is an important part of qPCR analytics – to obtain reliable results template loss during this step has to be minimized. We have developed an efficient method based on cell lysis by heating, which has been evaluated with four different cyanobacterial genera, including microcystin, saxitoxin and cylindrospermopsin producers.
Methods: Ten toxic and non-toxic strains of cyanobacteria belonging to the genera Anabaena, Cylindrospermopsis, Microcystis and Planktothrix were used in this study. Templates for qPCR from equal amounts of cells were produced using two sample preparation methods: 1) heat-treatment (80 °C, 10 min) of cells collected on fiberglass filters and 2) the standard phenol-chloroform extraction of DNA. Three qPCR methods were used to determine template yields; toxin-specific (mcyB for microcystin-producing cyanobacteria) and genus-specific (phycocyanin or RNA polymerase I genes for other toxin producers as well as non-toxic strains).
Results: In all the studied strains, the same amount of cells yielded either an equal (4/10) or higher (6/10) amount of template when using the simple heat-treatment method compared to the phenol-chloroform method. The biggest differences (up to 200-fold) were observed for Microcystis and Planktothrix. The average time to produce template from one sample was 25 minutes for heat-treatment and 10 hours for phenol-chloroform extraction.
Conclusions: The heat-treatment method improves template yields and allows for considerably faster sample preparation than phenol-chloroform-based DNA extraction. The number of steps needed to carry out the heat-treatment is minimal, and is likely to contribute to the increased template yields. The method is easy to use also in field conditions, and is well suited to both small and large scale studies, reducing the time needed to prepare samples for analysis.

T349
EVALUATION OF THE SERUM FREE LIGHT CHAIN (FLC) FREELITE™ ASSAY ON THE NEW SPAPLUS IMMUNOTURBIDIMETRY ANALYZER

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Background: Serum free light chain (FLC) assay has become a routine test available on a variety of chemistry analyzers. The International Myeloma Working Group (IMWG) has recommended to use this assay for monitoring oligosecretory myeloma and AL amyloidosis patients. Recent publications have shown several application limitations which may stem from the polyclonal nature of the assay antibodies. The assay lack of standardization may explain the non satisfactory harmonization between methods and should emphasis the importance of monitoring the patients by the same method.
Methods: We evaluated the performance of the serum FLC assay on the SPAplus immunoturbidimetry analyzer and compared the results to a Beckman-Coulter AU analyzer used in our lab.
Results: We analyzed 62 sera from out patient clinics and 3 external quality control materials. Regression analysis revealed good correlation between the two assays (kappa: y=0.91x-0.4, r=0.94, range: 3.6-112.9 mg/L, lambda: y=1.06x-1.5, r=0.98, range: 5.8-137.5 mg/L). Reproducibility varied from 1.6% to 4% and repeatability was less than 2.5% for both kappa and lambda fractions. Extremely high levels of free light chains could be correctly measured due to the SPAplus antigen excess detection ability. Serial dilutions showed mean recovery of 95% and 103% for the polyclonal and monoclonal kappa FLC. On the other hand, for the lambda FLC, increasing recovery was determined with increasing dilutions.
Conclusion: Laboratory staff should be aware of the lambda FLC non linearity although its clinical relevance for patient monitoring is not clear. Nevertheless, the SPAplus immunoturbidimetry analyzer is as an acceptable platform for the serum free light chain (FLC) Freelite™ assays.

T350
FIELD EVALUATION OF SPAPLUS SYSTEM FOR THE DETERMINATION OF FREE LIGHT CHAINS (FLC) IN SERUM

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Background: Measurements of serum immunoglobulin k and λ FLC and FLC ratio calculation are recommended for the evaluation of plasma cell disorders. Several practical issues for the analytical measurement of FLC have, however, been identified. Searching for a solution able to fulfil the performance goals for the effective use of FLC in clinical setting, we evaluated the suitability of SPAplus analyzer using Freelite reagents (both from The Binding Site) for FLC determination. Particularly, we compared the system performance with allowable goals for bias, imprecision (CV) and total error (TE) derived from biologic variation of FLC.
Methods: We evaluated the performance of SPAplus FLC using data collected during a six-month time period of routine use, employing two different reagent lots. The two-level (N and H) liquid SPAplus control material was used for bias estimate by comparing the obtained long-term experimental means (n=34, both levels) with the corresponding assigned values. The protocol for CV evaluation employed the liquid-frozen Bio-Rad Liquichek Unassayed Chemistry Control, measured in each performed run for a total of 29 runs. Inaccuracy was checked by results from three UK NEQAS exercises (system-specific (SPAplus) consensus value as reference). Goals (desirable/minimum quality levels) for bias, CV and TE were ±4.1%/6.1%, <4.0%/6.0% and ±10.7%/16.1% for κFLC and ±7.1%/10.6%, <3.5%/5.3% and ±12.9%/19.3% for λFLC, respectively. In addition, CV and TE for FLC ratio should be <2.3%/3.4% and ±7.7%/11.6%.
Results: Average cumulative bias was -6.0% (control N) and -6.2% (control H) for κFLC, and 4.3% (N) and 6.1% (H) for λFLC, respectively. Overall CV resulted in 10.8% for κFLC (mean 11.9 mg/L), 7.3% for λFLC (mean 13.6mg/L) and 8.8% for FLC ratio (mean 0.9). On EQAS evaluation all λFLC and two out of 3 results for κFLC were within the minimum allowable TE, while the FLC ratio achieved the minimum goal only in one exercise.
Conclusions: Considering our previous experience with other analytical systems, the SPAplus solution undoubtedly represents a significant step forward. A further improvement in measurement imprecision (priority) and method alignment is probably needed to fulfil the stringent analytical goals derived from biologic variation.

T351

INTEGRATION OF AN AUTOMATED SAMPLE PREPARATION WORKSTATION FOR THE ANALYSIS OF IMMUNOSUPPRESSANT DRUGS BY LC-MS/MS

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Background: For Research Use Only. Not For Use In Diagnostic Procedures. Liquid chromatography-tandem mass spectrometry technology provides laboratories with a powerful tool for robust, accurate, sensitive detection of a wide variety of analytes. Method automation reduces the possibility of human error at many different stages, including preparation of calibration standards, sample preparation, and data processing. The objective of this work was the automation of an LC-MS/MS method for the analysis of the immunosuppressant drugs Tacrolimus, Cyclosporine A, Sirolimus, and Everolimus, to eliminate human error, increase reproducibility, eliminate subjectivity during data processing, and save time.
Methods: An LC-MS/MS method for the analysis of immunosuppressant drugs was developed, making use of commercially available whole blood calibrators and controls. In addition to manual preparation, all steps of sample processing could be automated using a BioMek NXP platform. The sample preparation consisted of a simple protein precipitation using ZnSO4 solution. After centrifugation, the clear supernatant was injected directly onto the LC-MS/MS system. Samples were loaded in test tube format and the final samples were prepared in a 96-well plate format. The LC-MS/MS data acquisition, processing, and reporting were performed using the Cliquid® software.
Results: The reproducibility of the automated protocol versus manual protocol was assessed by preparing and analyzing replicates of each calibration standard. The measured %CVs were at least equivalent between protocols over the entire concentration range covered by the assay. The method displayed good linearity for all four immunosuppressant drugs, with R >0.999.
Conclusions: An automated sample preparation protocol has been developed for the analysis of four immunosuppressant drugs by LC-MS/MS, with performance better than or equal to the equivalent manual sample preparation.

T352

FULLY VALIDATED METHOD FOR RAPID AND SIMULTANEOUS MEASUREMENT OF SIX ANTIEPILEPTIC DRUGS IN SERUM AND PLASMA USING ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION TANDEM MASS SPECTROMETRY

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Background: Therapeutic drug monitoring (TDM) of antiepileptic drugs is very useful to establish optimal therapy regimes for individual patients and to study the variation in pharmacokinetics that occurs between individuals. Therefore, the development of a specific and sensitive method allowing the fast measurement of these drugs is of great interest in TDM. Here, we describe a simple rapid assay to measure the antiepileptic drugs lacosamide, lamotrigine, levetiracetam, primidone, topiramate, and zonisamide.
Methods: After the addition of internal standards (ISs) and protein precipitation of patient serum or plasma, 1 µl of supernatant sample was injected onto an Acquity UPLC system which was directly coupled to a Waters TQ tandem mass spectrometer. The chromatographic separation was performed on a 2.1 X 50 mm reverse phase column (Waters, Acquity UPLC BEH Phenyl, 1.7 µm). Elution occurred using a linear gradient of methanol and water, each containing 0.1% formic acid and 2 mmol/L ammonium acetate. Analytes were then ionized and detected by electrospray ionization mass spectrometry with multiple reaction monitoring. Runtime was 2.5 minutes per injection. Ion suppression was characterized using post-column infusion.
Results: The calibration curves of the 6 antiepileptic drugs were linear over the working range between 0.05 and 50 mg/L (r >0.99). The limit of detection (LOD), as well as the lower limit of quantification (LLOQ) of all drugs measured in the assay was <0.05 mg/L. The intraassay and interassay coefficients of variation for all compounds were <15% for very low concentration (0.1 mg/L) and <8% in the clinically relevant concentration range (>1.0 mg/L). Mean recoveries were between 85.0 and 110.7% for all drugs. There were no significant ion suppressions detected at the elution times of the analytes. The mean differences between serum and heparinized plasma values were less than 6% for the 6 antiepileptic drugs. All drugs were stable in serum at -20 °C, 4 °C, and even at RT for at least 2 months.
Conclusions: In summary, a specific and sensitive stable isotope dilution UPLC-MS/MS method was developed and validated for routine clinical monitoring of lacosamide, lamotrigine, levetiracetam, primidone, topiramate, and zonisamide.

T353
PERFORMANCE EVALUATION OF ELECTROLYTE MEASUREMENTS ON THERMO SCIENTIFIC INDIKO AND INDIKO PLUS CLINICAL CHEMISTRY ANALYZERS

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Background: Electrolytes help to maintain exchanges of osmotic pressure and have a central role in maintaining the normal distribution of water. Certain diseases can change the rate. Sodium, potassium and chloride are part of a screening malfunction of electrolytes, monitoring the ionic balance (acid or basic) states of hydration. Besides ISE measurements, the Indiko analyzers serve routine clinical chemistry tests and various dedicated specialty testing needs offering a complete system solution with the throughput of 200 photometric and 120 ISE tests/hour for Indiko and 350 photometric and 135 ISE tests/hour for Indiko Plus.
Methods: Electrolyte measurements in Indiko and Indiko Plus analyzers are made with ion selective electrodes (ISE) directly without any dilution of the sample. The measurement cell consists of sodium, potassium, chloride and reference electrodes. The measured potential between each ISE and the reference electrode is related to the natural logarithm of the ionic activity according to the Nernst equation. The changes in potential are developed across the ISE membrane / sample interface. Serum and Li-heparin plasma can be used as sample types. Calibrators used in the fully automated measurement are NIST traceable ready for use liquids.
Results: The measuring range is for sodium 100 - 200 mmol/L, for potassium 2.0 - 10.0 mmol/L and for chloride 60 - 150 mmol/L. The observed CV% for repeatability (within run precision) was 0.1% - 0.2% for Na (n = 84), 0.2% for K (n=84) and 0.2% for Cl (n = 84). The observed CV% for within device (total precision) was 0.8% for Na, 1.0% - 1.1% for K and 0.8 % - 1.1% for Cl. The method comparison studies were performed using Thermo Scientific Konelab PRIME 60i direct ISE methods as a reference.
Conclusion: The results demonstrate that ISE measurements can be done precisely and easily using Thermo Scientific Indiko and Indiko Plus clinical chemistry analyzers. Ion selective electrodes are maintenance free and they are easy to install.

T354
EVALUATION OF THE THERMO SCIENTIFIC INDIKO PLUS CLINICAL CHEMISTRY ANALYZER

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Background: Thermo Scientific Indiko Plus is a new bench top clinical chemistry analyzer, especially suitable for small and medium laboratories or as a back-up analyzer for bigger ones. It is intended for colorimetric and turbidimetric assays as well for electrolytes employing ISE technology. Throughput of the analyzer is maximum 350 photometric tests/hour. The Indiko Plus analyzer is a complete system including the instrument, system reagents, calibrators and controls as well the CE marked applications.
Methods: In this study we have evaluated the performance of one substrate assay Glucose (HK), one enzyme assay AST (IFCC) and one specific protein assay Immunoglobulin G (IgG). We have performed precision studies and method comparison studies for all of methods. The precision studies were made with three different Glucose and AST concentrations and with two different IgG concentrations. The method comparison studies were performed using Thermo Scientific Indiko clinical chemistry analyzer as a reference system.
Results: The observed CV% for repeatability (within run precision) was 0.5% - 0.7% for Glucose (n = 80), 0.3% - 0.8 % for AST (n = 20) and 1.2% -2.2% for IgG (n = 20). The observed CV% for within device (total precision) was 1.2% - 1.3% for Glucose, 1.2% - 1.6% for AST and 3.7% - 4.8% for IgG. In the method comparison studies the observed results showed excellent correlation for all the tested analytes between the evaluated and the existing routine analyzer.
Conclusion: The results in total demonstrate that the Indiko Plus is a precise, easy to use and reliable analyzer for routine biochemistry tests.

T355
NEW CK(IFCC)AND CK-MB ASSAYS FOR THERMO SCIENTIFIC INDIKO AND KONELAB CLINICAL CHEMISTRY ANALYZERS

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Background: Creatine kinase (CK) is an enzyme which consists of isoenzymes mainly of the muscle (CK-M) and the brain (CK-B). CK exists in the human body in dimeric forms as CK-MM, CK-MB and CK-BB. CK activity is elevated in many diseases, including those involving skeletal muscle, the heart, the central nervous system and the thyroid. CK-MB is present in low concentration in normal serum but it increases as a result of heart injury. Measurement of CK is used especially in conjunction with CK-MB for diagnosis and monitoring of myocardial infarction.
Methods: CK and CK-MB are two part liquid ready to use assays using the Thermo Scientific Indiko and Konelab clinical chemistry analyzers. CK is determined using IFCC recommended method. CK-MB reagent utilizes an immunoinhibition method employing monoclonal antibodies to the CK-M monomer. The activity of the non-inhibited CK-B monomer is assayed. The measuring range for CK is 10 –1000 U/l, up to 9000 U/l with automatic dilution, and for CK-MB 5–1000 U/l, up to 3000 U/l with automatic dilution.
Results: The repeatability (within-run precision) for CK is 0.6–1.8 % (CV; n=84), and for CK-MB 0.7–3.0 % (CV; n=84). The within device (total) precison for CK is 1.2–3.6 % (CV; n=84), and for CK-MB 2.0–5.4 % (CV; n=84). The methods were compared with the previous generation CK and CK-MB Konelab system methods (dry powder). Linear regression for CK was y (Indiko)=1.10x-4.7 and r=0.999, and y (Konelab)=1.07x-6.3, and r=0.999, n=95. Linear regression for CK-MB was y (Indiko)=0.99x-8.4 and r=0.996, and y (Konelab)=1.00x-10 and r=0.996, n=84. CK-MB results were slightly lower than previously probably because of the better immunoinhibition ability of the new method. On board stability time for both reagents is 21 days.
Conclusion: New liquid form CK(IFCC) and CK-MB reagents have a longer on-board stability than the dry powder format reagents. With these ready to use system reagents, CK and CK-MB analysis on Thermo Scientific Indiko and Konelab analyzers is quick and accurate.

T356
A NEW CALCIUM TEST BASED ON THE CHROMOPHORE NM-BAPTA

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Background: Calcium in serum must be measured with high accuracy and precision as its normal range is tightly controlled. The new test Calcium Gen.2 on Roche/Hitachi cobas c501 is presented. Compared to the preceding assay it shows improved stability, linearity and recovery of reference materials and reduced interference by magnesium and contrast media containing gadolinium.
Methods: The test uses the novel indicator NM-BAPTA on the automatic analyzer cobas c501 (Roche Diagnostics, Mannheim, Germany). Reagent 1 consists of a CAPSO buffered solution (at pH 10) of NM-BAPTA and reagent 2 of an EDTA solution. Firstly sample and reagent 1 are mixed to form a colored complex. Secondly reagent 2 is added. EDTA binds all calcium and discolors the calcium-dye complex. The difference in absorbance is measured at 340 nm. The method is traceable to standard reference material SRM 956 level 2 and is suitable for serum, plasma and urine. Each reagent lot is calibrated only once.
Results: The test is linear up to 5 mmol/L in serum and 7.5 mmol/L in urine. Typical within run and total precision are 1% CV in the normal range and 2 – 3% CV for very low samples (ca. 0.5 mmol/L). There is no significant magnesium interference up to 15mmol/L in serum and up to 60mmol/L in urine. Gadolinium containing contrast media do not interfere at therapeutic concentrations. Stability on board the instrument is 6 weeks without recalibration. The test shows no significant interference up to an I-index of 60 (60 mg/dL bilirubin), a H-index of 1000 (1000 mg/dL hemoglobin) and a L-index of 1000 (the L-index corresponds to turbidity, not to triglyceride concentration). The Passing Bablok regression of the method comparison to the previous calcium test on the same system is y =0.920x + 0.136 mmol/L. The serum based reference material SRM 956c level 2 is recovered in a range of 100 +-2%.
Conclusion: Compared to the preceding assay Calcium Gen.2 shows improved linearity and stability on board the analyzer without recalibration resulting in a more stable daily mean recovery. Due to the reduced interference to magnesium and to gadolinium containing contrast media results especially in urine become more reliable.

T357

HOMOGENEOUS SOLID PHASE HYBRIDIZATION ASSAY UTILIZING LANTHANIDE CHELATE COMPLEMENTATION: THE EFFECT OF LABEL MOIETY INTERCHANGE AND SPACER LENGTH

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Background: Homogeneous solid-phase hybridization assay is a wash-free proximity-based assay utilizing lanthanide chelate complementation technology. It consist of two oligonucleotide probes each carrying a part of the label, either the lanthanide ion carrier chelate (europium probe) or the light absorbing antenna ligand (antenna probe). A luminescent complex is formed by self-assembly when these two subunits are brought into close proximity by adjacent hybridization on the target sequence. We have investigated the effect of label moiety interchange and spacer length between the immobilized probe and substrate on assay performance using Pseudomonas aeruginosa heat shock protein gene groESEL as a model assay.
Methods: One of the complementary probes was immobilized via 3´ end onto a bottom of a microtiter well through a biotin-streptavidin linkage while the other was free in solution. For investigating the effect of label moiety interchange in immobilized and free oligonucleotide probes the immobilized probe was labeled either with europium or antenna and the solution phase probe with antenna or europium, respectively. In order to investigate the effect of the length of spacers up to 67 thymidines was introduced at the 3´ end of the immobilized antenna probe. The triethylene glycol (TEG) linker added additional 15 atoms into the spacer. After hybridization time-resolved fluorescence was measured by surface-scanning.
Results: A 60% signal increase was obtained with a 5 thymidine spacer (T5) compared to the probe containing the TEG linker only (T0). Signal was doubled with a 13 thymidine spacer (T13) and tripled with 67 thymidines (T67). Signal development rates were constant for up to 30 min during which the signals increased 23-41%, the largest increase occuring with the shortest spacers. After 30 min the signal development rate decreased 30% being constant for up to 420 min. Label moiety interchange between the immobilized and free probes didn't affect the signals.
Conclusions: A 13 thymidine spacer is sufficient for introducing enough spatial freedom for the probes in our assay. With longer spacers the signals can additionally be increased, but the increase is less drastic and the production costs of the long spacer probes increase remarkably.

T358

THE EFFECT OF THE SIZE OF UPCONVERTING PHOSPHORS IN HOMOGENEOUS ASSAY FOR ESTRADIOL

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Background: Upconverting phosphor (UCP) particles have the unique ability to convert low-energy infrared light into emission at visible wavelengths. This property enables autofluorescence free measurements, which makes UCPs ideal donors for homogeneous resonance energy transfer (RET) based assays. However, the performance of UCPs as donors in the assay depends on the size of the particle, which is affected by UCP synthesis method and different parameters used in the synthesis.
Methods: The effect of the size of UCP-particles was studied in upconversion resonance energy transfer (UC-RET) based assay for estradiol in which 25–250 nm sized UCPs were used as donors and Alexa Fluor 546 as an acceptor. UCPs were synthesized by co-precipitation or in organic oils. A functionalized silica layer was polymerized on the surface of UCPs and used for antibody conjugation. Upconversion luminescence and UC-RET-signal were measured with a modified plate reader equipped with an infrared laser diode as the excitation source.
Results: One of the requirements for RET is that the distance between the donor and the acceptor is below 10 nm. Whole volume of the particle produces upconversion luminescence, but only the parts of the particle that are in the required distance for RET can transfer energy to the acceptor. Thus, particles larger than 40 nm produced UC-RET signal-to-background ratios (S/B) less than 10, because of a high background signal originating from donor crosstalk and radiative energy transfer due to low surface-to-volume ratio. Particles smaller than 40 nm produced the most efficient energy transfer with S/Bs of 40–70. Even so, the smallest particles in this study (25 nm) produced the best S/B but the UC-RET-signal was relatively weak due to low upconversion luminescence.
Conclusions: The size of UCPs used as donors in a homogeneous assay for estradiol had a significant effect on assay performance. By reducing the size a bigger volume of the particle was in the required distance for RET and thus higher signal-to-background ratios were obtained.

T359
EVALUATION OF BECKMAN COULTER DXH800 HAEMATOLOGY ANALYSER

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Background: Our objective was to verify the analytical performance of Unicel DxH800, the newest generation haematologic analyser from Beckman Coulter, for routine CBC, 5-part WBC differential and NRBC count. The analyser is fully automated and uses individual cell volume, high-frequency conductivity and laser-light scatter to measure WBC differential and NRBC. The CBC analysis is based on Coulter Principle.
Methods: The analytical performance of the analyser was assessed for imprecision, linearity, carry-over and method comparison. Imprecision was studied by measuring three levels of QC material (Coulter 6C Cell Control) over five days in accordance to CLSI EP5-A2 guidelines. Linearity was determined using Coulter Linearity Control which consists of ten bottles of linearity material with different concentrations that span the assay. Carryover study was performed as per manufacturer's recommendation using whole EDTA blood (H) and diluent (L) in the following sequence H1 H2 H3 L1 L2 L3. The accuracy of CBC, WBC differential count and NRBC were compared with our current system, Sysmex XE-2100 using 82 patient samples.
Results: The total within-laboratory CV for all parameters ranging from 0.5% - 8.8% and were within manufacturer's specifications. Linearity was verified with recoveries of 98-102%. No significant carry-over was observed. The DxH800 provides accurate results for all parameters, which correlates well with Sysmex XE-2100.
Conclusions: The new generation automated haematology analyser, DxH800, showed satisfactory performance in method evaluation verification study.

T360
COMPARISON BETWEEN PERKIN-ELMER AND CHROMSYTEM VITAMIN D KIT ON TQ 5500 FROM AB SCIEX

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Background: Twenty-five hydroxy-vitamin D (25(OH) D) determination is now routinely prescribed in the Laboratory. Recently, different new methods have been available for this determination. Among them, LCMS/MS methods have emerged in some laboratories. However these methods are generally "home-brewed" and an important variability between them can be seen on different external quality controls, mainly due to a lack of standardization. Recently, Perkin-Elmer (PE) (Turku, Finland) and Chromsystem (CS) (Grafelfing, Germany) launched a standardised method for 25(OH)D determination on LCMS/MS. The aim of our study was to compare these methods on the AB SCIEX TQ5500 (Framingham, Massachusetts, USA) LCMS/MS to measure 25(OH) D3.
Methods: All the samples were treated according to our preanalyitical procedure: after sampling, they were spun at +4 °C at 3500G, aliquoted and kept frozen at -20 °C until determination. A method comparison was assessed with CS and PE for the measurement of the 25(OH)D3. We selected 110 remnant samples with 25(OH)D3 levels ranging from 1.6 to 136.7 ng/mL with the PE method to cover the range of usually values Slope and intercept were calculated using Passing and Bablock linear regression and we compared the methods with the Bland and Altman plots.
Results: For CS, the method is linear up to 250 μg/L, the LOQ is 3.6 μg/L, the intra-assay CV is <5% and the inter-assay is < 7%. For PE, the method is linear up to 314 μg/L, the LOQ is 3.4 μg/L, the intra-assay CV is <7.8% and the inter-assay is < 8.5%. On the whole range of measure (n=110), the regression equation is PE = 0.8521+0.9226 (CS) (95%CI of the intercept: (-0.0048;1.37) and 95% CI of the slope (0.89;0.95). The Bland and Altman plot does not show any bias between the two methods (mean difference CS-PE= -2.5 ng/mL) and the standard deviation of the mean is 3,98 ng/ml.
Conclusion: The performances of these methods are comparable on our new TQ 5500 from AB SCIEX. For now, there is no consensus on a "reference" method for vitamin D quantification. We notice only that the values obtained by CS are systematically a little bit lower than PE's values, especially for results below 20 ng/mL. However, we have no clear explanation for such behaviour.

T361

HOMOGENOUS HLA-DQA1*05 PCR ASSAY BASED ON SWITCHABLE LANTHANIDE FLUORESCENCE PROBES

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Background: Switchable lanthanide chelate complementation probe technology is a versatile tool for homogenous DNA detection assays due to low background fluorescence level and high specific signal generation. The complementation probes are able to detect low picomolar quantities of target DNA in a homogenous assay and enables remarkably high signal-to-background (S/B) ratio (max 300) in real-time PCR assay. The technology is based on two non-fluorescent oligonucleotide probes, one carrying a lanthanide ion carrier chelate and the other, a light absorbing antenna ligand. When the probes are hybridized into adjacent positions to the target DNA, a highly fluorescent lanthanide chelate complex is formed. Suitability of the technology for genotyping was studied developing complementation probes based homogenous PCR assay for the detection of HLA-DQA1*05 alleles used in assessing the risk for type 1 diabetes and celiac disease.
Methods: Probes were designed to detect DQA1*05 alleles according to two nucleotide adjacent polymorphism. One oligonucleotide probe was labeled with a nonfluorescent EuIII chelate and the other with an antenna ligand. The antenna probe contained the polymorphic site and was designed to hybridize only to the DQA1*05 alleles at the measurement temperature of 24 °C. Previously developed PCR primers were used and EuIII time-resolved fluorescence was measured after 40 cycle amplification. Performance of the assay was compared to the heterogeneous DQA1*05 PCR assay based on same primers testing 149 blood samples.
Results: The complementation probe based end-point DQA1*05 PCR assay correlated 100% with the reference assay. Samples with DQA1*05 allele (n=89) gave high signal-to-background ratio in average of 60.7 whereas the samples containing other alleles (n=60) yielded S/B in average of 1.5.
Conclusions: The developed DQA1*05 assay demonstrates the ability of the complementation probes to discriminate different alleles in closed-tube end-point PCR. Huge signal difference between DQA1*05 and non-target alleles led to definite results. Homogenous assay format is easy to use and eliminates the risk of PCR product contamination. Multiplexing should be possible using different lanthanide ions (TbIII, SmIII and DyIII) with suitable antenna ligands.

T362

PLASMA HOMOCYSTEINE MEASUREMENT BY KONELAB20XT : COMPARISON WITH IMMULITE2000 AND ION EXCHANGE CHROMATOGRAPHY (JEOL-AMINOTAC)

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Hyperhomocysteinemia is regarded as an independent risk factor for cardiovascular diseases. A wide range of methods are now available for total plasma homocysteine measurement. Lately, colorimetric assays adaptable to routine laboratory analyzers have been developed. We evaluated an enzymatic Diasys Homocysteine FS assay implemented on a biochemical analyzer Konelab 20XT (ENZ-Hcy) and compared its performance with our reference method, the ion exchange chromatography (IEC-Hcy) and an immunoassay (LIA-Hcy).
ENZ-Hcy is a tri-reagent assay in which free homocysteine produced is recycled to reinforce the signal. In the final step the decrease in NADH is measured by a colorimeter. In the IEC-Hcy method, homocysteine was measured by the amino acid analyzer Jeol-Aminotac after a two-step pretreatment: dithiothreitol reduction followed by sulfosalicylic acid deproteinisation. The LIA-Hcy method (Siemens-Immulite2000) is a competitive immunoassay using a built-in reduction step. 90 blood samples (lithium heparinate) collected from patients screened for hyperhomocysteinemia was used for comparaison studies. Within and between-run imprecisions were assessed not only in patient samples but also in commercial control samples. Test for linearity was performed on dilutions of 50 μmol/l L-homocysteine solution and a high concentration plasma sample (homocysteine=45 μmol/l). The within-run coefficient of variations (CVs) for medium and high levels were < 1.5% for ENZ-Hcy and <6% for LIA-Hcy. The between-run CVs were <6.5% for ENZ-Hcy and <9.5% for LIA-Hcy. ENZ-Hcy method is not linear in the concentrations around the upper reference limit of 15μmol/l and the correlation with IEC-Hcy method is better using non-linear regression (second degree polynomial curve, r^2>0.99) than linear regression (r^2=0.9685). In contrast LIA-Hcy method is linear and well correlated using Deming's linear regression (r^2>0.99). Using a cut-off value of 15 μmol/l to define hyperhomocysteinemia we report only one discrepancy for LIA-Hcy method and 9 for ENZ-Hcy method over 47 plasma samples. The Diasys Homocysteine FS assay adapted to Konelab 20XT is short of linearity and accuracy. Despite a low imprecision this method is not appropriate for screening mild hyperhomocysteinem

T363
HOMOGENOUS QUANTITATIVE ANALYSIS OF HUMAN PARATHYROID HORMONE BY USE OF MAGNETIC MARKERS AND SQUID MAGNETOMETER

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Background: Immunoassays are one main detection system used in the field of clinical chemistry. Recent developments of a new detection method utilizing a magnetic marker and magnetic sensor have enabled rapid and sensitive immunoassay without the need for bound/free (BF) separation. Recently, we successfully performed a quantitative evaluation of some proteins (immunoglobulin E, interleukin 8) in phosphate buffer, human serum and human hemolysate, without BF separation. In this time, we report the quantitative analysis of human parathyroid hormone (pTH) using this magnetic system.
Methods: Newly-synthesized conjugated streptavidin was used as the magnetic marker for quantitative analysis. Target antigens (human pTH) were catched to first anti-pTH antibodies immobilized to plate and were biotinylated by secondary antibodies. A superconducting quantum interference device (SQUID) sensor detected the magnetic fields from markers fixed to pTH by the sandwich method. Magnetic signals from unbound markers were nearly zero due to Brownian rotation.
Results: Our magnetic immunoassay could detect 10 pg of hormone (pTH) in phosphate buffer within 60 min. This technique has considerable potential for use as a biological immunoassay. Also, this homogeneous immunoassay could quantify three hundred cells from the fungus Candida albicans in phosphate buffer.
Conclusions: The present study demonstrates the ability of magnetic markers for measuring biological targets without BF separation. We newly indicated the useful for detection of hormones as well as proteins. This detection system has great potential for use as the next generation's analytical system.

T364
LANTHANIDE CHELATE COMPLEMENTATION FOR SENSITIVE HOMOGENEOUS PROTEIN DETECTION

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Background: Fluorescence-based homogeneous assays typically have limited sensitivity compared to heterogeneous assays because of interfering background signal. In a novel lanthanide chelate complementation technology the fluorescent lanthanide chelate is divided into two separate non-luminescent moieties: a lanthanide ion carrier chelate and a light harvesting antenna ligand. The fluorescent chelate complex is formed only when these two label moieties are brought together via two specific binding events. This results in a minimal background signal in the homogeneous assay enabling low limit of detection. In this study the feasibility of lanthanide chelate complementation for sensitive homogeneous protein detection was evaluated by using cardiac troponin I (cTnI) as a model analyte.
Methods: Two cTnI specific antibody fragments (Fabs) were conjugated to separate oligonucleotides. In the assay these Fab-conjugated oligonucleotides were hybridized with complementary signal oligonucleotides labeled either with Eu3+-carrier chelate or antenna ligand, thus forming two oligonucleotide complexes with Fab in one end and the label moiety in the other. In addition the signal oligonucleotides contained a short complementary terminal signal sequence. In the presence of cTnI the simultaneous binding of the Fabs brings the oligonucleotide complexes in close proximity and enables annealing of the signal sequence, which further results in the formation of long-lifetime fluorescent chelate complex. The total assay time was 6 min and the fluorescence was measured in time-resolved mode.
Results: The detection limit of the homogeneous cTnI-assay (0.37 ng/mL) was of the same order as in the heterogeneous reference assay based on the same Fabs (0.26 ng/mL). The linear range of the homogeneous assay was slightly over 2 orders of magnitude, which can potentially be further improved by shortening the signal sequence. The highest observed signal-to-background ratio was 50.
Conclusions: Here we have demonstrated that lanthanide chelate complementation technology enables sensitive and rapid method for homogeneous protein detection as a result of minimized background signal.

T365

MEASUREMENT OF IMMUNOSUPPRESSANTS BY LC-MS/MS: WORKFLOW OPTIMIZATION TROUGH AUTOMATED PROCESSING OF WHOLE BLOOD SAMPLES

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Background: Immunosuppressant TDM is a crucial requirement in providing optimal patient care following organ transplantation. LC-MS/MS is an efficient technology for routine determination of immunosuppressant in whole blood, due to its high specificity and sensibility. However, time-consuming manual sample preparation remains a significant limitation of this technique. Aim of the present study was to develop an automated sample-preparation protocol for quantification of sirolimus (SIR), everolimus (EVE) and tacrolimus (FK-506) by LC-MS/MS using a liquid handling platform Tecan Freedom EVO 100.
Methods: Six-level commercially available blood calibrators for SIR (0.2–29.0 µg/L), EVE (0.2–23.2 µg/L) and FK-506 (0.2–23.1 µg/L) were used for assay development, while four QC materials with different concentrations and three blood samples from patients under immunosuppressants treatment were employed for imprecision evaluation. Barcode reading, sample resuspension, transfer of whole blood samples into 96-well plates, addition of internal standard solution, mixing, and protein precipitation were performed by liquid handling platform. After plate filtration using vacuum block Te-VacS, the deproteinized supernatants were submitted to SPE on-line, using column switching prior to analysis. The only manual step within the entire process was the transfer of the well plate to the LC autosampler.
Results: Calibration curves were linear throughout the selected ranges. The intra- and inter-assay CVs (<16%), the LLOQ (0.2 µmol/L) and accuracy (bias%<10) for all analytes were highly satisfactory. Very good agreement between the results obtained after manual and automated sample preparation was observed (n=390, r^2=0.92, P <0.001). In daily routine (about 100 patient samples) a typical comprehensive total turnaround time is less than 6 hours (40 min for sample preparation using the automated protocol and 5 h for chromatographic analysis).
Conclusions: Our findings indicate that the proposed analytical system is suitable for routine analysis, since it is straightforward and precise. Furthermore, it minimizes substantial manual workload and the risk of mistakes in the quantification of whole blood immunosuppressant concentrations compared to conventional methods.

T366

ANALYTICAL VALIDATION AND DEVELOPMENT OF THE 25 OH VITAMIN D ASSAY FOR THE NEW UHPLC-MS/MS METHOD

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Background: 25-Hydroxy vitamin D (25-OH D) is an important marker for several diseases. Automated immunoassays from various manufactures have been available but they were unsatisfied. We developed and validated the new ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS/MS) method for the serum quantitative determination of 25-OH D2 and D3 .
Methods: Spiked serum bovine albumin samples were used in both method development and validation. Serum (150 µL) adding with 20 uL of internal standard (IS) were treated with zinc sulfate and acetonitrile for removing protein. Both 25-OH D2 and D3 were analyzed by UHPLC-MS/MS technique (Agilent Technology, Wilmington,DE,USA) which the chromatographic system consisted of an Agilent 1290 LC system with a 3.0x50 mm, 2.7 µm Poroshell 120 EC-C18 column and a 4.6x12.5 mm, 5 µm Zorbax Eclipse Plus-C18 guard column. A gradient of mobile phase A (water/ammonium formate (5M)/formic acid, 99.8/0.1/0.1,v/v/v) and mobile B methanol/ammonium formate (5M) / formic acid (99.8/0.1/0.1, v/v/v) was used at flow rate of 0.4 mL/min for pump A and 0.5 mL/min for pump B. The 3 µL injection volume was set. The Agilent 6460 Triple Quadrupole MS coupled to electrospray ionization (ESI) source was used. We validated the method in terms of linearity of range, limit of detection (LOD), limit of quantitation (LOQ), %recovery and precision.
Results: The mass (m)/charge (z) transitions of 413.3>355.3 (25-OH D2), 401.3>365.2 (25-OH D3), 416.3>358.3 (IS of 25-OH D2) and 404.3>368.2 (IS of 25-OH D3) were detected in the MRM and can be achieved at the time between 3.2 to 3.8 minutes. Total run time was 4.5 minutes per sample. Chromatogram of all analyzes showed symmetry peaks. The linearity over the range of 5-100 ng/mL with r^2 = 0.999 for both. The LODs and LOQs were 1.61,5.37 and 2.01,6.69 ng/mL for 25-OH D2 and D3, respectively. Recovery of 25-OH D2 and D3 from serum samples spiked with 10,30 and 80 ng/mL ranged between 94.83% to 100.76%. Inter and intra assay percentage relative standard deviation (%RSD) were less than 10%.
Conclusions: This new UHPLC-MS/MS method with a simple of sample preparation provides a rapid, accurate, precise and suitable assay in routine use as a useful tool for evaluation of individual vitamin D status.

T367

AUTOMATIC EVALUATION OF DNA DOUBLE STRAND BREAKS: WHEN RESEARCH MEETS DIAGNOSTIC LABORATORY

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Background: The number of DNA double-strand breaks (DSB) increases if a cell is exposed to ionizing radiation, chemotherapeutic substances or free radicals. H2AX is a histon protein, around which DNA wraps itself in the nucleus.In case of DSB,the histone gets phosphorylated (γ-H2AX) on a serin residue.γ-H2AX serves as highly sensitive marker for double-strand breaks and can be made visible by a specific antibody that is linked to fluorescent dye.
Aim: indirect immunofluorescent evaluation of γ-H2AX foci in cell nuclei is available as a complete manual procedure, but it is cost and time consuming and poorly reproducible. The method requires isolation and counting lymphocytes from whole blood, followed by immunostaining with primary and secondary antibodies prior to final microscopic evaluation of nuclear foci. In order to improve standardization of the method , taking advantage of the technology available in our diagnostic laboratories, we replaced the two manual steps requiring subjective microscopic evaluation (cell counting and analysis of nuclear foci)with automatic procedures.
Methods: the evaluation was conducted using peripheral lymphocytes of 5 healthy subjects .Isolated lymphocytes were counted manually and also with two automated differential counters: Beckman Coulter LH 780 and Sysmex XE-2100 .The measurement of γ-H2AX foci was performed on the AKLIDES platform and the associated software, developed for the automated analysis of cell-based immunofluorescence assays.
Results: the calculation of the lymphocytes with automated counters was more accurate and faster than those manual. The analysis on AKLIDES system with sensitive γ-H2AX signals yelded several quantitative parameters that characterized the degree of cell damage:number of cells (from 101 to 115), average number of foci per cell (from 0,000 to 0,049), percentage of cells with foci –total damage (from 0.000 to 3,883),standardizing analysis of γ-H2Ax foci in lymphocytes.
Conclusions: Our preliminary evaluation on a few healthy subjects where cell damage is light, leads to support the usefulness of automation . This improves standardization and enriches both quantity and quality of the obtained data and makes possible transferring it in clinical application areas.

T368

DETERMINATION OF INFLIXIMAB TROUGH LEVELS (IFX-TL) AND ANTIBODIES TO INFLIXIMAB (ATI) IN INFLAMMATORY BOWEL DISEASE

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Background: Anti-TNF blocker (Infliximab,IFX) is approved and used in the treatment of Crohn's disease (CD) and ulcerative colitis (UC). Therapeutic algorithms based on drug monitoring combined with anti-drug antibodies detection were proposed. Aim of this study was to compare IFX trough level (IFX-TL) and antibodies to IFX (ATI) with two different commercial kits. Furthermore, we evaluate association between IFX-TL and better disease outcomes and between ATI and adverse drug reactions.
Methods: 46 inflammatory bowel disease outpatients (27 CD and 19 UC;15 with active disease and 31 with quiescent disease), undergoing IFX i.v. dosing, was prospectively enrolled. Serum samples were taken before planned IFX infusion and detailed clinical history was collected. Two different ELISA-sandwich tests were used in order to determine IFX-TL and ATI concentrations: Promonitor IFX Determination of Drug and Anti-Drug Antibodies Concentration (Menarini) and TNF Blocker Monitoring/Antibodies against TNF Blocker (ImmunDiagnostik).
Results: 36 of 46 patients were tested, until now, with both methods. Good correlation (Spearman's rho 0.935, $p < 0.0001$) and discrete agreement ($y = 1.68x + 0.619$) between IFX-TL methods was found. As regards ATI detection (3/36 cases, 8%), both assays showed similar results, correlated with loss of clinical response to treatment. ATI levels determined with both Promonitor and ImmunDiagnostik methods resulted significantly associated with the degree of disease (OR 18.053, IC95% 0.85-383.93, $P < 0.05$ and OR 5.273, IC95% 0.84-33.01, $P < 0.05$, respectively). Regarding the results obtained with Promonitor, median IFX-TL in CD and UC patients were 1,5 and 4,8 μg/mL, respectively, while considering the results obtained with ImmunDiagnostik, median IFX-TL in CD and UC patients were 3.51 and 5.6 μg/mL respectively.
Conclusions: The results show similar performance for the two different assay methods. Measurement of IFX and ATI proves to be a useful tool to monitor IFX therapy, though clinical implications are still matter of debate before these tests can be proposed for routine clinical practice. Consolidation of results on a larger cohort of patients is warranted to confirm and extend the obtained results.

T369

A COMPARISON OF TWO DIFFERENT LABORATORY METHODS FOR DETECTION OF IGF-1

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Background: Insulin like growth factor I (IGF1) is also called Somatomedin C. Analysis of IGF1 in serum has become an integral component in the diagnosis of growth hormone related disorders and it is suggested as the most reliable component for monitoring the success of therapeutic interventions in acromegaly. There are no international accepted guidelines for the related age's ranges of results. The aim of this study is to compare two different laboratory methods for the evaluation of IGF-I serum levels.
Methods: Serum IGF1 has been measured in 44 samples of people with age between 15 and 80 years. All samples were analyzed by two different assays IGF1 Diasorin Liason and IGF1 IMMULITE 2000. The first is an automated 2-site sandwich immunoassay with a chemiluminescent detection (calibrators 1st WHO international Standard IGF1 NISBC code: 02/254; linearity between 3 and 1500 ng/mL, CV intra-assay < 4.4%, CV inter-assay <8.5%, hook effect >11.000 ng/mL) It is unknown any interferences. IGF1Immulite 2000 (Immulite 2000, Diagnostic Products Corp, Los Angeles, CA) is an automated 2-site sandwich immunocemiluminescent assay (calibrators WHO international Standard 87/518, sensitivity 25 µg/L, intra and inter-assay CV is <8). It is unknown any interferences.
Results: All 44 samples were measured by both two methods. We have registered a totally concordance by two system and the procedure was conducted in double-blind way.
Conclusions: No differences in IGF-1 levels was observed and there is an excellent correlation in order to give an equal diagnostic information to patients. So, to reduce the condition linked to an inappropriate detection of values of IGF-1 such as: an unknown subclinical thyroid dysfunction, use of medications like contraceptive drugs, and finally the body mass index of subjects, it is important to adopt the same calibrator in different system and it is desirable to create reference values of different commercial assays and for different ethnic groups. In our laboratory the attenction is focused, after these preliminary data, to collect all results for a possible match with the manifacturer's guidelines to observe if, in our population, there are differences.

T370

PERFORMANCE EVALUATION OF A NOVEL FULLY AUTOMATED HAEMATOLOGY ANALYZER MINDRAY BC-6800 (MEDICAL SYSTEMS S.P.A GENOVA-ITALY)

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Background: An accurate evaluation of analytical performance of a new hemocytometer is necessary before it can be introduced for routine testing. The aim of this study was a preliminary evaluation of complete blood cell (CBC) count on Mindray BC-6800, now available on the Italian market.
Methods: The imprecision of the BC-6800 was assessed according to the Clinical and Laboratory Standards Institute (CLSI) document EP5-A2 and haematology analyzers evaluation guidelines. Imprecision within-run was evaluated including 10 inpatient random samples from several wards of Rho Hospital (Milan), collected with various values of parameters and assayed for 20 times. For imprecision between-run three control materials were assayed in duplicate for 20 consecutive working days. The results were expressed as coefficient of variation, CV. All parameters of the Complete Blood Cell Counts (White blood cells,Red blood cells,Platelets, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscolar Haemoglobin Concentration and Red Distribution Width (WBC, RBC, PLT, Hb, Ht, MCV, MCH, MCHC, RDW) were investigated. The background check and carry over (in triplicate) in autoloading mode and open vial mode were also investigated, running a high concentration sample (high control) and then a low concentration sample (diluent). Throughput was evaluated by Chronometric measurement of the time passed from starting the instrument with the first sample to the last results produced on the 100th sample, performing all parameters.
Results: Optimal imprecision both in within-run and between-run was observed for all the parameters tested, with coefficients of variations (CVs) always below 3%, except for platelet count within run(4%). The blank count and the carry over demonstrated a performance (0.02% and 0.08% respectively) always under requirements. The throughput was approximately 120 tests/h processing 8 parameters, 93 tests/h for all parameters including reticulocytes and erythroblasts.
Conclusions: The preliminary evaluation of CBC on Mindray BC-6800 suggests that this novel hemocytometer may be suitable for routine hematological analysis of most blood specimens, including those of patients with haematological disorders

T371

CORRELATION BETWEEN A NEW FULLY AUTOMATED HEMATOLOGY ANALYZER MINDRAY BC-6800 (MEDICAL SYSTEMS S.P.A.) AND SYSMEX XE2100 ANALYZER (DASIT S.P.A.)

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Background: The aim of this study was a correlation between a complete blood cell (CBC) count on Mindray BC-6800 (Medical Systems S.p.A.) a novel hematological analyzer and our analyzer in use (Sysmex XE2100), looking for a new hematology analyzer that would allow quality results, sample stability performance, improvement of slides review rate, and efficiency

Methods: A total of 100 blood specimens were selected from inpatients of Garbagnate Milanese Hospital (Milan). Fresh samples of peripheral blood (<6 hours from collection), collected in Vacutainer tubes (Becton Dickinson) containing tripotassium EDTA–anticoagulant and stored at room temperature, were analysed both in XE2100 analyzer and BC 6800, for all parameters. Blood smears were evaluated and compared also with results of two instruments. For each smear, 2 independent technologists performed a 200-cell WBC differential according to the H-20 NCCLS guidelines. The comparison of results between BC 6800 and XE2100 for CBC count or manual microscopy for WBC differential, was assessed by Pearson's correlation. The bias [95% confidence interval (CI)] was calculated by means of the Bland-Altman analysis.

Results: The comparison of WBC, RBC, PLT counts and Haemoglobin between BC-6800 and XE2100 produced limited biases, i.e., 0.21x109/L (95% CI −0.10 to 0.52x109/L) for WBC, −0.15x1012/L (95% C −25 to −0.04x1012/L) for RBCs, 12 (95% CI 5 to 19x109/L) for PLT, and –2.1g/L (95% CI −2.5 to −1.7 g/L) for Hb. We have found excellent agreement and limited bias with the current gold standard for WBC differential (i.e., manual microscopy performed according to the CLSI guidelines) after analysis of 100 specimens with mean biases of 0.21x109/L (95% CI 0.05 to 0.45x109/L) for neutrophils, 0.14x109/L (95% CI −0.28 to 0.00x109/L) for lymphocytes, 0.25x109/L (95% CI 0.07 to 0.47x109/L) for monocytes, 0.03x10/L (95% CI 0.01 to 0.06 x 109/L) for eosinophils and 0.02 (95% CI −0.04 to 0.00 x 109/L) for basophils.

Conclusions:The preliminary correlation between Mindray BC-6800 and XE2100 analyzer suggests that this novel hemocytometer, for quality results, sample stability performance, slide review rates and efficiency may be suitable for routine hematological analysis.

T372

COMPARISON OF TWO EXTRACTION DEVICES FOR DETERMINATION OF FAECAL CALPROTECTIN ON IMMUNOCAP 250

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Background: Calprotectin is an acute-phase protein used in the assessment of inflammatory bowel diseases (IBD), such as Crohn's disease and Ulcerative Colitis. The aim of our study was to evaluate the performance of two different extraction devices for detection of faecal calprotectin (Roche vs. Thermofisher) o n the Phadia Immunocap 250.

Methods: We analysed 105 clinical samples, with a great diversity of stool form and covering a broad range of calprotectin levels (0-3112 μg/g). Within- and between-run precision of ten different extractions of the same sample were evaluated for both devices. According to the weighed stool captured with each device, the theoretical and expected (weight-corrected) concentration of calprotectin was calculated and compared with the measured value on Immunocap 250. Correlations of both devices over the whole measuring range (0-3112 μg/g) as well as in the low range (0-600 μg/g) were performed.

Results: Within-run coefficients of variation for the Roche (2,69%) and Thermofisher (3,01%) device were excellent. Acceptable between-run precisions were obtained; 14.25% (Roche) and 10.77% (Thermofisher). Pearson correlation coefficient of calprotectin levels obtained with both devices was 0,92 (intercept 0,27; slope 0,91). Comparison of measured and calculated value for each device showed a better correlation for the Roche than for the Thermofisher device; 0,99 (intercept - 6,75; slope 1,07) and 0,80 (Intercept -3,82; slope 1,98) respectively. Correlations between calculated and measured calprotectin levels in the low measuring range (<600 μg/g) were 0,99 (intercept -2,07; slope 1,09) with Roche and 0,45 (intercept 11,94; slope 1,92) with Thermofisher device.

Conclusion: We found discrepant results for the Thermofisher extraction device between measured and expected (weight-adjusted) values on Immunocap 250. Nevertheless, correlations for the Roche device were excellent. Enormous variations in weight with the Thermofisher device resulted in poor correlations between measured and calculated calprotectin levels, more specifically in the low range, and attributable to the different stool forms which were extracted. We therefore do not recommend the Thermofisher device to extract calprotectin for measurement on Immunocap 250.

T373

PROSTATE CANCER URINE PROTEIN PROFILING BY MALDI-TOF/MS: NORMALIZATION, REPRODUCIBILITY AND PRELIMINARY RESULTS

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Background: Only few studies have addressed MALDI-TOF/MS analytical variability for the urine matrix. We evaluated intra-(CVw) and inter-assay (CVb) variability of MALDI-TOF/MS urinary profiling using internal standard (IS), relative intensity (RI) and total ion current (TIC) normalizations. Results were applied on prostate cancer (PCa) protein profiling.
Methods: Fresh urine from 10 healthy subjects were pooled, aliquoted and frozen at -80 °C. To estimate CVb, every day and for 14 days one aliquot was thawed, dialyzed at 4 °C (1kDa) before MALDI-TOF/MS analysis (Bruker Daltonics). For CVw one aliquot was thawed, dialyzed and then spitted in 20 new aliquots, which were independently analysed. Before dialysis, each urine aliquot was added with a 1589.9 m/z peptide at 12 pmol/µL (IS). Flex Analysis was used for baseline subtraction and peaks detection. Peak intensities were divided by the intensity of the IS, the intensity of the most abundant peak (RI) or by TIC, obtained by averaging all spectra's TIC. 178 urine samples (106 Reference subjects, 72 PCa patients) were dialyzed, analysed by MALDI-TOF/MS and normalized.
Results: In a range of 1050 to 4000 m/z, we identified from the analysis of all 14 spectra for the CVb a total of 134 peaks, and a total of 81 peaks from the 20 spectra for CVw. With the IS, RI, TIC normalization, the mean CVb% [95%CI] were: 228.9 [214.1-243.6], 158.3 [137.0-179.5], 156.6 [135.3-177.8], while CVw% [95% CI] were: 199.9 [171.9-227.8], 151.0 [116.7-185.2], 149.4 [115.1-183.7], respectively. We evaluated, for each normalization method, whether a detection cut-off limit might improve CVs by checking a 0.2% to 5% range values. The best combination between CVs and the number of detectable features were 4%, 2.5% and 0.5 % for the IS, RI and TIC respectively.TIC performed better, being the mean CVb% 39.0 [33.6-45.2] and the mean CVw% 36.9 [31.1-42.8], respectively. On applying TIC normalization at the 178 collected spectra from the protein profiling study, 2 features (m/z 1161 and 2016) were statistically different between References and PCa patients.
Conclusions: For the MALDI-TOF/MS analysis of urine, TIC normalization perform better. Preliminary results from protein profiling identified 2 possible interesting features for PCa diagnosis

T374

STUDY OF THE INTERCHANGEABILITY OF NT-PROBNP RESULTS IN TWO ANALYZERS

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Background: BNP is a natriuretic peptide. In patients with left ventricle dysfunction its levels increase like those of the biologically inactive prohormone, in this process the proBNP splits into the biologically active BNP and the N-terminal fragment (NT-proBNP). The NT-proBNP is used in the differential diagnosis of dyspnea (cardiac or respiratory origin), helping in the diagnosis and prognosis of cardiac insufficiency. Our aim was to verify the interchangeability of the results obtained by means of two analyzers.
Methods: We determined the values of NT-proBNP in an analyzer Cobas e-411 (method in use) (immunoelectro chemiluminiscent assay) and in an Immulite 2000 (method in study) (immunochemiluminiscent assay). 102 patients' samples were processed in both analyzers. The origin of the patients was: cardiology (82%), internal medicine (13%), pneumology (3%), urology (1%) and emergencies (1%).
The results obtained were compared using the Spearman's correlation coefficient and the Passing-Bablock concordance test. Besides, the patients were classified according to their diagnosis of cardiac insufficiency, and ROC curves were performed for each analyzer. The statistical analysis was performed with the statistical program MedCal®.
Results: We obtained a correlation coefficient of 0.972 (CI 95 %: 0.959-0.981). The straight line of regression was obtained with arranged in the intercept: -37.7904 (CI 95%: -52.5302 to -23.6813) and slope: 0.9469 (CI 95%: 0.8966 to 0.9994). By means of ROC curves we obtained for diagnosis of cardiac insufficiency an area under the curve (AUC) for the method in use of 0.742 (CI 95%: 0.640-0.827). With a sensitivity of 82% and a specificity of 56% for a cutoff of 683 pg/mL. The AUC for the method in study was 0.727 (CI 95%. 0.624-0.815), with a sensitivity of 91% and a specificity of 47% for a cutoff of 361 pg/mL.
Conclusions: The comparison between the equipments provided a good correlation, but the values obtained in both analyzers are not transferable. In spite of this, the two have a good diagnostic capacity for cardiac insufficiency, though it is necessary to consider different cut-offs.

T375
EVALUATION OF A COMMERCIAL KIT FOR STEROIDS DETERMINATION BY LC-MS/MS

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Background: Steroids and their precursors have traditionally measured by immunoassay. These methods ensure adequate sensitivity but are strongly affected by interfering compounds. Analysis of steroids by LC-MS/MS offers much improved specificity and therefore sensitivity. The analysis of more steroids simultaneously, possible using mass spectrometry, can be useful to the clinician. In this work we evaluated a commercial kit by Perkinelmer for the simultaneous analysis of ten steroids (Aldosterone, Cortisol, Dehydroepiandrosterone sulfate, Corticosterone, 11-Deoxycortisol, 4-Androstene -3,17-dione Testosterone, 17 - a - Hydroxyprogesterone, Dehydroepiandrosterone, Progesterone) in serum, by liquid chromatography tandem mass spectrometry (LC-MS/MS).
Methods: The kit use a combined solvent extraction and protein precipitation method. Analyses were carried out using a ThermoFisher Scientific TSQ Quantum Access triple quadrupole mass spectrometer operating with atmospheric pressure chemical ionization in the positive mode for nine analytes and in negative mode for aldosterone. Separation was achieved, within 15 min, with a gradient elution with a Phenomenex Luna C8 (100x2.1 mm, 3μm) column maintained at 40 °C. MS conditions were optimized using the tuning solution, according to kit instructions. Two transitions were monitored for each analyte/internal standard.
Results: The method, used with our entry-level instrument, shows an adequate sensibility for all the steroids except for aldosterone. Calibration curves were linear (for all analytes except for aldosterone) in the calibration ranges. Inter and intra-assay imprecision, obtained by measuring in replicate (n=6) the QC solutions on the same day and in duplicate on seven different days, were lower than 10%.
Conclusions: The kit is suitable for clinical evaluation of all steroids. Only for aldosterone, sensitivity is not adequate because of the instrument characteristics. Although MS methods are time-consuming, and require skilful personnel, they have the specificity, imprecision, and sensitivity necessary for the reliable measurement of steroids, giving to the clinician a powerful diagnostic tool especially when a complete profile was provided.

T376
ION CHROMATOGRAPHIC-AMPEROMETRIC DETERMINATION OF IODIDE AND TOTAL IODINE IN URINE

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Background: The determination of total urinary iodine is a valuable tool to diagnosing iodine status before radioablative treatment of patients with differentiated thyroid carcinoma. The classic colorimetric method for detecting iodide is known as the Sandell-Kolthoff. In this reaction Ce(IV) is reduced by As(III), and iodide is a specific catalyst. Total iodine can be measured in urine in the same way by oxidative digestion and reduction procedure, before applying the classical colorimetric method.
We evaluated the ion chromatographic (IC) determination of total iodine in urine with electrochemical (EC) detection at the silver electrode after oxidative digestion and subsequent reduction. In order to obviate to the use of As(III), suspected as being unsafe, we used thiosulfate as the reducing agent. Iodide assay was performed on simply diluted urine.
Methods: Isocratic ion chromatography using a AS4A (Dionex) anion separator and a neutral phosphate buffer as the eluent. EC potential at -0.07 V vs SHE (Decade II, VT03 Ag flow cell, Antec, USA). Total Iodine: Urine is digested at 100°C with ClO3/ClO4 and then reduced with thiosulphate. 2 μL of mixture are injected. Iodide: 2 μL of tenfold water-diluted urine are injected. Run time: 8 min.
Results: Stability of Ag Electrode: electrode response was stable after hundreds urine assays (losses of some 5% could be observed after 400 runs).
Sensitivity: LOD: 5 μg/L, LOQ: 10 μg/L. Detection Selectivity: the symmetric peak obtained when urine samples were analyzed proved high specificity. Common reductants (cysteine, Fe (II), SCN-) do not interfere. Diagnostic use: Urine concentrations exceeding 50 μg/L correlate well with those obtained by using the colorimetric method. Imprecision (<4%) and accuracy (recovery 100%) made the procedure better than the colorimetric one.
Cost: This HPLC-EC procedure uses stable solid-phase electrode. Very few sample amounts are injected (0.2 μL of urine) i.e, very long column life. No further consumables are required. In summary it seems to be very cost-effective.
Conclusions: This analytical procedure is a simple, rapid, cost-effective tool for the assessment of iodide and total iodine in urine. The electrode response is stable, no interferences have been observed, no unsafe substances are required.

T377
BILE ACIDS INTERFERENCE IN PSEUDOCHOLINESTERASE DETERMINATION ON ROCHE COBAS 8000 SYSTEM

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Background: Bile Acids and Pseudocholinesterase (CHE) measurements in serum turn out to be overall useful as tests of liver function.
Methods:The assays of these molecules, in our laboratory, are carried out on Cobas 8000 system (Roche Diagnostics), composed by two lines. CHE is tested both on Cobas C502 of CC line, and on the second C502 line, named CCE, where are also determined bile acids, cardiac and sepsis markers. It is noteworthy that reagents used for Bile Acids determination are provided by Sentinel Diagnostics, while reagents used for CHE detection are supplied by Roche Diagnostics. Both analytes are subjected to internal quality control (IQC) previously carried out with controls provided by Roche. Actually, according to current guide lines IQC is performed using multiparameter third party control sera , at two concentration level (ChemGol1 and ChemGol2, BIO-DEV, Milan).
Results:From the observation of Levey-Jennings charts we noticed an increase of violation of Westgard rules only in the controls referred to CCE line. For this reason we proceeded to a general recognition of methods performed. It is shown, CHE determination immediately follows that of Bile Acids. This led to the hypothesis of analytical interference supported by chemical composition of reagents used for Bile Acids determination . In fact the reagent 1A contains Na3PO4 that added to pyrophosphate buffer, present in the reagent 1 used for CHE detection, inhibits the enzyme activity, because it increases pH solution. Therefore we decided to predispose an extra washing of the reagent needles with an acid solution (Cell Wash Solution). This device showed a significant improvement of quality control results as well as that of patient sera (n=40; CHE mean value before=6360UI/L; CHE mean value after extra washing =6872UI/L)
Conclusion: Multiparameter third party controls permitted us to individuate this new interference in a closed system, that generally limits the implementation of methods not provided by Roche.

T378
AGREEMENT BETWEEN SFRI4000 AND PROLYTE ISE ANALYZERS IN THE DETERMINATION OF PLASMA SODIUM AND POTASSIUM

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Background: Clinical decision making is confirmed by laboratory test results. When dealing with sick patients, the speed and accuracy of tests to detect metabolic derangements is very important. It is, therefore, required that laboratory results be timely, accurate, reliable and fit for the purpose. We evaluated agreement between two ion selective electrode analyzers (SFRI 4000 and PROLYTE) used for the quantitation of plasma sodium and potassium in our laboratory.
Materials and methods: 42 samples originally meant for electrolytes assay in our laboratory were employed for the study. After blood receipt the samples were spun at 4,000r.p.m immediately. Plasma sodium and potassium were analyzed simultaneously with Prolyte and SFRI ISE analyzers. The agreement between the two analyzers was assessed using Bland-Altman Method.
Results: Na+ Coefficient of variation: PROLYTE 0.9%, SFRI, 1.1%, Regression Analysis: Correlation Coefficient r2=0.93, slope=1.08, intercept,-13.24, maximum difference, 4mmol/L, minimum difference,-4mmol/L, Bland-Altman analysis mean difference between PROLYTE and SFRI ion selective method is 1.4±3.42 with 95% confidence interval of -2.02 to 4.82; 2. K+ Coeffient of variation: PROLYTE, 3.0% SFRI, 2.3%, Regression Analysis; Correlation Coefficient r2=0.98, slope=1.1, intercept ,-0.2, maximum difference = 0.2, minimum difference,-0.5, Bland-Altman analysis mean difference between PROLYTE and SFRI ion selective method is 0.21±0.26 with 95% confidence interval of -0.05±0.47.
Conclusion: Good degree of agreement was observed on comparing the two ISE analyzers for the measurement of sodium and potassium. The two analyzers can be used without fear of inaccuracy and imprecision.

T379

SINGLE-WALLED CARBON NANOTUBES AS POTENTIAL AGENTS FOR THE CIRCULATORY FUNCTION CORRECTION UNDER PATHOLOGICAL STATES OF CENTRAL GENESIS

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Background: The purpose of the work is to examine the hemodynamic effect of single-walled carbon nanotubes (SWCNTs) depending on the dose and technology of their administration in rats with genetically determined hypertension.
Methods: Spontaneously hypertensive rats represent an animal laboratory model of hypertension in humans that is widely used to compare response patterns in rats with persistently increased and normal arterial pressure. In urethane anesthetized rats, SWCNTs were administrated in the medullary nuclei (nucleus of solitary tract, NTS; paramedian nucleus, PMn; lateral reticular nucleus, LRN; nucleus ambiguus, AMB) that are directly involved in the nervous control of the vascular tone and cardiac activity. The effectiveness of applied antihypertensive technology was assessed by analyzing the changes in the systemic arterial pressure (SAP) and the heart rate. Also we have studied in detail the toxic effect by analyzing the stability of the erythrocytes to acid hemolysis depending on the dose and the way of SWCNT administration.
Results: SWCNT injections in the AMB resulted in the SAP drop by 14.4% ($P < 0.05$), in the LRN - by 22.8% and in the NTS - by 21.6%. Hypotensive responses were characterized by rapid development with maximum in 10-20 s and they lasted 3 min. SWCNT-evoked responses in the PMn were mostly hypertensive. In spontaneously hypertensive rats, SWCNT injections resulted in the SAP lowering in the studied nuclei by 22.8%, 21.0% and 13.0% in the AMB, LRN and PMn, responsively, that is, in those animals responses were usually more significant compared to those in the control rats. Hypotensive responses in spontaneously hypertensive rats developed more slowly compared to those in the control group. Changes in the heart rate were insignificant at left side SWCNT injections, while there right side administration resulted in heart rate reduction. Biochemical analysis of the blood samples to evaluate the erythrocyte stability to acid hemolysis after SWCNT either intravenous or intramedullary administration showed that SWCNTs did not have toxic effect on the cardiovascular system.
Conclusions: The data obtained suggest that SWCNTs are the promising class of pharmaceutical compounds to treat hypertension. Considering that the cardiovascular diseases, including hypertension, are often associated with excessive activation of the sympathetic nervous system, hypotensive effect of SWCNTs on the cardiovascular system can be realized via reducing the activity of the medullary sympathetic neurons through activation of nitric oxide synthesis. In addition, SWCNTs may be involved in inactivation of excessive free radical production observed in hypertension.

T380

COMPARISON OF LIQUID BASED CYTOLOGY WITH CONVENTIONAL CYTOLOGY IN THE EVALUATION OF ABDOMINAL MASSES

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Background: Liquid based preparations are used for gynaecologic and non-gynaecologic cytology including body fluids and FNA. These preparations have several preparatory, screening and diagnostic advantages making them an appropriate alternative to conventional cytology including uniform collection procedures, avoiding hazards of needle handling required and easy transportation to the laboratory. Immediate liquid fixation preserves morphology and residual material can be used to either process multiple slides & cell block for ancillary tests.Aims of this study were to study the role of Liquid Based Cytology in the diagnosis and characterization of abdominal masses,to compare the Liquid Based Cytology with Conventional smear Cytology and to correlate the results with either cell block preparations or histopathological sections and immunohistochemistry.
Methods: In the study total of 30 cases with abdominal masses were included. Ultrasound guided fine needle aspirate and cell block was collected. The aspirate was spread and fixed for conventional smears. A second pass was made and rinsed into liquid based collection vial and processed in Thin Prep 2000®(used for preparation of liquid based cytology smears). Slides from both the methods were compared for cellularity, adequacy, background (blood and necrotic cell debris), cellular architecture, maintenance of nuclear detail, cytoplasmic integrity and diagnostic accuracy.Cell block/biopsy correlation whichever available was done.
Observation and Results: Cellularity of Conventional smears was superior to Thin Prep ($P=0.025$). Difference not statistically significant ($P=0.112$) regarding adequacy of smears. Maintenance of architectural pattern in Conventional smears was superior to Thin Prep ($P < 0.001$). Reduction in background material of Thin Prep was superior to conventional smears ($P < 0.001$). Cytoplasmic integrity in Conventional smears was superior to Thin Prep ($P < 0.001$). Regarding nuclear details difference is not statistically significant ($P=0.091$) among the two preparations. The difference in the diagnostic accuracy between the two methods is not statistically significant (P value=0.226)
Conclusion:The liquid based cytology technique is expensive for routine use if used alone than in conjunction with conventional cytology.

T381

EVALUATION OF THE PERFORMANCE OF THE VIDAS® ATG ASSAY IN SUBJECTS WITH CONFIRMED OR SUSPECTED THYROID AUTOIMMUNE DISEASE

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Background: Anti-thyroglobulin (Anti-Tg) antibodies are often found in conjunction with anti-thyroid peroxidase antibodies in Hashimoto's thyroiditis and Graves' disease. The VIDAS® ATG (bioMérieux, France) assay is an automated quantitative test for the detection of IgG anti-Tg autoantibodies in serum or plasma for use as an aid in the diagnosis of autoimmune thyroid disease. The study objective was to establish the performance of the VIDAS® ATG assay compared to the Architect Anti-Tg assay (Abbott, USA) using samples from patients with Hashimoto's thyroiditis or Graves' disease, samples submitted for routine thyroid disease testing, and samples from subjects with non-thyroid autoimmune disease.
Methods: The cut-off used for each assay was determined by bioMérieux according to National Academy of Clinical Biochemistry criteria. Single replicates of 317 samples were tested using the VIDAS® ATG and Architect Anti-Tg assays. Sensitivity, positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were determined.
Results: The VIDAS® ATG and Architect Anti-Tg demonstrated PPA of 90%, NPA of 96%, and OPA of 94%. In samples from subjects with Hashimoto's thyroiditis, the VIDAS® ATG and Architect Anti-Tg demonstrated 98% PPA, 93% NPA and 97% OPA. In samples from subjects with Graves' disease, the VIDAS® ATG and Architect Anti-Tg assays demonstrated PPA of 97% and NPA of 93% and OPA of 95%. The sensitivity of both assays in Hashimoto's thyroiditis subjects was 77%. The sensitivities of the VIDAS® ATG and Architect Anti-Tg assay in Graves' disease subjects were 47% and 44%, respectively.
Conclusions: The VIDAS® ATG assay demonstrated equivalent sensitivity to the predicate device and high PPA, NPA, and OPA in all of the populations examined including subjects with Hashimoto's thyroiditis, Graves' disease, non-thyroid autoimmune disease and routine thyroid testing samples. Thus, the VIDAS® ATG assay provides a rapid (25 minute) test for the presence of Anti-TG in support of a diagnosis of autoimmune thyroid disease. This product has not been evaluated by the FDA, and is not intended for sale in the United States

T382

THERAPEUTIC DRUG MONITORING OF MYCOPHENOLIC ACID USING PHARMACOKINETIC MODELLING AND BAYESIAN ESTIMATION WITH THE MPAT DIMENSION® ASSAY

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Mycophenolic acid (MPA) is the antimetabolite of choice in immunosuppressive protocols. MPA exhibits large interindividual pharmacokinetic variability due to numerous factors, such as liver and renal functions, serum albumin levels or associated drugs; (ii) MPA AUC0-12 h is better correlated with patient outcomes than any single concentration timepoint. Different consensus conferences have advised: (i) to perform drug dosing based on MPA inter-dose AUC when obtained using limited sampling strategies (LSS); (ii) to target an MPA AUC0-12h between 30 and 60 mg.h/L. For MPA, multiple Maximum a Posteriori Bayesian estimators (MAP-BEs) were previously developed and routinely used for the dose adjustment of MPA in transplant patients based on a limited sampling strategy (LSS) using 3 concentrations. The aims of the present study were to further evaluate the new Dimension MPAT assay compared with a reference HPLC-DAD technique for MPA PK studies in three different populations of heart, liver and kidney allograft, and use BE models for optimizing TDM with these two analytical methods. Plasma samples were obtained from 103 transplant patients. Patients' plasma samples were thawed in batches and analyzed in parallel. A hybrid MAP-BEs specific to the MPAT assay was developed and validated. Precisely: (i)the error patterns of the MPAT assay were determined for each transplant type according to the recommendations, (ii)the equations derived from the regression analysis between the MPAT kit and HPLC were determined for each group of patients, (iii)100 MPA PK profiles were simulated for each group, (iv) each MAP-BE was evaluated by comparison of observed (ie trapezoidal rule) and estimated AUC using the predefined LSS and determination of the bias and Root Mean Squared Error (RMSE). 127 full kinetic profiles have been analysed. Correlation between different data were $r^2 = 0.9662$, $r^2 = 0.9442$ and $r^2 = 8766$ for heart graft, liver graft and renal graft respectively. For each MAP-BE the mean bias between the estimated AUC and AUCtrap did not exceed 6%, with RMSE values less than 15%. The number of patients with an imprecision greater than 20% was less than 15%. We concluded that HPLC-DAD and the Dimension MPAT assay could be used for routine dose adjustments of MPA with MPABEs.

T383

PERFORMANCE EVALUATION OF A NEW FERRITIN ASSAY FOR THERMO SCIENTIFIC KONELAB CLINICAL CHEMISTRY ANALYZER

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Background: The determination of ferritin is important when diagnosing iron metabolism disorders, monitoring iron therapy, ascertaining the iron reserves in groups at risk and in differential diagnosis of anemias. While very low serum ferritin values are always indicative of iron deficiency, very high serum ferritin values have many implications. Increased serum ferritin can be suggestive of iron overload but is also seen in conjunction with liver parenchymal damage, infections, inflammation and malignant diseases without any quantitative relationship to the iron reserve. Thermo Scientific Konelab clinical chemistry analyzers (20, 20XT, PRIME 30, PRIME 60) are random access, fully automated, clinical chemistry systems. Colorimetric, turbidimetric and ISE methods are applicable and the analyzers are capable of handling routine and stat requests.
Methods: Konelab (20XT, PRIME 30, PRIME 60) Ferritin method with system reagents, calibrators and controls is a particle enhanced immunoturbidimetric assay using latex particles coated with rabbit antibodies against human ferritin. Serum and Li-heparin can be used as sample types. The increase in absorbance caused by formation of immunocomplexes is recorded at 700 nm.
Results: The assay measuring range is 7 – 350 μg/L extended with automatic dilution up to 3500 μg/L. The determination limit of the assay is 7 μg/L. The repeatability (within-run precision) is from 2.5 to 4.4% (CV) for samples with ferritin concentrations from 28 to 270 μg/L (N=80). The within device (total) precision is from 5.7 to 6.6% (CV) for samples with ferritin concentrations from 28 to 270 μg/L (N=80). A method comparison study was performed using a commercially available particle enhanced immunoturbidimetric method as the reference. The Konelab method correlated well with the reference method. Linear regression was y = 1.02 x + 0.32 and r = 0.985 (N=98).
Conclusion: The results demonstrate that ferritin can be analyzed accurately and easily using Thermo Scientific Konelab clinical chemistry analyzer.

T384

PERFORMANCE EVALUATION OF A NEW D-DIMER ASSAY FOR THERMO SCIENTIFIC KONELAB CLINICAL CHEMISTRY ANALYZER

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Background: D-Dimer is a small protein fragment, present in the blood after a blood clot is degraded in fibrinolysis by plasmin. Although many clinical conditions are associated with increased blood concentrations of D-Dimer, its testing has become a useful laboratory tool for the diagnosis of venous thromboembolism (VTE) because it has high negative predictive value when used in combination with pretest clinical probability. Other clinical conditions related to increased concentrations of D-Dimer are arterial thrombosis (including myocardial infarction and stroke), disseminated intravascular coagulation (DIC), recurrent thrombotic risk following anticoagulation, post operative state, significant liver disease, malignancy and normal pregnancy. Thermo Scientific Konelab clinical chemistry analyzers (20, 20XT, PRIME 30, PRIME 60) are random access, fully automated, clinical chemistry systems. Colorimetric, turbidimetric and ISE methods are applicable and the analyzers are capable of handling routine and stat requests.
Methods: Konelab (20XT, PRIME 30, PRIME 60) D-Dimer method with system reagents, calibrators and controls is a particle enhanced immunoturbidimetric assay using latex particles coated with mouse anti-human D-Dimer monoclonal antibodies. Sample type is citrate plasma and the increase in absorbance caused by formation of immunocomplexes is recorded at 600nm.
Results: The assay measuring range is 0.2 – 5.0 mg FEU/l, extended with automatic dilution up to 20 mg FEU/l. The determination limit of the assay is 0.2 mg FEU/l. Expected values <0.5 mg FEU/l. The repeatability (within-run precision) is from 0.9 to 2.9% (CV) for samples with D-Dimer concentrations from 0.57 to 3.85 mg FEU/l (N=80). The within device (total) precision is from 3.9 to 6.5% (CV) for samples with D-Dimer concentrations from 0.57 to 3.85 mg FEU/l (N=80). A method comparison study was performed using a commercially available particle enhanced immunoturbidimetric method as the reference. The Konelab method correlated well with the reference method.
Conclusion: The results demonstrate that D-Dimer can be analyzed accurately and easily using Thermo Scientific Konelab clinical chemistry analyzer.

T385

EVALUATION OF THE PERFORMANCE OF THE VIDAS® ATPO ASSAY IN SUBJECTS WITH CONFIRMED OR SUSPECTED THYROID AUTOIMMUNE DISEASE

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Background: Anti-thyroid peroxidase (Anti-TPO) antibodies contribute to thyroid autoimmune disease, a major factor underlying hypothyroidism and hyperthyroidism. The VIDAS® ATPO (bioMérieux, France) assay is an automated quantitative test for the detection of IgG anti-TPO autoantibodies in serum or plasma for use as an aid in the diagnosis of autoimmune thyroid disease. The study objective was to establish the performance of the VIDAS® ATPO assay compared to the Architect Anti-TPO assay (Abbott, USA) using samples from patients with Hashimoto's thyroiditis or Graves' disease, samples submitted for routine thyroid disease testing, and samples from subjects with non-thyroid autoimmune disease.
Methods: Clinical cut-off for the VIDAS ATPO assay was determined according to National Academy of Clinical Biochemistry criteria (package insert value was used for Architect Anti-TPO assay). Single replicates of 317 samples were tested using the VIDAS® ATPO and Architect Anti-TPO assays. Sensitivity, positive percent agreement (PPA), negative percent agreement (NPA), and overall percent agreement (OPA) were determined.
Results: The VIDAS® ATPO and Architect Anti-TPO demonstrated PPA of 74%, NPA of 96%, and OPA of 95%. In samples from subjects with Hashimoto's thyroiditis, the VIDAS® ATPO and the Architect Anti-TPO demonstrated 98% PPA, 86% NPA, and 97% OPA. In samples from subjects with Graves' disease, the VIDAS® ATPO and Architect Anti-TPO assays demonstrated PPA of 94%, NPA of 96%, and OPA of 95%. The sensitivity of both assays in Hashimoto's thyroiditis subjects was 88%. The sensitivities of the VIDAS® ATPO and Architect Anti-TPO assay in Graves' disease subjects were 62% and 65%, respectively.
Conclusions: The VIDAS® ATPO assay demonstrated sensitivity comparable to that of the predicate device and high level agreement, in all of the populations examined including subjects with confirmed Hashimoto's thyroiditis, Graves' disease, or non-thyroid autoimmune disease and samples submitted for routine thyroid testing. Thus, the VIDAS® ATPO assay provides a rapid (25 minute) test for the presence of Anti-TPO in support of a diagnosis of autoimmune thyroid disease. This product has not been evaluated by the FDA, and is not intended for sale in the United States.

T386

HIGH GRADIENT MAGNETIC SEPARATION: A NOVEL METHOD IN THE SEPARATION OF UPCONVERTING LANTHANIDE-DOPED NANOPARTICLES

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Background: High gradient magnetic separation (HGMS) is a widely used method from mining industry to clinical research. In biotechnology it has been used to collect and concentrate biomolecules and cells by binding them onto magnetic nanoparticles. Upconverting nanoparticles (UCNPs) are unique reporters utilized in assay and imaging technologies. They have a luminescent property of converting low-energy infrared light into visible emission. However, as the particle size of the UCNPs approaches the size of biomolecules, the handling of reporters becomes cumbersome with traditional purification methods.
Methods: While the lanthanide-doped crystal structure of UCNPs is responsible for the upconversion luminescence, the lanthanide dopants also bring paramagnetic properties to the UCNPs enabling the use of HGMS as a method to capture them and their conjugates. The separation system consists of a pair of permanent neodymium super magnets and a column structure filled with ferromagnetic matrix (steel wool) placed in between them. The matrix is saturated with the magnetic field produced by the magnets and forms strong magnetic gradients on its surface. In these gradients the field is strong enough to capture even weakly paramagnetic UCNPs.
Results: The working principle of HGMS with UCNPs was demonstrated by injecting the UCNPs to the system and using different magnetic field strengths to capture the particles from the sample. The system was able to capture more UCNPs with a stronger magnetic field. The magnetic selectivity of HGMS was demonstrated by introducing a mixture of non-magnetic blue latex particles and UCNPs to the system, which resulted in latex particles flowing through in the presence of the magnetic field while the UCNPs were eluted after the magnets were removed.
Conclusions: By utilizing methods from industry applications we have developed a working solution for separation of UCNPs from liquids relying solely on the intrinsic paramagnetic properties of the lanthanide dopants. In this method there is no need to embed separate, optically inactive magnetic materials inside the UCNPs as the particle structure is paramagnetic by itself. The results indicate that HGMS could also be used with antibody-conjugated UCNPs used in bio-assays.

T387

VALIDATION OF TWO NEW PNEUMATIC TRANSPORTATION SYSTEMS FOR BLOOD SAMPLES, COVERING HIGHEST(13 METER) AND LONGEST(500 METER) DISTANCE TO DATE. THE SYSTEM IS EASY TO HANDLE, AND THE SAMPLES ARE IN FEW SECONDS DELIVERED, READY TO PLACE AT THE PREANALYTICAL SYSTEM, CONNECTED TO THE CHEMICAL ANALYZERS

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Background: The challenge is to quarantee fast analysis time for automated chemical analyzers. To optimize the proces rapid sample delivery via pneumatic tube transportation is attractive for reducing transport time to the laboratory.
Methods: After drawing the blood sample, the tubes are placed in the Tempus600® rack and loaded in the Tempus600® launch unit, without packaging compared to conventional systems. The launch unit is placed in the emergency department and the point of delivery is in the laboratory, Department of Clinical Biochemistry: The Tempus600® system was examined with a view to assess to what extend the blood samples were affected by the transport. 50 patients participated in the study, chosen randomly among patients in the department's phlebotomy. Two blood samples were drawn from each patient. One sample tube was sent by routine courier transport to the laboratory (reference) and the second one was sent with Tempus600®. The blood samples were tested for: Biochemical tests (potassium, lactatedehydrogenase, alkaline phoshatase, haemolytic index), Coagulation tests (international normalized ratio, activated partial tromboplastin time) and Haematology test (leucocytes, lymphocytes and trombocytes), using Roche Cobas6000, Stago STA-R and Sysmex XE2100 analyzers.
Results: No significant differences were found between routine transport and Tempus600® for the tests (P-value >0,05), except the haemolytic index. When transporting the blood sample tubes using Tempus600® the hemolytic-index went up approx. 2,3 mg/dL compared to routine transport (min. -7 to max. 10 mg/dL). This increase did not affect the results, and the haemolytic-index were accepted for the blood sample testing. Results from the 500 meter pneumatic system are still preliminary and will be presented at the congress.
Conclusions: The validation of Tempus600® has shown very satisfying results. Based on the findings it is recommended using Tempus600® for transporting blood sample tubes.

T388

NEW LIQUID STABLE NA+-, K+- AND CL- ASSAYS FOR THE FAST AND EASY ASSESSMENT OF ELECTROLYTES ON CLINICAL CHEMISTRY ANALYZERS

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In all higher life forms a precise maintenance of an electrolyte balance is crucial to regulate pH and hydration of the body and is critical for nerve and muscle function. In patients electrolytes such as Na+, K+ and Cl- are frequently measured as part of the clinical routine to evaluate acute or chronic diseases and to monitor treatment of certain problems. This includes high blood pressure, heart failure, liver and kidney diseases or diabetes-related complications. Here we present a new ready-to-use electrolyte reagent panel to measure Na+, K+ and Cl- on clinical chemistry analyzers (CCA). Established methods for the determination of electrolytes are flame emission spectroscopy (FES) and potentiometry with ion selective electrodes (ISE). These systems are cumbersome to integrate into routine testing or require a lot of regular maintenance to ensure a reliable performance. The liquid-stable DiaSys electrolyte tests are optimized for laboratories with small or mid-sized clinical analyzers without an ISE. The assessment of values presented here, are based on ion-dependent enzymes for Na+ and K+ and on a new colorimetric method for the detection of Cl- respectively. In particular sodium levels are detected using a Na+-dependent β-galactosidase. This reaction is assessed by enzymatic release of o-nitrophenol from its substrate. Potassium is detected by a K+-dependent pyruvate kinase connected to a lactate dehydrogenase/NADH system. In contrast chloride values are assessed by a new colorimetric method using a specific Cl—dependent iron(III) chloride-complex. All three tests show a wide linear range and allow the robust determination of electrolyte values in serum or plasma samples on routine CCA without prior dilution. Using a DiaSys respons®920 analyzer the tests demonstrated very strong correlation to the reference methods and an extraordinary precision of <2% within run and <3% between day. No significant interferences within ±3% (Na+) and ±4.5% limits (K+, Cl-) are given for all test assays. Our results demonstrate, that the DiaSys Na+, K+ and Cl- reagent panel offers a great opportunity for small and mid-sized labs to automate routine electrolyte diagnosis. The reagents can be used manually as well as on CCAs with a comparable performance to ISE or FES.

T389
IMMUNOSUPPRESSANT THERAPEUTIC DRUG MONITORING WITH HPLC-MS/MS – ARE ALL METHODS EQUAL?

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Background: HPLC-MS/MS is often considered as golden standard method in clinical biochemistry. Its selectivity is praised, its accuracy, precision and sensitivity is considered to be "equal or better" than clinical chemistry routine methodologies in endocrinology or therapeutic drug monitoring (TDM); especially compared to immunoassays. On the other side however, it has been shown in immunosuppressant drug TDM proficiency testing (PT) schemes, that most often locally designed LC-MS/MS platforms show a higher degree of inter - laboratory variability than automated commercial immunoassays. To allow a deeper insight into the factors contributing to the observed imprecision of LC-MS/MS assays, we combined published and widely used sample preparation protocols with the online-SPE-LC-MS/MS immunosuppressant drug TDM assay designed at our institute (ZIMCL method).
Methods: Both qualitative and quantitative experiments were performed. Anonymized leftover routine whole blood samples were used. Extraction yields were evaluated by peak area comparisons for cyclosporine A, tacrolimus, everolimus and sirolimus. The corrective action of the routinely used internal standards (IS) in a quantitative assay setup was investigated for cyclosporine A (IS cyclosporine D) and tacrolimus (IS ascomycin) using identical calibrator materials.
Results: The peak area comparison experiment did clearly show that several published sample preparation protocols had significantly lower analyte yields compared to the ZIMCL method. Comparison of extraction yields from calibrator and patient sample materials did unveil, that only two of eight protocols investigated did not discriminate these materials. Quantitative results comparison to the ZIMCL method did show significant deviations (±10% to ±30%) for some but not all of the sample preparation protocols.
Conclusion: The increased inter-laboratory variability observed in PT schemes stems most likely from different extraction yields in the whole blood sample preparation and not from an inter-laboratory calibration bias. Erythrocyte lysis prior to protein precipitation seems to be mandatory to achieve an identical analyte yield from patient and calibrator samples.

T390
SERUM FREE LIGHT CHAIN ASSESSMENTS: COMPARISON OF PRECISION AND LINEARITY

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Background: Serum free light chain measurements utilising the polyclonal antisera based immunoassay (FreeliteTM, Binding Site, UK) have changed the diagnostic and monitoring paradigm for patients with B cell disorders; culminating in their inclusion in international guidelines. Recently, a monoclonal antibody immunoassay has become available (N latex FLC, Siemens, Germany). Here we compare the within batch imprecision and linearity.
Methods: Comparison of the Freelite and N latex FLC assays was performed on a BN™II nephelometer (Siemens, Germany). Precision was measured at 140-200% of the lower calibration range and 80-95% of the upper calibration points at standard dilutions. Due to different dynamic measuring ranges the concentrations of the analytes measured were: for FreeliteTM low κ 8.75-12.5 mg/L and λ 7.1-10.1mg/L, high κ 160-190 mg/L and λ 130.24-154.7 mg/L; N latex low κ 5.36-7.66 mg/L and λ 2.62-3.75 mg/L, high κ 48-57 mg/L and λ 98-116.4 mg/L. To assess linearity, monoclonal samples were analysed in their undiluted states in triplicate, and allowed to repeat as required (start dilutions for Freelite κ and λ were 1/100 and N latex κ and λ were 1/100 and 1/20 respectively). Dilutions were performed with Siemens N Diluent to achieve a dilution series of 0-100%.
Results: Within batch CVs for lower end and upper end precision respectively were: for Freelite κ 5.1% and 4.1%, for λ 6.3% and 3.5%; for N latex FLC κ 1.3% and 3.3% and for λ 3.8% and 1.9%. Following linearity analysis the R2 values for Freelite κ and λ were 0.9881 and 0.9929 whereas for N latex R2 values were 0.9429 and 0.94. The maximum difference between the observed and expected values was -38.3% for N latex but only -18.3% for Freelite. The change between the observed and expected values shifted from -38.3% to 25.1% as measured concentrations exceeded the standard dilution on the N latex λ assay.
Conclusion: Both the FreeliteTM and N latex FLC κ and λ assays demonstrate acceptable precision at the lower end of their respective measuring ranges at the standard dilution. Freelite showed a greater degree of linearity than the N latex assay, possibly reflecting matrix interference.

T391

EVALUATION OF A C1 INACTIVATOR ASSAY FOR USE ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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Background: C1 Inactivator is an important regulator of the classical complement pathway. It acts by inhibiting the activity of C1s and C1r serine proteases, therefore limiting any potentially harmful effects of over-activation. Measurement of C1 Inactivator aids in the diagnosis of hereditary and acquired angioedema which are the most common complement deficiencies. Here we describe the performance characteristics of a C1 Inactivator assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay has a measuring range of 0.06 – 0.4 g/L at the standard 1/10 sample dilution, with sensitivity of 0.06 g/L. High samples are remeasured at a dilution of 1/20 with an upper measuring range of 0.12 – 0.8 g/L.
Methods: Correlation to the Binding Site C1 Inactivator assay for the SPA PLUS was performed using 28 samples (range 0.081 – 0.406 g/L). Intra-run precision was assessed by measurement of ten replicates of samples at 0.108 g/L and 0.343 g/L. Furthermore, precision was assessed at the clinically relevant decision point of 0.169 g/L. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range and comparing expected versus observed results (0.068 – 0.338 g/L).
Results: Correlation with the C1 Inactivator SPA PLUS assay demonstrated good agreement when analysed by Passing-Bablok regression; y=0.94x + 0.01. The assay was shown to be linear over the range of 0.068 – 0.338 g/L; y = 0.9526x + 0.01795 (R2 = 0.9961), maximum deviation from expected result was 15.8%. Intra-run precision produced the following results: sample 1 (0.108 g/L) CV of 0.85%, sample 2 (0.169 g/L), CV of 0.98% and sample 3 (0.343 g/L), CV of 0.59%.
Conclusions: We conclude that the C1 Inactivator assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

T392

EVALUATION OF A CYSTATIN C ASSAY ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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The Binding Site Group Ltd, UK

Background. Cystatin C is a 13kDa, non-glycosylated endogenous cysteine protease inhibitor. Here we describe the performance characteristics of a Cystatin C assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems. The assay has a measuring range of 0.402 – 8.04 mg/L at the standard 1/10 sample dilution, with a sensitivity of 0.04 mg/L. High samples are remeasured at a dilution of 1/20 with an upper measuring range of 0.804 - 16.08 mg/L.
Methods. Correlation to the Binding Site Cystatin C assay for the SPA PLUS was performed using 35 samples (range 0.619 – 7.910). Intra-run precision was assessed by measurement of twenty replicates of samples at 6.2, 3.4, 1.0, 0.75 and 0.64 mg/L. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range and comparing expected versus observed results (0.402 – 8.04 mg/L). Antigen excess capacity was determined by extending the width of the curve through increasing calibrator concentration and looking for the hook effect.
Results. Correlation with the Cystatin C SPA PLUS assay demonstrated good agreement when analysed by linear regression; y=0.94x + 0.004, R^2 =0.996. The assay was shown to be linear over the range of 0.342 – 8.451 mg/L; y=0.9929x – 0.1262, R^2 =0.996, maximum deviation from expected result was 10.0%. Intra-run precision produced the following results: sample 1 (6.275 mg/L) CV of 1.81%, sample 2 (3.420 mg/L), CV of 1.50%, sample 3 (1.040 mg/L), CV of 1.99%, sample 4 (0.748 mg/L), CV of 2.26% and sample 5 (0.636), CV of 4.04%. The assay was proven to be antigen-excess proof to 46.53 mg/L (6 times higher than seen in chronic kidney disease stage 5).
Conclusions. We conclude that the Cystatin C assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

T393
EVALUATION OF AN IGG ASSAY FOR USE ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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The Binding Site Group Ltd, Uk

Background: In normal adults, IgG constitutes approximately 75% of total serum immunoglobulin. IgG levels are decreased in primary immunodeficiency conditions, whilst levels are elevated in conditions such as multiple myeloma. Here we describe the performance characteristics of an IgG assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems.
Methods: The assay has a measuring range of 1.65-36.40 g/L at the standard 1/10 sample dilution, with a sensitivity of 0.16 g/L. High samples are remeasured at a dilution of 1/40 with an upper measuring range of 6.60-145.60 g/L. Correlation to the Binding Site IgG assay for the SPA PLUS was performed using 31 samples (range 1.04-31.8 g/L). Intra-run precision was assessed by measurement of twenty replicates of samples at 33.1, 15.5, 6.3 and 3.1 g/L. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range and comparing expected versus observed results. Antigen excess capacity was determined by extending the width of the curve through increasing the calibrator concentration and looking for the hook effect.
Results: Correlation with the IgG SPA PLUS assay demonstrated good agreement when analysed by Passing-Bablok regression: y=0.95x + 0.07. The assay was shown to be linear over the range of 1.9-39.3 g/L; y=1.02x + 0.03 (R^2=0.9989), maximum deviation from expected result was 9.4%. Intra-run precision produced the following results: sample 1 (33.1 g/L) CV of 1.16%, sample 2 (15.5 g/L), CV of 1.98%, sample 3 (3.1 g/L), CV of 2.15% and sample 4 (6.3 g/L), CV of 1.55%. The assay was antigen-excess proof to 195.63 g/L.
Conclusions: We conclude that the IgG assay for the Binding Site Next Gen is reliable, accurate and precise and shows good agreement with existing assays.

T394
EVALUATION OF A C3C ASSAY FOR USE ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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Introduction: Serum complement consists of around 30 proteins that have a fundamental role in immune system functionality. Inherited deficiencies in C3c are associated with an increased risk of developing systemic lupus erythematosus (SLE). C3c deficiency may present with recurrent infections such as pneumonia, septicaemia and meningitis. Here we describe the development of serum C3c assay for Binding Site's Next Generation Protein Analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems.The assay has a measuring range of 0.25–3.0 g/L at the standard 1/10 sample dilution, with sensitivity of 0.025 g/L. High samples are remeasured at a dilution of 1/20 with an upper measuring range of 0.5-6 g/L
Method: Correlation to the Binding Site C3c assay for the SPA PLUS was performed using 49 samples (mean 1.214; range 0.49–2.15 g/L). Precision studies (CLSI EP5-A2) were performed at three levels in duplicate over 21 working days. Antigen levels of 2.418, 0.778 and 0.38 g/L were assessed for total, within-run, between-run and between-day precision, using one lot of reagent on three analysers. Linearity was assessed by assaying a serially-diluted patient sample pool across the width of the measuring range (0.303–3.036 g/L)and comparing expected versus observed results.
Results: Correlation with the C3c SPA PLUS assay demonstrated good agreement when analysed by Passing-Bablok regression; 1.02x + 0.05 g/L. The assay was shown to be linear over the range of 0.303–3.036g/L; y=1.03x+0.017 g/L (R^2 = 0.998). Precision produced the following results for total, within-run, between-run and between-day precision respectively: sample 1 (2.418 g/) 3.1%, 1.0%, 1.3% and 2.6%, sample 2 (0.778 g/L) 0.4%, 2.0%, 2.4% and 1.4%, sample 3 (0.38 g/L) 3.3%, 1.1% 1.3% and 2.8%.
Conclusions: We conclude that the C3c assay for the Binding Site next generation protein analyser is reliable, accurate and precise and shows good agreement with existing assays.

T395
EVALUATION OF AN IGG4 ASSAY FOR USE ON THE BINDING SITE NEXT GENERATION PROTEIN ANALYSER

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The Binding Site Group Ltd, UK

Background: Recent research has shown that high polyclonal IgG4 levels are associated with autoimmune pancreatitis (AIP). Here we describe the performance characteristics of an IgG4 assay for use on the Binding Site's next generation protein analyser. The instrument is a random-access bench top turbidimetric analyser capable of a wide range of on-board sample dilutions (up to 1/10,000) and throughput of up to 160 tests per hour. Precision is promoted by single-use cuvettes which are automatically loaded and disposed of, whilst the utility is enhanced through host interface capability, primary sample ID and bar coded reagent management systems.
Methods: The assay measuring range was 0.03–3 g/L at the standard 1/25 sample dilution, with sensitivity of 0.003 g/L. High samples were remeasured at a dilution of 1/500 with a measuring range of 1.5–60 g/L. Correlation to the Binding Site IgG4 assay for the SPA PLUS was performed using 16 samples (range 0.04–2.39 g/L). Antigen excess protection was assessed by measuring 18 analyte concentrations ranging 0.038–2.37 g/L at the minimum sample dilution (equivalent to 0.96–59.2 g/L at the standard 1/25 sample dilution). Intra-run precision was assessed by measurement of twenty replicates of samples; low (0.04 g/L), medium (1.23 g/L) and high (2.39 g/L). Linearity was assessed by assaying a serially-diluted patient sample pool and comparing expected versus observed results (range 0.03–2.39 g/L).
Results: There was good agreement between assays (mean 0.56 g/L; range 0.04–2.37 g/L v mean 0.55 g/L; range 0.02–2.39 g/L), Passing-Bablok regression; y=0.98x + 12.44. The assay was linear over the range of 0.03–2.39 g/L; y=1.07x + 1.01 (R^2=0.99), maximum deviation from expected was 15.9%. Intra-run precision produced the following results: low (0.04 g/L) CV of 6.82%, medium (1.23 g/L) CV of 2.66% and high (2.39 g/L) CV of 2.61%. All samples up to 2.37 g/L (equivalent to 59.2 g/L at the standard sample dilution) were correctly flagged by the antigen excess protection function and did not report results in antigen excess.
Conclusions: We conclude that the IgG4 assay for the Binding Site next generation protein analyser is reliable, accurate and precise and is protected from antigen excess when challenged with the high IgG4 levels seen in AIP.

T396
EVALUATION OF CUTOFF VALUE FOR HIGH-SENSITIVE TROPONIN T ASSAY IN RELATION TO PREVIOUSLY USED TROPONIN I ASSAY

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Background: Cardiac troponin is considered a leading laboratory diagnostic tool for detection of myocardial damage. Until recently main diagnostic assay was measuring cardiac troponin I (cTnI) levels. However, development of high-sensitive troponin T (hsTnT) assay has made its use available. The aim of this study was to determine cutoff value for hsTnT assay, which was introduced in our laboratory, in comparison to previously used cTnI assay.
Methods: Total of 401 patient samples from routine laboratory workload was simultaneously analyzed for hsTnT (Roche, Basel, Switzerland) and cTnI (Siemens, Newark, USA) levels. Blood samples were collected in BD Vacutainerä tube with gel separator and centrifugated for 10 minutes at 3500 rpm at least 30 minutes after collection and before analysis. Cutoff value of 0.2 μg/L for cTnI was determined upon introducing method into routine laboratory work according to manufacturer recommendation and our internal validation study. Cutoff value for hsTnT was evaluated using receiver operating characteristic (ROC) curve with MedCalc statistical software (Mariakerke, Belgium) using cTnI cutoff value as differential criteria.
Results: Pearson's correlation coefficient for hsTnT and cTnI measurements was r=0.939 (P <0.001; 95% CI 0.927-0.949) which indicates excellent correlation between tests. The area under the ROC curve (AUC) was 0.982 (P <0.001; 95% CI 0.964-0.993) for hsTnT assay. The ROC curve gained cutoff value for hsTnT of 0.049 μg/L, with sensitivity of 96.2% (95% CI 89.2-99.2) and specificity of 91% (95% CI 87.4-93.9).
Conclusion: Results demonstrated that the hsTnT concentration of 0.049 μg/L could be used as cutoff value for detection of myocardial damage in emergency department. We find this cutoff in accordance with manufacturer recommendation (0.053 μg/L). Therefore, introduction of new hsTnT method using proposed cutoff will not influence consistency of medical decisions.

T397
VALIDATION OF A METHOD TO RULE OUT URINE ALBUMIN DETERMINATIONS

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Aim: New generation dipsticks offer the albumin/protein-to-creatine ratio. We need to know their analytical behavior and cost-effectiveness.
Question: Is it possible to avoid the exact (reference) determination of ACR/PrCR if dipstick value (field) is the semiquantitative maximum result?
Methods (Siemens Healthcare Diagnostics): 1. Strip (protein-to-creatinine ratio, sqPrCR): Multistix PRO-12 in a Clinitek-ATLAS reader. Maximum category: ≥500 mg protein/g creatinine. 2. Creatinine: photometry in an ADVIA-2400. Jaffé kinetic method with ID-MS calibration. 3. Albumin (albumin-to-creatinine ratio, ACR): nephelometry in a BN-II. Mouse monoclonal antibody. 4. Protein (protein-to-creatinine ratio, PrCR): nephelometry in a BN-II. TCA/20% aqueous solution. SAMPLES (from 01.01.09 until 31.10.12). Number of urine with sqPrCR strip results: 474932. Number or urine with PrCR/ACR results (reference): 8475. Female: 3597. Male: 4878. Number of urine with sqPrCR ≥500 mg/g: 1285. Female: 431. Male: 854. Results: a. JNC7 criteria number of urine samples with maximum sqPrCR by dipstick n=1285 PrCR ACR Normal <31 mg/g 0 0% 71 5.5% High 31-299 187 14.6% 14.6% 263 20.5% 26.0%. Very high >299 1098 85.4% 100% 951 74.0% 100%. b. ESH/ESC criteria i. female number of urine samples with maximum sqPrCR by dipstick n=431 PrCR ACR Normal <31mg/g 0 0% 45 10.4% High 31-299 79 18.3% 18.3% 103 23.9% 34.3% Very high >299 352 81.7% 100% 286 65.7% 100%. ii. male number of urine samples with maximum sqPrCR by dipstick n=854 PrCR ACR Normal <22 mg/g 0 0% 35 4.1% High 22-299 108 12.6% 163 19.1% 23.2% Very high >299 746 87.4% 100% 665 76.8% 100%
Conclusions: 1. The goodness-of-strip is shown with 0% of normal PrCR if spPrCR≥500mg/g. 2. Nevertheless, this approach would not detect 26.0%, 34.3% or 23.2%, according to criteria, of urine samples with normal or high ACR. This is clearly unacceptable regardless of the criteria used. 3. In short, the answer is that we advocate to do not.

T398
DEVELOPMENT OF A PHOTON UPCONVERSION READER WITH NEAR-INFRARED LASER EXCITATION

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Upconverting nanoparticles (UCNPs) have attracted an immense attention for their possible applications in biotechnology. These trivalent lanthanide ion doped materials are capable of absorbing near-infrared light and converting it into luminescence at visible wavelengths. The advantages of upconversion luminescence are the avoidance of autofluorescence at measurement wavelengths, reduced sensitivity to scattering and absorption as well as narrow emission peaks of lanthanide ions. Hindrance with the upconversion technology thus far has been the lack of commercial upconverting nano particles as well as a well-established detection platform with infrared laser irradiation source for the excitation of the UCNPs. Recently commercial UCNPs have been introduced so the only problem remains with the instrumentation. To solve this problem a new detection platform was developed in association with Hidex, Turku, Finland. This was based on Plate Chameleon V reader (Hidex) from which the light source was replaced with 976 nm laser system (4 W). To enhance the performance of the device the laser itself was equipped with a peltier element for temperature control. In this epifluorometer the laser light was collimated to 0.8 mm diameter beam, which was directed to a well with an aluminum mirror. Emitted light from the well was gathered with a lens, filtered to block the excitation light and to select a correct emission band, e.g. 550 nm and then detected with a photomultiplier tube (PMT). UCNP detection limit, with streptavidin coated Ø 25 nm UCNPs, with this new device was measured to be approximately 500 000 particles/ml, which allows the performance of high sensitivity immunoassays. Also the dynamic range was 5 orders of magnitude. With the development of this detection platform, the first steps to standardization of UCNP assays have been taken and this is also one of the key issues in acquainting the technology to common knowledge.

T399
ASSESSMENT OF EFFICACY OF DISINFECTION AND STERILIZATION PROCEDURES

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Background: Surgical procedures and larger number of invasive medical procedures involve contact by a medical device or surgical instrument with a patient's sterile tissue or mucous membranes. A major risk of all such procedures is the introduction of pathogenic microbes, which can lead to infection. Aim of this study was assessment of the efficacy of disinfection and sterilization procedures in University Clinical Center in Skopje, Macedonia.
Methods: For the period of one year in 18 departments of Clinical Center efficacy of disinfection and sterilization procedures was assessed with application of physical, chemical and biological indicators. Influencing factors that affect efficacy of disinfection/sterilization were cleaning of the object, presence of organic and inorganic load, type and level of microbial contamination, concentration and exposure time to disinfectant/sterilzant, nature of the object, temperature and relative humidity. Steam, heat, ethylene oxide and hydrogen peroxide were commonly used as sterilizing agents.
Results: According obtained results 98.81% of sterilization procedures were performed successfully. From 7242 sterilization procedures in total, only 86 procedures were unsuccessfully (56 because of electric current failure, and 30 because of technical failure). Biological indicators were recognized as reliable sterility indicators. As indicator were used spores inoculated on carrier. Growth of microorganisms was determined through the color change in a pH indicator due to a pH change resulting from byproducts of microbial cell growth in the medium.
Conclusions: Achieving disinfection and sterilization through the use of disinfectants and sterilization practices is essential for ensuring that medical and surgical instruments do not transmit infectious pathogens to patients. Biological indicators provide confidence that a sterilization process has been done successfully. Inactivation of biological indicator strongly implies that other potential pathogens during sterilization have been killed.

T400
MEASUREMENTS OF MINERAL ELEMENTS AND HEAVY METALS FROM HAIR CELLS BY IPC-MASS SPECTROMETRY

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Background: Aim of this work was to identify intracellular levels of principal mineral nutritive elements (Ca, Mg., Cu, Zn) and trace elements (Al, Pb, Hg), measured by inductively coupled plasma– mass spectrometry (ICP-MS) instrument, to apparent healthy persons, with a good nutritional status and gold standard of lifestyle.
Material and method: At a number of 75 persons, all adult females, in range age 30-55 years, from different regions of the country was taken the samples of 100mg hair, obtained by cutting the first 3 cm closest to the scalp. Protocol of work Was delivered 100milligrams hair, cutting from back scalp of subjects, in the special cuvettes IPCMS and, results were measured and interpreted after graphics IPC –MS.
Results: A number of 12 patients (16%) displayed high levels of intracellular Mg, (mean value = 1.2 mmol /L, high values of Ca, (mean value =0.72 mmol/L), but low values of report Ca/Mg, (mean value = 0.58) and six patients (8%) were exhibited low levels Mg (mean value = 0.004 mmol/L low values of Ca (0.04 mmoli/ L) but high report Ca/Mg (10). Any persons not presented signs of acute or chronic intoxication with havey metals.
Conclusions: Environmental of life to habitants, from different regions of country, is reflected in hair cells, as so is in all cells of body, in concentrations from last months.

T401

ANALYTICAL VALIDATION AND DEVELOPMENT OF A 25 OH VITAMIN D ASSAY BY UHPLC-MS/MS

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Background: 25-Hydroxy vitamin D (25-OH D) is an important marker for several diseases. Although various automated immunoassays are available they are not satisfactory in certain aspects. We developed and validated a new ultra-high-performance liquid chromatography mass spectrometry (UHPLC-MS/MS) method for determination of 25-OH D2 and D3.
Methods: Aliquots of 150 uL spiked bovine serum albumin were prepared by adding 20 uL of internal standard (IS) followed by treatment with zinc sulfate and then by acetonitrile to remove protein. Samples were then analyzed for 25-OH D2 and D3 using UHPLC-MS/MS. The chromatographic system consisted of an Agilent (Santa Clara, USA) 1290 LC system with a Poroshell 120 EC-C18 column (3.0x50 mm, 2.7 μm) and a Zorbax Eclipse Plus-C18 guard column (4.6x12.5 mm, 5 μm). A gradient of mobile phase A (water/5M ammonium formate/formic acid, 99.8/0.1/0.1, v/v/v) and mobile phase B (methanol/5M ammonium formate/formic acid, 99.8/0.1/0.1, v/v/v) was used at flow rate of 0.4 mL/min for pump A and 0.5 mL/min for pump B. The injection volume was 3uL. An Agilent 6460 triple quadrupole MS, with an electrospray ionization (ESI) source, was used in positive mode. The method was validated in terms of linearity of range, limit of detection (LOD), limit of quantitation (LOQ), recovery, and precision.
Results: The mass /charge transitions of 413.3>355.3 (25-OH D2), 401.3>365.2 (25-OH D3), 416.3>358.3 (IS of 25-OH D2) and 404.3>368.2 (IS of 25-OH D3) were monitored by multiple-reaction monitoring (MRM) between the time from 3.2 to 3.8 min (total run time, 4.5 min). The linearity was 5-100 ng/mL with r^2 = 0.999 for both analytes. The LODs were 1.61 and 2.01 ng/mL while the LOQs were 5.37 and 6.69 ng/mL for 25-OH D2 and D3, respectively. Recovery of both 25-OH D forms ranged from 94.8% to 100.7% at concentrations of 10, 30 and 80 ng/mL. Inter- and intra- assay precision value were less than 10%.
Conclusions: This new UHPLC-MS/MS method, with a simple sample preparation, provides a rapid, accurate, and precise assay suitable for routine testing as a tool for evaluation of individual vitamin D status.

T402

COMPARISON OF TWO METHODS FOR MEASUREMENT OF 25-HYDROXYVITAMIN D

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Background: Measurement of circulating 25-hydroxyvitamin D (25(OH)D) in blood is the most reliable indicator of vitamin D status in organism. There are currently two main types of measurement used routinely for measuring the main circulating metabolites of vitamin D, 25 (OH)D2 and 25 (OH)D3. These are competitive immunoassay and methods based on chromatographic separation followed by non-immunological direct detection. With increasing clinical demand for 25(OH)D assays, fast automated platform with immunoassays are attractive. We evaluated an automated immunoassay for the quantitative determination of total serum 25(OH)D and compared it with a selective and sensitive HPLC method with UV detection.
Methods: 25 (OH)D from human sera (routine serum samples, N=48) was measured using two methods: fully automated competitive electrochemiluminescence immunoassay (Roche Elecsys Vitamin D total assay) and Chromsystems HPLC method for 25 (OH) D3/D2. The evaluation protocol for immunoassay consisted of within-run imprecision (10 sequential runs, two samples) and between-run imprecision (10 consecutive working days, 2 sequential runs) with commercial controls PreciControl Bone 1 and PreciControl Bone 2, inaccuracy (N=20), and method comparison. Methods were compared by Passing and Bablok regression and Bland-Altman analyses.
Results: Within-run imprecision for Roche Elecsys Vitamin D total assay was 2.56%, and between-run imprecision was 2.99% and 3.79%. Quality requirement for inaccuracy was fulfilled. The comparison with HPLC method demonstrated strong correlation (r=0.9581; y=0.918x-4.5082) and good agreement (bias = ±1.96 SD).
Conclusion: Roche Elecsys Vitamin D total assay showed good correlation and agreement with HPLC-UV method and represents an accurate and precise automated tool for serum total 25 (OH)D determinations. Measurement of 25 (OH)D by immunoassay is also the method of choice for reasons of convenience, speed, turnaround, and cost.

T403
GENETICALLY ENCODED PROTEASE SUBSTRATE BASED ON LANTHANIDE-BINDING PEPTIDE FOR TIME-GATED FLUORESCENCE DETECTION

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Background: The study of biomolecular interactions is at the heart of biomedical research. Fluorescence and Förster resonance energy-transfer (FRET) are potent and versatile tools in studying these interactions. Fluorescent proteins are genetically encodable, which facilitates their use in recombinant protein and in vivo applications. To eliminate the autofluorescence background encountered in applications based on fluorescent proteins, lanthanide labels can be used as donor fluorophores. Their long emission lifetime enables the use of time-gating that significantly improves assay sensitivity. The commonly used lanthanide chelate and cryptate labels, however, are not genetically encodable. This problem can be circumvented by using a lanthanide-binding peptide, which is a small genetically encoded peptide sequence that selectively binds lanthanide ions with high affinity.
Methods: In this work we combined the favorable characteristics of an intrinsically fluorescent terbium-ion containing lanthanide-binding peptide (Tb3+-LBP) and green fluorescent protein (GFP) in a FRET-based homogeneous caspase-3 activity assay. Caspase-3 is a key member mediating the proteolytic cleavage cascade that is activated during apoptosis. The genetically engineered construct used in the assay had LBP and GFP sequences at adjacent ends of a linker that comprised a recognition sequence for caspase-3.
Results: In the assay both the time-gated terbium donor signal at 545nm and the time-gated sensitized GFP acceptor emission at 520nm were monitored. The inhibitor dose-response curves gave IC50 values of approximately 60 nM. Signal-to-background ratios were maximally 33. The coefficients of variation between replicate reactions were small, maximally 4.2%.
Conclusions: We were able to demonstrate, for the first time, the applicability of a Tb3+-LBP–GFP energy-transfer pair in a protease activity assay. The intrinsically fluorescent and genetically encodable components enable easy expression of the construct without the need of cumbersome chemical labeling. By varying the fluorescent protein and the protease specificity of the internal linker sequence the method can be applied for the detection of a wide variety of proteases.

T404
EVALUATION OF AN AUTOMATED ASSAY FOR GALECTIN-3

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Background: Replacement of functional myocytes with crosslinked collagen is a final common pathway that is central to the progression of heart failure (HF), irrespective of etiology. In response to different mechanical and neurohormonal stimuli, macrophages secrete Galectin-3, that stimulates cell proliferation and secretion of procollagen I. This protein is then irreversibly crosslinked to form collagen and result in cardiac fibrosis. We report an initial assessment of a new assay for Galectin-3.
Methods: The analytical characteristics of a fully automated immunoassay for Galectin-3 on the Abbott ARCHITECT platform have been evaluated and the assay has been compared with a conventional enzyme immunoassay (EIA, BG Medicine) on a reference population of healthy subjects and on patients with heart failure.
Results: The limit of blank (LOB), limit detection (LOD) and limit of quantitation (LOQ) of the ARCHITECT assay were 0.58 ng/mL, 1.32 ng/mL and 4.33 ng/mL (5.57% CV), respectively. The total imprecison on three levels of assay controls ranged from 1.60% to 3.75% and the serum/ plasma equivalency on 44 samples from patients with myocardial infarction was satisfactory, with an average difference of 13.76%. On 136 healthy subjects the 97.5th percentile was 19.56 ng/mL (21.03 in males, 19.00 in females). On a 274 healthy and diseased subjects the correlation between ARCHITECT and EIA was very high (r2=0.94), with a positive proportional bias of the automated assay. Galectin-3 levels correlated with the severity of HF in 177 patients, with median values of 17.05ng/mL in NYHA-I, 28.10ng/mL in NYHA-II, 27.40ng/mL in NYHA III and 42.95 in NYHA IV patients. By the Mann-Whitney test the differences between NYHA I and II/III/IV and NYHA III and IV were statistically significant. By ROC curve analysis, the best discriminating value between healthy and diseased subjects corresponds to 16.40 ng/mL (sensitivity: 90.4%; specificity: 73.4%).
Conclusions: The automated assay for Galectin-3 has shown good analytical performances and a good correlation with another method. Though Galectin-3 values appear to correlate with the severity of HF, additional clinical data are needed in order to introduce this new assay in clinical practice.

W001
BONE TURNOVER MARKERS IN POSTMENOPAUSAL WOMEN WITH DEFICIENT CALCIUM INTAKE

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Primary osteoporosis is a metabolic bone disease characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and increased fracture risk. It also has normal mineral-to-collagen ratio. Primary osteoporosis represents bone mass loss that is unassociated with any other illness and is related to aging and loss of the gonadal function in women. Secondary osteoporosis can result from a variety of the chronic conditions that significantly contribute to bone mineral loss, or it can result from the effects of medications and nutritional deficiencies like low calcium intake. The aim of this study was to determinate relationship between Ca intake and bone turnover markers, β-Cross Laps (B) as a bone resorption marker and N-MID Osteocalcin (O) as a bone formation marker. Material and methods: The Elecsys β-Cross Laps serum assay is a sandwich immunoassay with two monoclonal antibodies specific for the β–isomerized 8-amino acid sequence. As bone resorption markers, serum osteocalcin (OC) was determined by the Elecsys N-MID OC assay.the relationship between Ca intake and bone turnover markers was determinated in 116 postmenopausal woman divided in 3 groups according to their Ca intake: 1st Ca intake less then 500 mg/day , 2nd 500-1000 mg/day, 3th more than 1000 mg/day. For Macedonian population we found significant differences in serum concentrations of β-Cross Laps concentration were observed between groups: 0.59+-0.26 ng/mL in the 1st,0.46+-20 ng/mL in the 2nd, and 0.40+-0.22ng/mL in the 3th group, and it is closelly associated with the Ca intake. Serum osteocalcin levels were not significantly different among the groups. Long-term lower Ca intake induces increased bone turnover with significantly higher β-Cross Laps concentration. This condition is caracterised with higher bone turnover, increased bone resorption and insufficient bone formation,indicating higher osteoporotic risk. Bone turnover markers are of a great importance for determination the risk of the osteoporosys in posmenopausal women with low CA intake.

W002
LABORATORY PARAMETERS OF METABOLIC BONE DISEASE IN CKD PATIENTS FROM EARLY THIRD STAGE UNTIL TERMINAL STAGE AND THEIR SIGNIFICANCE IN CLINICAL PRACTICE

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Background: Secondary hyperparathyroidism (SHPT) is a common disorder in patients with progressive chronic kidney disease (CKD). An increase in parathyroid hormone (PTH) is early detected with GFR <60 mL/min/1.73 m² and is particularly reported in hemodialysis patients. The results of the disorder are accompanied by metabolic mineral bone diseases, complications in cardiovascular system function and other. The objective of the paper was to establish and monitor the values of PHT, serum calcium (Ca), serum phosphorus (P), alkaline phosphatase (AF) being significant for estimation of SHPT progression, complications development and substitution therapy.
Method: 31 CKD patients with stages 3-5 were assayed as well as 38 hemodialysis patients of both sexes. Ca, P, AF and intact PTH(iPTH) levels were determined using commercial assays in the integrated biochemical analyzer Architect ci 8200.
Results: Ca, P, AF values in CKD patients with stages 3-5 were within the reference ranges while in hemodialysis patients the values were in accordance with the KDIGO guidelines. iPTH values with progression of kidney disease showed significant increase (P <0.01). Average iPTH values in CKD patients with stage 3 is 140.4±71 pg/mL, with stage 4 is 241.5±156.9 pg/mL and with stage 5 is 400.1± 218 pg/mL. Hemodialysis patients had iPTH levels 2-9 times higher than upper reference limit for the assay and were in accordance with KDIGO guidelines.
Conclusion: The assay showed that in early stages of CKD and during hemodialysis treatment iPTH, Ca, P and AF determination is important to estimate SHPT, metabolic bone disorder, timely use of substitution therapy.

W003

THE RELATIONSHIP BETWEEN OSTEOPROTEGERIN, RANK SYSTEM AND OBESITY IN POSTMENOPAUSAL WOMEN

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Background: Obesity and bone metabolism are interrelated. Both obesity and osteoporosis are associated with increased production of proinflammatory cytokines, which may promote osteoclast activity and bone resorption through modifying the receptor activator of NF-kb (RANK)/RANK ligand /osteoprotegerin pathway. Furthermore, excessive secretion of leptin in obesity may either affect bone formatıon directly or indirectly affect bone resorption through up-regulated proinflammatory cytokine production. The aim of this study is to investigate the relationship between obesity and osteoclastic (TRAP), osteoblastic (bone ALP) and osteoclastogenic (RANKL, osteoprotegerin) markers and leptin and insulin resistance in postmenopausal osteoporosis.
Material and methods: Four study groups were included in the study. Group 1 (n=19): osteoporosis (T Score <2.5 at the lumbar spine and/or femoral neck) +obese, group 2 (n=29): non-osteoporosis+obese, group 3 (n=25): osteoporosis+non-obese, group 4 (n=14): non-osteoporosis+ non-obese as control. The serum levels of osteoprotegerin, RANKL, TRAP, bone ALP and leptin were measured by ELISA. Glucose and insulin levels were analyzed by colorimetric and electrochemiluminescence methods respectively.
Results: Osteoprotegerin (P <0.05) and bone ALP (P <0.01) levels were significantly lower in group 1 compared to group 2. Comparison of group 1 with group 3 revealed significantly higher (P <0.001) leptin levels in the former. Osteoprotegerin (P <0.001), TRAP (P <0.001), RANKL (P <0.01), bone ALP (P <0.01) and leptin (P <0.05) were significantly lower in group 1 compared to control group. Comparison of group 3 with the control group revealed significantly lower values of osteoprotegerin (P <0.001), RANKL (P <0.01), bone ALP (P<0.01), TRAP (P<0.001) values for patient group. Osteoprotegerin (P <0.001), RANKL (P <0.01), bone ALP (P <0.01), TRAP levels were significantly lower, leptin levels (P <0.05) significantly higher in group 2 vs. control group.
Conclusion: Osteoprotegerin, TRAP, RANKL, bone ALP and leptin levels are significant predictors in the development of osteoporosis and/or obesity in postmenopausal women.

W004

ASSESSMENT OF BONE TURNOVER MARKERS FOR THE EARLY DETECTION OF MYELOMA BONE DISEASE RELAPSE

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Background: Myeloma Bone Disease (MBD), comprising osteolytic lesions secondary to uncoupling of osteoblast and osteoclast activity, is a significant cause of morbidity in Multiple Myeloma (MM). Early detection of progressive osteolysis upon relapse is crucial to allow preventative therapeutic intervention which could significantly impact on quality of life. Imaging techniques have limitations in that they cannot provide a real-time assessment of bone turnover. The aim of this study was to assess the potential utility of bone markers, C-terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-propeptide (P1NP) in the early detection of MBD relapse.
Methods: CTX-1 and P1NP were measured by chemiluminescent immunoassay on fasting plasma samples from MM patients at regular intervals over a 30 month study period. Relapse was identified by routine paraprotein (PP) and serum free light chain (SFLC) monitoring, and confirmed by imaging and bone marrow biopsy. In a subset of patients with disease relapse, bone markers pre-relapse and at relapse were compared. Results are median (IQR) unless stated.
Results: There were 24 relapse episodes in 21 patients with prior bone markers. The median time between the pre-relapse sample and disease relapse was 14.5 weeks (range 2-53 weeks). CTX-1 rose from 0.127 (0.112-0.232) μg/L pre-relapse to 0.247 (0.173-0.525) μg/L at relapse (p=0.0156). The change in P1NP from 17.2 (11.9-32.2) μg/L pre-relapse to 30.8 (18.2-42.5) μg/L at relapse was not significant (p=0.0764). Six patients showed evidence of a rise in CTX-1 up to 6 months before the pre-relapse sample, which was prior to SFLC or PP rising in 3 cases. In one further case, CTX-1 was the only biochemical parameter to rise significantly on relapse.
Conclusion: CTX-1 is a useful adjunct to SFLC and PP in the early detection of MBD relapse. In 17% of relapses, CTX-1 rises before SFLC or PP. P1NP is a less robust marker and did not decrease as expected, meriting further investigation. CTX-1 is more cost effective and accessible than imaging and should be used routinely when monitoring bone disease activity in MM patients, facilitating early intervention when relapse occurs.

W005

MARKERS OF BONE RESORPTION IN WOMEN WITH SURGICAL MENOPAUSE TREATED WITH HRT

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Background: Bone alkaline phosphatase(BALP), acid phosphatase(sAcP) sera activity and urine N-terminal telopeptide(NTx) were measured in 60post menopausal women with surgical intervention and compared with result in 47 healthy pre menopausal women.
Methods and results: We propose individual prognostic osteoporotic index (IPOI) as a half of the sum of serum alkaline phosphatase (BALP) and serum acid phosphatase (sAcP) for early prediction of the next osteoporosis in the postmenopause. We estimated 60 women with surgical menopause, divided in two groups: group A which started hormone replacement therapy (HRT) within a period of one to six months after the operation (n=30). The biochemical indicators of one turnover: BALP, sAcP and a new parameter, individual prognostic osteoporotic index (IPOI), as a half of the sum of BALP and sAcP, were estimated before and after first and sixth cycle of the therapy in group A, and before, and after one and six months after the operation in group B. At the end of the study, all parameters decreased significantly in group A ($P < 0.001$) and increased in group B ($P < 0.001$). On the beginning, all parameters were significantly higher in group A ($P < 0.001$), perhaps from the reason that HRT started after one to six months of the operation. We propose IPOI for determination of future osteoporosis. If IPOI amounts between 28.5 and 37, this woman enter into the group of slow losers of bone mass-candidates of rigorous control for endometrial and breast cancer, but no HRT, because of increasing conversion of estrone into the fatty tissue. If IPOI amounts 42.5 and more, this woman enter into a group of fast losers-candidates for HRT and additional therapy. Markers of osteoresorption, NTx and sAcP were significantly increased in women with surgical menopause without HRT (82.13±46.75 vs 41.60±21.58 BCE/mmol cretinine) and 3.87 ± 1.19 vs 2.35 ±0.87 U/L. We also found significantly stimulated osteosyntesis trough BALP values (99.14±21.36 vs 46.12±13.1 U/L) in women on HRT.
Conclusion: IPOI, could be a good parameter for early prediction of the next osteoporosis in the postmenopause.NTx is the most responsive and specific indicator of the bone resorption.

W006

IS THE LACTOSE INTOLERANCE A RISK FACTOR TO DEVELOPMENT OF OSTEOPOROSIS AND BONE FRACTURES? GENETIC STUDY IN PERIMENOPAUSAL SPANISH WOMEN

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Background: Lactose intolerance (LI), a widespread condition throughout the world, is a genetically determined decline of lactase-phlorizin hydrolase activity, is inherited through an autosomal recessive gene. Results of studies on the role of LI in the development of bone mass are controversial. The CC genotype of the lactase gene at chromosome 2q21-22 is associated with LI and the CT and TT genotypes with lactase persistence. We hypothesized that the CC genotype is associated with a decreased bone mineral density (BMD) and increased fracture risk. Our aim was to evaluate the influence of LI on bone fracture risk in Spanish perimenopausal women.
Methods: We studied 60 perimenopausal women (mean 54 years), with or without osteoporosis/osteopenia. BMD (g/cm2) was measured by dual energy X-ray absorptiometry at lumbar spine and femoral neck and results were expressed as Z-score. In all subjects fractures were documented. Moreover, none of the participants was taking calcium or vitamin D supplementation or any drug interfering with bone metabolism. For genetic analysis, a Real-time PCR followed by melting curve analysis was performed on the LightCycler 2.0 (Roche Applied Science).
Results: The prevalence of CC, CT y TT genotypes was 26,6%, 50% and 23,4% respectively. The number of pre-menopausal bone fractures were 7 (11.9%):2 (3.4%) in CC genotype and 5 (8.5%) in CT genotype. Women with CC genotype did not report more fractures than those with the other genotypes. We didn't found association between CC genotype and pre-menopausal bone fractures ($P=0.276$). The relationship between bone fractures after menopause and genotype was not significant ($P=0.672$). 6 fractures occurred (12.5%), of which only 1 (2.08%) were CC genotype, 3 (6.25%) were CT genotype and 2 (4.1%) were TT genotype. There was an associations between the presence of the C allele (homozygous and heterozygous) and pre-menopausal bone fractures ($P < 0.05$).
Conclusion: Molecularly defined LI doesn't significantly contribute to the development of osteoporosis and the occurrence of osteoporotic fractures in Spanish perimenopausal women. However, the presence of the C allele implies an increased risk of bone fractures.

W007

AUGMENTATION OF ADAMTS9 GENE EXPRESSION BY IL-1BETA IS REVERSED BY NF-KAPPAB AND MAPK INHIBITORS, BUT NOT PI3 KINASE INHIBITORS

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The pathways involved in the regulation of a disintegrin and metalloproteinase with thrombospondin motifs 9 (ADAMTS9) expression have not yet been elucidated. Therefore, the aim of this study was to investigate the involvement of nuclear factor-kappaB (NF-kappaB), mitogen activated protein kinases (MAPK) and phosphatidylinositol 3-kinases (PI3 kinase) in ADAMTS9 gene regulation, with special focus on the involvement of NF-kappaB in interleukin-1 beta-induced ADAMTS9 expression. The OUMS-27 chondrosarcoma cells were exposed to IL-1beta. They were pretreated with PD98059 (specific inhibitor of p44/42 kinase), SB203580 (specific inhibitor of p38 kinase), SB600125 (MAPK inhibitor), and Wortmannin and LY294002 (specific inhibitors of PI3 kinase) for 30min and subsequently incubated with IL-1beta. For the effects of NF-kappaB inhibitors, cells were pretreated with curcumin or BAY117085 for 30 min and subsequently incubated with IL-1beta. BAY117085 and different concentrations of curcumin were applied to the cells just after the first experiment to determine their concentration effect on ADAMTS9 gene expression. After total RNA was extracted, they were reversely transcribed with random primers and then real-time polymerase chain reaction (PCR) was performed on cDNA samples. There was a significant difference between control and stimulated cells in terms of ADAMTS9/beta-actin ratio.Wortmannin and LY294002 did not have any repressive effect on the OUMS-27 whereas SB203580 and SP600125 were found to decrease the expression of ADAMTS9 gene. BAY117085 and curcumin, which are two NF-kappaB inhibitors, led to a decrease in the ratio of ADAMTS9/beta-actin. As a conclusion, the pathways MAPK and NF-kappaB were thought to be responsible pathways for the induction of ADAMTS9 gene.

W008

DEVELOPMENT OF AN ASSAY TO DETECT TOTAL 25(OH) VITAMIN D ON THE BECKMAN COULTER ACCESS® FAMILY OF IMMUNOASSAY SYSTEMS

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Background: The role of vitamin D in maintaining calcium homeostasis is well established. Increasing evidence also suggests that vitamin D may play a role in decreasing the risk of many chronic illnesses. 25-hydroxyvitamin D [25(OH)D] is the major circulating metabolite of vitamin D in the body and reflects inputs from cutaneous synthesis and dietary intake. For this reason, serum concentration of 25(OH)D is considered the standard clinical measure of vitamin D status. Beckman Coulter is developing an automated assay to measure total 25(OH)D levels in human serum and plasma on its Access and DxI immunoassay platforms.
Methods: The prototype Access 25(OH)D assay is a competitive chemiluminescent immunoassay. Sample is added to a reaction vessel with vitamin D binding protein (VDBP) releasing agent and paramagnetic particles coated with monoclonal anti-25(OH)D antibody. 25(OH)D-alkaline phosphatase conjugate is then added and competes for binding to the immobilized monoclonal anti-25(OH)D. After incubation, bound materials are held in a magnetic field and unbound materials are washed away. Chemiluminescent substrate, Lumi-Phos 530, is added and light generated imeasured. Light production is inversely proportional to the concentration of 25(OH)D in the sample, which is determined from a stored, multi-point calibration curve.
Results: Feasibility studies have been completed on a prototype assay with the following preliminary performance characteristics: A limit of detection (LoD) of 1.8 ng/mL and a limit of quantification (LoQ) equal to 6 ng/mL with a measuring range up to 180 ng/mL. Total CVs of approximately 10% for samples at 10 ng/mL and ≤5% for samples ranging from 30-180 ng/mL. A correlation with LC-MS/MS was performed.
Conclusions: This evaluation demonstrates that the prototype Access 25 (OH)D Total assay exhibits good precision and sensitivity with acceptable correlation to LC-MS/MS. In addition, the assay displayed satisfactory equimolar recognition of both 25 (OH)D2 and 25 (OH)D3. These characteristics demonstrate the potential for this assay to be used in the quantitative determination of total 25(OH)D levels in a routine laboratory environment.

W009

EVALUATION OF THE PERFORMANCE OF THE NEW AUTOMATED IDS-ISYS 1,25-DIHYDROXY VITAMIN D ASSAY

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Background: 1,25-Dihydroxyvitamin D (1,25D) is one of the major regulators of calcium metabolism. Measurement has always been difficult due to its lipophilic nature, its low circulating concentration and the presence of higher concentrations of other Vitamin D compounds. Recently, IDS has commercialised the IVD CE marked automated 1,25D assay on the iSYS platform. In this study, we aimed to validate the performance of this new automated assay.
Methods: The accuracy profile was determined with 5 serum pool levels (16.9-175.4 pg/mL). The method linearity and recovery were verified with high/low samples. A total of 65 remnant serum samples were assayed by IDS EIA and IDS-iSYS for method comparison. In addition to establish the reference intervals with specimens from apparently healthy Caucasians subjects with normal Calcium, Phosphate, PTH and eGFR > 60ml/min/1.73m², we also established ranges for paediatric, pregnant women and haemodialysis population.
Results: The IDS-iSYS 1,25D total time to first result (immunoextraction and immunoassay) is 4h30min. The assay range is 6.5 to 210 pg/mL with precision of 1.3%, 2.2%, 3.6%, 7.8% and 16.0% for samples at 16.9, 33.2, 52.9, 100.3 and 175.4 pg/mL, respectively. Method comparison against the IDS- EIA yields a Passsing Bablok regression of: IDS-iSYS = 1.09 x (IDS EIA)+3.9; Pearson r = 0.97 (P<0.0001). The median (5-95 percentile range, pg/mL) for paediatric (statified by every 2 years), pregnant women and haemodialysis population are: 0-2 years, 52.4(34.8 - 139.8); >2-4, 53.8(26.5 - 108.8); >4-6, 47.0(20.2 - 84.1); >6-8, 48.2(19.0 – 78.3); >8-10, 47.0(20.2 - 84.1); >10-12, 35.9(19.7 – 69.4); >12-14, 57.4(26.1 – 88.1); >14-16, 55.1(32.3 – 86.8); >16-18, 54.5(30.5 – 78.6); >14-16, 55.1(32.3 – 86.8); 1st trimester, 55.2(33.8-111.6); 3rd trimester, 140.4(85.5-196.0) and haemodialysis, 11.3(7.3-27.8).
Conclusion: The IDS-iSYS 1,25-Dihydroxy Vitamin D kit is a complete test system with the proven immunocapsule extraction and automated chemiluminescence immunoassay. With excellent low end precision and good correlation to current method, the automated IDS-iSYS 1,25D assay will be a valuable tool for clinical laboratories to accurate measure larger number of 1,25D samples in a single working day.

W010

ANALYSIS OF CELLULAR AND SPECIFIC HUMORAL IMMUNE RESPONSE IN PATIENTS WITH SURGICAL PATHOLOGY OF JOINTS AND CHLAMYDIAL INFECTION

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Background: We studied 116 patients (51 males and 65 females) aged 37.0±12.5 years. For the comparison of immune parameters patients were divided into 4 groups. Group 1 – patients with acute pathology of the knee joint (n=12). Group 2 – post operated patients with degenerative-dystrophic diseases of joints/other joint damages (n=22). Group 3 – patients after revision surgery (n=42). Group 4 – patients with chronic Chlamydia-induced reactive arthritis (n=40) with characteristic symptoms and laboratory markers of Chlamydial infection.
Methods: To detect the human antibodies IgG and IgA against antigens of Chlamydia trachomatis (Ctr) in serum we used serological tests "MEDAC" (Germany). The characteristics of cellular immune response and definitions of lymphocyte subpopulations were identified using flow cytometry «Beckman Coulter» (USA). Cytokine levels were determined by ELISA (RF).
Results: The presence of IgG against antigens of Ctr was found in 73 patients. Specific IgG were detected significantly more rapidly in serum of patients of groups 2 and 4 (38.4% и 71.5% respectively). IgG were positive more rapidly in women (58%), than in men (42%) (P=0.016). Specific IgA were positive in 15% and were detected more rapidly in serum of patients of group 4 (13.1% − in men and 7.2% − in women). Only one person from group 1 was seropositive to Ctr. Mean level of absolute number of lymphocytes in peripheral blood and lymphocytes subpopulation (CD3, CD4, CD16, CD20) for the patients of groups 2,3 and 4 was lower than for the group 1 (P <0.05). The patients of group 3 had significantly lower levels of CD3+ T cells - (56.3±1.7)%, CD4+ T cells - (29.8±1.8)% and CD16+ T cells - (9.5±0.7)% (P <0.05). IFN-gamma level in serum of patients of group 4 was (8.5±1.1) pg/mL. It was significantly (P <0.05) lower than in patients of group 1 (23.1±1.6) pg/mL. Patients of group 4 had the lowest level of IL-1 (64.0±9.7) pg/mL and significantly highest level of IL-10 (3195±123) pg/mL (P <0.05).
Conclusions: Conducted immunological studies have shown that immune disorders and cytokine network malfunctions in case of knee joint pathology associated with silent infection cause immunosuppression. Inhibition of T-cells subpopulations affects all studied subpopulations of T-lymphocytes.

W011

TENSION FORCE-INDUCED ATP PROMOTES BONE FORMATION THROUGH P2X7 RECEPTOR IN OSTEOBLASTS

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Background: The skeletal system develops and adapts throughout life in response to hormones, cytokines, and external factors such as mechanical loading. Maintenance of bone mass in adults is a dynamic process that involves a balance of resorption by osteoclasts and formation by osteoblasts. Orthodontic tooth movement induces alveolar bone resorption and formation by mechanical stimuli. In particular, the force exerted on the traction side promotes bone formation. Nucleotides such as Adenosine triphosphate (ATP), is thought to be a one of the key mediator in the response of bone cells to mechanical stimuli. It has been reported that fluid shear stress induces ATP release in the osteoblastic MC3T3-E1 cells, and also that ATP promotes bone formation through P2X7 receptor in rat calvarial cells. However, the effect of tension force (TF)-induced ATP on osteogenesis is poorly understood. Thus, we determined the effect of TF on ATP production, the expression of osteogenesis-related transcription factors, P2X7, extracellular matrix proteins, alkaline phosphotase (ALP) activity and osteogenesis in osteoblasts.
Methods: MC3T3-E1 cells were plated on flexible-bottom plate, incubated in the presence or absence of P2X7 antagonist A438079, and then stimulate with cyclic TF (6 or 18%, that is 6 cycles/min: 5 sec strain, 5 sec relaxation) for maximum 24 hours using a Flexercell Strain Unit 3000. ATP release in the culture medium was measured by the luciferin/luciferase assay. The expression of osteogenesis-related transcription factors, P2X7, and extracellular matrix proteins was determined at mRNA level by real-time PCR, and at protein level by Western blotting or ELISA. ALP activity was estimated by ALP staining indicating of the enzyme activity. The calcium content in mineralized nodules was determined by using a calcium E-test kit.
Results: TF at 6% induced ATP production, the expression of P2X7, osteogenesis-related transcription factor and extracellular matrix protein. TF at 6% also increased the calcium content in mineralized nodules and ALP activity. Moreover, P2X7 antagonist A438079 blocked TF-induced these phenomenon.
Conclusions: These results suggest that ATP stimulated by TF promotes osteogenesis through P2X7 receptor in osteoblasts.

W012

HYDROGEN SULFIDE SUPPRESSES MINERALIZED NODULE FORMATION BY OSTEOBLASTIC ROS17/2.8 CELLS

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Background: Hydrogen sulfide, which is the main component of the volatile sulfur compounds (VSCs) produced by putrefactive bacteria, plays a role in not only oral malodor, but also the initiation and progress of periodontitis. The loss of alveolar bone associated with periodontitis appears to be related to local factors that change the balance between bone formation and resorption. Previous studies have indicated that VSCs, including hydrogen sulfide, induce bone resorption by stimulating the differentiation and activation of osteoclasts; however, there is little information about the effect of VSCs on bone formation by osteoblasts. Therefore, the study was conducted to examine the effect of hydrogen sulfide on cell proliferation, alkaline phosphatase (ALPase) activity, extracellular matrix protein (ECMP) expression, and mineralized nodule formation using osteoblastic cell line, ROS17/2.8.
Methods: Cells were cultured with 0 (control), 0.1, 1, or 10 mM sodium hydrogen sulfide (NaHS; hydrogen sulfide donor). Mineralized nodule formation was detected by alizarin red staining. The expression of ECMP, including type I collagen, bone sialoprotein (BSP) and osteopontin (OPN), was examined at the mRNA and protein levels using real-time PCR and Western blotting, respectively.
Results: Cell proliferation was suppressed by the addition of 10 mM NaHS, but was unaffected by 1 and 0.1 M NaHS. The expression of type I collagen was not affected by the addition of NaHS. ALPase activity and the expression of BSP and OPN at the mRNA and protein levels were decreased when cells were cultured with 0.1 and/or 1 mM NaHS. In addition, mineralized nodule formation was strongly inhibited by 0.1 and 1 mM NaHS.
Conclusions: These results suggest that hydrogen sulfide suppresses mineralized nodule formation by decreasing ALPase activity and the production of BSP and OPN by osteoblasts.

W013

IS THERE A GENETIC PREDISPOSITION IN THE DEVELOPMENT OF OSTEOPOROSIS? ANALYSIS OF POLYMORPHISMS OF BONE METABOLISM GENES, COL1A1-SP1, ESR1X-XBAI AND VDR-BSMI, IN RELATION TO BONE MINERAL DENSITY IN PERIMENOPAUSAL WOMEN

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Background: Osteoporosis is characterized by bone mass decrease and an alteration of bone microarchitecture which leads to a greater susceptibility to fractures. One of the most important factors involved is the presence of family antecedents of osteoporosis. The type I Colagenous gene (Col1A1-Sp1: “SS”, ”Ss”, ”ss” genotypes) encodes for type 1 collagen, the main bone protein. The “s” allele is associated with increased vertebral fracture risk. Estrogens Receptor gene (ESR1X-XbaI: “XX”, “Xx”, “xx” genotypes) has an important effect on the regulation of the bone mass maintenance. The “xx” genotype predisposes to a bone density reduction. The “B” allele of the Vitamin D Receptor gene (VDR-BsmI: “BB”, “Bb”, “bb” genotypes) is associated with a lower bone mineral density and the “BB” genotype with a decrease in the intestinal absorption of calcium and lower BMD in menopausal women. Our aim is to assess possible contribution of the genetic polymorphisms to the bone mass loss increased risk measured by bone densitometry in perimenopausal women.
Methods: We studied 224 perimenopausal women from University Clinic of Navarra. Polymorphism genotyping: Amplification of 5 regions of the human genome and posterior detection of the amplified product (Clinical Arrays® MetaBone, Genomica) Bone mineral density: Dual-Energy X-ray Absortiometry at lumbar spine and femoral neck, measuring the Z-score.
Results: Patients with “ss” genotype have less bone mass in femoral neck (z-score= -0.411 vs -0.108, P <0.05). 80% of the patients with “BB” genotype, have osteopenia (P <0.05). Patients with “xx” genotype have fewer years of fertile life (P=0.010). In the calculation of risks: “BB” genotype leads to 2.59-fold odds of being affected by osteopenia/osteoporosis (ExpB=2.599).
Conclusion: Genetic markers could be used to screen individuals who are at most risk of fracture, allowing not only measure the genetic susceptibility of patients, but also initiating preventive measures for bone loss.

W014

HUMAN ERYTHROCYTE’S MEMBRANE AND CYTOSOLIC GLYCOHYDROLASES AS NEW TOOL FOR THE EVALUATION OF OXIDATIVE STRESS IN PATIENTS WITH PROSTHETIC-JOINT-ASSOCIATED INFECTION

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Background: The numbers of primary total hip and total knee arthroplasties have been increasing over the past decade. Prosthetic joints improve the quality of life, but they may fail, necessitating revision or resection arthroplasty. Infection is the most serious complication, occurring in 0.8 to 1.9% of knee arthroplasties and 0.3 to 1.7% of hip arthroplasties. A central role in the pathophysiologic “vicious cycle” of inflammation, deeply related to infection, is played by oxidative stress (OS) due to an over production of ROS, commonly produced during inflammatory processes. Recently has been showed that the degree of proteins O-GlcNAcylation may influence the stress response pathway; cellular levels of O-GlcNAc, regulated by O-GlcNActransferase (OGT) and O-β-N-Acetyl Glucosaminidase (OGA), are considered as OS sensor and are implicated in the aetiology of various diseases. It was demonstrated that an increased production of ROS leads to an increase of O-GlcNAcylation levels. It is known that, in inflammation conditions, erythrocyte’s (RBC) membrane is altered; indeed the overproduction of ROS cause peroxidation of cell membranes, with consequent alterations in their components. Human erythrocytes are considered as useful model for investigating physiopathological conditions and some glycohydrolases and OGA, present on RBC plasma membrane and cytosol, have been proposed as new and sensitive oxidative stress markers.
Methods. To compare the oxidative status of 11 patients with Prosthetic-joint-associated Infection (PJI) (60.2±16 years) and 30 matched controls (66.8±13 years), plasma antioxidant total defences (by Lag-time method), OGA and membrane Hexosaminidase (Hex), β-D-Glucuronidase (GCR) and α-D-Glucosidase (αGLU) activities (by fluorimetric assay) were evaluated.
Results. Compared to controls, PJI plasma membrane Hex, GCR and αGLU activities were significantly higher (P <0.05, P <0.01 and P <0.001); Lagtime values and OGA activities were significantly lower (P <0.05).
Conclusions. Data confirmed the strong OS in PJI; the role of considered enzymes as sensitive OS biomarkers suggesting their possible use to monitor conditions of PJI patients under therapies in order to manage and/or prevent the debridement and retention of the prosthesis.

W015

OPTIMIZATION OF A METHOD FOR QUANTIFICATION OF SERUM ALLOPURINOL AND OXYPURINOL CONCENTRATION USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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Background: Allopurinol, a hypouricemic agent used for the treatment of gout, inhibits xanthine oxidase, which forms uric acid from xanthine and hypoxanthine. It is rapidly and extensively metabolized to oxypurinol, which also inhibits xanthine oxidase. The metabolism of these drugs varies among individuals; thus, therapeutic drug monitoring is important for treatment optimization, especially in the case of renal impairment due to interaction with uricosuric drugs. Drug monitoring can be achieved through accurate measurement of allopurinol and its metabolite oxypurinol. The aim of study was to develop an accurate method for quantification of allopurinol and oxypurinol in serum using LC-MS/MS.
Methods: Serum samples (20 μL) were diluted with 200 μL of internal standard solution (chlorzoxazone diluted with 80% ACN) and centrifuged. Fifty microliters of the supernatant was mixed with 1% formic acid after incubation at 70 °C for 30 minutes. Chromatography was performed on an Agilent Poroshell 120 EC C-18 column (2.7 μm, 3 x 50 mm) using a mobile phase comprising formic acid in DW or in methanol. Mass spectrometric detection was performed using Triple Quad LC-MS/MS (Agilent Technologies 6490) in negative electrospray ionization mode. Serum concentrations of allopurinol and oxypurinol were quantified using a six-point standard curve in multiple reaction monitoring. For validation of the performance of the method, linearity, precision, accuracy, limit of detection, carryover, and stability were evaluated.
Results: The assay was linear over a concentration range of 471–41,598 ng/mL for allopurinol and 439–42,384 ng/mL for oxypurinol when using diluted serum which was spiked with each drugs. The imprecision tests were done with 3 levels of QC samples in quadruplicate for 5 consecutive days. The CVs of intra- and inter-day imprecision were less than 15% over the entire concentration range. The accuracy was more than 90% both for allopurinol and oxypurinol. The limit of detection was 44.9 ng/mL for allopurinol and 0.5 ng/mL for oxypurinol. The carryover rates were less than 1% for both analytes.
Conclusion: The method was suitable for quantification of serum allopurinol and oxypurinol using LC-MS/MS, and may be used for therapeutic monitoring of the patients with gout with renal impairment or poor compliance.

W016

ANGIOTENSIN II INDUCES THE PRODUCTION OF MMP-3 AND MMP-13 THROUGH THE MAPK SIGNALING PATHWAYS VIA THE AT1 RECEPTOR IN OSTEOBLASTS

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Background: Angiotensin II (Ang II) activates mitogen-activated protein kinases (MAPKs) via Ang II type 1 (AT1) and/or type 2 (AT2) receptors. Ang II not only regulates systemic blood pressure through a direct vasoconstrictive effect, but also promotes bone resorption by stimulating osteoclasts. However, the role of Ang II in the turnover of extracellular matrix (ECM) proteins in osteoid by osteoblasts is unclear. Therefore, we examined the effect of Ang II on the expression of matrix metalloproteinases (MMPs), plasminogen activators (PAs), and their inhibitors [i.e., tissue inhibitors of metalloproteinases (TIMPs) and PA inhibitor-1 (PAI-1)] using osteoblastic ROS17/2.8 cells.
Methods: The cells were cultured with or without Ang II in the presence or absence of the AT1 receptor blocker losartan, or the AT2 receptor blocker PD123319. Expression of the AT1 and AT2 receptors, MMPs, TIMPs, PAs, and PAI-1 was examined at the mRNA and protein levels by real-time PCR and Western blotting, respectively.
Results: While cell number increased following Ang II treatment, alkaline phosphatase activity decreased. The expression of MMP-3 and -13 increased markedly following Ang II treatment, whereas the expression of MMP-2, -9, -14, urokinase-type PA, tissue-type PA, TIMP-1, -2, -3, and PAI-1 was unaffected. Expression of MMP-1 and TIMP-4 was not detected. Both the AT1 and AT2 receptors were expressed in ROS17/2.8 cells. Phosphorylation of extracellular signal-regulated kinase (ERK)1/2, p38 MAPK, and stress activated protein kinases/c-jun N-terminal kinases (SAPK/JNK) increased following Ang II treatment. Losartan blocked Ang II-induced expression of MMP-3 and -13, whereas PD123319 did not completely block these responses. Losartan also blocked the Ang II-induced phosphorylation of ERK1/2, p38 MAPK, and SAPK/JNK. MAPK kinase 1/2 inhibitor PD98059 and JNK inhibitor SP600125 suppressed Ang II-induced expression of MMP-3 and -13.
Conclusions: These results suggest that Ang II stimulates the degradation process that occurs during ECM turnover in osteoid by increasing the production of MMP-3 and -13 through MAPK signaling pathways via the AT1 receptor in osteoblasts. Furthermore, our findings suggest that Ang II does not influence the plasminogen/plasmin pathway in osteoblasts.

W017

IS THERE AN ASSOCIATION OF THYROID-STIMULATING HORMONE WITHIN NORMAL RANGE WITH FRACTURES IN EUTHYROID POSTMENOPAUSAL WOMEN?

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Background: The aim of study was to verify the association of serum TSH and biochemical bone turnover markers in postmenopausal women with normal thyroid function and to answer whether the differences in TSH concentration within reference range may affect bone metabolism. Patients, Material and Methods: 52 women (55-99 years old) admitted to the hospital after osteoporotic fracture participated in the study. Serum propeptide of type 1 procollagen -P1NP (ELISA Kit for Procollagen I N-Terminal Propeptide PINP; Uscn, Life Science Inc.; detection limit was 6.9 pg/mL) as a bone formation marker and crosslinked C-terminal telopeptides - CTX (CTx ELISA, Immunodiagnostic Systems Ltd; detection limit 0.02 ng/mL), as bone resorption marker, TSH, fT4 (ARCHITECT , Abbott Diagnostics) and vitamin 25(OH)D total (Vitamin D total, Cobas e-411, Roche) were assayed. Study group was divided according to age into two subgroups: 55-70 yrs and 71-99 yrs. Results: Significantly higher median P1NP (36.4 vs 29.3 ng/mL; P <0.002), similar CTX (0.468 vs 0.439 ng/mL) and significantly lower 25(OH)D (6.2 vs 14.7 ng/mL; P <0.001) were found in patients aged 71-99 yrs than in the younger subgroup, at similar TSH (0.89 vs 0.79 mIU/mL) and fT4 (1.29 vs 1.23 ng/dL) values. Most of women with fractures (n=43; 82.7%), independently of age, had low-normal TSH concentration at first tertile (0.36-1.38 mIU/mL) whereas only 5.8% had TSH in the highest tertile (2.4-3.41 mIU/mL). The lower TSH value the higher P1NP concentration was observed in both subgroups. Elevated P1NP concentration (>45 ng/mL) was found in 21% of all cases with low-normal TSH. On the contrary, CTX concentration was within normal range in study patients with low-normal TSH, except two, in which was highly elevated.
Conclusion: In postmenopausal women, independent of age, low-normal TSH concentration seems to be related to higher prevalence of fractures, however the association with bone turnover needs further explanation.

W018

DETERMINATION OF PARATHYROID HORMONE LEVELS BY TWO AND THIRD GENERATION ASSAY.

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Background: Most methods for the determination of parathyroid hormone (PTH) showed cross-reactivity with N-truncated forms of PTH. The aim of the study was to determine the PTH levels using third generation bio-intact PTH or PTH(1-84) assay (Roche, Elecsys®) in comparison to second generation intact PTH assay (Roche, Elecsys®).
Methods: Serum PTH levels were measured by an electro-chemiluminescent immunometric assay (Elecsys® 2010, Roche).The subjects are 43 patients (26 chronic Kidney Disease (CKD), 17 non CKD). The statistical analysis used method validator and SPSS software.
Results: The intra- and interassay imprecision was measured with Precicontrol Varia® 1 and 2 (CV intra (N=7): 1.14 and 1.46 % respectively; CV inter (N=4): 1.22 and 1.58 % respectively). The intraassay imprecision on 4 patients levels (17 pg/mL, 38 pg/mL, 70 pg/mL, 113 pg/mL, N=5) were respectively 2.29 %, 1.25 %, 1.75 %, 0.65 %. PTH(1-84) was significantly correlated with intact PTH [T-Test (SPSS): r=0.998, P <0.000]. With Method validator software, the regression equation (Passing Bablock) was calculated (y=0.719X + 4.621, r=0.988, n=43). Regression equations were also calculated for CKD vs non CKD: (y=0.666X + 4.634, r=0.987, n=26) vs (y=0.795X + 3.064, r=0.795, n=17). There are no significant analytical differences between both populations (CKD and non CKD). The difference diagram (Bland and Altman) showed a linear increase between the both assays: the increase is more important the higher the values are. Correlation studies between the both methods showed a slope of about 0.6 meaning that the results with PTH(1-84) were about 60% of these from intact PTH.
Conclusion: Our study showed that PTH(1-84) levels were lower than PTH results generated by a second generation intact assay. The PTH (1-84) assay shows less variability regarding the interpretation of the results for haemodialysis patients due to the fact that there is no interference with fragments such as 7-84. PTH (1-84) can be used for haemodialysis patients because the results are lower and easier to interpret for the clinical follow-up. There might be other interferences that result in higher results; further investigations would be necessary to determine the origin of variation for high values.

W019
THE INFLUENCE OF IL1A AND RANK GENE-GENE INTERACTION ON BONE PHENOTYPES

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Osteoporosis, the bone disease with enhanced bone fragility and increased fracture risk, has a strong genetic background with up to 80% genetic influence on bone mineral density (BMD). A lot of candidate genes and later genome-wide association studies were performed to find genes and/or genetic variations involved in disease etiology. In two of our previous studies we showed that polymorphisms in genes IL1A and RANK are significantly associated with BMD or biochemical markers of bone turnover. In addition the gene-gene interactions between RANK and OPG, and RANK and RANKL genes showed to influence BMD in postmenopausal women. For this reason we wanted to test whether there is any influence of gene-gene interaction of IL1A and RANK genes on BMD or biochemical markers of bone turnover in postmenopausal osteoporosis. The genotype-phenotype association study was performed in 593 Slovenian participants, 106 elderly men, 431 postmenopausal and 56 premenopausal women. All participants were genotyped for three single nucleotide polymorphisms (SNP) in RANK gene: +34694C>T, +34901G>A and +35966insdelC and for one SNP in IL1A gene: +12534G>A. BMD at the lumbar spine, femoral neck and total hip, and biochemical markers of bone turnover were measured. The assessment of gene-gene interactions association with BMD or biochemical markers of bone turnover was tested using general linear model adjusted for age and body mass index. Interaction between IL1A +12534G>A and RANK gene +34901G>A SNPs was significantly associated with concentration of osteocalcin in the group of all participants (P=0.030), in the group of women (P=0.015) and in the group of postmenopausal women (P=0.004). For the interaction of IL1A +12534G>A and RANK gene +35966insdelC we found a significant association with concentration of osteocalcin in the group of women (P=0.022) and postmenopausal women (P=0.013). For this interaction a significant association with concentration of bone alkaline phosphatase in the groups of women (P=0.019) and postmenopausal women (P=0.013) was also found. Our results suggest that interactions between IL1A and RANK genes may play a role in the complex genetic background of osteoporosis showing interplay between inflammation (IL1A) and RANK regulation of bone remodeling process.

W020
DEVELOPMENT OF A PARATHYROID HORMONE BIOCHIP BASED IMMUNOASSAY

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Background: Mammalian parathyroid hormone (PTH) is secreted from the parathyroid glands in response to low calcium and is the key mechanism by which the body regulates calcium levels. Hyperparathyroidism, an excessive secretion of PTH by the parathyroid glands, results in excess calcium in the bloodstream and increased bone resorption. Elevated levels of PTH are observed in chronic kidney disease (CKD) and are used as a marker to assess CKD associated skeletal disorders. Many publications highlight the lack of comparability between PTH immunoassays suggesting a robust PTH assay is required. This study reports the development of a biochip based PTH assay, traceable to a WHO international PTH standard, which will facilitate the determination of this compound in clinical settings.
Methods: The reported biochip array based immunoassay utilised a sandwich format for the determination of intact PTH. This involves the simultaneous reaction of a capture antibody immobilised on the biochip surface, PTH in the sample and a horseradish peroxidase labelled antibody. Following removal of unbound material, chemiluminescent substrate is added and the intensity of the resulting signal is proportional to the PTH concentration in the original sample. The assay was applied to the Evidence Investigator analyser.
Results: The PTH biochip immunoassay covered an assay range up to 2500 pg/mL with calibrators traceable to WHO international standard, NIBSC material 95/646. Prozone effect was not observed at PTH concentrations up to 150,000 pg/mL. 35 patient serum samples were assessed for intact PTH (concentration range 0-214 pg/mL) using the biochip immunoassay and a commercially available immunoassay and the subsequent linear regression analysis demonstrated a correlation coefficient of 0.92 and a y-intercept of -14 pg/mL.
Conclusions: The results indicate the developed biochip based intact PTH immunoassay exhibits optimal analytical performance and compares favourably with another commercially available immunoassay. The inclusion of this immunoassay on the fully automated random access immunoassay analyser, Evidence Evolution, will result in a high throughput assay for the quantitative determination of intact PTH in human serum.

W021

ANALYTICAL EVALUATION OF AN EVIDENCE BIOCHIP BASED IMMUNOASSAY FOR THE EQUIMOLAR DETERMINATION OF 25(OH)D2 AND 25(OH)D3 IN SERUM SAMPLES

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Background: The fat soluble Vitamin D exists in two forms: Vitamin D2 and Vitamin D3. Measurement of the tightly regulated physiologically active 1alpha,25(OH)2D does not reflect vitamin D storage levels in the body, therefore 25(OH)D concentrations in either serum/ plasma are measured. Low concentrations of 25(OH)D result in the debilitating bone condition, rickets, and epidemiological studies implicate 25(OH)D with an increased risk of various cancers, diabetes, multiple sclerosis, depression, Alzheimer's and cardiovascular events. This study reports the analytical evaluation of a biochip based immunoassay which can accurately measure total 25(OH)D [i.e. both 25(OH)D2 and 25(OH)D3] and offers the potential for high throughput applications with advantages for the clinical settings.
Methods: This biochip based assay utilises a competitive chemiluminescent immunoassay applied to the Evidence Investigator analyser. Assessment of percent cross-reactivity was estimated by comparison of the concentration yielding 50% inhibition in human 25(OH)D depleted serum spiked with 25(OH)D2 or 25(OH)D3. 40 serum samples comprising clinically relevant range of 25(OH)D concentrations were assessed by this method and by LC-MS/MS and the results compared. In addition, UTAK controls and 10 Vitamin D external quality assessment samples (DEQAS) were also tested.
Results: Total 25(OH)D assay calibrator values are traceable to LC-MS/MS with an assay range up to 128 ng/mL. The percent cross reactivity of 25(OH)D2 and 25(OH)D3) spiked serum was 90%. Assessment of serum samples comparing the biochip assay and LC-MS/MS, resulted in a slope of 0.98 and regression coefficient of 0.91. Measurement of UTAK controls and DEQAS met the organisations acceptance criteria.
Conclusions: Based on the data obtained, the biochip based immunoassay exhibits equimolar recognition of 25(OH)D2 and 25(OH)D3. This method compares favourably with LC-MS/MS when serum samples were assessed. This total 25(OH)D assay will be available on the fully automated random access immunoassay analyser, Evidence Evolution for straightforward high throughput testing.

W022

NICOTINE REDUCES BONE RESORPTION DUE TO THE SUPPRESSION OF CATHEPTHIN K, MMP-9 AND VACUOLAR-TYPE-ATPASE D2 PRODUCTION AND ACTIN ORGANIZATION IN OSTEOCLASTS.

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Background: Tobacco smoking is an important risk factor for the development of several cancers, osteoporosis, and inflammatory diseases such as periodontitis. Nicotine is one of the major components of tobacco. In a previous study, we showed that nicotine inhibited mineralized nodule formation by osteoblasts, and that nicotine and lipopolysaccharide indirectly enhanced osteoclast differentiation via osteoblasts. However, the direct effect of nicotine on the differentiation and function of osteoclasts is poorly understood.
Methods: We examined the direct effects of nicotine on mineral resorption activity, bone resorption activity, actin organization, and the expression of nicotine receptors and bone resorption-related enzymes using RAW264.7 cells or bone marrow cells as osteoclast precursors. Cells were cultured with 0.01 mM, 0.1 mM or 1mM nicotine and/or 50 uM alpha-bungarotoxin (btx) an alpha 7 nicotine receptor antagonist—in differentiation medium containing the soluble receptor activator of NF-kappa B ligand (RANKL) for up 7 days.
Results: Alpha-5, 7, 9, and 10 nicotine receptors are expressed on RAW264.7 cells. Expression of the alpha 7 nicotine receptor was increased by the addition of nicotine. Nicotine suppressed the number of tartrate-resistant acid phosphatase (TRAP)-positive multinuclear osteoclasts that have the large numbers of nuclei (≥10 nuclei), and decreased the planar area of each cell. Nicotine decreased expression of cathepsin K and matrix metalloproteinase-9 (MMP-9). Moreover, btx inhibited the effect of nicotine on bone resorption-related enzyme expression. However, nicotine increased carbonic anhydrase II (CA II) expression although it decreased vacuolar-type-ATPase subtype D2 (V-ATPase D2) expression and suppressed the distribution of F-actin. Furthermore, nicotine suppressed bone resorption by osteoclasts, but had no effect on mineral resorption.
Conclusions: These results suggest that direct stimulation with nicotine of RAW264.7 cells or bone marrow derived osteoclast precursors suppressed osteoclasts with the large number of nuclei and bone resorption due to the suppression of V-ATPase D2, cathepsin K and MMP-9 expression and actin organization.

W023
HYPERHOMOCYSTEINEMIA: A POSSIBLE CAUSAL EFFECT TO OSTEOPOROSIS

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Background: Elevated levels of plasma total homocysteine due to low levels of folic acid, vitamins B12 and B6 in persons with osteoporosis have been associated with impaired collagen cross-linking, low BMD and subsequent fractures. However, there is paucity of information on these associations in patients at risk of fragility fractures due to osteoporosis in Nigeria. This study was therefore designed to evaluate plasma total homocysteine, folic acid, vitamins B12 and B6, in patients with primary osteoporosis.
Methods: Fifty (50) osteoporotic patients with mean age of 57±1.9years were selected for this study. Fifty (50) apparently healthy volunteers with mean age of 42.8±1.5 years were also included as controls. Bone mineral density (BMD) measurement was based on the World Health Organization criterion for the diagnosis of osteoporosis using the Dual energy X-ray absorptiometry (DEXA) and scans were measured at the distal radius of the arm, anthropometric indices, fasting plasma total homocysteine, folic acid, vitamins B12 and B6, were determined using standard procedures.
Result: The results showed remarkable significant increases in plasma tHcy (P <0.001) (200%) compared with the control value. Notable significant decreases were obtained in folic acid, vitamins B12 (42%), B6, (59%) and BMI P <0.001) compared with the control values. Significant correlation was obtained between vitamin B12 and BMD (r =0.311, P <0.05). Total plasma homocysteine, although was observed to be increased in patients with decreased BMD but showed no significant correlation.
Conclusion: The main findings of the present study were significant increase in tHcy with corresponding decreases in BMD, folic acid, vitamins B12 and B6 in osteoporotic patients. The mechanism underlying the association between the homocysteine level and risk of osteoporotic fracture due to low BMD may be attributed to interference by homocysteine in collagen cross-linking. Collagen cross links is important for the stability and strength of the collagen network. The mechanism by which elevated homocysteine interferes with collagen cross-linking could be as a result of protein homocysteinylation giving rise to the formation of Hcy thiolactone when the normal remethylation or trans-sulphuration pathways are affected. Hcy thiolactone is highly reactive and conditions such as elevated tHcy as obtained in the osteoporotic group in the present study favours its production. Homocysteine thiolactone may lead to protein functional damage by the inactivation of lysyl oxidase which is required for collagen cross linking and modification. Thus, increased level of plasma tHcy could lead to increase in the risk of fracture through interference in collagen cross-linking. Thus suggesting that elevated plasma homocysteine may interfere with the development of the microarchitecture of bone independently of the amount of minerals in the bone. . These therefore provide evidence that this abnormality is an associated feature of osteoporosis and that Homocysteine has a possible causal effect on osteoporosis and such patients are at increased risk of fragility fractures.

W024
MYELOPEROXIDASE AND OXIDATIVE STRESS PARAMETERS IN INFLAMMATORY ARTHRITIDES

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Aim: Ankylosing spondylitis (AS) and Rheumatoid arthritis (RA) are inflammatory arthritides and their etiology is not clear. The major symptoms of them are joint pain, swelling and stiffness. Persistent inflammation results in destruction of cartilage and bone. This occurs with oxidative and proteolytic disruption of collagen and proteoglycans. During the inflammation, neutrophils degranulate and release a variety of potentially harmful enzymes (such as myeloperoxidase) and peptides. Reactive oxygen species are occured with respiratory burst. Because of their potentially damaging effects, several potent antioxidant defence mechanisms have evolved to provent cells and organisms from damage by enormous amounts of these reactive oxygen species. Oxidative stress (OS) is defined as the imbalance between oxidants and antioxidants in the body. In this study, we aimed to assess oxidative stress and myeloperoxidase activity (MPO)in RA and AS. For this, total antioxidant capacity (TAC), Total Oxidant Status (TOS), MPO, paraoxonase (PON) and total sulphydryl (t-SH) levels are measured in AS and RA groups. Also these parameters were assessed for the disease activity.
Material and method: A hundred and twelve patients (52(AS), 60 (RA)) and thirty one healthy controls (C) were included in the study. Measurements were performed using spectrophotometric techniques. Findings: MPO levels were significantly higher in patients with RA and AS compared with C (40.97±24.25 U/L and 38.25±19.05 vs 31.09 ±7.64, P <0.05). There was no significant difference in PON levels between AS, RA and C. TOS was significantly higher in patients with RA and AS compared with C (57.84±23.48 umol/L and 59.75±30.31 vs 34.63 ±6.35, P <0.05). TAC levels were significantly higher in C than RA and AS patients (2.05±0.34 mmol/L vs 1.84±0.14 and 1.82±0.18 P <0.05). t-SH levels were significantly higher in C compared with RA and AS patients (747.53±166.12 umol/L vs 498.68±97.39 and 550.50±114.20, P <0.05). There were significant correlations between MPO and TAC, MPO and t-SH, TAC and t-SH in AS and RA patients.
Conclusion: OS and higher MPO concentration play important role in AS and RA pathogenesis. Our results suggest that addition of antioxidants are a potentially useful approach in the treatment of AS and RA

W025

CORRELATION BETWEEN LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 AND HIGH SENTIVITY C-REACTIVE PROTEIN IN TUNISAN CORONARY ARTERY DISEASE PATIENTS

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Background: It well establishes that inflammation play an important role in atherosclerosis. Investigation of biomarkers inflammation was interesting to evaluate their prognostic value. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a new biomarker that has been proposed to be more specific to vascular inflammation. The purpose of study was to evaluate the levels of Lp-PLA2 and hsCRP in CAD patients and explore their correlation.
Methods: our study included 100 CAD patients (mean age: 59.76±11.23), the presence of one or more stenoses ≥50% was determined by angiography. Control group consisted of 50 healthy subjects (mean age: 41.50±7.86). Lp-PLA2 was assessed by a dual monoclonal antibody immunoassay standardized to recombinant Lp-PLA2 (PLAC test, diaDexus, Inc). hsCRP was measured by immunonephelometry (DadeBehring, Marburg, Siemens,Germany).
Results: Mean values of plasma Lp-PLA2 were found to be statistically significantly higher in patients with CAD compared to controls (452.49±163.39 ng/mL vs 174.0±34.12 ng/mL; P < 10-3). We noted statistically significant increase of hsCRP was in CAD patients compared to controls (3.23±1.08 mg / L vs. 1.1 ±10.6 mg/L, P <10-3). Also, Lp-PLA2 and hsCRP were statisticly higher in CAD patients with triple vessel disease compared to CAD patients with single- vessel disease. In patients, Lipoprotein-associated phospholipase A2 was positive correlated with hsCRP (r: 0.551; P: 0.01).
Conclusion: our results showed that serum levels Lp-PLA2 and hsCRP were increased in CAD patients and these 2 inflammatory biomarkers were associated with severity of CAD. The correlation between Lp-PLA2 and hsCRP illustrate their interaction role in inflammation. The user of CRP and Lp-PLA2 together was better prediction of the presence of CAD and CAD death.

W026

PLASMA FIBRINOGEN CONCENTRATION AND BLOOD VISCOSITY IN OBESE AFRICANS

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Background: There are vast research literature's on the biochemical,hematological,immunological,molecular interactions/activities and haemorheological changes in obese caucasians. However, research literature's are very scanty on the effects of obesity on plasma fibrinogen and blood viscosity in obese Africans.
Objectives: This research work is aim at estimating the level of plasma fibrinogen concentration and blood viscosity in obese Africans.Is there any correlation between Basal Metabolic Index (BMI) and plasma fibrinogen concentration and blood viscosity.
Methodology: A total of 121 apparently healthy individual. 41 obese,47 overweight and 33 controls age and sex matched were used for this study.Their Packed Cell Volume(PCV),Total Leukocytes count (WBC),Relative Whole Blood Viscosity (RWBV),Relative Plasma Viscosity(RPV),Plasma Fibrinogen Concentration(PFC), Euglobulin Lysis Time (ELT) and BMI were analyzed using reference methods.
Results: The results show a significant increase in RWBV, PFC, ELT and BMI (P <0.005) between controls and obese.We also observed a significant increase in all the parameters (P <0.005) between control and overweight.However,there was no significant difference in PFC and ELT (P >0.005) between obese and overweight.We recorded a significant difference in PCV,WBC,RWBV AND RPV (P <0.005) between obese and overweight.There was a positive correlation between BMI AND PFC (r=0.2424) (P <0.005) in overweight and obese.
Conclusion: The results from this study shows elevated values of RWBV,PFC,ELT and BMI in overweight and obese,and a positive correlation exist between BMI and PFC in overweight and obese Africans.We therefore conclude, that overweight and obese Africans are predispose to thrombosis complications due to hyper-blood viscosity and delay fibrin clearing mechanism.

W027

HYPERURICEMIA AND METABOLIC SYNDROME IN NON DIABETIC AND NON HYPERTENSIVE IN A TUNISIAN POPULATION

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Background: Serum uric acid (UA) is reported as an important marker of hypertension, coronary heart disease, and diabetes. We examined the association of serum uric acid (UA) with metabolic syndrome (MS) in a Tunisian population.
Materiel & Methods: The study included 2712 subjects (1228 men and 1484 women), aged from 35 to 70 years and living in the Great Tunis region. Patients with a history of CVD and Chronic Kidney disease (CKD) were excluded from the study. The MS was defined according to ATPIII. Hyperuricemia was defined as a serum UA value >7.0 mg/dL for males or > 6.0 mg/dL for females.
Results: The prevalence of hyperuricemia, and metabolic syndrome, were 6.1% (9.8% in men and 2.8% in women), and 12.8% (10.2% in men and 15.2% in women), respectively. Serum uric acid concentrations were significantly and positively correlated with body mass index, diastolic blood pressure and serum triglyceride concentrations; and statistically significant and inverse correlations were noted for serum uric acid and serum HDL-C concentrations. Multivariate logistic regression analysis revealed that there was a significant association between third-quartile uric acid levels and prevalence of metabolic syndrome in men and in women.
Conclusion: An increase of uric acid constitutes a risk factor for metabolic syndrome in Tunisian population. Uric acid may be a useful index for initial risk stratification of patient non-diabetic non hypertensive.

W028

SERUM LIPID LEVEL IN TUNISIAN PATIENTS WITH PSORIASIS

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Background: Psoriasis is a common chronic inflammatory skin disorder that has been associated with oxidative stress, abnormal plasma lipid metabolism and high frequency of cardiovascular events. This later seems to be strongly related to intensity of inflammation and severity of psoriasis.The aim of this study was to determine lipid profile variation in Tunisian psoriatic patients.
Methods: This study was designed and conducted as a case–control assay with 91 psoriatic patients and 91 controls. Patients with diseases that could cause secondary hyperlipidaemia, such as diabetes mellitus, hypertension, obesity (BMI ≥30), nephrotic syndrome, infectious or autoimmune diseases and chronic renal insufficiency were also excluded. The lipid profile, including serum level of triglyceride, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were assessed in both groups. A glucose tolerance test was performed to calculate the homeostasis model assessment of insulin resistance (HOMA).
Results: The two groups had included the same effective: 91 patients and 91 controls with (45 male and 46 female). In psoriatic group, serum triglycerides, low density lipoprotein and very low density lipoprotein cholesterol were significantly higher than those in control group ($P < 0.05$). While the high density lipoprotein cholesterol (HDL-cholesterol) was significant decreased in patients with psoriasis than in controls ($P < 0.001$). There were no significant differences concerning insulin or insulin resistance and total cholesterol between two groups. The insulin secretion was significantly higher in patients psoriasis than in control group ($P=0.003$). However, there was no significant correlation between severity of psoriasis and serum lipid and insulin secretion. A negative correlation ($r= -0.253$, $P=0.019$) was found between PASI index and HDL-C.
Conclusion: A high serum lipid level is significantly more common in psoriatic patients. This could be responsible for higher prevalence of cardiovascular accidents in psoriatic patients. It may be useful to do early screening and treatment of hyperlipidaemia in psoriatic patients to prevent atherosclerosis and its complications.

W029

TOTAL HOMOCYSTEINE CORRELATED WITH CLASSIC CARDIOVASCULAR RISK FACTORS IN TUNISIAN HEALTHY GROUP

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Background: Hyperhomocysteinemia is considered as an important independent risk factor for atherosclerosis and thrombotic disease.
Aims: To determine the distribution of homocysteine levels in healthy Tunisian subjects and to evaluate the relationship between Hcy levels and cardiovascular risk factors.
Method: Randomly selected subjects (592 men and 114 women) were recruited from different regions of Tunisia and divided into groups according to their geographical origins. G1 from North, G2 from the Capital, G3 from Center and G4) from South. Total Hcy levels, VitB12, folates, TChol, LDLc, HDLc, TG, ApoA, ApoB, Lp a were assessed for all subjects.
Results: The distribution of Hcy levels in hyperhomocysteinemics was different according to the geographical origins ($p < 0.01$). Hyperhomocysteinemic subjects have higher TChol, LDL, Apo A and Apo B but lower VitB12 compared to normohomocysteinemic subjects. Hcy levels correlated with TChol ($r = 0.09$; $P < 0.05$), Apo A ($r = 0.012$; $P < 0.01$), Apo B ($r = 0.013$; $P < 0.01$) levels and TChol/HDL ratio ($r = -0.085$; $P < 0.05$).
Conclusion: Heterogeneous distribution of Hcy in Tunisian subjects was found. Elevated Hcy concentrations may interact with the classic risk factors to accelerate the process of developing cardiovascular diseases.

W030

RELATION BETWEEN HOMOCYSTEINE, INFLAMMATORY BIOMARKERS AND SEVERITY OF ACUTE CORONARY SYNDROME

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Background: Inflammation and hyperhomocysteinemia are demonstrated to be involved in the development and the progression of atherosclerosis.
Aims: In this study we investigated the distribution of homocysteine and inflammatory markers in patients with acute coronary syndrome (ACS) and evaluated the association between these biomarkers and severity of the disease.
Methods: The present study was carried on 122 patients with ACS and 80 control subjects. Total homocysteine (Thcy), folic acid, Vit B12, Hs CRP, IL6 and TNF α concentrations were determined for all participants. The distribution of these parameters was compared between groups and according to the number of diseased vessels in patients with ACS.
Results: ACS patients had significantly elevated levels of Thcy (17.67 ± 8.32 μmol/L vs 13.95 ± 6.09 μmol/L; $P < 0.01$), Hs CRP (14.6 ± 9.8 g/L vs 3.7 ± 1.3 g/L; $P < 0.001$), IL6 (11.56 ± 8.23 pg/L vs 2.32 ± 1.42 pg/L; $P < 0.001$) and TNF α (11.18 ± 6.83 pg/mL vs 6.81 ± 4.67 pg/L; $P < 0.001$). Patients with three affected vessels showed a significant elevated Thcy, Hs CRP, IL6 and TNF α compared to those with one and those with two affected vessels. Homocysteine (OR=1.14; 95% IC: 1.04-1.25; P=0.006), TNF α (OR=1.27; 95% IC: 1.13-1.44; P=10-3) were significant predictors of severity of the disease.
Conclusion: The results of the study suggest an association between inflammation, hyperhomocysteinemia and development of ACS. The cited biomarkers appear to enhance the degree of affected arteries and so the severity of coronary artery disease.

W031
HOMOCYSTEINE AS A MARKER FOR CARDIOVASCULAR RISK ASSESSMENT IN WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine.Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin.Smaller amounts of reduced homocysteine and the disulfide homocystine are present. Total homocysteine (tHCY) represents the sum of all HCY species found in serum or plasma (free plus protein bound).Epidemiological studies have investigated the relationship between elevated homocysteine concentrations and cardiovascular disease.Metabolic and endocrine dysfunctions that may occur with polycystic ovary syndrome (PCOS) can be associated with future comorbidities such as diabetes, cardiovascular disease, and endometrial cancer. Although a definitive link between PCOS and these chronic illnesses has not been demonstrated, there is significant overlap in the clinical characteristics of these disorders. Consequently, the issue of identifying and measuring potential conditions that may be associated with PCOS is a priority and should be the standard of practice in its management. The aim of our study was to determinate levels of homocysteine in woman with polycystic ovary syndrome compared with healthy woman.Thirty patients (age, 23, 5 ± 5.5) with PCOS and twenty four (age, 25.5± 4.3) healthy woman were involved in the study. Blood samples were collected in early follicular phase. Homocysteine assay is a Fluorescence Polarization Immunoassay (FPIA) for the quantitative measurement of total L-homocysteine in human serum or plasma. Statistically significant differences in serum concentration of homocysteine were observed between groups. Mean homocysteine level we found as (10.2±2.9 vs. 7.0±1.5) in PCOS and normal group respectively (P <0.05). For Macedonian population we found statistically significant increased homocysteine levels in woman with PCOS. Although the mean homocysteine levels are within normal limits, there are significant higher mean homocysteine concentrations between these two groups.Because an increased concentration of tHcy has been shown as and independent risk factor for cardiovascular alterations, it is essential in this group of woman to be taken measures for early prevention

W032
MR-PROANP, COPEPTINE AND ATRIAL FIBRILLATION DURATION

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Evaluating the duration of Atrial Fibrillation (AF) remains difficult in clinical practice since most AF episodes are asymptomatic. According to guidelines, patient with an AF onset <48 h could be safely cardioverted without prolonged anticoagulant treatment. We investigated whether MR-proANP and copeptine could be of interest to further evaluate the duration of AF (< vs ≥48 h). 460 consecutive patients with an ECG documented AF were admitted in our clinical institution. Blood samples were collected at the time of admission and stored at - 80 °C. MR-proANP and copeptine were retrospectively measured using immunoenzymatic assays (TRACE technology on Kryptor from ThermoFisher) after controlling for each series of measures. Patients with an AF index episode associated with heart failure, acute coronary syndrome, hyper- or hypothyroidism as well as patients with AF of undetermined duration were excluded leaving 163 patients for the purpose of this analysis. 105 patients (63%) had an episode of AF <48 h. MR-proANP level was significantly lower in patients with an AF onset <48 h (m=143.6 pmol/L, 81.0-250.1) vs ≥48 h (m=186.9 pmol/L, 131.3-256.5), with P <0.02. Copeptine level was comparable in the 2 groups. The values of area under curve for MR-proANP and copeptine was 0.61 and 0.56, respectively. None of these biomarkers were predictors of AF duration < or ≥ 48 h in multivariable analysis. The significant predictors were Persistent AF (OR=5.98, 95% CI: 2.58-13.84), and Permanent AF (OR=10.60, 95% CI: 3.18-35.40) with P <0.001. MR-proANP or copeptine were not able to accurately identify patients with an AF onset <48 h.

W033

LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 AS A MARKER FOR CORONARY DISEASE IN TUNISIA POPULATION

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Background: Increasing evidence suggests that the enzyme lipoprotein-associated phospholipase A2 (LpPLA2), also known as PAF-AH, is a potential useful plasma biomarker for cardiovascular disease. However, it is unclear whether it plays a pro or anti-atherogenic role in atherosclerosis. LpPLA2 is responsible for the generation of two bioactive inflammatory markers: non esterified Fatty acid and Lysophosphatidylcholine. At the same time, it is implicated in the degradation of the platelet activating factor (PAF), a potent mediator of inflammation. We investigated associations of circulating Lp-PLA2 mass and activity with coronary vessel status; and with the severity of coronary artery diseases (CAD) in a Tunisian population.
Methods: 187 patients with CAD (mean age 61±11years) (and free from renal failure, thyroid illness, inflammatory diseases (rheumatoid arthiris) and Alzheimer) were recruited from the cardiology clinic at Fattouma Bourguiba Hospital. They were subdivided into 4 groups according to vessel diseases: normal status (n=14), single vessel (n=65), double vessel (n=47) and three vessels (n=61). In addition, they had been classified following the severity of coronary artery disease into 4 groups: stable angina (n=23), ST-Trop- (n=81), ST-Trop+ (n=47) and ST+ (n=36). LpPLA2 mass was measured by ELISA test (PLAC III, Diadexus), and LpPLA2 activity using a colorimetric activity assay (CAM, Diadexus).
Results: Circulating LpPLA2mass and activity levels were higher in patients with three vessel coronary disease compared to patient with single vessel Coronary disease(mass: 556±164 vs 307 ±93 ng/mL; P <0.001 and activity:122± 25 vs 98±25 nmol/min/mL; P <0.001). In addition, we observed that LpPLA2 level was higher in ST-Trop+ group compared to ST-Trop+ (450 ±185vs 375±139; P=0, 01).
Conclusion: In a Tunisian population, both LpPLA2 mass and activity are markers of coronary status (0, 1, 2, 3 vessel disease). However, further studies in this population are needed if to judge if LpPLA2 is related to the severity of coronary artery disease.

W034

TOTAL BILIRUBIN AND CARDIOVASCULAR RISK IN CLINICALLY HEALTHY NON-DIABETIC INDIVIDUALS

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Background: Bilirubin possesses potent antioxidant effects. Elevated bilirubin concentration is strongly associated with protection against many immune and inflammatory diseases. The aim of study is to present the relationship between serum total bilirubin concentration and traditional and novel cardiovascular risk factors in clinically healthy non-obese individuals.
Methods: Study included 145 healthy normoglycemic, non-obese subjects, aged 25-40 (73 women and 72 men). Serum total bilirubin (BilT), lipid profile, insulin, C-reactive protein (CRP), apoliporoteins B100 and AI (apoB, apoAI), plasma fasting glucose and glycated hemoglobin (HbA1c) measurements were performed. LDL cholesterol (LDL-C), non-HDL cholesterol (non-HDL-C), body mass index (BMI) waist-hip ratio (WHR), Homeostasis Model Assessment - Insulin Resistance (HOMA-IR) and atherogenic indexes (TC:HDL-C, LDL-C:HDL-C, apoB:apoAI) were calculated. Carotid intima-media thickness (IMT) was measured using ultrasound method. Subjects were divided into quartiles of BilT concentration.
Results: Significant differences in BilT concentration between women and men (0.65 vs. 0.85 mg/d; P <0.05) were found. All anthropometric and biochemical indicators, except HDL-C, and apoAI, were lower in women. BilT was inversely correlated with non-HDL-C (R=-0.29; P=0.01), total cholesterol (R=-0.29; p=0,01), LDL-C (R=-0.27; P=0.02), apoB (R=-0.28; P=0.01), apoB:apoAI (R=-0.20; P=0.02) and IMT (R=-0.30; P=0.003). Interestingly, in men BilT was also associated with insulin (R=-0.35; P=0.001) and HOMA-IR (R=-0.35; P=0.001). Subjects with elevated BilT (>1.2 mg/dL) had significantly lower concentration of LDL-C, non-HDL-C, apoB, HOMA-IR and atherogenic indexes. TC, non-HDL-C, LDL-C and apoB values were significantly lower with increase of BilT in quartiles. Systolic and diastolic blood pressure increased sequentially in 1st, 2nd and 3rd quartile of BilT, but in the 4th quartile were lower then in 3rd quartile (122±11 and 82±9 vs. 129±10 and 86±6 mmHg; P <0.05).
Conclusions: Observed relationship between serum total bilirubin and cardiometabolic risk factors suggest its potential role in preventing cardiovascular disease in healthy non-diabetic non-obese subjects, however this issue needs further investigation.

W035

NON-HDL CHOLESTEROL VERSUS TRADITIONAL LIPID PROFILE AS PREDICTOR OF INSULIN RESISTANCE AND CARDIOVASCULAR RISK IN YOUNG CLINICALLY HEALTHY NON-OBESE INDIVIDUALS

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Background: Non-HDL cholesterol (non-HDL-C), a sum of cholesterol accumulated in all potentially atherogenic lipoproteins, has been proposed by Adult Treatment Panel III as a second (after LDL-cholesterol) goal of lipid-lowering therapy. The aim of study was to compare non-HDL-C and traditional lipid profile with novel markers of cardiovascular disease and insulin resistance in clinically healthy non-diabetic individuals.
Methods: Study included 145 healthy non-obese normoglycemic subjects, aged 25-40 (73 women and 72 men). Serum total cholesterol (TC), HDL-cholesterol (HDL-C), triglycerides (TG) insulin, C-reactive protein (CRP), total bilirubin, apolipoproteins B100 and AI (apoB, apoAI), adiponectin, 25-hydroxyvitamin D (25(OH)D), plasma fasting glucose and glycated hemoglobin (HbA1c) measurements were performed. LDL cholesterol (LDL-C), non-HDL cholesterol (non-HDL-C), body mass index (BMI) waist-hip ratio (WHR), Homeostasis Model Assessment - Insulin Resistance (HOMA-IR) and atherogenic indexes (TC:HDL-C, LDL-C:HDL-C, apoB:apoAI) were calculated. Carotid intima-media thickness (IMT) was measured using ultrasound method.
Results: Anthropometric and biochemical indicators, except non-HDL-C, HDL-C, apoAI, adiponectin and 25(OH)D, were lower in women than in men. Non-HDL-C was positively related with BMI (R=0,38; P <0,001), WHR (R=0,40; P <0,001), lipids, insulin (R=0,21; P=0,015), adiponectin (R=-0,39; P <0,001), HOMA-IR (R=0,21; P=0,02), IMT (R=0,21; P=0,03) and atherogenic indexes. Compared to LDL-C, non-HDL-C showed higher correlation with apoB (R=0,96 vs. 0,89; P <0,001) and apoB:apoAI (R=0,79 vs. 0,70; P <0,001). Only TG and HDL-C were superior to non-HDL-C in correlation with CRP, adiponectin and HOMA-IR. Additionally, non-HDL-C was significantly inversely associated with 25(OH)D in women, positively with HOMA-IR in men and inversely correlated with total bilirubin in both groups.
Conclusions: Non-HDL-C reflects proatherogenic profile better than LDL-C, therefore its use in a routine lipid profile is valuable for better assessment of cardiovascular risk. Presented relationship of non-HDL-C and markers of insulin resistance seems to be interesting, but remains further investigation.

W036

SERUM URIC ACID AS PREDICTOR OF MORTALITY IN CORONARY ARTERY DISEASE PATIENTS

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Introduction: The role of serum uric acid (UA) as an independent risk factor for cardiovascular disease remains still controversial.
Aim: To evaluate the predictive value of UA (Beckman Coulter, Brea, USA) for cardiovascular events in a large population of patients with angiographycally proven coronary artery disease (CAD), with an extended follow-up.
Design and patients: We studied 2592 (2051 males, age: 67±10 years, mean±SD) CAD patients. Patients data were collected from the Institute electronic databank which saves demographic, clinical, instrumental and follow-up data of all patients admitted to our Coronary Unit.
Results: During a mean follow-up period of 35±25 months, 239 (9%) patients died; there were 145 (6%) cardiac deaths, and 239 (9%) total death. UA (≥7 mg/dL, 75th percentile) was a strong predictor of cardiac and overall mortality (hazard ratio at the univariate analysis, HR=2.1, confidence intervals, CI 1.5–3, P <0.001; and HR=1.7, CI 1.3–2.2, P <0.001, respectively) in the whole population. The Kaplan–Meier survival estimates showed a significantly worst outcome in patients presenting high UA (log-rank test P <0.001 for both cardiac and overall mortality). UA remained significant predictor of cardiac and overall mortality also after adjustment for known prognostic factors in a Cox multivariate proportional hazard model (HR=2.0, 95% CI=1.4-2.8, P <0.001; HR=1.6, 95% CI=1.3-2.1 P ≤0.001).
Conclusion: High UA is low-tech, and easily accessible biomarker that may be helpful for stratifying mortality risk in CAD.

W037

COMPARISON OF CALCULATED LDL CHOLESTEROL VERSUS MEASURED LDL CHOLESTEROL AND IMPACT IN TERMS OF THERAPEUTIC MANAGEMENT

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Background: In France the recommendations for hypercholesterolemia treatment are based on the value of LDL-cholesterol (LDL). Different threshold values are used, dependent on other cardiovascular risks factors and pre-existing stroke or heart attack (1.0; 1.3; 1.6 or 1.9 g/L). In this study, we compared LDL values calculated (LDL-C) to those obtained through direct measurement (LDL-M). We then compared the two sets for inaccuracies in LDL threshold values.
Methods: This study examined 444 subjects for whom a lipid exploration was prescribed. Total cholesterol (CT), triglycerides (TG), and both HDL and LDL cholesterols were measured by a Cobas 6000 Roche analyzer with dedicated reagents (LDL-M). We then calculated LDL-C using the Friedewald Formula. Statistical analysis was performed using Graph Pad Prism.
Results: There was a good correlation between LDL-C and LDL-M on the all data. The correlation coefficient r was equal to 0.97 with a slope of 0.936 and an intercept of 0.156. However depending on TG levels: <1g/L (n=146), between 1 and 2 g/L (n= 243), between 2 and 3 g/L (n=46) or between 3 and 3.4g/L (n=9) we noted differences in the slope and intercept respectively: y=0.94x+0.08, y=0.92x+0.24, y=0.90x+0.43 and y=0.93x+0.38. Despite the correct correlation, using a xy paired test there was a significant difference (P <0.0001) between the LDL-C and LDL-M. In fact, in looking at the threshold values of LDL, 17 % of subjects' TG were incorrectly valued, while LDL-C consistently returned higher LDL values than LDL-M tests. The ICC of LDL-C and LDL-M equalled 0.96, which was very good. The inter-class Kappa concordance assessment, however, was equal to 0.75, which signified that the calculated value was most likely correct, but the discordance was important and needed to be taken into account if the direct measure of LDL is to be extended on a larger scale.
Conclusion: This study demonstrates that a significant difference in the results returned exists between LDL-C and LDL-M, with LDL-C consistently returning inaccurately high values for LDL. Moreover, 17% of subjects are misclassified by LDL-C and therefore undergo unnecessary treatment, a problem which can be rectified by switching to the LDL-M method for LDL value calculation.

W038

MONITORING LABORATORY TESTS IN PATIENTS WITH CHRONIC HEART FAILURE AND OBESITY

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Background: Recent data show increasing role of brain natriuretic peptide (NT-proBNP), high sensitive cardiac troponin I (hs cTn I), high sensitive C-reactive protein (hs CRP) in patients with chronic heart failure (CHF), the relationship between levels of this markers, the stage of CHF and outcomes may help in the diagnosis, treatment and prediction adverse outcomes. The aim of the study was to evaluate in the dynamics the clinical significance of laboratory tests: NT-proBNP, hs cTnI and hs CRP in patients with chronic heart failure and obesity.
Methods: The study covered 72 patients aged 45 to 75 years with chronic heart failure II and III NYHA class. It was 3 consecutive time points: on admission to hospital, at discharge and 6 months after discharge. According to clinical outcomes patients were divided into 2 groups: 1st group — patients II and III NYHA class with clinical improvement of the disease; 2nd group — patients with adverse outcomes (III NYHA class with the worsening of the disease or cardiovascular death within 6 months). Concentrations of NT-proBNP, hs cTnI and hsCRP were detected by ELISA, also analyzed body mass index (BMI), NYHA class and left ventricular ejection fraction (LVEF).
Results. Differences in BMI (P <0,05) were identified between patients in 1st and 2nd groups on admission to hospital and 6 months after discharge in group 1 (27,8 and 26,9) and group 2 (28,7 and 28,3) respectively. LVEF was significantly related to NT-proBNP level (rs=-0,475, P <0,05). In the first group average level hs cTnI after 6 months after discharge significantly decrease (P <0,05) (from 0,031 to 0,021 ng/mL). The all groups demonstrated higher than normal levels of CRP (>5 mg/L). It was revealed the significant (P <0,05) decrease the averaged levels of hsCRP in patients with CHF after 6 months after discharge (from 22,5 to 11,2 mg/L in group 1 and from 24,9 to 8,4 mg/L in group 2).
Conclusions: The decrease in BMI and hs cTnI levels in patients with CHF and obesity is associated with positive clinical outcomes. CRP levels are elevated in patients with CHF and obesity and decreased significantly after discharge from hospital independently of outcomes. NT-proBNP levels correlate with LVEF and demonstrate a functional condition of the heart.

W039

CARDIAC BIOMARKERS MEASUREMENT IN PLASMA FOR EARLY DETECTION OF CARDIOTOXICITY IN PATIENTS RECEIVING CANCER THERAPY

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Background: Cardiotoxicity is a complication of cancer therapy. Image techniques can measure left ventricular ejection fraction (LVEF) and parameters for early detection like global strain longitudinal (GSL). Cardiac biomarkers can detect myocardial injury and dysfunction and limited evidence suggests that may be important predictors of cardiotoxicity during cancer therapy.
Methods: We included 37 patients (mean 58.3 years) undergoing chemotherapy with potential cardiotoxicity. Ethical approval was obtained. Transthoracic echocardiograms were performed at baseline and 6 months to measure LVEF and GSL. Blood samples were obtained at baseline, 21d, 3m and 6m. Chemioluminiscence assays were used for biomarkers: hs-cTnT (Roche Elecsys) and c-TnI and NT-proBNP (Siemens Vista) and 99th percentile used as cut-off (hs-cTnT: 14 pg/mL CV=10% and c-TnI 27 pg/mL CV=7.7%). Criteria for cardiotoxicity were: LVEF (decrease >10% without or >5% with heart failure to a value <50%) and GSL (decrease >10% to a value < -18%).
Results: 37 patients with lymphoma(n=15) or breast cancer(n=22) were enrolled. The incidence of cardiotoxicity using LVEF was 5.4% (decreased from 65.6% to 59.5% from baseline to 6m) and GSL 36% (-18.6% to -16.5%). Maximum value of troponins occurred at third month; hs-cTnT always identified additional patients than c-TnI although no significant differences were found. Percentage of positive troponin values(>P99) were higher in patients with cardiotoxicity(LVEF and GSL). Concordance at 6m between hs-cTnT and LVEF and SGL were 51.4% and 55.5%.In both cases hs-cTnT was positive in 48.6% and 46.1% without cardiotoxicity. For c-TnI we observed no positive results in cardiotoxicity group defined by LVEF and a 55.6% concordance in the cardiotoxicity group defined by GSL. No significant relation was observed with NT-proBNP and cardiotoxicity.
Conclusions: • Cardiotoxicity incidence using GSL is higher than LVEF • Increased troponins concentrations occurred in the cardiotoxicity groups with a maximum concentration at 3m • Hs-cTnT assays identifies more patients than conventional c-TnI • We couldn´t established a relation between NT-proBNP and cardiotoxicity • Use of image techniques (GSL) and hs-cTnT may enable early detection of cardiotoxicity in subclinic patients

W040

CONTRIBUTION OF ELEVATED CONCENTRATION OF HIGH SENSITIVITY C REACTIVE PROTEIN AND HOMOCYSTEIN TO THE LIPID PROFILE TO ASSESS THE RISK OF CARDIOVASCULAR EVENTS

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Background: Atherosclerosis is a major reason for developing cardiovascular events (CE). In primary prevention it is recommended to measure the concentrations of cholesterol (cCHOL), low density lipoprotein cholesterol (cLDL), high density lipoprotein cholesterol (cHDL) and triglycerides (cTg). Nevertheless, other quantities related to ischemic events, such as concentrations of C-reactive protein measured by high sensitivity methods (hsCRP) and homocysteine (HCYS) are usually reserved to secondary prevention. This study aims to know how elevated concentration of hsCRP and HCYS contribute to the lipid profile to assess the risk of any CE in a population with several risk factors.
Methods: Variables potentially influencing in developing CE (age, sex, body mass index, alcohol consumption, smoking, diabetes mellitus, cCHOL, cHDL, cLDL, cTg, family history, exercise and systolic blood pressure) were collected from 212 patients attending on the Cardiovascular Risk Prevention Unity. The evolution of patients was followed during 4 years. The variables were included in multiple regression analysis to assess the risk of cardiovascular events explained by them. The risk of CE was evaluated by logistic regression analysis and by Hazard ratio (HR). We study the contribution of the elevated hsCRP and HCYS to the model, independently and together, in order to know each one's effect and the joint effect on developing CE.
Results The logistic regression with control variables provided a HR of 0.43 (0.38 -0.47). The logistic regression (adjusted by control variables) which included the hsCRP provided a HR of 0.59 (0.46-0.66); the regression which included the HCYS provided a HR of 0.45 (0.39-0.47); finally, the regression which included both (hsPCR and HCYS) provided a HR of 0.62 (0.47-0.76).
Conclusion: Elevated concentration of hsCRP and HCYS increase the risk provided by the lipid profile to assess the risk of developing CE. In fact, the risk is a 16% higher if the case of the hsCRP, and a 2% in the case of HCYS. The risk if both were elevated was about 19%. Taking into account these results, measuring these quantities could be useful in individuals with cardiovascular risk in order to optimize risk stratification and clinical management.

W041

INFLUENCE OF APOA5, APOC3 AND APOE GENE VARIANTS ON THE RESPONSE TO FIBRATE TREATMENT IN PATIENTS WITH CARDIOVASCULAR RISK

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Background: Fibrates are used to reduce the plasma triglyceride concentration (cTg) and to raise the HDL cholesterol. They act inhibiting the expression of the APOC3 gene, which decreases the concentration of apolipoprotein and favours the lipase activity. Nevertheless, a wide heterogeneity has been described in the response to the treatment, and several polymorphisms of genes involved in the development of hypertriglyceridemia (HTG) have been proposed to also influence this response.
Methods: The variants -1131T>C and c.56G>C from APOA5, c*40C>G from APOC3 and E2 and E4 from APOE were analyzed in 145 patients with HTG (concentration in serum greater than 1.7 mmol/L according NCEP*). The response to treatment was assessed using the relative percentage difference between cTg at the beginning of the treatment and 3 months afterwards. In addition, change variables that could be responsible for variation in the concentration of triglycerides were collected: age, sex, diet, body mass index, blood pressure, and alcoholism. All statistically significant control variables in univariate models were analyzed together with the genetic polymorphisms with multiple regression in the adjusted model.
Results: We have found a weak non-significant correlation between the dose and the relative differences of triglyceride concentration; therefore, the model did not take into account the dose received in the decrease of the cTg. In univariate studies, the non-genetic variables were not significant. Regarding the genetic variables, after the study of multiple linear regression, which included age, sex, and each one of the minor alleles, it was found that just the effect of the minor variant in APOC3 was statistically significant and that it explains an additional 11% variation in response to fibrate treatment with respect to the non-carriers in this population (CI 95%: 3 to 22).
Conclusions: The effect of the decrease of the cTG following administration of fibrate is mostly attributable to the drug. Despite the fact that all individuals respond to treatment with fibrate, there is wide variability in this response, suggesting that there may be genetic factors involved, such as the variant c.*40 C>G of the APOC3 gene.
*NCEP (National Cholesterol Education Program)

W042

AGE-RELATED PENETRANCE IN GENETIC CARRIERS OF HYPERTROPHIC CARDIOMYOPATHY

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Introduction and Purpose: Hypertrophic cardiomyopathy (HCM) was initially considered to have an early onset in the life. The last advances in genetic area have showed new carriers without clinical diagnosis. The aim is study the age-related penetrance of HCM in patients with different MYBPC3, MYH7 and TNNT2 mutations to determinate whether the age at diagnostic depends on genetic background.
Methods: We included 195 HCM causal mutation carriers (55% males, age 40±16 years); 64.8% had clinical manifestations of the disease. All patients were diagnosed in inheritance cardiomyopathy consultation, in a reference hospital. 146 patients were carriers of at least one mutation in MYBPC3 [IVS23+1G>A (72), Arg891fs (37), A107fsX116 (26), A216T (11), V896M (4)], 21 were carriers of a mutation in MYH7 (T1377M (21), D928N (4), E1348Q (8), E1356Q (4), R1382Q (4)) and 8 patients were carriers of R278C in TNNT2. IVS23+1G>A, the most prevalent mutation, was present in 18 unrelated families. We performed time-to-diagnosis analysis according to the affected gene and the most prevalent mutations.
Results: No differences in time to diagnosis were detected between the most prevalent mutations. Median age at diagnosis was 46±2 years old for IVS23+1G>A, 44±3 years old (Arg891fs), 43±2 years old (A107fsX116), 44±7 years old (T1377M) and 51±9 years old (A216T); log rank P=0.963. Similarly, there were no differences according to the 3 analyzed genes (log rank P=0.935). Median age at diagnosis for the whole was 47±2 yrs.
Conclusions: Mutations in MYBPC3 encoding myosin binding protein C could be considered more benign form of HCM than initially was considered. Now, genetic diagnosis reveals that HCM-phenotype can appear later in life, reaching near full penetrance in the elderly.

W043

INFLUENCE OF TIME-DEPENDENT DEGRADATION OF CARDIAC TROPONIN T ON INFARCT SIZE ESTIMATION

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Background: Cardiac troponin T (cTnT) is widely used for the diagnosis of acute myocardial infarction (AMI). However, it is still unclear whether degraded cTnT forms circulate in the patient's blood. We therefore aim to elucidate which cTnT forms are detected by the clinical assay and investigate its influence on infarct size estimation.
Methods: First, sera of 13 AMI patients were separated by gel filtration chromatography (GFC) to examine cTnT degradation. These eluates were subjected to Western blot analysis employing the antibodies of the Roche immunoassay. Secondly, standardized serum samples of 18 AMI patients collected 0-72 hours post-admission were subjected directly to this Western blot analysis. For these patients, data on infarct size estimated by MRI, creatine kinase (CK) and lactate dehydrogase (LD) were also available.
Results: GFC analysis of AMI patients' sera revealed two cTnT peaks. Western blot analysis identified these peaks as cTnT fragments of 29 and 14-18 kDa, respectively. Western blotting of the standardized serum samples demonstrated a time-dependent degradation pattern (14-40 kDa) with intact cTnT (40 kDa) only present in 3 patients within the first 8 hours. Nevertheless, the area-under-the-curve (AUC) of conventional cTnT measurements correlated well with infarct size determined by MRI ($r=0.671$; $P=0.006$) and with AUC of CK and LD (both $r>0.9$; $P<0.001$).
Conclusions: This study reveals for the first time that the clinical immunoassay detects intact but mainly degraded cTnT forms in AMI patients' sera, although the consequences for the use of cTnT measurements to estimate infarct size remain limited.

W044

EFFECT OF A MOUNTAIN BIKE RACE ON CARDIAC BIOMARKERS CIRCULATING CONCENTRATIONS

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Background: Cardiac markers may be released, in athletes, during strenuous exercise but with a doubtful meaning. This phenomenon has been clearly demonstrated in marathon races but it is not clearly known during cycling, especially mountain biking.
Methods: 15 male runners between 32 and 55 years of age, were examined in a race of 44 Km long and with a difference in altitude of 1640 m in a mean time of 178 minutes (range 146 – 235). All competitors were trained, in good health, with a low cardiovascular risk and regularly checked at the service of sports medicine. Blood samples were collected immediately before the start and after the end of the race. Cardiac troponin I (cTnI on Dimension EXL, Siemens Healthcare Diagnostics, Milan, I), cardiac troponin T (hs-cTnT, Modular, Roche Diagnostics, Monza, I), NT-proBrain Natriuretic Peptide (NT-proBNP, Dimension EXL, Siemens Healthcare Diagnostics, Milan, I) and a metabolic biochemical profile were determined with routine methods.
Results: 5 out of 15 cases (33%) showed a significant increase of cTnI, 14 cases (93%) of hs-cTnT, and also, in all cases, we observe an increase in NT-proBNP. In 2 cases the increase of cTnI has exceeded the 99th percentile of normal levels and in 5 cases that of hs-cTnT. There is an inverse correlation between the athletes' degree of training and the release of markers. Urea and creatinine were also increased significantly at the end of the race, also after data correction for estimated dehydration degree detected by the albumin measurement before and after the race. We found a significant correlation between the release of the two cardiac markers hs-cTnT and NT-proBNP and the increase of urea at the end of the run, with Spearman coefficients $r=0.70$ and $r=0.72$. A significant correlation ($r=0.60$) has been observed between hs-cTnT and the increase of creatinine after race.
Conclusions: The study confirms that following a cycling race physical stress, there was a release of cardiac markers. Furthermore this release appears, in our assessment, to be proportional to the increase of urea and creatinine.

W045
SALIVARY NITRITE AND NITRATE IN FIBROMYALGIA PATIENTS

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Nitric oxide plays a major role in cardiovascular protection. Its formation occurs from exogenous and endogenous nitrates involving the entero-salivary cycle. Salivary nitrates are converted into nitrites acting as anti-infectious agents against periodontal infections and fungal infections. Drugs, including those prescribed in fibromyalgia patients can disturb the biological balance of the mouth.
Objectives. The aim of our study was i/to validate the quantification of nitrite/nitrate (NOx) in saliva by capillary electrophoresis/UV and ii / to quantify NOx in control subjects and in treated or untreated fibromyalgia patients.
Methods: We included patients referred to Pain Centre : treated (n = 18) or untreated (n = 6) fibromyalgia patients (F 21/3 M, mean age 51.5±8.54 years) and 24 healthy controls ((F 19 / M 5; 46.6±14.1 years). Saliva was collected in the morning using a Salivette Kit (cotton, Sarstedt), then placed on ice until analysis. Data from the buccal examination and clinical questionnaire were recorded. The salivary ultra-filtrate were analyzed by capillary electrophoresis/UV.
Results: i/The method we developed allows quantification of nitrite / nitrate (NOx) in 10 minutes. The use of an internal standard (Molybdate) improved linearity. ii / Our findings in salivary extracts show a high inter-individual variability as reported in the literature. NOx concentrations in fibromyalgia patients (n=24) and controls were comparable (nitrites : 132.7 ±95.5 μmol/L vs 98.0±70.9 μmol/L ; nitrates : 222.0±211.8 μmol/L vs 220.0±326.1 μmol/L). In treated patients, no major changes in the effect of drugs (anticholinergics) we found. On the other hand, untreated patients exhibited significantly lower nitrite concentrations (37.0±15.4 μmol/L, P <0.03). This suggests that untreated patients were at risk for developing an infectious lesion requiring clinical monitoring. An inverted profile was found in a patient with an abnormal parotid gland confirming the biological interest of salivary analyses.
Conclusion: We propose a standardized method of saliva collection and an easy, inexpensive quantification of biological parameters that may help control the effect of drugs or diagnose buccal and systemic diseases.

W046
THE POC ASSAY PATHFAST NTPROBNP FOR RISK STRATIFICATION AND DECISION MAKING IN THE EMERGENCY DEPARTMENT

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Background: NT-proBNP testing in the emergency department (ED) appear to improve stratification and clinical management of patients presenting with acute dyspnea. Questions remain about the value of NT-proBNP in emergency patients with other clinical signs. Aim of our study was to examine the utility of NT-proBNP in patients admitted to the medical ED for any symptom or disease by use of the point of care (POC) assay PATHFAST NTproBNP.
Methods: A prospective multicentre trial was conducted in 8 romanian county hospitals (Arad, Oradea, Tg. Mures, Sibiu, Neamt, Craiova, Botosani) to examine the effect of NT-proBNP on hospitalization rate, intensive care unit (ICU) admission rate, mortality and re-hospitalization during 6 month follow up (FU). NT-proBNP was measured immediately in whole blood samples obtained from patients at presentation in the ED after inclusion by use of the POC assay PATHFAST NTproBNP. Inclusion criteria were age >50 years without limitations with respect to the presenting symptoms.
Results: 282 patients were enrolled in the study. Mean age was 69 years. Median NT-proBNP concentration was 1390 (95% CI: 1080-1992) pg/mL. The patients were assigned into 4 diagnosis groups: ischemic heart disease (e.g. ACS) (N=45; 15.9%), non-ischemic heart disease (e.g. heart failure) N=123; 43.5%), lung disorders (e.g. POCD) (79; 28%) and others (36; 12.7%) with NT-proBNP median values of 990, 2170, 1524, and 674 pg/ml, respectively. 17% of the patients presented with NT-proBNP <300 pg/mL (low), 25% between 300 and 1000 pg/ml, and 58% with NT-proBNP >1000 pg/mL (high). All different study endpoints were associated with significantly increased NT-proBNP values. The combined endpoint death or readmission during FU was 31% and 15% in patients with high and low NT-proBNP, respectively.
Conclusion: Death or readmission was 31% in patients with high NT-proBNP compared to 15% with low NT-proBNP. NT-pro BNP <300 ng/mL might help to identify low risk patients, whereas patients with NT-proBNP >1000 ng/mL need intensified care. The PATHFAST point-of-care assay allows NT-proBNP determination within 16 min from whole blood and may improve risk stratification and management of emergency patients regarding hospital and ICU admission.

W047

MASS SPECTROMETRY-BASED PEPTIDE MAPPING REVEALS TEMPERATURE-INDUCED CLEAVAGES IN THE BACKBONE OF TROPONIN I

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Background: Cardiac troponin (cTn) I and T are structural proteins unique to the heart. In patients with acute myocardial infarction (AMI) cardiomyocyte necrosis can be detected from increased cTn levels in peripheral blood. Currently, cTns have become an integral part in the diagnosis of AMI. Roche Diagnostics has patented a cTnT assay whereas a variety of commercial immunoassays is used for cTnI quantification, each assay having specific 99th percentiles of a healthy reference population. To overcome the lack of cTnI standardization, the International Federation of Clinical Chemistry's Working Group for the Standardization of Troponin I (IFCC WG-TNI) was established. A "stable midregion" in the cTnI peptide sequence has been defined, which implies the presence of a less stable part within the protein sequence. Indeed data on time- and temperature-dependent instability of cTnI have been reported, potentially influencing assay standardization. From a standardization viewpoint, knowledge about instability of a protein biomarker and monitoring its structural integrity over time is of great importance.
Methods: Standard Reference Material (SRM) 2921, a primary reference material obtained from the National Institute of Standards & Technology (NIST), and a cTnI protein standard from Calbiochem (identical amino acid sequence) were used to monitor possible cleavages in the backbone of cTnI. The material was incubated at both 4 °C and 37 °C. Tryptic peptides were identified using a liquid chromatography ion trap mass spectrometry system in combination with a semi-tryptic database search.
Results: A peptide backbone sequence coverage of 58% was determined for cTnI. It was found that two peptide backbone cleavages had occurred in NIST SRM2921 material, namely between amino acids at 148/149 and 194/195. The Calbiochem standard did not show increased levels of "unexpected" peptides in tryptic peptide maps.
Conclusions: Peptide backbone instability of reference material prohibits standardization of a quantitative assay. The instability of cTnI in SRM 2921 material will be further investigated using isotopically labelled peptide standards that allow accurate quantification of the backbone cleavages.

W048

SERUM TROPONIN T ASSAYED AT 24 H AFTER ONSET OF CHEST PAIN WITH A HIGHLY SENSITIVE ASSAY IS NOT STRONGLY CORRELATED WITH INFARCT SIZE IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background: High sensitivity cardiac troponin T (hs-TnT) test (Roche Diagnostics, 5th generation) is useful in early diagnosis of acute myocardial infarction (AMI). Its clinical utility as a measure of infarct size is insufficiently known.
Methods: In 188 patients with first ST-segment elevation myocardial infarction (STEMI) treated with primary percutaneous coronary intervention (pPCI) serial creatine kinase (CK) and hs-TnT in serum were assayed. Cumulatively released quantities (Q) of CK in the first 24 and 48 h (Q24(CK) and Q48(CK), resp.) and Q of hs-TnT in the first 24 h (Q24(hs-TnT)) were calculated and expressed as gram-equivalents myocardium/m2 body surface are (g-eq/m2). Q48(CK) was chosen as an established measure of infarct size. Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine, age and gender. Time-to-peak CK (TTP CK) was used as indicator of quality of reperfusion.
Results: In the first 24 h released quantities of hs-TnT and CK, both expressed as g-eq/m2, are in the proportion of 1 : 8, demonstrating only fractional release of hs-TnT compared to CK. Serum hs-TnT at 24 h (hs -TnT24h) is correlated with Q48(CK): r=0.72 (n=175). However, the 13 outliers at the top (hs-TnT24h/Q48(CK) > mean value +SD) had lower eGFR than the whole population (49.2±4.4 vs. 65.0±1.6 mL/min/1.73m^2; P <0.05), suggesting that renal insufficiency is responsible for diminished TnT clearance in this subset of patients. The 13 outliers below (hs-TnT24h/Q48(CK)< mean value -SD) had TTP CK higher than the whole population (20.5±3.5 h vs. 13.8±0.50 h; P <0.05), suggesting that slow or incomplete reperfusion may lead to reduced release of TnT compared to release of CK. This may indicate local TnT degradation in myocardium that is ischemic for several h. For the group of patients with eGFR≥70 mL/min/1.73m^2 correlation of hs-TnT24h with Q48(CK) had r=0.84 (n=61) and further selection based on TTP CK <13.8h improved this correlation to r=0.89 (n=44).
Conclusions: Hs-TnT24h assayed with Roche hs-TnT assay in pPCI-treated STEMI patients is theoretically well correlated with infarct size, but factors as renal insufficiency and poor reperfusion affect this correlation which limits the value of hs-TnT24h as an accurate measure of infarct size.

W049

BASIC BIOCHEMISTRY ANALYSIS IN LITGEN (GENETIC DIVERSITY OF THE POPULATION OF LITHUANIA AND CHANGES OF ITS GENETIC STRUCTURE RELATED WITH EVOLUTION AND COMMON DISEASES) PROJECT

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Background: In 2011 a four year project – LITGEN has started. Analysis of basic biochemical phenotype of ethnic Lithuanians is part of this project.
Methods: Total cholesterol (TChol), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), triglycerides (TG), glucose (Glu) and hsCRP were investigated on Architect ci8200 (Abbott) system.
Results. 1344 volunteers (617 males (age x=39) and 727 females (age x=40)) from 32 cities were enrolled into the study. 217 were individual persons, 1127 from triads, altogether 332 triads. At least three generations of 256 triads are ethnic Lithuanian. Triads were divided into three generations, male M1 (n=376, age x=50), M2 (n=232, age x=21), M3 (n=9, age x=17) and female W1 (n=475, age x=49), W2 (n=243, age x=31), W3 (n=12, age x=18). In M1 group 78.72% TChol, 31.91% TG, 70.48% LDL-C, in M2 31.47% TChol, 9.91% TG, 25.86% LDL-C, in M3 22.22% TChol, 11.11% TG and LDL-C of results were above normal range. In W1 64.00% TChol, 12.21% TG, 55.58% LDL-C, in W2 37.86% TChol, 1.65% TG, 23.87% LDL-C, in W3 8.33% TChol, TG and LDL-C of results were above normal range. As expected, statistically significant difference was observed for all analytes (P <0.01), except HDL-C between M1 and M2, M1 and M3 groups, with higher values in M1. In contrast statistically significant difference just for TChol, LDL-C and hsCRP (P <0.001) was found between W1 and W2, W1 and W3, and additionally for TG (P <0.01) in W1 and W3 group, with higher values in W1. There was no significant difference between M2 and M3, W2 and W3. Comparing M1 and W1, M2 and W2 groups, statistically significant difference was observed for HDL-C (P <0.001) with higher values in W1 and W2 respectively. Comparing M1 and W1, Glu (P <0.005) was higher in M1. Interestingly in M2 and W2 groups TChol (P <0.01) was higher in W2. There was no difference between M3 and W3 groups.
Conclusions: Most significant differences were found between different age groups within the same gender. Only differences of TChol, Glu and HDL-C concentrations were found between gender groups. Next step of the study will include investigation of ApoAI, ApoB, Lp(a), IL-1b, MMP-9. The study is supported by LITGEN Project (VP1-3.1-SMM-07-K-01-013).

W050

HIGH SENSITIVE CARDIAC TROPONIN T AND COPEPTIN ASSAYS IN THE MANAGEMENT OF PATIENTS PRESENTING WITH CHEST PAIN OF LESS THAN 6 HOURS

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Background: The use of high sensitive troponin T (hsTnT) alone or associated with other biomarker in the management of chest pain patient is not yet precisely described. The diagnostic performances of hsTnT and copeptin assays in unselected patients presenting to the emergency department with acute chest pain were evaluated and compared to sensitive-contemporary troponin T (4th generation) (TnT4g), Creatine kinase and myoglobin assays.
Methods: Patients with chest pain and without ST-segment Elevation Myocardial Infarction (NSTEMI) were included. The final diagnosis of NSTEMI was based on TnT4g concentration (cut-off ≥0.03 μg/L) and standard clinical history. Cardiac markers were measured at admission (T0), 2 h (T2) and 3 (T3) hours later. The diagnostic performances of these assays were calculated for early diagnosis of NSTEMI. Receiver-operating characteristic (ROC) analysis was used to compare their diagnostic performances for the prediction of NSTEMI. Results: 89 patients (age range, 60±16 years; 72% male) were selected. NSTEMI was found in 10 patients (11%). When NSTEMI were compared to other diagnosis, CK and copeptin were not significantly different at any time considered. TnT4g was significantly different at T2 and T3 but myoglobin and hsTnT were significantly increased at admission, T2 and T3. To identify an acute myocardial injury, the difference between T0 and T2 (relative delta change in %) was calculated. Only TnT4g delta change was significantly increased in NSTEMI patients (P=0.002). At admission, significant area under ROC curve were found with hsTnT (0.881: 95% CI 0,793 to 0.940) and myoglobin (0.735 : 95% CI 0.631 to 0.823), but not with other biomarkers. For ruling out NSTEMI (after excluding STEMI), an hsTnT concentration <12 ng/L yielded a diagnostic sensitivity of 90%, a diagnostic specificity of 84.4%. Copeptin give no added value to hsTnT for ruling out NSTEMI.
Conclusions: The introduction of hsTnT assay displays an excellent diagnostic performance for the workup of patients with chest pain at the time of their initial presentation. Using the 99th percentile cutoff for hs-cTnT enable prediction of earlier diagnosis of NSTEMI.

W051

GENETIC CARDIAC SCREENING AMONG HYPERTROPHIC CARDIOMYOPATHY FAMILIES FROM SOUTHERN ITALY

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Background: Hypertrophic cardiomyopathy (HCM) is the most common genetic cardiovascular disorder: it is the most frequent cause of sudden cardiac-related death in young people and a major cause of cardiac failure and death in the elderly. However, HCM frequently goes undiagnosed until the appearance of overt signs and symptoms. Recent guidelines provide recommendations for genetic cardiac screening in at-risk families, but the impact of these measures in Italy is unknown.
Methods: We analyzed, by PCR and direct sequencing, 93 independent HCM patients from Southern Italy for MYH7, MYBPC3, TNNT2, TNNI3, TPM1, MYL2, MYL3 and ACTC1 genes. We also analyzed 119 subjects from 31 HCM families to evaluate the mutation segregation in the family.
Results: Mutations were identified in 47 of 93 patients (50%). The most frequent genes altered in the genotyped patients were MYH7 and MYBPC3, which were mutated in 43% and 42 % of genetic positive cases, respectively. Genes TNNT2, TPM1 and TNNI3 were altered in 11%, 2% and 2% of cases, respectively. We found 11 novel mutations: 6 in MYBPC3, 4 in MYH7 and 1 novel mutation in TNNT2. Five patients at HCM end-stage phase had double mutations: two had double mutations in MYBPC3 gene, and one patient had 2 mutations in MYH7 gene; the other were compound heterozygous, harboring 1 mutation in MYH7 and 1 in MYBPC3 genes and 1 mutation in MYH7 and one in TPM1 genes. Seventy (59%) family subjects, of whom 26 were asymptomatic, carried a mutation. Of note, within the group of at-risk, asymptomatic relatives of probands, genetic testing were positive in 35% of cases.
Conclusion: Our data agree with HCM molecular epidemiology present in the literature, reporting genes most frequently associated with HCM are MYH7, MYBPC3 and TNNT2. In our cohort complex genotypes, characterized by double mutations, were present in HCM end-stage patients, suggesting that genetic screening may play a role in the identification of HCM patients at risk of disease progression. Molecular screening of first-degree resulted very important for characterize family members at risk for developing disease and excluding unaffected relatives, which is information not achievable otherwise.

W052

EFFECTS OF BERBERINE AND RED YEAST ON PROINFLAMMATORY CYTOKINES IL-6 AND TNF-ALPHA IN MONONUCLEAR CELLS (MNCS) OF HUMAN SUBJECTS

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Purpose: Obesity is a condition characterised by diffuse, chronic inflammation involved in the pathogenesis of diseases associated with obesity (insulin resistance, cardiovascular complications and dyslipidemias). Although the response to inflammation starts in adipose tissue, recent studies demonstrate the involvement of MNCs in raising levels of proinflammatory cytokines, particularly IL-6 and TNF-α, in obese subjects. The immunomodulating action of natural substances such as berberine and monacolin K, a red yeast metabolically active compound, is assessed in MNCs extracted from peripheral blood activated with lipopolysaccharide (LPS) by determining the levels of expression and transcription of IL-6 and TNF-α.
Methods: MNCs extracted from whole blood of healthly donors was placed in culture and incubated at 37 °C. To induce the production of proinflammatory cytokines, the cells were stimulated with 50 ng/mL LPS (from E. coli Lipopolisaccarides - Sigma) and simultaneously treated with increasing doses (1, 10 and 50 μg/mL) of berberine (BRB) and red yeast 1,5% S.C.(Specific Concentration) monacolin K (RY), alone or in association for 3, 6 and 24 h. The expression and transcription levels of IL-6 and TNF- a were assessed by ELISA (R&D Systems ELISA kit) and real time PCR.
Results. Berberine plus red yeast treatment carries out a synergic inhibitory effect on TNF-α expression at concentration of 50 μg/mL during all the time-course experiment. Moreover berberine plus red yeast treatment show a synergistic inhibitory effect on IL-6 expression at very low concentration just after 3 hr of incubation. In addition BRB plus RY significantly reduce the transcription of genes coding for IL-6 and TNF-α in MNCs from peripheral blood at 3, 6 and 24 h of incubation.
Conclusions. The results of our study show that both berberine and red yeast carry out a strong anti-inflammatory action trough an inhibition of proinflammatory proteins concentration IL-6 and TNF-α and their gene expression in MNCs activated by LPS. According to some literatures data, our results show that induction of IL-6 and TNF-α production, induced in MNCs activated by LPS, is similar to that observed in obese subjects.

W053

DETERMINATION OF REFERENCE VALUES FOR HEART-TYPE FATTY-ACID BINDING PROTEIN IN AN ITALIAN COHORT

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Background: Heart-type fatty acid-binding protein (H-FABP) is a low molecular-weight cytoplasmic protein that has been recently used as an early marker of acute myocardial infarction. As reference intervals are the most common decision support tool for interpretation of laboratory results, this study aimed to define the upper reference value for H-FABP determined with the recent Randox Laboratories immunoturbidimetric assay on Roche Cobas 6000 analyzer in our laboratory for italian subjects.
Methods: Plasma EDTA samples from 306 blood donors (153 females and 153 males) were collected and frozen at -80 °C. H-FABP was measured in all samples in a single run with reagents from the same lot and properly calibrated; quality control (two levels) was performed at the beginning and at the end of the run and after every batch of 75 samples. All data were collected and analysed in MedCalc software Ver. 12.3.0.0 for Windows 7. The statistical analysis was performed following CLSI guidelines C28-A3 and IFCC's Expert Panel on Theory of Reference Values. Outliers were detected and the upper reference value (97,5th centile) was calculated by nonparametric method for all subjects together and for each gender subclass after outlier exclusion.
Results: The age range of all subjects was 20-55 years with a mean age of 33 for female subclass, 35 for male subclass, and 34 the whole. After outlier exclusion, the 97,5th centile for the entire cohort was 3,4 μg/L and the upper reference values obtained for the two gender subclasses were statistically compared showing no significant difference.
Conclusions: As the two reference values obtained for the two gender subclasses were not significantly different, it is appropriate to use the reference value calculated on all subjects, that is 3,4 μg/L as 97,5th centile.

W054

A STUDY OF LIPOPROTEIN ASSOCIATED PHOSPHOLIPASE A2 (LPPLA2) AS AN EMERGING BIOMARKER IN CORONARY ARTERY DISEASE

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Background: Lipoprotein-associated phospholipase A2 (Lp-PLA2), also known as platelet-activating factor acetylhydrolase, is an enzyme that hydrolyzes phospholipids and is primarily associated with low-density lipoproteins (LDLs). Accumulating evidence has suggested Lp-PLA2 as predictor of coronary artery disease (CAD) and may have a proinflammatory role in the progression of atherosclerosis. However its role has not been documented clearly in Indians.The aim of this study was to explore the relationship of serum lipoprotein-associated phospholipase A2 (LpPLA2) in coronary artery disease in an Indian Population.
Methods: 100 patients with angiographically proven CAD were studied of which 50 patients were of stable angina (Group I), 50 patients with Acute Coronary Syndrome (Group II) [35 patients with unstable angina and 15 patients with MI] from a tertiary health center, New Delhi and a third group comprising of 50 age and sex matched healthy controls were also studied over a period of 1 year The LpPLA2 levels were measured by ELISA technique and angiographic clinical vessel scoring was done for all patients. Data is presented as Mean ± S.D. and relationships were determined by Pearson correlations.
Results : The mean age of the patients 49±8.8 years (84% men, 16%women). The mean serum LpPLA2 levels for stable angina (Group I) [274.30±33.16 ng/mL], acute coronary syndrome (Group II) [287.39±35.61 ng/mL] were significantly higher in CAD patients than controls [196.64±21.4 ng/mL] [P <0.001]. High LpPLA2 values correlated with higher vessel scores indicating a more severe CAD both in stable angina patients [r=0.384, P <0.001] and unstable angina patients[r=0.459, P <0.001].
Conclusion: Lipoprotein associated phospholipase A2 were found significantly higher in unstable angina than stable angina and also correlated with higher angiographic vessel scores. High serum levels of LpPLA2 is associated with both the presence and the severity of angiographically proven CAD patients Our results suggest that LpPLA2 is an emerging marker of Coronary Artery Disease and can also predict the disease severity.

W055

THE RELATIONSHIP BETWEEN OSTEOPROTEGERIN, MATRIX GLA PROTEIN, AND HBA1C IN CONTROLLED AND UNCONTROLLED TYPE 2 DIABETES MELLITUS PATIENT

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Background: Many studies have reported that diabetes mellitus correlates with vascular calcification event and increases progressively in uncontrolled diabetes. Osteoprotegerin (OPG) is known to act as a promoter in vascular calcification, contrary Matrix Gla Protein (MGP) is an inhibitor in vascular calcification. The aim of this study is to observe progress of vascular calcification in uncontrolled diabetes patient by biochemical markers OPG as promoter and MGP as inhibitor in vascular calcification.
Methods: This study is an observational study with cross sectional design on adult type 2 diabetes male that is defined with DM Consensus Criteria Indonesia.
Results: The results of this study show that there is a positive significant correlation between OPG and HbA1c (r=0.261, P=0.030), despite MGP shows no significant correlation with HbA1C. OPG also correlates significantly with Fasting Plasma Glucose (r=0.261, P=0.014). In uncontrolled diabetes group there is positive significant correlation between OPG and HbA1C (r=0.397, P=0.014). There is no significant difference in level of OPG in controlled and uncontrolled diabetes group (P=0.567), but OPG/MGP index has higher difference (P=0.259). The OPG/MGP index also has positive significant correlation with HbA1c (r=0.285, P=0.018) and Fasting Plasma Glucose (r=0.313, P=0.009).
Conclusion: This study suggest progress to vascular calcification in uncontrolled type 2 diabetes mellitus. The use of vascular calcification biomarkers are recommended to predict /detect vascular calcification event in type 2 diabetes patients.

W056

DETERMINATION OF HEAT SHOCK PROTEIN 70 HIDDEN IN CIRCULATING IMMUNE COMPLEXES: A MARKER FOR PREDICTION OF CORONARY HEART DISEASE

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Background: The 70 kDa heat shock protein (Hsp70) and/or autoantibodies to this protein are considered as endogenous factors implicated in development of atherosclerosis and acute coronary disease. However, the reports about correlations between the plasma Hsp70 or anti-Hsp70 autoantibody levels and a risk of cardiovascular pathologies seem rather conflicting. Here we propose to assess the prognostic value of Hsp70 which is a “hidden” constituent of circulating immune complexes (CIC); according to our observations, the high level of Hsp70-containing CIC is better correlated to the risk of coronary diseases than the high plasma levels of “free” Hsp70 or anti-Hsp70 autoantibodies.
Methods: Using precipitation with 2.5% polyethylenglycol (PEG) we isolated CIC from samples of sera of healthy donors and patients with a different degree of atherosclerosis and coronary disease. After washing the isolated CIC, Hsp70 was released from its complexes with the autoantibodies by means of destruction of immunoglobulin molecules with dithiothreitol and iodoacetamide (the successive two-step treatment); then both the low molecular agents were removed by dialysis. In the obtained samples of dialyzed protein mixture, the amount of Hsp70 was quantified by routine enzyme - linked immunosorbent assays (ELISA) with commercial (rabbit and mouse) antibodies against human Hsc70/Hsp70.
Results: We compared the Hsp70 levels in CIC fractions obtained from healthy individuals (no established atherosclerosis or other cardiovascular disorders, n=51) and cohorts of patients with early coronary atherosclerosis (n=68), stable angina (n=72), acute coronary syndrome (n=83) or acute myocardial infarction (n=75). In all the examined cases, no or only slight Hsp70 amount was determined in the samples of CIC from healthy donors, whereas the large fractions of Hsp70-enriched CIC (hidden Hsp70: >median, 2.05 ng/mL) were always determined in sera of patients with the above diseases. Importantly, the significant increase in Hsp70-containing CIC was found to correlate to the severity of cardiovascular pathology (P for trend = 0.001).
Conclusions: The level of Hsp70-enriched CIC in human serum seems to be a predictive biomarker indicating a risk of the coronary heart disease development.

W057

ANTIENDOTHELIAL CELLS ANTIBODIES (AECA) IN PATIENTS WITH SYSTEMIC SCLEROSIS (SSC) IN RELATION TO THE PRESENCE OF PULMONARY HYPERTENSION AND LUNG FIBROSIS

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Although scleroderma is generally considered a fibrosing disease, it is now recognized that underlying vascular pathology is playing a fundamental role in its pathogenesis. The present study was aimed at testing the prevalence of AECA in Ssc patients with and without pulmonary hypertension (PH) and in relation to the presence of pulmonary fibrosis. 54 SSc patients (50 females and 4 males, mean age 55.7±16.3 years) were prospectively screened. Healthy control group was also included (n=27; 7 men and 20 women, mean age 49.8±12.1 years). The study of AECA was performed using the indirect immunofluorescence method on commercially available human umbilical vein endothelial cells. The HRCT-scans revealed signs of lung fibrosis in 15 (out of 36 examined patients). Tricuspid gradient (TRPG) at rest of 31mmHg was demonstrated in 14 (21%) patients. During cardiac catheterisation arterial PH was found in 2 patients. At the baseline 14/54 patients (26%) were positive for AECA. In the control group the frequency of the antibodies was 3/27 (11%) None of the negative patients at the baseline was positive after one year of the observation and only in 2 cases the antibodies disappeared. No statistical correlation between antibody titter and presentation of the disease existed. Only one of the patients with TRPG above 31mmHg was positive for AECA. This was a patient with pulmonary arterial hypertension confirmed by right heart catheterization. AECA were highly prevalent in the subgroup of patients suffering from interstitial pulmonary fibrosis. Out of 15 patients suffering from lung fibrosis 7 were AECA positive. The presence of AECA correlated very well with antinuclear antibodies (ANA), but was not related to the profile of ANA. Our findings supports evidence that endothelial cell damage is involved in Ssc as there is increased prevalence of circulating AECA of the IgG isotype in patients with Ssc. AECA may be related to severe complications of Ssc like pulmonary fibrosis. The relation between the presence of AECA and different types of pulmonary hypertension is unresolved.

W058

CORRELATION OF LIPID PARAMETERS WITH THE LEVELS OF VCAM-1, ICAM-1 AND ADMA IN HYPERCHOLESTEROLEMIA

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Background: Hypercholesterolemia with high levels of total cholesterol and LDL cholesterol are associated with endothelium-dependent vasodilation in the forearm and in the coronary vasculature, indicating impaired endothelial function. In this study we investigated the correlation of some markers of endothelial dysfunction (ED) with lipid parameters in patient with hypercholesterolemia.
Material and methods: Seventy clinically asymptomatic hypercholesterolemic patients without other cardiovascular risk factors and 70 controls were evaluated. We measured the plasma levels of ICAM-1, VCAM-1 and ADMA by ELISA assay and lipid profile by conventional methods.
Results: Significant difference was found between patients and controls ($P < 0.001$) regarding the serum levels of total cholesterol, triglycerides, HDL cholesterol and LDL-cholesterol. The mean values of ICAM-1, VCAM-1 and ADMA calculated at baseline in both groups differed significantly ($P < 0.001$). We found a very strong positive correlation, statistically significance, between the ADMA, ICAM-1 VCAM-1 and LDL cholesterol and the total cholesterol levels ($P < 0.001$). Multiple regression analysis revealed that LDL- and total cholesterol levels are powerful predictors of variations in the ADMA, ICAM-1 and VCAM-1 levels.
Conclusion: The LDL cholesterol and total cholesterol are basic modulators of concentration of ADMA, ICAM-1 and VCAM-1 in hypercholesterolemia. The significant positive correlation between the ADMA and LDL cholesterol confirm potential role of hypercholesterolemia in ADMA metabolism. Impaired endothelial function under conditions of elevated cholesterol in the circulation may be an important link between hypercholesterolemia and increased rates cardiovascular diseases.

W059

KINETIC OF B-TYPE NATRIURETIC PEPTIDE PLASMA LEVELS IN PATIENTS WITH LEFT SIDED BREAST CANCER TREATED WITH RADIATION THERAPY: A PROSPECTIVE STUDY

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Background. Biochemical parameters could provide support for identifying patients at risk for radiotherapy treatment (RT)-induced cardiotoxicity in addition to other diagnostic procedures. We monitored B-type natriuretic peptide (BNP) as cardiac biomarker in patients with left-sided breast cancer during one year after RT and we investigated its relationship with different dosimetric constraints.
Methods. Plasma parameters (BNP, troponin) and dose-volume parameters for heart and ventricle were collected in 59 left-sided breast cancer patients (median age 57.0 years, range 34-78 years) during a follow up of one year. Measurements were performed before the RT treatment (T0), at 15 days during RT (T15day), at the end of RT (Tend), and then at 1, 3, 6, 9 and 12 months (T1, T3, T6, T9, and T12).
Results. The mean value of BNP concentrations analysed in all patients increased after RT (P=0.06); the ratio between BNPT12 values and the pre-RT baseline value (ratio BNPT12/BNPT0) ranged from 0.20 to 5.00 (mean 1.38, SD 0.90); considering all patients, the ratio BNPT12/BNPT0 showed a statistical significant increase (P <0.01). TnI levels remained below the cut off threshold in all patients. No association was observed between BNP ratio increase and age, cardiologic risk factors and chemotherapy, except for hormone-therapy (HT): women underwent HT and women without HT showed, respectively, mean ratio BNPT12/BNPT0 values 1.47 vs 0.92 (P=0.04). Twelve patients showed at the end of the follow up BNP pathological values, as BNP >50 pg/mL and ratio BNPT12/BNPT0 increase above 25%; in these subjects there were significant associations with V3Gy(cm3), V3Gy(%), V5Gy(%), D10cm3(Gy), D20cm3(Gy), D30cm3(Gy), D3%(Gy), D15%(Gy), D20%(Gy) for the heart, D15cm3(Gy), D30cm3(Gy), D40cm3(Gy), D5%(Gy), D25%(Gy), D30%(Gy) for ventricle, but this effect was not confirmed after adjustment for risk factors. Considering patients with only ratio BNPT12/BNPT0 >25%, there were significant associations with median V3Gy(cm3), V5Gy(%), D10cm3(Gy), D20cm3(Gy) in the heart, D10cm3(Gy), D15cm3(Gy) in left ventricle.
Conclusions. The results of this prospective study showed a possible role of BNP as early marker of heart irradiation also in its plasmatic normality range.

W060

GENETIC PRE-PARTICIPATION SCREENING IN SELECTED ATHLETES: A NEW TOOL FOR THE PREVENTION OF SUDDEN CARDIAC DEATH?

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Background: Sudden cardiac death (SCD) of athletes is a topical issue. "Borderline cardiac abnormalities", which occur in ~2% of elite male athletes, may result in SCD, which may have a genetic base. Genetic analysis may help identify pathological cardiac abnormalities. We performed phenotype-guided genetic analysis in athletes who, pre-participation, showed ECG and/or echo "borderline" abnormalities, to discriminate subjects at a greater risk of SCD.
Methods: We studied 24 elite athletes referred by the National Federation of Olympic sports; and 25 subjects seeking eligibility to practice agonistic sport referred by the Osservatorio Epidemiologico della Medicina dello Sport della Regione Campania. Inclusion criteria: a) ECG repolarization borderline abnormalities; b) benign ventricular arrhythmias; c) left ventricular wall thickness in the grey zone of physiology versus pathology (max wall thickness 12-15 mm in females; 13-16 mm in males). Based on the suspected phenotype, we screened subjects for the LMNA gene, for 8 sarcomeric genes, 5 desmosomal genes, and cardiac calcium, sodium and potassium channel disease genes.
Results: Genetic analysis was completed in 37/49 athletes, 22 competitive and 27 non-competitive athletes, showing "borderline" clinical markers suggestive of hypertrophic cardiomyopathy (HCM,n. 24), dilated cardiomyopathy (n. 4), arrhythmogenic right ventricular dysplasia/cathecholaminergic polymorphic ventricular tachycardia (ARVD/CPVT, n. 11), long QT syndrome (LQTS, n. 4), sick sinus syndrome (SSS, n. 5), Brugada syndrome (BrS, n. 1). We identifyed 11 mutations in 9 athletes (an ARVD athlete was compound heterozygote for the PKP2 gene and an HCM athlete was double heterozygote for the MYBPC3 and TNNT2 genes): 3 known mutations related to LQTS, HCM and ARVD, respectively, and 8 novel mutations, located in the SCN5A, RyR2, PKP2, MYBPC3 and ACTC1 genes. The new mutations were absent in ~800 normal chromosomes and were predicted "probably damaging" by in silico analysis. Patch clamp analysis in channelopathies indicated for some mutation abnormal biophysical behavior of the corresponding mutant protein.
Conclusion: Genetic analysis may help distinguish between physiology and pathology in athletes with clinically suspected heart disease.

W061

A PILOT MODEL FOR RISK STRATIFICATION OF BRUGADA PATIENTS BASED ON GENOTYPE

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Background: Brugada Syndrome (BS) is a cardiac disorder characterized by electrical ventricular instability that can lead to sudden death at young age. To date the only available treatment is implantation of a cardioverter defibrillator, however, actual guidelines are only partially efficient in predicting the predisposition of these patients to develop ventricular arrhythmias, especially for asymptomatic subjects. New criteria for risk stratification are therefore needed. According to the emerging concept of "arrhythmia genomics", the co-segregation of different variants may modulate the presentation of the clinical phenotype. However, in BS genetic bases have been only partially understood, with less than 25% associated with mutations in the SCN5A gene, encoding the cardiac sodium channel. Our main goal is to correlate genotype with clinical phenotype, in order to improve risk stratification and management of asymptomatic patients.
Methods: We collected 92 BS patients, who were characterized from the clinical, electrophysiological and genetic point of view and followed 32±17 months. We performed the molecular analysis of the SCN5A gene using the ABI 3730 DNA analyzer and we genotyped BS patients for a panel of 71 candidate SNPs localized in genes involved in cardiac excitability, using the SNPstream-Beckman Coulter system.
Results: Molecular analysis of the SCN5A gene identified 18 mutations, 7 of which were novel. This enabled us to extend the family screening and to diagnose BS in 18 asymptomatic relatives who may be at risk of sudden death. Interestingly, statistical analysis suggested that SCN5A-positive patients were more likely to experience major arrhythmic events (MAE). In an effort to identify variables able to improve risk stratification, Kaplan-Meier and Log-Rank analysis showed 5 SNPs significantly associated with MAE. We tried to elaborate a pilot risk stratification algorithm for MAE susceptibility including genetic variables using a weighted genetic risk score. Indeed using this model, patients experiencing MAE had mean wGRS =6.36±2.5, while event-free patients =2.87±1.99 (Mann-Whitney P=1.5*10-5).
Conclusions: In perspective, this method may allow to integrate genetic data with clinical variables to improve risk stratification in BS.

W062

GENE EXPRESSION OF ANTIOXIDANT ENZYMES IN PATIENTS WITH CHAGAS CARDIOMYOPATHY DISEASE

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Background: The pathogenesis of chronic chagasic cardiomyopathy (CCM) is controversial; there are no definitive proofs of which are the necessary factors to reach the determinate stage. Each host genetic factors could actively participate in the evolution of Chagas disease. Whereas the variability of phenotypic expression of the CCM could be because of genetic components of the patient, we decided to do a descriptive study of genotype frequencies (GF) of SOD-Mn Ala-9-Val and the enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (KAT) in chagasic patients with cardiomyopathy (CcC) and without cardiomyopathy (CsC) compared with healthy controls (CN).
Methods: The molecular characterization was performed by PCR-RFLP. Enzyme activities were determined by spectrophotometric techniques. The hypothesis test under normal theory proportions and Kruskal Wallis test were carried out.
Results: The SOD-Mn FG (IC 95%) were CN: Ala/Ala 0.54 (0.40-0.67), Ala/Val 0.33 (0.20-0.45), Val/Val 0.13 (0.04-0.21); CsinC: Ala/Ala 0.36 (0.07-0.64), Ala/Val 0.46 (0.16-0.75), Val/Val 0.18 (0.00-0.40); CconC: Ala/Ala 0.35 (0.14-0.56), Ala/Val 0.30 (0.10 - 0.50), Val/Val 0.35 (0.14 - 0.56). The enzime activities were: KAT(K/gHb): CconC 316±68, CsinC 332± 41, CN 185±28; GPx(U/gHb): CconC 98±17, CsinC 102±20, CN 61±11; SOD(USOD/gHb): CconC 3270±833, CsinC 2590±188, CN 895±314. The study of SOD-Mn GF of chagasic patients and CN showed significant differences (P <0.01) between them. The activities of KAT, SOD and GPx showed significant differences (P <0.01) between chagasic patients and CN.
Conclusions: The data suggest that polymorphisms involved in oxidative stress may have implications in the pathogenesis of CCM, modifying individual risk in the development of cardiomyopathies.

W063

INTERLEUKIN-15, CORONARY ARTHERY DISEASE AND EPICARDIAL ADIPOSE TISSUE: POSSIBILE CORRELATIONS

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Background: Obesity is a risk factor for cardiovascular diseases. Many of the adverse health consequences of excessive fat accumulation are caused by increased secretion of pro-inflammatory adipokines and cytokines by the adipose tissue leading to a chronic low-grade inflammatory status. Among different fat depots, epicardial adipose tissue (EAT) has been shown to increase in visceral obesity and to play a potential role in the development of coronary artery disease (CAD). The observation that interleukin-15 (IL-15) is up-regulated in atherosclerosis and is produced by the inflammatory cells located at vulnerable atherosclerotic plaques suggests that this cytokine may have a pathogenic significance in CAD. In our study we measured IL-15 circulating level in patients affected by CAD undergoing open-heart surgery both to elective coronary artery bypass grafting (CABG) surgery and in patients without CAD undergoing open-heart surgery to valve replacement (VR). We also compared gene expression levels of IL-15 and its receptor (IL-15RA) in EAT samples isolated from CABG and VR patients.
Methods: Blood samples of patients undergoing elective CABG or VR surgery and of healthy controls were collected after an overnight fasting to measure IL-15 level by immune-enzymatic assay. IL-15 and IL-15RA gene expression in EAT was evaluated by one colour microarray platform. EAT thickness was measured by echocardiography.
Results: IL-15 plasma levels resulted higher in CABG than in VR patients. After patients classification according to their body mass index, IL-15 levels resulted higher in overweight/obese (OB) CABG compared to OB VR patients. After patients classification according to metabolic syndrome (MS) criteria (IDF 2005), IL-15 level resulted higher in CABG patients affected by MS compared to MS-VR patients. A great increase in the gene expression of IL-15 and IL-15RA was observed in CABG vs. VR patients.
Conclusions: EAT is a potential source of IL-15. IL-15 circulating protein is increased in OB CABG compared to VR patients and related to the presence of MS. Whether EAT may significantly contribute to increase IL-15 circulating levels in these patients need further investigation and could give more information on the relationship between IL-15 and CAD.

W064

COMPARISON OF THE DYNAMICS OF CARDIAC TROPONIN I IN CORONARY ARTERY BYPASS GRAFTING WITH OR WITHOUT LEFT VENTRICLE ANEURISM REPAIR

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Background: The aim of study was to determine the dynamics of cardiac troponin I (cTnI) levels before and after coronary artery bypass grafting (CABG) with or without left ventricle aneurism repair (LVAR) in patients with coronary artery disease (CAD).
Methods: 40 patients (100% male, age 54,3 ± 7,5 years) were enrolled in the study. For further evaluation, patients were divided in two groups according to operation type: 20 patients underwent first time elective CABG, 20 patients - CABG with LVAR. The operations were performed using cardiopulmonary bypass and isothermal blood cardioplegia. cTnI levels, were measured before operation, and 12, 24, 48, and 120 h after declamping of the aorta. cTnI levels was measured using immunochemical analyzer «ARCHITECT i2000» («Abbott», USA). Twelve lead electrocardiograms (ECGs) were recorded preoperatively and at days 1, 2, and 5. 99th percentile was determined to be 32 ng/l (male, 18-63 yrs). Diagnosis of perioperative myocardial infarction was performed According to the Third Universal Definition of Myocardial Infarction ESC/ACCF/AHA/WHF 2012.
Results: No complications were observed after operations. Before surgery, all patients' cTnI levels ranged from 0 to 15 ng/l. The cTnI serum levels after elective CABG were less than 100 ng/L in 5% of patients, from 100 to 250 ng/L in 80% and did not exceed 290 ng/L in all patients in the present study (12-24 hours after surgery). In CABG with LVAR group minimal cTnI level was 116 ng/L. It was 600-1000 ng/L in 50% of patients and more than 1000 ng/L in 10% of patients (12-24 h after surgery). In the CABG group cTnI did not exceed the diagnostic level of perioperative myocardial infarction (type 5). In CABG with LVAR group cTnI levels were 25 times more than 99th percentile in the majority of patients and up to 50 times in some cases due to the aneurism resection amount.
Conclusions: In present study no clinical evidence for myocardial ischemia was found, and only increase in cTnI levels could be diagnosed. Measurement standards of cTnI levels and the correct time periods of blood sampling should be applied for the definition of perioperative myocardial infarction for each type of cardiac surgery operations.

W065

IL-18 LEVEL IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING SURGERY OR VALVE REPLACEMENT: WHICH LINK WITH EPICARDIAL FAT DEPOT?

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Background: Interleukin-18 (IL-18), a member of the interleukin -1 family cytokines, is produced constitutively in many different cell types, such as macrophages/monocytes, endothelial cells, smooth muscle cells and cardiomyocytes. In addition, since IL-18 is elevated in human obesity and weight loss is associated with its reduction, it could also be produced by adipose tissue. Due to the link between obesity, inflammation and cardiovascular diseases, we measured IL-18 circulating level in patients undergoing open-heart surgery both to elective coronary artery bypass grafting (CABG) surgery or to valve replacement (VR). We also evaluated whether epicardial adipose tissue (EAT), a fat depot closely apposed on the myocardium, may be a potential source of IL-18.
Method: Blood samples of patients undergoing elective CABG or VR surgery and of healthy controls were collected after an overnight fasting to measure IL-18 level by immune-enzymatic assay. IL-18, IL-18 receptor 1 (IL-18R1) and IL-18-receptor accessory protein (IL-18RAP) gene expression in EAT depot were evaluated by one colour microarray platform. EAT thickness was measured by echocardiography.
Results: Quantification of circulating IL-18 indicated that patients, considered both together and after subdivision in CABG and VR patients, had higher level than controls. After patients classification in normal-weight, over-weight and obese, in each group IL-18 level resulted higher than in controls but not different between the groups. In addition, we did not observe any difference in IL-18 circulating level after patients classification both according to waist circumference cut-off value of ≥94 cm or ≥102 cm and the median echocardiographic EAT thickness. A great increase in the gene expression of IL-18, IL-18 R1 and IL-18 RAP was observed in EAT samples obtained from CABG vs. VR patients.
Conclusions: CABG and VR patients had similar increased level of circulating IL-18 protein, independently to the adiposity status, but in EAT depots isolated from CABG patients gene expression of IL-18, IL-18 R1 and IL-18-RAP resulted higher than in VR patients. Future investigation on local IL-18 protein production, could give more information on the relationship between IL-18 and coronary artery disease.

W066

IMMEDIATE POST-OPERATIVE FALL IN SERUM ALBUMIN IN PATIENTS UNDERGOING CARDIAC SURGERY PREDICTS POOR OUTCOME

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Background: Cardiac surgery while successful in the vast majority of cases has nevertheless an underlying medium term mortality rate of around 6% associated with it. The acute phase response may itself be an important marker of subsequent outcome however it does not usually become evident for 24-48h. A fall in serum albumin post-operatively however occurs within 24 h and may be a much earlier indicator of developing risk of morbidity and mortality.
Methods: 1010 consecutive patients who had undergone cardiac surgery had their albumin measured before and immediately post surgery (within 6hrs). The percentage drop in albumin was calculated and related to all-cause mortality at three, six and twelve months following surgery.
Results: Patients who died within 30 days of surgery showed a larger percentage drop in albumin level (54.5%; IQR 48.8 – 63.2%) than those still alive (48.9%; IQR 41.9 – 55.6%; $P < 0.001$). A similar relationship was observed for patients who died within 6 months (51.1%; IQR 47.8 – 59.2%) and within one year (51.1%; IQR 47.8 – 59.8%) compared to those still alive at this time point (48.9%, IQR 41.9 – 55.7%, $P = 0.002$); and (48.9%, IQR 41.9 – 55.6%, $P = 0.002$) respectively.
Conclusion: Early signs of significant acute phase response following cardiac surgery may predict subsequent short and medium term mortality in patients undergoing cardiac surgery. The immediate post operative fall in serum albumin may allow early identification of patients who could benefit from an accelerated care pathway.

W067

BUTYRYLCHOLINESTERASE IS POSITIVELY ASSOCIATED WITH LDL-CHOLESTEROL, TOTAL CHOLESTEROL AND TRIGLYCERIDES, AMONG APPARENTLY HEALTHY ADULTS

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Objective: Although butyrylcholinesterase is widely distributed in different tissues of the body, its physiologic role has not yet been defined. This study aimed to explore the relationship between butyrylcholinesterase and lipids levels, among apparently healthy adults.
Design and Methods: During 2009, 490 volunteers (46±16 years, 40% males) that visited the outpatients' office of our Hospital for routine examinations, were consecutively enrolled in the study (participation rate 85%). Biochemical analyses were performed through established procedures and haematological as well as biochemical parameters were measured. Anthropometric, lifestyle and dietary characteristics were also recorded to account for potential confounding.
Results: Butyrylcholinesterase activity was positively correlated with glucose, LDL-cholesterol, total cholesterol, triglycerides, uric acid, haptoglobin, and platelets count, after age-sex adjustments (all P's <0.05). Further adjustment for BMI, waist circumference, physical activity, cigarette smoking, diet, alcohol consumption, diabetes, overweight/obesity, hypertension, hyperlipidaemia revealed that only LDL-cholesterol, total cholesterol and triglycerides were positively associated with serum butyrylcholinesterase activity.
Conclusions: This study demonstrated the positive association of serum butyrylcholinesterase activity with LDL-cholesterol, total cholesterol and triglycerides, a fact that could state a hypothesis for a novel marker of atherosclerotic disease that could -together with other biomarkers- improve our potential to assess cardiovascular disease risk.

W068

EXPANSION OF NECROTIC CORE AND SHEDDING OF EXTRACELLULAR DOMAIN OF MERTK RECEPTOR IN HUMAN CAROTID PLAQUES: A ROLE FOR OXIDATIVE DERIVATIVES OF POLYUNSATURATED FATTY ACIDS?

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Background: Tyro3, Axl and Mer tyrosine kinase (Merkt) (TAM receptors) are surface receptors that have been implicated in the process of apoptotic cell recognition and engulfment.
Methods: In the present study, we investigated the relationships between apoptosis, Mertk and metallopeptidase domain 17 (Adam17) in the area surrounding the lipid core (LC) and in the periphery (P) of human carotid plaques. Further, in macrophages-like THP-1 cells we studied the effect of plaque extract (PE) on the expression of Adam17 and Merkt and on the cleavage of its extra cellular domain (sMer) that antagonizes the grown arrest-specific protein 6 (Gas6), a bridging molecule between TAM receptors and apoptotic cells.
Results: Among the TAM receptors only Merkt resulted significantly higher in LC than in P (P <0.01). Also Adam17 but not Gas6 was higher in LC than in P (P <0.01). By immunoistochemistry, there was an opposite trend of Merkt and Adam17 expression from the outer edge of LC out, Adam17 being higher in the area closest to the edge of LC. While the incubation of THP-1 with PE increased the mRNA and protein of ADAM17 (P <0.001), the rise of Merkt mRNA was followed by a reduction of its extra cellular domain (P <0.01). This phenomenon was associated with the increase of sMer in the culture medium (P <0.01).
Conclusions: The ex vivo and in vitro results suggest that the area closest to the lipid core may be a strong inducer of ADAM17 which in turn may release the extra cellular domain of Merkt producing sMer, an inhibitor of Gas6 activity.

W069

ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISMS AND THE RISK OF DEVELOPING RESTENOSIS AFTER CORONARY ANGIOPLASTY

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Background: The clinical treatment most frequently used for the revascularization of vessels affected by coronary artery disease is percutaneous transluminal coronary angioplasty (PTCA). Reduced or impaired synthesis of nitric oxide promotes the proliferation of vascular smooth muscle cells, and thus may induce the neointimal formation leading to coronary in-stent restenosis. The object is to evaluate the role of endothelial NO-synthase (eNOS) gene polymorphisms in developing restenosis after PTCA.
Methods: Case-control studies evaluating the association between the G894T, C774T, 4b/a eNOS polymorphisms and in-stent restenosis were searched. The study involved 635 patients with restenosis developed after PTCA, the control group consisted of 790 healthy donors. The diagnosis of coronary artery disease was calculated from clinical examination, laboratory data and angiography. Isolation of genomic DNA was performed by phenol-chloroform extraction. The study was carried out of the genotype by PCR followed by enzymatic restriction.
Results: We found that homozygosity for the 4a allele and heterozygosity for the a/b allele of the eNOS gene polymorphysms (VNTR) were associated with increase in the risk of in-stent restenosis (OR = 2.3; CI (95%) =1.1-7.8; P= 0.001; OR = 2.1; CI (95%) =1.3-3.7; P=0.001), association was observed for homozygosity for the 894T allele with restenosis (OR = 1.4; CI(95%) = 1.1-4.8; P=0.002). There was no association between the C774T eNOS polymorphisms and risk of restenosis after PTCA.
Conclusions: These data support a role for the NOS3 gene polymorhisms as a genetic determinant of the risk of developing restenosis after the stent implantation.

W070

NEW INSIGHTS IN THE PATHOPHYSIOLOGY OF ACUTE MYOCARDIAL INFARCTION (MI) DETECTABLE BY A NEW GENERATION TROPONIN I ASSAY

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Background: Several studies evaluated the clinical value of cardiovascular biomarkers measured in the acute phase of MI on case series including both patients with ST-elevation MI (STEMI) and MI with no ST-elevation at electrocardiogram (NSTEMI). This contrasts with the definition of MI, considering STEMI and NSTEMI as two distinct pathophysiologic entities. According to the advancement in the pathophysiology of myocardial injury gained by cardiac troponin I (cTnI) assays with improved sensitivity, we sough to evaluate cTnI release in STEMI and NSTEMI patients undergoing early percutaneous coronary intervention (PCI).
Methods: From 856 individuals with suspected acute coronary syndrome (ACS) consecutively admitted to the ED, STEMI (n=225) and NSTEMI (n=135) patients were selected whether undergoing early (≤4 h from admission) and successful PCI, and cTnI measurements (Siemens Advia Centaur TnI-Ultra) at ED presentation and within 24 h. The influence of MI type on cTnI concentrations at baseline and after PCI and on the rate of marker increase (RMI) were studied by multiple regression analysis, adjusting for patient features.
Results: A statistically significant (P <0.0001) interaction between MI type and time from symptoms was reported on cTnI concentrations: STEMI and NSTEMI differed for cTnI release at admission (after 5h from symptoms) and after revascularisation (within 24h from symptoms). A higher RMI in STEMI was detectable in patients admitted within 6h from symptoms. Baseline cTnI concentrations were lower in patients with history of ACS and increased with aging (P <0.0001). In the elderly (>75 years) the RMI significantly increased.
Conclusions: STEMI and NSTEMI patients have different patterns and kinetics of cTnI release, influenced by the interaction with time from symptoms, aging and previous history of ACS. In STEMI a rapid cTnI increase is likely to mirror the sharp washout of necrosis markers after an early successful PCI, whereas in NSTEMI later PCI seems to cause the slow ongoing cTnI increase, even persisting for several hours. Stating different biochemical substrates, STEMI and NSTEMI should be investigated separately for reporting on cardiovascular biomarkers to avoid biased estimate of diagnostic and prognostic performances.

W071

MULTI-MARKER NETWORK IN ST-ELEVATION MYOCARDIAL INFARCTION (STEMI) PATIENTS UNDERGOING PRIMARY PERCUTANEOUS CORONARY INTERVENTION: WHEN AND WHAT TO MEASURE

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Background: A multi-marker strategy including biomarkers contributing independent information is expected to improve the prognostic evaluation of STEMI patients. We sought to explore the relationship between cardiac troponin I (cTnI), C-reactive protein (CRP), B-type natriuretic peptide (BNP) and chromogranin A (CgA), accounting for biomarker profiles detected within 48 h from successful primary percutaneous coronary intervention (PPCI).
Methods: We evaluated cTnI, CRP, BNP and CgA profiles based on measurements performed before PPCI and 6, 24 and 48 h later in 73 STEMI patients. DIRECT STATIS and DUAL STATIS based on Principal Components Analysis (PCA) were employed to assess similarities between average profiles of patients and the relationship between markers measured at different times of sampling.
Results: In evaluating 'similarities between patients', measurements at 24 h and 48 h contributed most variability to subject profiles (score =0.942 and 0.915, respectively) as highly associated with the first principal component (accounting for 77.4% of explained variance). Patients' profiles at these times resulted highly correlated (correlation coefficient, 0.91). In evaluating 'similarities between patterns of markers', the sampling time mainly contributing to average biomarker profiles was 24 h. Concerning different biomarkers, BNP and cTnI were highly correlated and mainly explained the first component (accounting for 40.1% of explained variance). The second component (accounting for 26.3% of variance) was mainly explained by CgA, contributing independent information to previous markers, and partially by CRP. CRP as inflammatory marker partially overlapped BNP and cTnI and seemed to have correlated but opposite effects with respect to CgA.
Conclusions: The sampling time contributing most information from biomarker measurements to the definition of patients' profile was 24 h after PPCI. BNP and cTnI resulted interchangeable in a multi-marker panel for reporting about the extent of necrosis, whereas independent information derived from CgA. This marker may reflect a pathophysiologic mechanism opposite to inflammatory response and orthogonal to the one explained by BNP, which resulted the most informative marker.

W072

CYSTATIN C AND NOT LP-PLA2 PREDICTS OUTCOME AFTER ACUTE MYOCARDIAL INFARCTION

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Background: Increasing amount of evidence links cystatin C and cardiovascular risk. Lipoprotein-associated phospholipase A2 (Lp-PLA2) is a good predictor of unstable plaque prone to rupture. The purpose of our study was to compare prognostic potential of cystatin C and Lp-PLA2 in patients after acute myocardial infarction (AMI).
Methods: 981 patients after AMI were evaluated in the follow-up study lasting 5.5 years. 892 patients survived, 89 died. Cystatin C (Abbott Architect), Lp-PLA2 activity and concentration (DiaDexus) were evaluated at admission.
Results: No difference in cystatin C concentrations was found at admission between patients with or without history of kidney disease. Cystatin C was higher in patients who died (median 1.16 mg/L) during follow-up than in survivors (median 0.92, P <0.0001). ROC analysis revealed the best prediction of death by cystatin C (AUC 0.75, CI: 0.72-0.78) or age (AUC 0.66, CI: 0.63-0.69). AUCs for both Lp-PLA2 activity and concentration were less than 0.55. Cystatin C above 1,4 mg/L was connected with shortened survival probability (56%) in comparison to patients with cystatin C bellow 1.4 mg/L (survival probability 91 %, P <0.0001, Kaplan-Meier analysis). Predictors of death in logistic regression were increased cystatin C, higher age and female sex (P <0.0001, 91% of patients correctly classified).
Conclusions: Cystatin C should be monitored during follow-up after AMI, increased concentrations are connected with worse prognosis. Lp-PLA2 measured immediately after AMI (both concentration and activity) hasn´t any additional prognostic role in these patients.

W073

HIGH SENSITIVITY CARDIAC TROPONIN ASSAYS: PROGNOSTIC VALUE PATIENTS WITH HEART FAILURE

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Background: New methods have been developed for measuring high-sensitivity troponin in the diagnosis of acute coronary syndrome (ACS).These tests improve detection and quantification limits of this protein, detecting lower serum concentrations than conventional methods. This analytical improvement would permit a better management in chronic or subacute processes and other cardiac disorders such as myocarditis or congestive heart failure (HF).
Objective: To assess the prognostic significance of two high sensitiviy Troponin methods in a HF outpatient population.
Patients: 876 patients with chronic cardiac disease were studied during 3 months (72% men, median age 70.3 (60.5-77.2). Median follow-up was 3.45 (1.8-5.0) years.
Methods: High-sensitivity Troponin T (hsTnT) (Roche 05092744) was processed on a Modular Analytics E170 (Roche Diagnostics). The reference values at the 99th percentile with a CV <10% was 13 ng/L . Troponin I (LOCI TnI) (Siemens RF621) was processed on Dimension® EXL™ (Siemens Diagnostics). The cut-off for LOCI TnI method at the 99th percentile with a CV <10% was 50 ng/L .
Results: 69% of the patients studied showed higher hs-Tn T levels than the reference value of the method (13 ng/L). Only 12% of the patients with HF showed higher LOCI TnI levels than 50 ng/ L. The incidence of mortality during the evaluated period was 27%. The diagnostic sensitivity of hsTnT was 88% and the specificity 38% (11% with hsTnT <13 ng/L and 89% with hsTnT >13 ng/L). The diagnostic sensitivity for LOCI TnI was 17.6% and the specificity 87.8% (82% with LOCI TnI <50 ng/L and 18% with LOCI TnI>50 ng/L). In Cox regression multivariable analysis the both methods remained independent predictors of mortality, although hs-cTnT (HR 3.90 (1.69-8.98), P=0.001) was more sensitive predictor of mortality that LOCI TnI (HR 1.52 (1.11-2.08), P=0.008).
Conclusions: The Troponin is detected in a significant proportion of patients with HF. The incidence of detection depends of the analytical sensitivity of troponin methods and their cut-offs. Hs-cTnT and LOCI TnI are predictors of mortality in a real-life cohort of patients with HF. However hs-cTnT is more sensitive and shows better measurements of performance in mortality prediction.

W074

GAMMA-GLUTAMYLTRANSFERASE LEVELS ARE ASSOCIATED WITH INFLAMMATORY ACTIVATION, MYOCARDIAL DYSFUNCTION AND MORTALITY IN PATIENTS WITH ST-ELEVATION MYOCARDIAL INFARCTION

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Introduction: Gamma-glutamyltransferase (GGT) is a well-known prognostic marker in patients with heart failure and stable coronary artery disease. Aim of the study was to define the relationship between GGT activity, systemic inflammation, myocardial function and mortality in patients with ST-elevation myocardial infarction (STEMI).
Methods: 1007 patients (male: 74%; mean age: 66.1±12.5 years) admitted for STEMI and subjected to early reperfusion therapy were included in this study. Routine biohumoral markers, including GGT activity, fibrinogen and C-reactive protein (CRP) dosage were performed at admission. Brain natriuretic peptide (BNP) was determined at admission and each day during hospitalization. Ejection fraction was determined by echocardiography within 48 h from admission. An 18±13 months follow-up was performed and all cause mortality were reported.
Results: An elevation of GGT activity (>65 UI/L) was found in 105 (10%) patients at admission. This group of subjects had also higher fibrinogen (322.5 ±133.9 vs 299.7 ±92.1 mg/dL; P=0.03), CRP (4.2±6.8 vs 1.8±3.4 mg/dL; P <0.0001), basal BNP (414.6±631.7 vs 299.4±516.4 ng/mL; P=0.04) and peak BNP levels (888.9±1403.7 vs 555.7±827.7 ng/mL; P=0.0005). A significantly lower ejection fraction was also found in these patients (42.0±10.5 vs 44.5±9.7%, P=0.01). At follow-up, higher GGT levels were associated with a significant increase all cause mortality (Log-Rank 21.9; P <0.0001).
Conclusions: elevated GGT activity is associated with a higher degree of inflammation, neuroendocrine activation, myocardial dysfunction and all cause mortality at follow-up in patients with STEMI.

W075

HIGH-SENSITIVITY TNT FOR EARLY RULE-OUT OF NON-ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION IN PATIENTS PRESENTING WITH SUSPECTED ACUTE CORONARY SYNDROME

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Background: We conducted a study to asses the diagnostic accuracy of a high sensitivity cTnT (hsTnT) assay in the early evaluation of patients with suspected ACS, particularly non-ST-segment elevation myocardial infarction (non-STEMI).
Methods: We enrolled 122 patients admitted in ED with suspected ACS and onset of signs and/or symptoms ocurring within the previous 12 h. AMI was diagnosed according to the criteria of the Universal Definition, using a "Guideline acceptable" and "contemporary" assay as biochemical standard (cTnI LOCI Dimension Vista, 99th percentile: 45 ng/L; CV ≤ 0%: 40 ng/L, measured serially at baseline and after 3 to 8 hours). Patients with final diagnosis STEMI were excluded. hsTnT on presentation was measured with an electrochemiluminescence immunoassay (99th percentile: 14 ng/L) on Cobas 411 analyzer (Roche Diagnostic). Diagnostic accuracy of hsTnT on admission for ruling out of non-STEMI was evaluated by analyzing the receiver operating characteristic (ROC) curve and diagnostic sensitivity (S), negative predictive values (NPV) and likehood ratio (-) were calculated for prespecified cutoffs.
Results: Non-STEMI was the adjudicated final diagnosis in 36 patients (29,5%). For samples obtained on admission, the diagnostic accuracy was similar with the hsTnT assay as compared with the troponin I Dimension Vista assay (AUC ROC: 0.886 (CI 95%: 0.827-0.945) vs 0.890 (CI 95%: 0.821-0.959). For ruling-out non-STEMI, an hsTnT on admission < 14 ng/L yielded a diagnostic sensitivity of 80.6% (CI 95%: 66.2%-94.9%), a NPV of 91.0% (CI 95%: 84.0%-98.0%) and a LR(-) of 0.24 (0.12-0.46) and TnI Dimension Vista < 45 ng/L on admission yielded a diagnostic sensitivity of 58.3% (CI 95%: 40.8%-75.8%), a NPV of 84,9 (77.3%-92.4%) and a LR(-) of 0.43 (0.29-0.63).
onclusions: Performance of hsTnT for ruling-out non-STEMI on admission is higher than with a "contemporary" and "Guideline acceptable" assay. However, an strategy based in only one measurement on presentation doesn´t allow to exclude safely non-STEMI with no assay. In patients with suspected ACS a strategy based in serial measurements of cTn is needed to exclude non-STEMI, although a high-sensitivity test is used.

W076

ANALYTICAL PERFORMANCE EVALUATION OF THE ABBOTT DIAGNOSTICS ARCHITECT I2000SR GALECTIN-3

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Background: Galectin-3, secreted from macrophages, is a novel marker for the management of patients with heart failure. We evaluated the analytical performance of the two-step routine and STAT Galectin-3 immunoassays on the Architect i2000SR (Abbott Diagnostics).
Methods: Assay precision, limits of blank (LoB), detection (LoD), quantification (LoQ) and linearity were derived for both routine and STAT assays. Serum samples with concentrations of Galectin-3 across the measuring range were compared between the routine and STAT assay and a Galectin-3 ELISA (BG Medicine Inc.). The reference interval was investigated using 120 serum samples from apparently healthy individuals (60 male, 60 female) with no evidence of cardiac disease.
Results: The between day coefficient of variation (CV%) was 2.26-5.46% in the range 9.89-74.54 ng/mL and 1.88-5.35% in the range 9.81-74.52 ng/mL for the routine and STAT assays respectively. The LoB, LoD and LoQ were 0.26, 0.67, 4.2 ng/mL and 0.04, 0.45, 4.3 ng/mL for the routine and STAT assays respectively. Both assays were linear up to 105 ng/mL. There was excellent correlation between the routine and stat assays (n=225, bias -0.62, 95%CI -4.51-3.27 ng/mL) in the range 6.9-107 ng/mL. Both the routine (n=225, bias -1.93, 95%CI -2.28 to -1.57 ng/mL) and STAT (n=225 bias -2.552, 95%CI -2.959 to -2.145 ng/mL) assays correlated well with the Galectin-3 ELISA. 120 samples were obtained from apparently healthy individuals. The median age 42, (interquartile range 18-69) years. The upper 97.5 percentile was 34.42 and 33.46 ng/mL for the routine and STAT assays respectively. There was no significant difference in concentrations between males and females for either the routine (P=0.772) or STAT (P=0.835) assay.
Conclusions: Both the Galectin-3 Routine and STAT assays determined on the Abbott Architect i2000SR demonstrate excellent analytical performance for the determination of Galectin-3. Clinical studies are required to demonstrate the prognostic potential of this novel marker in heart failure.

W077

ASSOCIATION OF CALCIFICATION AND LABORATORY MARKERS OF CARDIOVASCULAR RISK

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Background: The purpose of this study was to investigate the relationship between the vascular calcification and known cardiovascular risk factors. 65 male patients with atherosclerosis and vascular calcification in age (49±11) years were studied.
Methods. Vascular calcification in patients was measured and quantified using multi-slice spiral computed tomography (MSCT) with calcium index (CI). To detect the human fetuin-A in serum we used ELISA (Bio Vendor Laboratory Medicine, Inc, Czech Republic). For the evaluation of human endotelin (1-21) we used enzyme immunoassay (Biomedica Medizinprodukte GmbH & Co. KG, Austria). For measurement of high sensitive C-reactive protein (hs-CRP) and homocysteine (HCY) was used analyzer IMMULITE 2000 (Siemens Healthcare Diagnostics, USA). Lipid parameters were measured using Synchron CX9 Pro biochemistry analyzer (Beckman Coulter, USA).
Results: Statistically significant relationships between CI and age (r_T=0.45, P <0.05) were found. The homocysteine level was (14.9 [12.9; 17.8]) mkmol/L and it was significantly (P <0.05) higher than reference range (5÷12) mkmol/L. Conducted analysis revealed statistically significant relationships between HCY and CI (P=0.05; r_T =0.48). High levels of cholesterol (6.1 [5.7: 6.9]) mmol/L were detected. Level of low-density lipoprotein was (3.9 [3.4: 4.4]) mmol/L. In patients with atherosclerosis and vascular calcification low-density lipoprotein levels in serum showed linear relationship with level of CRP (P <0.05; r_T=0.29). Interrelation CI with the levels of fetuine was shown. Level of endotelin was (0.33 [0.24; 0.48]) fmol/mL and level of fetuin was (33.7 [24.4; 38.2]) mg/L. The inverse correlation of endotelin with fetuin (P=0.05; r_T=-0.29) was found.
Conclusions: Age is the major risk factor of vascular calcification. We detected endothelial dysfunction association with fetuin-A level reduction. The use of laboratory markers may help to estimate the level of vascular inflammation and degradation of patients with atherosclerosis and vascular calcification.

W078

INTACT AND BIOACTIVE PARATHYROID HORMONE ASSAYS: ARE THEY RELATED TO CARDIAC BIOMARKERS IN PATIENTS WITH SYSTOLIC HEART FAILURE?

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Background: Parathyroid hormone (PTH) is a major systemic calcium-regulating hormone. Recent evidence has suggested that measurement of PTH might provide complementary information for the diagnosis and risk stratification of patients with heart failure (HF). The aim of our study was to evaluate serum concentrations of intact and bioactive PTH in heart failure patients with reduced ejection fraction and to determine their relationships with established biomarkers of the disease severity.
Methods: The following measurements were carried out in blood samples from 73 patients with systolic heart failure (left ventricular ejection fraction <40%): bioactive PTH (1-84) assay, intact PTH assay, pro B-type natriuretic peptide 1-108 (proBNP), Galectin-3, Chromogranin-A (CgA), Myostatin, Urotensin II (UII) and high sensitive troponin T (hsTnT).
Results: In patients with systolic HF, intact and bioactive PTH assays were highly correlated but serum levels of intact PTH were significantly higher than bioactive PTH. Intact and bioactive PTH levels were significantly and positively correlated to proBNP, Galectin-3, CgA and UII but not to hsTnT and Myostatin. The strongest associations were observed between intact and bioactive PTH and proBNP (r =0.46 and r =0.49; P <0.001, respectively) and Galectin-3 (r =0.34 and r =0.99; P <0.005, respectively).
Conclusions: Serum concentrations of intact and bioactive PTH are significantly related to biomarkers of cardiac remodeling and disease severity in patients with systolic heart failure. Monitoring of intact and bioactive PTH might therefore be relevant for the monitoring of HF patients and for selection of treatment.

W079

C242T POLYMORPHISM OF NADPH OXIDASE P22 PHOX GENE REDUCES THE RISK OF CORONARY ARTERY DISEASE IN EGYPTIAN POPULATION

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Background: The p22phox protein subunit is essential for NADPH oxidase activity. It is encoded by the cytochrome b-245 a (CYBA) gene. The C242T polymorphism of p22phox gene has been previously found to influence NADPH oxidase expression. This CYBA C242T gene variant is in exon 4 and causes a structural modification in the protein from the histidine-to-tyrosine in the heme binding site. This can lead to functional changes in the protein. Thus, the aim of this study is to examine the prevalence of the C242T polymorphism of the NADPH oxidase in a representative sample of Egyptian population and to investigate its relationship with the occurrence of early onset acute myocardial infarction (AMI). The contribution of oxidative stress, represented by oxidized low density lipoprotein (ox-LDL), in the development of AMI was also studied and correlated with the different C242T gene variants.
Methods:The study subjects consisted of 104 AMI patients and 101 age-matched volunteers. Genotyping was done by the PCR-RFLP method. Serum levels of ox-LDL were determined quantitatively using ELISA technique.
Results: Data showed that wild type CC genotype is prevalent in 27% of the control subjects while CT and TT are present in 72% and 1%, respectively. The allele frequencies of the C and T alleles were 63% and 37%, respectively. In the AMI patients, the wild type CC genotype is prevalent in 40.2% of the AMI subjects while CT and TT are present in 59.8% and 0%, respectively showing a significant difference in the NADPH oxidase genotype compared to the control subjects. The allele frequencies of the C and T alleles were 71% and 29%, respectively showing no significant difference from the control subjects. The serum levels of ox-LDL were significantly elevated in the AMI patients as compared to controls ($P \leq 0.0001$). Subjects having CT genotype had significantly lower levels of serum ox LDL than CC genotypes suggesting the protective role of the C242T polymorphism in this study population.
Conclusion: The C242T polymorphism of the p22 phox gene of NADPH oxidase is a novel genetic marker that is associated with reduced susceptibility to AMI... Our results also revealed the association of oxidative stress with the incidence of AMI.

W080

HYPER-CHOLESTEROLAEMIA (DYSLIPIDAEMIA) IN AN URBANIZED MIXED ANCESTRY POPULATION FROM SOUTH AFRICA

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Background: Cardiovascular management guidelines primarily promote control of low-density lipoprotein cholesterol but both low high-density lipoprotein cholesterol and elevated triglyceride concentrations have been reported to be risk factors for cardiovascular disease. Lipid-modifying treatment has shown little effect on the prevalence of low high-density lipoprotein cholesterol or hypertriglyceridaemia while a reduction in abdominal obesity has been associated with increases in high-density lipoprotein cholesterol. Therefore, the aim of this study was to investigate the prevalence of hypercholesterolaemia/dyslipidaemia in an overweight urbanized mixed ancestry population from Bellville-South, Cape Town, South Africa.
Methods: Subjects (244 males and 802 females) were randomly recruited from the study population. Blood sample analysis included total cholesterol, low- and high density lipoprotein cholesterol and triglycerides, while anthropometric measurements included waist and hip circumference.
Results: Hypercholesterolaemia was present in 53.7% of males and 62.3% of females. Of these, 41.2% of the males and 52.3% of the females were undiagnosed. Of those aware of their cholesterol status, only 4.6% of males and 10.4% of females took treatment for the condition. Those who took the medication had significantly lower low-density lipoprotein cholesterol in both the male and female subjects ($P < 0.04$). Low high-density lipoprotein cholesterol was present in 31.6% males and 51.9% in females. Obesity prevalence was 51.5% in males and 78.3% in females, while hypertriglyceridaemia was present in 25.9% of males and in 24.6% of females.
Conclusions: Results showed a high prevalence rate for hypercholesterolaemia in the study subjects and this was highly significantly positively associated with plasma triglyceride levels and body measurements. Undetected hypercholesterolaemic cases were high and adherence to prescribed medication was extremely poor. Currently, evaluating dyslipidaemia as a combination of abnormal concentrations of cholesterol and the lipo-protein-cholesterols, in this study 70.9% of males and 96.0% of females were dyslipidaemic.

W081

LATEX ENHANCED IMMUNOTURBIDIMETRIC ASSAY FOR THE MEASUREMENT OF LIPOPROTEIN(A) ON THE RX MONACO ANALYSER

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Background: Lipoprotein (a) determination is intended for use in conjunction with clinical evaluation, patient risk assessment and other lipid tests to evaluate disorders of lipid metabolism and to assess coronary heart disease in specific populations. Elevated concentrations of Lp(a) are a risk factor for coronary heart disease (CHD) and monitoring its levels may be useful to guide the management of individuals diagnosed with CHD, or who have a family history of CHD. This study reports the development of an immunoturbidimetric assay kit with enhanced precision and accuracy for the measurement of Lp(a) in human serum and plasma applied to the fully automated bench top/floor standing analyser RX monaco.
Methods: Agglutination occurs due to an antigen-antibody reaction between Lp(a) in a sample and anti-Lp(a) antibody adsorbed to latex particles. This is detected as an absorbance change at 700 nm proportional to the concentration of Lp(a) in the sample. Concentrations are calculated from a multi-point calibration. On-board and calibration stabilities were tested by storing the reagents uncapped on the analyser for a period of 28 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 4 replicates twice a day for 20 days. Correlation studies were conducted using another commercially available clinical chemistry system.
Results: The Lp(a) reagent presents an on-board and calibration stability of 28 days. The assay was found to be functionally sensitive to 9.43 mg/dL and be linear up to 103mg/dL. The within-run and total precision for three different concentration levels typically had %C.V.'s of ≤6.0%. In the correlation study 42 serum patient samples were tested and the following linear regression equation was achieved: Y = 0.98x + 1.47; r =0.99.
Conclusions: This immunoturbidimetric assay kit, in conjuction with use on the bench top/floor standing RX monaco analyser, exhibits high sensitivity and reproducibility with the added advantage of using liquid reagents with good stability. This is of value in the accurate determination of this analyte in human serum/plasma for clinical and research applications.

W082

LIQUID IMMUNOINHIBITION ASSAY FOR THE MEASUREMENT OF CK-MB ON THE RX MONACO ANALYSER

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Background: Creatine kinase (CK) is internationally accepted as a sensitive and specific indicator of acute myocardial infarction (AMI). There are 3 iso-enzymes of CK, CK-MM, CK-MB and CK-BB. CK-BB is produced by the brain in very small insignificant amounts. CK-MM is produced by the skeletal and heart tissue and CK-MB is produced by the heart muscle. CK-MB determination is therefore an important element in the diagnosis of myocardial ischemia. In the vast majority of cases the CK activity rises within 6 hours of an acute infarction. This study reports the development of an immunoinhibition assay kit with enhanced precision and assay range for the measurement of CK-MB in human serum and plasma applied to the fully automated bench top/floor standing RX monaco analyser. This is of value in the rapid diagnosis of myocardial ischemia.
Methods: An antibody is incorporated into the CK reagent which will bind to and inhibit the activity of the M subunit of CK MB. This means that only the activity of the B subunit is measured via the Total CK assay UV test principle. Concentrations are calculated from a two point calibration. On-board and calibration stabilities were tested by storing the reagents uncapped on the analyser for a period of 28 days. Within-run and total precision were assessed by testing serum samples at defined medical decision levels, 4 replicates twice a day for 20 days. Correlation studies were conducted using another commercially available clinical chemistry system.
Results: The reagent presents an on-board and calibration stability of 28 days. The assay was found to be functionally sensitive to 19.16 U/L and be linear up to 2289 U/L. The within-run and total precision for three different concentration levels typically had %C.V. ≤7.1%. In the correlation study 43 serum patient samples were tested and the following linear regression equation was equation was achieved: Y = 1.02x–1.11; r = 0.99.
Conclusions: This immunoinhibition assay kit exhibits good sensitivity and reproducibility with the added advantage of using liquid reagents with good stability. In conjuction with the use of the fully automated analyser RX monaco, this assay is of value for the rapid and accurate determination of CK-MB in human serum/plasma for clinical applications.

W083

SERUM LIPID PROFILE IN NIGERIAN PATIENTS WITH ISCHAEMIC CEREBROVASCULAR ACCIDENT

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Backgroud of study: Ischaemic cerebrovascular accident (CVA) has been shown to be associated with abnormal lipid profile (dyslipidaemia) as a risk factor. There is paucity of data regarding this in this environment hence this study.
Method: In this study, a total number of 63 subjects were recruited comprised of 33 patients with cerebrovascular accident (stroke) and 30 apparently healthy volunteers as control. A complete lipid profile which included total serum cholesterol (TC), serum triglyceride (TG), high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) were assayed for both patients and controls. The lipid profile was determined using standard methods.
Results: The TC for both patients and control were 212+53 mg/dl and 196+46 mg/dL respectively, TG for both patients and controls were 159+9 mg/dL and 79+13 mg/dL respectively, HDL-c was 55+9 mg/dL for patients and 61+7 mg/dL for controls and LDL-c was 151+34 mg/dL for patients and 117+51 mg/dL for control subjects. TC, TG and LDL-c were higher in the ischaemic CVA patients than the controls; however only TG showed a significant increase while HDL-c although not significant was lower than control.
Conclusion: The study therefore showed that ischaemic CVA is associated with hypercholesterolaemia, hypertriglyceridaemia and high LDL-cholesterol. We thus suggest preventive and management strategies that will reduce lipid levels (TC, TG and LDL) and enhance HDL- cholesterol in Nigeria patients that are prone to or diagnosed of ischaemic CVA.

W084

DETERMINATION OF ABSOLUTE CONCENTRATION CHANGES IN HIGH-SENSITIVITY CARDIAC TROPONIN T DURING SERIAL TESTING OF PATIENTS WITHOUT ACUTE CORONARY SYNDROME

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Background: New, high-sensitivity assays for cardiac troponin have lower detection limits and less imprecision than earlier assays. Reference 99th percentile cut-off values for these new assays are also lower leading to higher frequencies of positive test results. When cardiac troponin concentrations are only minimally increased, serial testing allows discrimination of myocardial infarction from other causes of increased cardiac troponin concentration. Absolute concentration changes in high-sensitivity cardiac troponin T were determined on population of emergency department patients from Belgrade's Zvezdara University Medical Center.
Methods: Blood was collected from 258 patients presenting with cardiac chest pain. Blood was taken immediately upon arrival in hospital and 3, 6, and 12 h later. Cardiac troponin was measured with the high-sensitivity cardiac troponin T assay (Cobas e411 analyzer, Roche Diagnostics). Cardiac troponin results from patients without acute coronary syndrome or stable angina were used in calculating absolute changes in cardiac troponin T.
Results: The 95th percentile for absolute change in high-sensitivity cardiac troponin T was 9 ng/L. Within-individual coefficient of variation (CV) and total CV were 13% and 16% respectively.
Conclusions: Absolute concentration change in high-sensitivity cardiac troponin T was low in emergency department patients without ischemic myocardial necrosis. Detection of high-sensitivity troponin T absolute change above estimated 95th percentile value can help to identify patients with acute myocardial necrosis. Contrary, absolute concentration change in high-sensitivity troponin T below 95th percentile value renders acute coronary syndrome less likely.

W085

DECREASED POSTPRANDIAL TRIGLYCERIDE LIPOPROTEIN CLEARANCE IN NORMOLIPIDEMIC ANGIOGRAPHICALLY DEFINED CORONARY ARTERY DISEASE PATIENTS

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Background: Atherosclerosis is the main reason for coronary artery disease (CAD) which is one of the most frequent causes for mortality and morbidity rates among adult population. Although numerous theories about the progression and etiology of atherosclerosis were defined, none of them brought a satisfactory explanation for all cases. The most widely accepted risk factor for CAD is increased blood cholesterol levels. However it has also long been hypothesized to be a disorder influenced by postprandial effects. Thus we aimed to investigate blood lipoprotein and lipid changes after a standard oral fat loading test in normolipidemic CAD patients and compare their controls.
Methods: The study groups were coronary arterial disease positive (CAD+) group (20 patients; 14 men and 6 women) and CAD- (10 patients; 6 men and 4 women) group according to their coronary arteriogram assessments. After drinking oral fat loading solution, blood samples were taken at 2nd , 4th , 6th and 8th hours. Fasting and postprandial lipoprotein subfractions were isolated by sequential micro-ultracentrifugation technique. Apo A1 and Apo B levels were measured by immunonephelometric kits.
Results: While there were not any statistical differences between patient groups in all lipid parameters, CAD+ group had higher blood triglycerides at 4th ,6th and 8th hours of the test , elevated 6th and 8th triglyceride-rich lipoproteins cholesterol concentrations than the CAD- group, IDL levels were remained elevated at 8th hour. Also at 8th hour, total HDL-C and HDL2-C levels of CAD+ group were significantly lower than those in the CAD- group.
Conclusions: This study showed a diminished postprandial TRL cholesterol and triglyceride clearance in a normolipidemic and angiographically defined CAD patient group compared to their controls. Because the only difference between groups was CAD occurrence, the results could be related to the coroner atherosclerosis progression. Interestingly, total cholesterol levels were minimally increased after fat loading. Therefore, it could be suggested that cholesterol levels might tend to be more stable and not effected by acute diet changes.

W086

B-TYPE NATRIURETIC PEPTIDE VERSUS AMINO TERMINAL PRO-B TYPE NATRIURETIC PEPTIDE: CHOOSING THE OPTIMAL HEART FAILURE MARKER IN PATIENTS WITH IMPAIRED KIDNEY FUNCTION

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Background: The effect of impaired kidney function on the heart failure markers, B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) is not clear. This study was performed to examine the influence of kidney dysfunction on these markers and determine appropriate cutoffs for heart failure.
Methods: Adults with estimated glomerular filtration rate (eGFR) <60 mL/min using the Cockcroft Gault formula for ≥3 months were identified in consulting clinics from June 2009 to March 2010. Heart failure was defined as documented by a cardiologist with ejection fraction < 40% and assessed by New York Heart Association classification (NYHA). Plasma from each participating subject was assayed for creatinine (Cr), BNP and NT-proBNP.
Results: The mean age of patients (n=190) was 58 (±15) years, 67.4% being males. BNP (r= -0.3) and NT-proBNP (r = -0.5) showed an inverse relationship with eGFR. Mean BNP levels showed a 2.5 fold and 1.5 fold increase from chronic kidney disease (CKD) stage 3 to stage 5 in patients with and without heart failure respectively. NT-proBNP levels in non-heart failure group were 3 fold higher in CKD stage 5 compared to stage 3. Similarly mean NT-proBNP levels were 4 times higher in CKD stage 5 compared to stage 3 in patients with heart failure. The diagnostic value of BNP and NT-proBNP were assessed by area under the ROC curve. The overall area under the curve for BNP and NT-proBNP was 0.7 and 0.86 respectively. Higher BNP cutoff was required to diagnose heart failure in CKD stage 5 (491 pg/mL) as compared to CKD stage 3 (146 pg/mL). Plasma NT-proBNP cutoff of 1500 pg/mL in CKD stage 3 gave a sensitivity of 72% with 92.7% specificity. Whereas in CKD stage 5 a cutoff of 11200 pg/mL diagnosed heart failure with 94.7% sensitivity and 100% specificity.
Conclusions: BNP and NT-proBNP were elevated in kidney dysfunction even in the absence of heart failure. However the magnitude of increase in NT-proBNP was greater than that of BNP. Both can be useful in diagnosing heart failure; however by using higher cutoffs stratified according to kidney dysfunction, NT-proBNP appears to predict heart failure better than BNP.

W087

CORRELATION OF C-REACTIVE PROTEIN WITH AMINO-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE, HIGH SENSITIVITY TROPONIN T AND BIOMARKERS OF RENAL FUNCTION IN CARDIOVASCULAR RISK ASSESSMENT IN PRIMARY PREVENTION SETTING

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Background: C-reactive protein determined using high-sensitivity methods (hsCRP) is the most widely studied biomarker for cardiovascular disease (CVD) risk assessment in primary prevention. There is evolving body of evidence that shows also the significance of high-sensitivity troponin T (hsTnT), amino-terminal pro-B-type natriuretic peptide (NT-proBNP), cystatin C (Cys C) and estimated glomerular filtration rate (eGFR) in predicting adverse cardiovascular events in general population. The aim of this study was to examine correlation between these parameters of cardiovascular risk assessment in primary prevention setting.
Methods: Concentrations of hsCRP, hsTnT, NT-proBNP, creatinine and Cys C were measured in fasting serum samples of 255 healthy volunteers (108 men, 147 women), 25–80 years old. Creatinine derived eGFR was calculated using Modification of Diet in Renal Disease (MDRD) equation and Chronic Kidney Disease–Epidemiology equation (CKD-EPI), and Cys C–derived eGFR was calculated with Cys C–PENIA equation.
Results. Examined participants were classified into tertiles of CVD risk according to their hsCRP concentration <1 mg/L, in the range 1–3 mg/L and >3 mg/L. Kruskal-Wallis test indicated significant increase in Cys C and decrease in eGFR (CKD-EPI) ($P < 0.0001$) and eGFR(Cys C) ($P = 0.0122$) across tertiles of hsCRP concentrations. Spearman's rank correlation coefficients were significant between hsCRP and Cys C ($\rho = 0.360$, $P < 0.0001$), eGFR(MDRD) ($\rho = -0.130$, $P = 0.039$), eGFR (CKD-EPI) ($\rho = -0.184$, $P = 0.003$) and eGFR (Cys C) ($\rho = -0.360$, $P < 0.0001$). However, in multiple linear regression analysis only hsTnT ($P = 0.030$) and Cys C ($P = 0.001$) remained independently correlated with hsCRP concentration with coefficient of determination $R2 = 0.225$ and adjusted $R2 = 0.203$.
Conclusions: Out of examined emerging biomarkers of atherosclerosis, after the series of regression analysis, only hsTnT and Cys C remained correlated with hsCRP. The additional information they provide may complement the predicting value of hsCRP. Complex processes in atherosclerosis demand multimarker approach for appropriate evaluation of its progression.

W088

PROPERTIES OF RED BLOOD CELLS ISOLATED FROM BLOOD OF HYPERTENSIVE DONORS

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Essential hypertension is a major risk factor for cardiovascular morbidity and mortality. It is a complex condition supposedly originating from the interactions of multiple genetic and environmental factors, in which also epigenetic inheritance plays a significant role. Using absorption spectroscopy we studied the degree of hemolysis as a function of various concentrations of K+ and Na+ ions. Applying atomic force microscopy we monitored topography and mechanical properties of isolated RBC in their native environment. The permeability of the RBC membrane for O2 and its binding affinity to hemoglobin inside the cells we examined by use of Mössbauer spectroscopy. The results show that the skeleton protein interactions are modified in the case of hypertensives. Under our experimental conditions, at honeycomb structure of the cytoskeleton network, that is usually observed in healthy RBC's, was changed into at corncob structure in erythrocytes from hypertensive patients. This effect seems to be associated with a modification of the erythrocyte membrane O2 permeability what leads to at decreased ability of the oxygen release at lower O2 partial pressure in comparison to the healthy cases. We have observed a correlation between the phenomena mentioned above and the stability of RBCs isolated from patients with hypertension under various conditions of incubation in the presence of Na+ and K+ cations. They significantly differ from that one detected for the normotensives.
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W089
EFFECTS OF HYPOXIC ADAPTATION ON THE ENDOTHELIAL DYSFUNCTION IN THE ISCHEMIC CARDIOMYOPATHY PATIENTS

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Background:The patients with ischemic cardiomyopathy (ICMP) and chronic heart failure(CHF)are characterized by nitric oxide imbalance and endothelial dysfunction (EtD)progression.Nowadays EtD severity is an independent predictor of early cardiovascular events.
The purpose of this research was to estimate the influence of normobaric intermittent hypoxia adaptation (NIHA) on the endothelium functional (EF) state in the ICMP patients.
Materials and Methods: We studied 35 ICMP patients (57.01± 1.38 years) with depressed left ventricle (LV) systolic function less than 35% and CHF class II-III by NYHA treated with the 10-days NIHA course against CHF drug management with no significant differences.EF state was assessed by endothelium-dependent flow-mediated vasodilation (FMD) as measured by using ultrasonography of the brachial artery with reactive hyperemia test. We also analysed the changes of the biochemical EtD markers levels, such as endothelin-1 (ET-1, pg/mL), tumor necrosis factor-alpha (TNF-α, ng/mL), homocysteine (HCY, mcmol/L), brain natriuretic peptide (BNP, pg/mL).
Results:The initial CHF severity was determined by depressed LV contractile function against maladaptive LV remodeling, multiple myocardial perfusion defectes and the presence of EtD with the high level of the biochemical EtD and CHF markers. We marked the reduction of endothelium-dependent vasodilatation. After the 10-days NIHA course we revealed significant changes in the EF state. The number of the patients with normal endothelium-dependent FMD increased from 28.57% to 60% ($P < 0.05$) and the number of vasoconstrictor reactions decreased from 29% to 6%. We noted the increase in the endothelium-depend FMD from 4.09±1.49 to 9.42±1.11 ($P < 0.05$).The concentration of ET-1 after NIHA decreased from 2.04±0.23 to 1.03±0.14 ($P < 0.05$), TNF-α-from 19.59±2.67 to 10.43±3.28 ($P < 0.05$), HCY – from 15.46±0.90 to 11.77±0.73 ($P < 0.05$). The EF state improvement reflected on the clinical CHF course. The BNP concentration decreased from 612.20± 136.19 to 399.79±139.16 ($P < 0.05$).
Conclusion: The use of NIHA helps to improve the EF state and provides a more favorable course of the main pathological process in the ICMP patients with CHF thus allowed to assume a more adequate protection from early cardiovascular events.

W090
UNDERSTANDING CARDIAC TROPONIN INTERPRETATION IN THE LABORATORY

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Understanding Cardiac Troponin interpretation in the laboratory needs constant cross talk with the clinician in these times of high sensitivity assays which are said have increased sensitivity with a concomitant loss of specificity resulting in unnecessary invasive procedures and hospitalization. Hence a clinician's feedback prior to any standardization to a reference range for each lab is absolutely necessary. Fifty samples were compared on Roche platform Elecsys 2010 between Troponin T & HsTroponin T. Assay showed a concordance of 98% and paired sample statistics, a p value of 0.319 inferring that the two assays did not differ significantly at 5% level. Seventy five samples were validated on Roche, Elecsys 2010 & Beckman Coulter Access2, for HsTroponin T and HsTroponin I. Concordance was 89% and paired sample statistics had a p value of 0.008 inferring that the two variables of instrumentation and methodology differ significantly at 5% level. Further change of platform in the laboratory to Cobas e 601 had concordance studies done with Access2 for twenty samples which showed 90%. Validation between Elecsys 2010 and Cobas e 601 for Hs Troponin T had a concordance of 100% (The numbers in the study are small and hence a prospective with greater numbers are being considered). Interpretation of Cardiac Troponin results need correlation with the clinical review. Whether the elevation is due to "supply-demand imbalance" resulting due to many signs and symptoms of cardiac manifestation like tachycardia, hypotension or hypertension and thereby ischemia or to direct effect of hormonal influence, drugs, sepsis or other underlying conditions. Also the pattern of rise and fall of the biomarkers is very relevant in diagnosis. Major initiatives are occurring to standardize Troponin assays. Numbers generated with any given assay should not be related to another because of the variable modes of measurement and differences in antibodies used. The latest trends of considering reportable numerical by "actual" or "percentage variation" should be validated in association with clinical and bed side reality. However, the future not only would emphasize on a larger number of patients at risk but definitely would also increase the rapidity of diagnosis on an acute coronary event.

W091

PREDICTION OF SHORT-TERM ADVERSE EVENTS BY HIGH-SENSITIVITY CARDIAC TROPONIN I AND HIGH-SENSITIVITY CARDIAC TROPONIN T BASED ON THE ORIGINAL SAMPLE OBTAINED WITHIN SIX HOURS OF THE ONSET OF PAIN

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Background: Recent studies with high-sensitivity cardiac troponin (hs-cTn) assays have focused mainly on the diagnosis of myocardial infarction (MI) in emergency department (ED) patients, and comparative studies have been rare. This study compared the ability of two hs-cTn assays to identify prognostically relevant short-term cardiovascular events in ED patients.
Methods: After ethics approval, patients ≥18y, with suspected acute coronary syndrome who presented within 6h after pain onset were enrolled. Patients that were referred to surgery; those with an MI diagnosis prior to the first cTn result (e.g., ST segment elevation MI); and patients who refused were excluded. Blood (EDTA plasma) was obtained at presentation and stored below -80C prior to testing with the Roche hs-cTnT assay and an Abbott pre-commercial hs-cTnI assay. 72h outcomes were collected prospectively to define those who underwent percutaneous coronary intervention, coronary artery bypass graft, hospital admission for refractory ischemic symptoms, significant arrhythmia, heart failure, stroke, non-fatal cardiac arrest, MI, and/or death. An ED physician and an Internal Medicine physician evaluated all cases blinded to the hs-cTn data. The composite outcome (all serious cardiac outcomes) was used to determine hs-cTn test performance (Analyze-it software).
Results: The median (interquartile) age of the population (n=152) was 60y (49-69). The concentration of hs-cTn in those with adverse outcomes (n=24; median concentrations: hs-cTnI=36 ng/L; hs-cTnT=32 ng/L) was significantly higher than those without adverse outcomes (n=128; median concentrations: hs-cTnI=2 ng/L; hs-cTnT=5ng/L; P <0.0001). Using the 99th percentile (Abbott ≥26 ng/L; Roche ≥14 ng/L) yielded the sensitivity/specificity/positive LR/negative LR for adverse outcomes of 63%/94%/10.0/0.4 for hs-cTnI and 71%/77%/3.0/0.4 for hs-cTnT. The use of sex-specific 99th percentile cutoffs did not change the results.
Conclusions: Different hs-cTn assays predict short-term adverse events differently when the analysis is restricted to the initial sample obtained at the time of presentation. These data suggest these hs-cTn assays cannot be used interchangeably and that there is a need for large studies to establish optimal thresholds.

W092

TEMPORAL CHANGES IN ESTIMATED GLOMERULAR FILTRATION RATE (DELTA EGFR) MAY BE A PREDICTOR OF PROGRESSION TO CORONARY HEART DISEASE

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Background: Chronic kidney disease and cardiovascular disease are major public health problems worldwide and often share the same pathophysiological mechanisms. Indeed, the prevalence of traditional cardiovascular risk factors can be higher in those with impaired kidney function, and most patients with a lower estimated glomerular filtration rate (eGFR) die of cardiovascular causes and not due to progression to end stage renal disease. However, the effect of reduced eGFR on coronary heart disease has not been well delineated among Korean population. Here, we reviewed temporal changes in eGFR (delta eGFR, ΔeGFR) of the healthy Korean population, and then, carried out a study to investigate the link between a ΔeGFR and the risk of coronary heart disease.
Methods: We reviewed data of 7676 healthy outpatients selected from a population of - 10,000 individuals referred to the health promotion center of the ASAN medical center between January 2010 and December 2010. At the time of the first evaluation, data of patients` age, sex, blood pressure, creatinine, total cholesterol, LDL, HDL and triglycerides as well as history of smoking, hypertension or familial coronary heart disease were collected by chart review. The % risk of coronary heart disease were calculated by Framingham score (Score:www.nhlbi.nih.gov/guidelines/cholesterol). We classified ΔeGFR % into 4 groups according to Gaussian distribution, ±2 standard deviation (SD); below -2SD, -2SD to mean, mean to +2SD, over +2SD (mean=1.559, SD=9.5837).
Results: Among the enrolled patients, 5.9% (456 patients) were over 15 of the Framingham score, and this is equivalent of coronary heart disease risk over 20% which means they have high risk for occurence of coronary heart disease in ten yrs. A group of over +2SD (ΔeGFR>19.17%) had odds ratio of 3.656, and it was related to incident % risk of coronary heart disease rather than other ΔeGFR groups.
Conclusions: A decline of eGFR >19.17% was independently related to incident % risk of coronary heart disease. ΔeGFR could be a possible indicator of progression to the coronary heart disease in general population. Therefore, this parameter may be potentially used for screening subjects with higher risk of coronary heart disease.

W093

WITHIN-DAY AND WEEKLY VARIATION OF CARDIAC TROPONIN IN PATIENTS WITH CHRONIC CARDIAC TROPONIN ELEVATIONS

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Background: Acute myocardial infarction is defined by a cardiac troponin concentration above the 99th percentile with an acute increase and/or decrease. Knowledge of the biological and analytical variation is required to distinguish significant changes. Although these measurements by definition can only be performed with healthy individuals, this population may not be representative for patients where cardiac troponin measurement is of clinical interest. Therefore, we evaluated within-day and weekly variation of cardiac troponin in patients with chronic elevations of cardiac troponin under highly standardized conditions.
Methods: Twenty-three diabetes type 2 patients were studied on three occasions with intervals of one week. Within-day variation was assessed under inactive conditions, as well as under daily physical activity and blood samples were collected at standardized times (9 occasions between 0830 and 1930). Cardiac troponin T was measured with a high-sensitivity assay and reference change values (RCV) were computed according to the analytical and intra-individual variances (CVa, CVi).
Results: Within-day CVa and CVi values were 1.6% and 9.5% under inactive conditions, and 1.7%, 6.5% under physical activity, respectively. Inactive and active within-day RCVs were 26.4% and 18.6%, respectively. Weekly CVa and CVi values were 1.5% and 5.6%, resulting in a RCV of 16.8%.
Conclusions: Within-day biological variation of cardiac troponin in patients with chronic cardiac troponin elevations is lower compared to the biological variation previously demonstrated in healthy individuals. Our results suggest that a short-term change of >30% can be used in attempting to diagnose acute myocardial injury.

W094

RELATIONSHIP OF PAI-1 4G/5G POLYMORPHISM AND RISK FACTORS FOR CORONARY ARTERY DISEASE IN ELDERLY

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Background: Coronary artery disease (CAD) is the major cause of death among the population in Thailand, especially in the elderly. Moreover, it has been reported that PAI-1 4G/5G polymorphism is associated with CAD. Therefore, the aims of this study were to determine the genotype distribution of the polymorphism in elderly as well as its association with risk factors for CAD including male gender, lack of exercise, smoking, alcohol consumption, family history of CAD, dyslipidemia, diabetes, hypertension, increased body mass index and metabolic syndrome.
Methods: A total of 166 subjects at the age ≥ 60 years were recruited in this study. Multiplex allele specific polymerase chain reaction was applied to determine the PAI-1 4G/5G polymorphism.
Results: The results showed that the genotype frequencies of 4G/4G, 4G/5G and 5G/5G in the elderly were 32.5, 59.6 and 7.8%, respectively. The protective associations of 4G allele carrier with hypertension [Odds ratio (OR) = 0.28, 95% confidence interva1 (CI) =0.08, 0.95 and P = 0.042] and high levels of low density lipoprotein-cholesterol (LDL-C) (OR = 0.20, 95% CI = 0.05, 0.76 and P = 0.018) were found. However, the protective association was found only with high levels of LDL-C after adjustment of OR (adjusted OR = 0.21, 95% CI = 0.05, 0.80 and P = 0.023).
Conclusions: These results suggested that 4G allele carriers were associated with a lower risk of increased levels of plasma LDL-C which is a risk factor for CAD in elderly.

W095

SERUM AMYLOID A IS INDEPENDENTLY RELATED TO APOLIPOPROTEIN A-I BUT NOT TO HDL-CHOLESTEROL AND CORONARY STENOSIS IN PATIENTS WITH ANGINA PECTORIS

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Background: Inflammation processes are considered important links between classical risk factors and the progression of atherosclerosis. The interrelationship of high-density lipoproteins and apolipoprotein apoA-1 with inflammation markers was examined in patients with angina pectoris.
Methods: On exclusion criteria, 198 patients were recruited and subdivided according to the degree of angiographically documented stenosis <50% vs. ≥50% according to CASS guidelines. Lipids, apoA-1 and apoB, acute phase proteins, cytokines were measured.
Results: Low HDL-C (and apoA-I) is associated with advanced coronary stenosis (>50%) and with the number of diseased vessels, independently of age, gender, diabetes, smoking, and lipid-lowering therapy. In contrast to hs-CRP and fibrinogen, SAA and cytokine levels were not significantly associated with stenosis. Acute phase proteins negatively correlated with HDL-C and apoA-I. SAA (P=0.0003) and diabetes (P=0.0002) were strong predictors of apoA-I concentration independently of age, gender, BMI, smoking, CRP, and IL-6 (P=0.006), in a multiple regression model. SAA was not independently related to HDL-C.
Conclusions: SAA is independently and inversely related to apoA-I but not to HDL-C in patients with angina pectoris, reflecting the effect of SAA on the quality of HDL particles. However, HDL-C but not SAA is inversely related to the degree of coronary artery stenosis.

W096

INVESTIGATION OF THE PRESENCE AND SEVERITY OF CORONARY HEART DISEASE BY THE 1H NMR-BASED LIPID PROFILING OF ATHEROGENIC AND ATHEROPROTECTIVE PLASMA LIPOPROTEINS

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Background: Disturbances in the metabolism of plasma lipoproteins have been considered as risk factor for coronary heart disease (CHD). We investigated the ability of the NMR-based lipid profiling of atherogenic (non-HDL) and atheroprotective (HDL) plasma lipoproteins to detect the presence and severity of CHD.
Methods: Serum samples from 99 patients with CHD [30 patients with one (mild), 29 with two (moderate) and 40 with triple (severe) vessel disease], and 60 patients with normal coronary arteries (NCA), all angiographically determined, were collected after an overnight fast. Lipid content of the HDL and non-HDL lipoproteins was extracted according to a standard procedure. Pattern recognition analysis was applied on the 1H NMR HDL and non-HDL lipidomic data recorded on a Bruker DRX-600 Spectrometer.
Results: The lipidomic analysis showed that CHD patients at any disease stage presented different lipid profiles of atherogenic and atheroprotective plasma lipoproteins from those recorded from NCA patients. The alterations occurring in lipid profiling of atherogenic lipoproteins were able to distinguish gradually patients with mild, moderate, and severe disease from those with NCA, whereas in atheroprotective lipoproteins lipid profiling was significantly altered only in patients with severe CHD. The lipid components of plasma lipoproteins that characterized the initial stages of disease were the high levels of saturated fatty acids in both HDL and non-HDL lipoproteins, the low levels of HDL-sphingomyelin and HDL-phosphatidylcholine, and omega-3 fatty acids and linoleic acid in non-HDL lipoproteins. Moreover, the low levels of HDL-cholesterol and the high levels of non-HDL cholesterol contributed, as expected, to the onset of disease but to a lesser extent. Finally, a trend for separation among CHD subgroups was observed that was more obvious and statistically significant in models constructed based on lipid data of atherogenic lipoproteins.
Conclusions: 1H NMR-based lipidomic analysis of plasma lipoproteins could contribute to the identification of lipid biomarkers for the early evaluation of the onset of CHD and establishment an appropriate therapeutic option.

W097
HOMOCYSTEINEMIA AND EXTEND OF CORONARY ARTERIES WITH SIGNIFICANT STENOSIS

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Introduction: The role of hyperhomocysteinemia in the development of coronary artery disease (CAD) is well known. However, an association between hiperhomocysteinemia and extend of coronary artery disease (CAD) remains controversial.
Objective: This study looks at the relationship between serum total homocysteine (tHcy) concentration and the extent of coronary atherosclerosis, expressed by the number of coronary arteries with significant stenosis (>50%) in 165 patients divided into 3 groups based on 10 years risk for CAD established according ATP III and Framingham criteria.
Method: Plasma tHcy concentration was determined with enzyme cycling method.
Results: Mean plasma tHcy levels in group with high CAD risk, above 20%, were 16.0 micromole/L (P <0.001), in the group with proven CAD, 15.3 micromole/L respectively (P <0.008) vs. control, CAD risk less than 10%, (13.0 micromole/L). There was significant correlation between tHcy and total CAD risk (r = 0,629, P <0.004), white blood cells count (r = 0,783, P <0.002), cholesterol (r = 823, P <0.001) and hypertension (r = 412, P <0.003) in high risk patients compared with CAD group and control. There was no found correlation between tHcy and the number of coronary arteries with significant stenosis. Positive significant correlation was found only between tHcy and high grade of coronary artery stenosis (>95% of arterial lumen) in group with proven CAD (r = 0.543, P <0.004).
Conclusion: We concluded that elevated tHcy correlated with the total CAD risk, cholesterol, blood cells count, hypertension and the stage of coronary artery disease in CAD patients.

W098
PROGNOSTIC SIGNIFICANCE OF MATRIX METALLOPROTEINASES, TISSUE INHIBITOR OF METALLOPROTEINASE-1 AND N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE FOR POSTINFARCTIONAL REMODELING AND ADVERSE PROGNOSIS

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Purpose: To evaluate the relationships with left ventricular remodeling and outcome, of high-sensitivity C - reactive protein (hsCRP), N-terminal pro-B-type natriuretic peptide (NT-proBNP), pro-matrix metalloprotease -1 (proMMP-1), matrix metalloprotease -9 (MMP -9), and their tissue inhibitor (TIMP-1) after acute myocardial infarction (AMI).
Methods: 175 patients with AMI were taken to investigation. All of them underwent coronarography followed by angioplasty and stenting of the infarct-relating coronary artery. All patients got a common standard therapy. The levels of hsCRP were measured on the 4-5th day by immunoturbidimetric method, NTproBNP – by electrochemiluminescence immunoassay. The levels of proMMP-1, MMP-9 and TIMP-1 were measured also on the 4-5th day by enzyme-linked immunosorbent assay (ELISA kit). Echocardiography for estimating parametrs of myocardial remodeling (index of end diastolic volume – EDVi, ejection fraction - EF, index of end sistolic volume - ESVi etc.) was presented on 5-7 days and over 3 -12 months after AMI.
Results: The levels of biomarkers (proMMP-1, MMP9, NTproBNP, hsCRP) were significantly above in patients with ventricular remodeling (P <0.05). Plasma MMP9, NT-proBNP correlated with infarction size as assessed via troponin T(r = 0.37, P <0.05; and r=0.42, P <0.05, respectively). Plasma MMP9, TIMP-1, NT-proBNP each correlated with greater left ventricular (LV) disfunction over 3 and 12 months after AMI, as indicated by direct correlation with left ventricular EDVi and ESVi; and inverse correlation with LV EF. During follow-up 8 patients died and 13 experienced recurrent AMI or unstable angina. Factors with independent association with death were MMP9 (HR 3.1, 95%CI 1.7-4.2, P <0.02), NT-proBNP (HR 2.2,95% CI1.8-2.77, P <0.01), TIMP (HR 1.6, 95%CI 1.14-2.3, P <0.05). Plasma concentrations of MMP-9 or NT-proBNP having best combination of sensitivity and specifity for prediction of the primary end-point (death, unstable angina or heart failure episode) from ROC curves were 283.7 ng/mL (95%CI 264.2-301.4 ng/mL) and 1258pg/mL (95% CI1380 - 1647 pg/mL) respectively.
Conclusion: Plasma concentrations MMP9, NTproBNP, TIMP -1 correlated with echocardiographic parameters of LV remodeling after AMI and may identify patients with adverse prognosis.

W099

THE CONCENTRATIONS OF HIGH-SENSITIVITY C-REACTIVE PROTEIN AND N-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE IN PATIENTS WITH ACUTE CORONARY SYNDROME

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Purpose: To compare concentrations of High-sensitivity C-reactive Protein (HsCRP) and N-terminal pro-B-type natriuretic peptide (NTproBNP) in patients with Acute Coronary Syndrome (ACS) depending on the times and revascularization methods.
Methods: 195 patients with ACS have been included in investigation at the age from 24 till 78 years (middle age of 56 years). Concentrarions of NTproBNP and HsCRP were defined for 3-4 days of disease. Results of measurements are presented as Median value [interquartile range].
Results: ST-elevation was observed in 107 (54.9%) patients with ACS and median of HsCRP in this group was 12.1 mg/L [6.0; 34.4], NTproBNP - 667.4 [295.2; 1744] pg/mL. Plasma concentrations of HsCRP and NTproBNP were above in case of revascularization more than 12 h, than at the first 12 h - 17.61 [9.3; 30.7] mg/L and 967 [469.6; 645.3] pg/mL versus 7.3 [5.0; 35.5] mg/L and 566 [266.7; 1286] pg/mL respectively. Also level of these markers was above in group of patients with Q-myocardial infarction (MI): median of HsCRP was 13.91 [6.38; 38] mg/L and NTproBNP - 800,6 [502; 1876] pg/mL, than in patients with non Q-MI - 8.43 [3.4; 18.12] mg/L and 319 [223.4; 1493] pg/mL respectively. In group of ACS without ST elevation HsCRP and NTproBNP levels also were above in patients with early revascularization – 26.3 [9.6; 60.44] mg/l and 891 [286.2; 3716] pg/mL respectively, because indications for revascularization in this group were big size of necrosis, high concentrations of troponin T and adverse prognosis . The HsCRP and NTproBNP levels in patients with unstable angina were 1.7 [0.9; 6.2] mg/L and 122.1 [68.4; 258.9] pg/mL accordingly.
Conclusion: The HsCRP and NTproBNP levels in patients with acute coronary syndrome were associated with variant of acute coronary syndrome (with ore without ST segment elevation) and time of revascularization.

W100

IMMUNOASSAY FOR DIRECT MEASUREMENT OF CARDIAC RISK BIOMARKER FREE PAPP-A

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Background: Elevated pregnancy-associated plasma protein A (PAPP-A) in blood is associated with increased risk for cardiac events. Usually PAPP-A is measured as total PAPP-A (PAPP-A in complex with the proform of eosinophil major basic protein and noncomplexed free PAPP-A together). Free PAPP-A has been reported to better predict the cardiac risk. The only means to determine free PAPP-A has been the calculation of the difference of the results of total PAPP-A assay and complexed PAPP-A assay. We have developed an immunofluorometric assay for direct measurement of free PAPP-A.
Methods: Mice were immunized with recombinant PAPP-A that resembles the endogenous free PAPP-A. Antibody producing hybridoma cell lines were created from the mouse spleen cells. The antibodies were tested in noncompetitive sandwich assays on streptavidin coated microtitration plates. The capture antibodies were biotinylated and the tracer antibodies were conjugated to inherently fluorescent europium chelates. The antibodies were evaluated for the detection of recombinant PAPP-A, complexed PAPP-A and endogenous free PAPP-A in various combinations between each other and with some commercial total PAPP-A antibodies.
Results: Thirty free PAPP-A specific monoclonal antibodies were produced. One promising free PAPP-A antibody was found that provided well performing candidate assays with total PAPP-A antibodies. The most promising candidate assay detects well recombinant PAPP-A (linear range 0.3-3000 mIU/L, y=178.54x, r^2=0.999) and endogenous free PAPP-A in patient samples (using recombinant PAPP-A for calibration, the patient sample results of the new assay on average 166% vs. the traditional method - complexed PAPP-A subtracted from total PAPP-A). The assay has a low cross-reactivity towards complexed PAPP-A (4.2% for total PAPP-A in third trimester pregnancy serum). Matrix effects from serum or heparin plasma in comparison to the standard diluent buffer were insignificant (recovery 86%).
Conclusions: The developed novel direct free PAPP-A assay provides simpler and presumably more accurate detection of free PAPP-A levels in serum and heparin plasma samples. Future studies will reveal whether the assay can contribute to improved estimation of the future risk of adverse cardiac events.

W101
RACE CYCLING: BIOLOGICAL EVOLUTION

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Background: The metabolic and cardiac impact of a cycling effort on blood biology is not very well described in the literature. We aimed to measure the concentration of different biomarkers (cardiac and metabolic) released during an international cycling race.
Methods: Venous blood samples of 15 young men (25.1 ± 6.4 y.o.) were collected just before (T1), just after (T2), 3 h (T3) after an international cycling race of 179.6 kilometers in Belgium for the determination of cardiac and metabolic biomarkers: red blood cell (RBC), haemoglobin (HgB), creatinin (Cr), highly sensitive troponin T (hsTnT), myoglobin (MYO) and NT-proBNP. All automated assays were performed according to the manufacter's specifications. For the statistical analysis, an Anova calculated with the Statistica Software version 9.1 was used.
Results: RBC and HgB levels varied significantly between T0 and T3 (respectively P=0.0026, and P=0.002). Cr concentration also varied significantly between all times (T0-T1: P <0.0001, T1-T3:P=0.0326 and T0-T3 P=0.0001). These changes might be related to renal flow depletion during exercice. MYO increased significantly between T0 and T1 (P <0.0001), but quickly decreased between T1 and T3, however the T3 level stay higher than T0 (P=0.014). The stress delivered from the physical activity performed during the race induced a significant variation of hsTnT which increased significantly between T0 and T1 (P <0.0001) and stayed higher 3 hours after the end of the exercise (T0-T3: P <0.0001) .The intense exercise delivery by the race induced a significant variation of NT-proBNP, that followed the same kinetic of hsTnT but in smaller proportion. We noticed variations statistically significant between T0 and T1 and between T0 and T3 for NT-proBNP. These increases of cardiac biomarkers were significant but reasonable and could not allow us to talk about cellular necrosis or irreversible injury.
Conclusion: Our results show that stress generated by a cycling race could be the cause for the different metabolic variations observed. Troponin T stays without a doubt the most specific marker for stress related to myocardial tissue. Its increase can then be considered as being of interest.

W102
COMPARISON OF THE HEART-TYPE FATTY ACID-BINDING PROTEIN (H-FABP) WITH THE HIGH SENSITIVE CARDIAC TROPONIN T IN HEALTHY RUNNERS

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Background: Heart-type fatty acid-binding protein (H-FABP) is a low molecular weight protein involved in the intracellular uptake and buffering of long chain fatty in the myocardium. It is an early marker for acute coronary syndrome. Troponin T (TnT) is a component of the contractile apparatus of the striated musculature. Cardiac TnT is a cardio-specific, highly sensitive marker for myocardial damage. The aim of our study was to compare the results obtained with the H-FABP and the highly sensitive cardiac troponins (hsTnT) and to test their cardiospecificity in healthy runners.
Methods: Twenty three runners (marathon) were enrolled. We drowned samples at three times: just before (T0), just after (T1), and three hours after the end of the race (T3). H-FABP was determined with a Randox immunoturbidimetric assay and hs-TnT with a Roche electrochemiluminescence immunoassay, both on Cobas 6000. A linear regression was calculated to observe if there is any correlation between the two biomarkers. Values above the 95th percentile for H-FABP (2.5ng/mL) and the 99th percentile for hsTnT (14ng/L) were considered as positive.
Results: At T0, none of the subjects were positive for hsTnT but 35% were positive for H-FABP; at T1, 83% for hsTnT and 100% for H-FABP; at T3, 83% for hsTnT and 96% for H-FABP. At T0, the regression equation was H-FABP T0 = 3.9454 – 0.1001 x hsTnT T0; at T1: H-FABP T1 = 51.838 – 1.7026 x hsTnT T1; at T3: H-FABP T3 = 47.977 – 1.6193 x hsTnT T3. No correlation was observed between the two biomarkers at the different time.
Conclusions: We observed a significant increase of H-FABP and hsTnT in runners. These markers are independent to each other. These values could biologically correspond to a heart ischemia. However, we suggested that exercise-induced cardiac hsTnT and H-FABP release is not a marker of exercise-induced pathology but likely a physiologic response to effort or an exercise-induced cardiac remodelling.

W103

IMPACT OF STRENUOUS EXERCISE ON THE RELEASE OF CARDIAC BIOMARKERS

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Background: Cardiac troponins (cTn) are considered as the best biomarkers for detection of myocardial cell injury and NT-proBNP as the best for the cardiac insufficiency. In this study, cTnT was measured by new commercially available high-sensitive methods in subjects un-dergoing the Maasmarathon. Our aim was to compare cTnT and NT-proBNP levels before and after the stress tests, in sportive subjects.
Methods: Twenty eight subjects (26 ♂, 42.5±11yo) underwent a race of 42.195 kilometers be-tween Visé (Belgium) and Maastricht (The Netherlands). We drowned blood sample before (T0), just after (T1) and three hours after the race (T3). In all patients, cTnT concentrations were measured by high sensitive methods (hsTnT, Roche Diagnostics) on heparin plasma. The NT-proBNP was also determined with the kit Roche on heparin plasma. The protocol was approved by the ethics committee of the University of Liège (Belgium). All subjects gave their informed consent. All statistical analyses were performed using Medcalc version 8.1 for Windows. p-value <0.01 was regarded as statistically significant.
Results: There was a significant difference between hsTnT concentrations at T0 and T1 (P <0.0001), and between T0 and T3 (P <0.001) for NT-proBNP, but not between T1 and T3. This observation appeared only after a strenuous exercise but today this type of exercise is not reproduce easier in a laboratory of sport. Moreover, at this moment, nobody knows if these observations would have cardiac consequences at long terms.
Conclusions: Measurement of cardiac troponins by high sensitive methods allows detecting significant release of biomarkers from the heart during exercise. The value of NT-proBNP are also significant but less than TnThs. We think that the TnThs could be an interesting tool in the future to help sport medicine to detect risk of developing a cardiac problem in the future or a sudden death.

W104

TWO SITES ANALYTICAL AND CLINICAL EVALUATION OF TOSOH AIA NATRIURETIC PEPTIDE TYPE B ASSAY

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Background: Since Type B Natriuretic peptide (BNP) assays are not standardized, analytical evaluation concerning new BNP assays are crucial for validating their clinical use. The aim of our work was to realize an analytical and clinical evaluation of the BNP assay developed by Tosoh BioSciences for AIA analyser Methods : Analytical evaluation was realized with AIA-1800 (Calais : Site 1) and with AIA-360 (Tenon: Site 2) according to COFRAC (Lab SH GT A 04) protocol. BNP Tosoh assay was compared to NT-proBNP assay (Vitros 5600 OCD: site1) and BNP assay (Architect Abbott site 2). Utility of Tosoh BNP assay in the diagnosis of acute heart failure (AHF) was evaluated by analysis of dyspneic patients presenting at emergency department.Results : BNP Tosoh AIA analytical characteristics were the following: Limit of detection : 1.22ng/L; 20% CV (precision profile) 12.2 ng/L; Within run and between run precisions demonstrated CV below 4.3%. Linearity was checked between 1.22 and 300 ng/l with recovery ranging from 92 to 98%. Maximal storage times of BNP Tosoh (significant change: + 15%) at ambient temperature, +4 °C and -20 °C were 18 h, 57 h and one month, respectively. Method comparison (Passing Bablok) gave the following results: BNP AIA = 0,669 BNP Architect + 0.3093 r = 0,97 (n =229), and BNP AIA = 0,059 NT-proBNP + 11,44 r = 0,83 (n =99). Analytical concordances between BNP Architect and Tosoh tests were 96% (at 100 ng/L) and 87% (at 400 ng/L). The use of Tosoh BNP in the diagnosis of AHF was tested in 88 dyspneic patients. Area under ROC curves found with Tosoh BNP, Architect BNP and Vitros NT-proBNP were 0.88 (CI 95% : 0,81 to 0,95), 0.89 and 0.95 and were not significantly different. For Tosoh BNP, at the cut-off of 100 ng/L sensibility was 91% (95%IC: 76 to 98%) and specificity 65% (95CI: 51 to 77%). At 400 ng/L sensibility was 56% (95CI 37 to 72%) and specificity 94% (95 CI: 85 to 99%).Conclusion : This work demonstrated the good analytical performances of BNP Tosobh AIA assay evaluated by lab SH GT A 04 protocol. Clinical results demonstrated a diagnostic value near from other BNP assays. Recommended cut-off suggested for exclusion and inclusion diagnosis of AHF in patients presenting with dyspnea can be used with BNP AIA Tosoh assay.

W105
CORRELATION LEVELS OF SERUM CARDIAC MARKERS IN PARAGUAYAN PATIENTS

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Background: Among cardiovascular diseases, ischemic cardiopathy is the first cause of death in men and the second in women and it is an important element of mortality in Paraguay. In the laboratory, the most valuable assay is the serial measure of cardiac enzymes and new evaluations have been developed including the quantitative determination of CK-MB and Troponin I. Our objective was to determine correlation indexes between CK-MB and Troponin I, CK and Troponin I, CK and CK-MB in patients that attended the Emergency Room of the Hospital de Clínicas in Asunción, Paraguay from January to August 2011.
Methods: An observational, descriptive, cross-sectional study was carried out in male and female patients admitted to the Hospital de Clínicas in Asunción, Paraguay or that attended the Emergency Room of the same hospital from January to August, 2012 in whom measures of coronary profile were made. The measurement of TnI was made using an immunoenzimatic assay. The determination of total CK and CK-MB was performed by the kinetic method using the Spinreact reagent in the Selectra multianalyzer.
Results: This study included 141 patients and the values determined were for Tn I: a mean of 0.02 (UNITS) with a range of 0.01 to 88 (UNITS), for CK-MB a mean of 20 (UNITS) with a range of 1 to 566 (UNITS) and for CK a mean of 141 and a range of 15 to 9180 (UNITS). The correlation between Trop I and CK-MB was low and not significant r2:0.0998, between Tn I and CK r2:0.0585 and between CK-MB and CK r2:0.4375.
Conclusion: An adequate risk stratification would allow a more precise therapeutic management of these patients. However, the problem is the heterogeneity of this syndrome that includes patients with diverse clinical profiles. This study verified the lack of correlation between TnI and CK-MB as the first has more efficiency and sensitivity that in some cases indicate cardiac damage. The use of TnI is important as its elevation is rapid and contrarily to other enzymes, it offers additional information which is useful at the time of evaluating the repercussion of the chest pain. In the case of CK-MB, sometimes it increases due to damages in non-cardiac areas and its correlation to CK is naturally observed in this case. The other major contribution made by Tn I is that is an excellent prognosis indicator per se from the first evaluation, stratifying fatal event risks at 30 days. Based on this, the use of these markers should be estimated in the cost-benefit aspect of the cardiac damage.

W106
DEVELOPMENT OF A HIGHLY SENSITIVE IMMUNOASSAY FOR CARDIAC TROPONIN I FOR THE ARCHITECT® I2000SR AND I1000SR ANALYZERS

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Background: Troponin I is a 24 kD modulatory protein that is part of the troponin I-C-T complex which serves to regulate actin and myosin filament interactions. Troponin I is measured in the blood as an indication of cardiac or microvascular damage. The ARCHITECT STAT High Sensitive Troponin-I (hsTnI) assay is a two-step, double-monoclonal CMIA immunoassay that can determine the presence of cTnI in human serum and plasma at concentrations a magnitude lower than the previous generation assay. Two significant design differences between the new ARCHITECT STAT hsTnI assay and the current ARCHITECT STAT TnI (List #2K41) assay are sample volume and antibody clone construction. The ARCHITECT STAT hsTnI assay has a calibration curve spanning 0 – 50,000 pg/mL (ng/L).
Methods: During development, total precision, limit of blank (LOB), limit of detection (LOD) and limit of quantitation (LOQ), linearity, and interference from potentially interfering drugs were determined following recommendations from CLSI documents EP5-A2, EP17-A, EP6-A, and EP7-A2. A 99th percentile cutoff was established from a healthy population (n=1531). Interference from HAMA and RF antibodies, and analytical specificity were determined. The AUC value for the diagnosis of myocardial infarction was determined using a minimum of 71 acute myocardial infarction (AMI) and 780 non-AMI specimens collected in 3 different tube types at 3 different time points.
Results: Precision CVs ranged from 1.6 to 8.0%, the LoQ ranged from 4.0 to 10.0 pg/mL and the LOD ranged from 0.5 to 1.9 pg/mL. The calculated 10%CV is at 4.7 pg/mL. The range of linearity was <10.0 to >50,000 pg/mL. Potentially interfering drugs had less than 10% interference. The 99th percentile cutoff was 26.2 pg/mL. The AUC values were >0.92 from a Receiver Operating Characteristic (ROC) curve for all 3 tube types at each time point tested. The mean interference with HAMA and RF samples was -2.8% and -3.4% respectively, and cross–reactivity with skeletal troponin I was <0.1% and was <1% with both troponin C and troponin T.
Conclusions: These results demonstrate that the ARCHITECT STAT High Sensitive Troponin-I assay is a precise and highly sensitive method for measuring troponin I in human plasma or serum on a high throughput analyzer.

W107

CONCENTRATION OF MG IN SERUM AND ERYTHROCYTES IN PATIENTS WITH CARDIOVASCULAR DISEASES

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Beckground: Cardiovascular effects of magnesium include coronary and systemic vasodilatation, inhibition of platelet and antiarrhythmic action and protection from myocardial ischemia. Because of the importance of magnesium in cardiovascular diseases, our goal was to review the imbalance Mg2+ ions in serum and erythrocytes, and the determination of significant differences in specific disease groups.
Method: The study included 65 patients (35 patients with acute myocardial infarction, 15 with diagnosed hypertensio arterialis and 15 diagnosed with cardiomyopathy. Determination of Mg2+ concentration in serum was provided by spectrophotometric method and concentration of erythrocytes by indirect method.
Results: Statistical analysis demonstrated a significant difference of magnesium in serum for patients with acute myocardial infarction (P=0.01). Lowering of Mg2+ concentrations in serum for patients with hypertension also showed a statistically significant difference (P‹0.01). In patients with cardiomyopathy, there is also a statistically significant (P‹ 0.05). Mg2+ concentration of erythrocytes in patients with acute myocardial infarction was significantly lower (P‹0.001), with arterial hypertension is also in (P‹0.05) and in patients with dilated cardiomyopathy (P ‹0.05).
Conclusion: There is a significant decrease of Mg2+ in serum and erythrocytes in patients, compared to the control group. The reduction of Mg2+ in serum is accompanied by decrease of Mg2+ in erythrocytes. The most striking statistical difference compared to the control group, is in patients with acute myocardial infarction. For other diseases, there is a difference but not as pronounced and therefore the determination of this and cannot be used in the diagnosis of cardiovascular diseases. Having in mind that importance of biochemical Mg2+, is not negligible giving of magnesium per os in the treatment of cardiovascular diseases in the case of the verified reduced concentration of Mg2+.

W108

SERUM ADIPOCYTE FATTY ACID BINDING PROTEIN IS MORE ASSOCIATED WITH ATHEROGENIC RISK PROFILE THAN ADIPONECTIN IN WOMEN WITH INCREASED BODY MASS.

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Background: A-FABP (adipocyte fatty acid-binding protein) one of the most abundant proteins in adipocytes, plays a key role in obesity-related insulin resistance, inflammation and atherosclerosis. In the present study, we investigated the association of A-FABP with proatherogenic profile and insulin resistance in young non-diabetic overweight/obese women.
Methods: A-FABP, hsCRP, adiponectin, glucose, insulin, lipids and apolipoproteins were measured in 104 women (20-45 yrs) with BMI ≥25 kg/m^2 and age-matched healthy controls (n=76; BMI <25 kg/m^2). All underwent blood pressure and anthropometric measurements. Serum CRP (hsCRP) concentration was measured (BN II, Dade Behring) and human A-FABP and Adiponectin was determined by ELISA (BioVendor Laboratory Medicine Inc; DRG MedTek, R@D, respectively).
Result: Median A-FABP in women with excessive body mass (mean BMI 32.6±6.1 kg/m^2) was significantly higher than in normal-weight women, and higher already in women with overweight (17.5 vs 10.1 ng/mL; P <0.0001). In the whole study group serum A-FABP concentrations correlated positively with hsCRP whereas no significant association was found between adiponectin and hsCRP. A-FABP correlated with BMI and HOMA-IR (P <0.003), insulin (P <0.005) and atherogenic indices apoB/apoA-I, TC/HDL-C, TG/HDL-C (P <0.007). On the other hand A-FABP had the negative association with two anti-inflammatory indicators like HDL-C and ApoA1 (R=- 0.30, P <0.04; R=- 0.34, P <0.02, respectively). Moreover, A-FABP was an independent predictor of TG/HDL (the index of LDL particle size and surrogate for insulin resistance) explaing 47% of TG/HDL variation in women with excessive body weight. Our results showed that A-FABP was of excellent diagnostic utility for discrimination between controls and women with excessive body mass, whereas the diagnostic utility of adiponectin was only sufficient (AUC 0.96 vs 0,67). A-FABP >16 ng/mL predicted atherogenic risk with OR 11.2 (95% CI 3.7-34.2), 7.1 (1.9-27.2), 4.6 (95% CI 1.2-17.4), 6.7 (2.6-17.2) for having elevated TG/HDL-C, apoB, HDL-C/ApoA-I, CRP and insulin resistance with OR 5.6 (1.8-17.2).
Conclusion: A-FABP seems to be a better predictor of atherogenic risk profile than adiponectin in young non-diabetic overweight/obese women.

W109

SERUM ADIPOCYTE FATTY ACID-BINDING PROTEIN – IS IT USEFUL IN ISCHEMIC STROKE?

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Background: Adipocyte fatty acid-binding protein (A-FABP) is an adipokine shown to have adverse metabolic and proinflammatory effects. In this study, we investigated the association of serum A-FABP with ischemic stroke.
Methods. Serum A-FABP was measured, using ELISA, in 46 subjects with acute ischemic stroke and 46 controls free of cardiovascular diseases.
Results: Serum A-FABP was higher in subjects with ischemic stroke as compared to controls (21.9 ng/mL [13.4-32.7 ng/mL] vs 14.7 ng/mL [8.6-21.2 ng/mL] in men and 35.9 ng/mL [21.7-50.8 ng/mL] vs 18.7 ng/mL [9.3-29.6 ng/mL] in women, stroke vs control, P <0.001). In a panel which includes valuation of hypertension, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, lipid-lowering treatment, smoking, and A-FABP, serum A-FABP was independently associated with stroke (odds ratio 2.02, 95% confidence interval 1.45-2.77, P <0.001), and the associations of A-FABP with ischemic stroke were parallel to conventional risk factors.
Conclusions: Serum A-FABP was significantly associated with ischemic stroke in this study, and may be used to predict early mortality.

W110

URINARY NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN (NGAL) IN PATIENTS WITH CHRONIC HEART FAILURE

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Background. Renal impairment, as measured by reduced glomerular filtration rate (GFR) and increased urinary albumin excretion (UAE), is prevalent in patients with chronic heart failure (CHF) and is associated with reduced survival. The prevalence of structural tubular damage in CHF is unknown.
Methods. We investigated 50 CHF patients and 20 age and sex matched healthy controls, and determined estimated GFR, UAE, N terminal-pro brain natriuretic peptide (NT-proBNP) and urinary neutrophil gelatinase associated lipocalin (NGAL) as a marker for tubular damage.
Results. CHF patients had significantly lower averaged estimated GFR (64±17 vs 90 ±12 mL/min/1.73 m^2, P <0.0001), but higher NT-proBNP and UAE levels (both P <0.0001). Median urinary NGAL levels were markedly increased in CHF patients compared to controls (175 (70–346) vs 37 (6–58) μg/gCr, P <0.0001). Both serum creatinine (r=0.26, P=0.006) and eGFR (r=−0.29, P=0.002) were significantly associated with urinary NGAL levels as were NT-proBNP and UAE but to a lesser extent.
Conclusions. Renal impairment in CHF patients is not only characterised by decreased eGFR and increased UAE, but also by the presence of tubular damage, as measured by increased urinary NGAL concentrations.

W111
PERFORMANCE EVALUATION OF THE ARCHITECT GALECTINE-3 ASSAY

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Background: Galectin-3 is a structurally unique member of a family of multifunctional beta-galactoside-binding lectins that play many important regulatory roles in inflammation, immunity, and cancer. The expression elevation of galectin-3 has been associated with inflammation and fibrosis processes that are pivotal contributing pathophysiological mechanisms to the development and progression of heart failure. Architect Galectine-3 assay is a chemiluminiscent microparticle immunoassay (CMIA) for the quantitative determination of galectine-3.
Methods: Analytical performance of the ARCHITECT Galectin-3 assay following CLSI guidelines EP5-A2 (precision), EP17-A (LoB, LoD, LoQ), linearity and normal references ranges with 120 samples collected from apparently healthy individual. Also a comparison between instrumentation (Architect i1000 and i2000) was included.
Results: Within run, run to run and total CVs are for low level (mean 9 ng/mL) 4.6%, 4.6% and 4.6%; for medium level (mean 20,1 ng/mL) 1.4%, 2.0% and 2.4%; and for high level (mean 73,6 ng/mL) 1.5%, 1.6% and 2.5% respectively. The LoB is 0.40 ng/mL; the LoD is 0.68 ng/mL; the LoQ is around 4ng/mL. In the dilution study for linearity we found that the differences range from -0.1% to 2.2% or -0.3% to 2.6%. Expected ranges were evaluated by age (under and over 40 years old) and by gender. For females the mean value was 13.91 ng/mL (12.88 to 14.94 95% CI) (Min 5.6 and Max 23,9 (ng/mL)); for males the mean value was 14,16 ng/mL (13.88 to 14.93 95% CI) (Min 6.5 and Max 24.4). For health individuals under 40 the mean value was 13.22 ng/mL (12.43 to 14.01 95% CI) (Min 5.6 and Max 23.4), for health individuals over 40 mean value was 15.01 (13.99 to 16.03 95% CI) (Min 8.9 and Max 24.4 (ng/mL)). No significant differences were found between genders, but the Galectine-3 levels are associated to age. There were no statistical significant differences between the two instruments (Passing Bablok - 0.41 + 1.03x; and bias plot study bias 0.4 ng/mL(-0.2 to 2.8 95% CI).
Conclusions: Architect Galectine 3 assay provides a precise and convenient automated method for the measurement of Galectine 3. This assay is ready for further clinical evaluation of patients at risk for major adverse cardiac events.

W112
PLATELET STIFFNESS IN PATIENTS WITH ARTIFICIAL HEART VENTRICLES

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Superficial morhpology and stiffness of platelets plays significant role in thrombus formation especially in patients with the implanted artificial heart ventricles (VAD).
Aim: Local elastic properties of platelets were investigated during atomic force microscopy (AFM) in patients with end-stage ischemic heart failure (ESHF), whom were selected for emergency open heart surgery. Groups of 16 ESHF VAD patients (52,4 years, LVEF 16,1±3,1%) and 12 Non VAD pts were estimated prospectively before surgery (0), after bypass starting (1), immediately after VAD implantation/open heart surgery (2), 1 (3) and 6 (4) month after operation. Heparin infusion with subsequent warfarin treatment had all VAD patients. Minor and major thromboembolic events were analyzed carefully after surgery. AFM NT-206 ("MicroTestMachines", Belarus) in contact mode with the standard cantilevers CSC38 ("MikroMasch" Co., Estonia, spring constant of 0.03 N/m) were used for local elastic properties of platelets in quantitatively determined force spectroscopy regime. By recording the cantilever deflection while the tip is brought in contact at the fixed point and retracted, we obtain force curve. The Young's modulus of inactivated platelet was calculated using the Hertz model describing the elastic deformation of the two bodies in contact under load. Spontaneous, ADP- and adrenalin-stimulated platelets aggregations and coagulogramma were assessed simultaneously.
Results. All patients with ESHF characterised low platelet elastisity with very high Young's modulus (VAD pts 319.7±37.2 kPa and Non VAD pts 284.1±44.9 kPa, $p < 0,0001$ vs donors, $p < 0,05$ between groups). Patient with higher Young's modulus had higher rate of thromboembolic events (OR 2.1, CI 1.6;2.9).
Conclusions. Drammatic high platelet stiffness followed by higher incidence of dyscoagulable state and support the idea of multifactorial nature of thromboembolic formation in patients with artificial heart ventricle.

W113

EVALUATION OF THE ANALYTICAL PERFORMANCE OF A NOVEL IMMUNOENZYMOMETRIC ASSAY FOR BRAIN NATRIURETIC PEPTIDE

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Background: We evaluated the analytical performance of a novel immunoenzymometric assay for brain natriuretic peptide (BNP) set up for the automated AIA-2000 platform (Tosoh).
Methods: The ST AIA-Pack method for plasma BNP assay was performed according to the recommendations made by the manufacturer, using the AIA-2000 platform (TOSOH Corporation). The limits of blank (LoB) and detection (LoD) for the ST AIA-Pack BNP method were determined according to the CLSI EP17-A protocol. The assay reproducibility was evaluated in accordance with the CLSI EP5-A2 protocol: 3 different EDTA-plasma samples, containing on average 8.7 ng/L (sample A), 21.7 ng/L (sample B) and 38.7 ng/L (sample C) of BNP, respectively, were repeatedly measured for 20 consecutive working days, using two distinct reagent lots. The between-runs imprecision profile was performed by repeatedly measuring 8 EDTA-plasma samples, collected from healthy subjects and patients with heart failure, in 20 different runs and using two reagent lots. For comparison, plasma concentrations of BNP were also measured in 100 consecutive EDTA-plasma samples, using the Alere-Triage BNP test for Beckman-Coulter with the UniCell DxI 800 platform (Beckman-Coulter Diagnostics).
Results: The calculated LoB and LoD values were 2.2 ng/L and 6.7 ng/L, respectively. The LoB value, estimated in the present study, was very similar to that reported by the manufacturer (i.e. 1.9 ng/L). Within run and total imprecision (CV%) of the assay were: 11.25, 19.36 for sample A (8.7 ng/L); 4.27, 9.27 for sample B (21.7 ng/L); 2.56, 4.95 for sample C (ng/L). In particular the BNP concentration of sample B was close to the reference limit reported by the manufacturer (i.e. 26.5 ng/L). The calculated limit of quantitation (LoQ) at 20% CV and 10% CV were 11.7 and 24.0 ng/L, respectively. A very close linear regression was found when BNP concentration values were obtained with Tosoh and Beckman-Coulter methods (R=0.983, y=0.6255x -1.1934).
Conclusions: our data indicate that the analytical performances of the ST AIA-Pack BNP method make this assay suitable for the clinical evaluation of patients with cardiac diseases.

W114

UPCONVERTING NANOPARTICLES AS REPORTERS IN A HETEROGENEOUS CARDIAC TROPONIN I IMMUNOASSAY

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Background: In contrast to conventional photoluminescent reporter molecules, upconverting nanoparticles (UCNPs) have a unique ability to emit high energy visible light when exposed to low energy infrared radiation. This anti-Stokes emission can be measured free of autofluorescence without time-resolved detection. In this study UCNPs were used as reporters in a heterogeneous immunoassay for cardiac troponin I (cTnI), which is a biomarker used in the diagnosis of myocardial injury.
Methods. The UCNPs with structural composition of NaYF4:Yb3+, Er3+ and diameter of 20–30 nm were functionalized by polymerizing a silica shell on the surface and the particles were conjugated with monoclonal antibody (Mab) specific to the C-terminus of cTnI. Two biotinylated capture antibodies, capture-Mab and a humanized Fab-fragment (hFab), were immobilized on streptavidin coated microtiter wells. The capture antibodies had specificity against different epitopes of cTnI, capture-Mab recognizing an epitope in the stable mid-fragment and hFab in the C-terminus. In heterogeneous sandwich type immunoassay human cardiac troponin I-T-C complex was detected with the Mab-conjugated UCNPs. Upconversion luminescence was measured from dry well surface with a modified plate reader equipped with an infrared laser diode as excitation source.
Results: The lowest analyte concentration 0.005 ng/mL was detected with a signal to background ratio of 3.5. The analytical sensitivity of the assay was 0.002 ng/mL.
Conclusions: A highly sensitive immunoassay for cTnI was developed utilizing the UCNP reporter technology. The performance of the assay may be further improved by reducing the nonspecific binding and optimizing the assay conditions.

W115
CARDIOVASCULAR MARKER IN PREECLAMPTIC WOMEN : TUNISIEN EXPERIENCE

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Background: Preeclampsia remains a major cause of maternal mortality and morbidity. This maternal disorder was associated with an increased risk of atherosclerosis. The aim of this study was to evaluate same cardiac risk factors in preeclamptic women.
Methods: Our study included 40 preeclamptic patients (PE) and 30 matched healthy pregnant women. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) were measured by autoanalyzer (Synchron CX9, Beckmann coulter), serum apolipoprotein (Apo) A-I, Apo B, serum Lp(a) and high sensitivity C-reactive protein (hsCRP) were measured by nephelometric assay (DadeBehring, Marburg, Siemens, Germany). Serum levels of total homocysteine were assessed using enzyme immunoassay method (Axsym, Abbot). Serum low-density lipoprotein (LDL) was calculated using Friedwald equation.
Results: Serum levels of LDL-C, Lp(a), hsCRP and homocysteine were higher in the PE patients than in the normal pregnant women (P <10-3). HDL-C level was lower in the patient group compared to normal pregnant women (P <10-3).
Conclusion: Our findings suggest that high LDL-C, Lp(a), hsCRP and homocysteine , and low HDL-C , are important risk factors for atherosclerosis among preeclamptic women. It is indispensable to screening and monitoring women with PE for these cardiac risk factors during pregnancy.

W116
INFLAMMATION AND LIPID PEROXYDATION IN TUNISIAN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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Background: Inflammation and oxidative stress play an important role in the pathogenesis of many chronic inflammatory lung disorders such as chronic obstructive pulmonary disease (COPD). In the present study, we aimed to evaluate hsCRP and thiobarbituric acid reactive substances (TBARS) in patients with COPD.
Methods: 22 smokers patients (19 males) with COPD (mean age 69±8 years) were recruited in department of pulmonary diseases in CHU Farhat Hached, and 23 healthy non smokers (20males, mean age 59±7 years) were studied, hsCRP was measured by immunonephelometry (DadeBehring, Marburg, Siemens, Germany), Serum malondialdehyde levels were measured by the reaction with thiobarbituric acid reactive substances (TBARS) based on spectrophotometric measurement (Yagi method).
Results: We noted statistically significant increase of hsCRP in patients with COPD compared to controls (6.35 ± 4.42 mg /L vs 1.47±0.9 mg/L, P: 0.004). While, plasma TBARS was significantly higher in patients than control groups (1.29±0.35 μmol/L vs 0.74±0.16 μmol/L P <10-3).
Conclusion: Our data showed increased levels of inflammation biomarkers and oxidative stress in patients with COPD. These results suggested that patients with COPD have a significantly higher risk of cardiovascular diseases.

W117
INFLAMMATORY CYTOKINES IN OBSTRUCTIVE SLEEP APNOEA

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Obstructive sleep apnoea (OSA) is a common disorder and associated with several CV risk factors and inflammation. The gold standard for confirmation of OSA is polysomnography, which is labour intensive, expensive and time consuming. Simple non-invasive blood tests which could be used for screening/diagnosis of patients would be invaluable in high risk patients. This was a preliminary study aimed to determine if serum cytokines like tumour necrosis factor alpha (TNF-α), and interleukins (IL) namely IL1A, IL1B, IL2, IL4, IL6, IL8, IL10, monocyte chemotactic protein 1 (MCP1), vascular endothelial growth factor (VEGF), interferon gamma (IFNG), epidermal growth factor (EGF) are different in subjects with and without OSA, with a view to determining their value as a screening tool to identify OSA. All subjects 25 to 70 years underwent overnight oximeter monitoring to determine apnoea hypopnoea index (AHI) and classified into three groups: a) normal (AHI<5 events/hour, n=20) b) mild OSA (AHI 5 to 19, n=15) c) moderate and severe OSA (AHI >20, n=29). All subjects on steroid therapy or any acute illness in the last month were excluded from the study. The average age (years) was 43.5, 48.3, 54.9 and BMI was 26.2, 32.1, 33.5 respectively in normal, mild and moderate to severe OSA. Hypertension (20%, 15%, 58.6%), type 2 DM (0%, 13.3%, 24.1%) and CHD (0%, 0%, 17.2%) were present in normal, mild and moderate to severe OSA. EGF and TNF-α are significantly different in normal and mild OSA before (P=0.002 and 0.001 respectively) and after adjustment of BMI, age, sex, statins, aspirin, diabetes and hypertension (P=0.004 and 0.002 respectively). IL6 and IL8 are only significantly different in normal and mild OSA before adjustment of BMI (P=0.048 and 0.035 respectively) but not after. All other markers do not show any consistent differences in the three groups. Our study shows only differences in EGF and TNF-α between normal and mild OSA before and after adjustment of BMI and other variables. This is likely to be because OSA does not increase inflammation much beyond what is already present in severe OSA with co-morbidities. Further work needs to be done to elucidate the role of inflammatory cytokines as a screening tool in the investigation of OSA.

W118
DOES CANCER ANTIGEN 125 (CA 125) PLAY A ROLE IN PROGNOSIS OF CHRONIC HEART FAILURE PATIENTS?

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Background: Cancer antigen 125 (CA 125) is a high-molecular weight glycoprotein routinely used as tumor marker. CA 125 concentrations are elevated when serous effusions exist. In heart failure (HF), the existence of pleural effusions is associated with increased CA 125 values; elevated CA 125 levels have been associated with poor prognosis in acute and chronic HF.
Aim: To assess whether CA 125 has prognostic value in stable chronic HF (CHF) patients. Patients and outcomes: Blood samples of 157 stable CHF patients were obtained at the time of a routine visit. Patients were followed during 18±8 months; worsening of HF (new HF) requiring hospital admission and death were registered as outcomes.
Results: Patients' mean age was 72±12 years, 61% were men. Ischemic heart disease (37%) and hypertension (29%) were the predominant causes of the HF. At the time of the visit, all subjects were in NYHA functional class II-III and 68% had preserved ejection fraction. Patients were at their maximum tolerated doses of diuretics and antihypertensive drugs. During follow-up, 104 poor outcomes occurred: 76 new HF (48%) and 28 deaths (18%). Median CA 125 and NT-proBNP concentrations in new HF cases (31.1 KU/L and 3310 ng/L) and deaths (40.7 KU/L and 3966 ng/L) were significantly higher than in patients without poor outcomes (16.3 KU/L and 919 ng/L, P <0.001). In a multivariate Cox regression model including as factors the existence of diabetes, hypertension or atrial fibrillation, left ventricle ejection fraction, telediastolic diameter of left ventricle and NT-proBNP and CA 125 concentrations at 75th percentile, CA-125 concentrations higher than the 75th% percentile (43.9 KU/L) were the most powerful predictor of death (P <0.005). Survival free of death or new HF was significantly reduced in patients with CA 125 higher than the 75th percentile compared to their counterparts (64% vs. 82% at 24 months, Long Rank test P=0.0001).
Conclusion: Circulating concentrations of CA 125 higher than the 75th of a population of stable CHF patients are prognostic of new admissions by HF and mortality.

W119

IMPACT OF CYP2C8*3 AND CYP2J2*7 POLYMORPHISMS ON MYOCARDIAL INFARCTION IN THE BULGARIAN POPULATION

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Cytochrome P450 2C8 is a polymorphic enzyme responsible for the biosynthesis of vasoactive substances from arachidonic acid. Cytochrome P450 (CYP) 2J2 is expressed in the vascular endothelium and it metabolizes arachidonic acid to biologically active epoxyeicosatrienoic acids (EETs). The EETs are potent endogenous vasodilators and inhibitors of vascular inflammation. Inter-individual differences in the action of these substances might be important in the pathogenesis of cardiovascular diseases such as coronary artery disease (CAD) and myocardial infarction (MI). In the present study we analyzed the impact of a genetic variant in CYP2C8 on CAD and of MI in Bulgarian population. We conducted a case-control study to determine whether the common genetic variation rs890293 (CYP2J2*7) in CYP2J2 gene was associated with the risk of CAD and MI. We analyzed 99 patients with MI and 377 controls for a potential correlation of the CYP2J2 polymorphism G-50T and a history of MI. 96 of these 99 patients were tested for the presence of polymorphisms CYP2C8 To evaluate the genotypes of the samples real time PCR with predesigned TaqMan SNP Genotyping Assays (Applied Biosystem) for rs890293 (Assay ID: C___9581699_20) was used. Studied the variation of allele polymorphism CYP2J2 *7 and CYP2C8 *3 on the balance of Hardy-Weinberg (Hardy-Weinberg) and the frequency of the T allele with X2 test. The resulting p-values for both polymorphism (for CYP2C8 *3, P=0.7901 and P=0,0670 CYP2J2*7) indicates that the distribution of T allele CYP2C8 * 3 with high probability close to balance Hardy Weinberg, than in the CYP2J2 *7. The chances for people with T-allele polymorphism in the CYP2C8 *3, MI occur on average 1.7 times higher than those who did not carry this allele. CI of OR (2,8746-1,0334) with 95% probability. CI indicated that it could be argued with a 95% probability that the presence of T allele in CYP2C8 *3 increases the risk of MI. The analysis of data obtained P=0,9489, OR=0,9717, CI (0,4034-2,3404) showed no indications T allele CYP2C8 * 3 has completely different shanosove for the occurrence of stroke in men and women. The analysis of results shows that polymorphism CYP2C8 * 3 is important for the occurrence of MI compared with CYP2J2 * 7 participants in the survey.

W120

ASSOCIATION BETWEEN RENAL FUNCTION AND MORTALITY IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Background: Low glomerular filtration rate (GFR) has been associated with adverse clinical outcomes in patients with broad range of cardiovascular diseases, including heart failure, stable coronary artery disease and acute coronary syndromes. Therefore, the aim of this study was assess the effect of kidney function on in-hospital mortality in patients with acute myocardial infarction (AMI).
Methods: This historic cohort included 103 consecutive patients admitted with final diagnosis of AMI at the University Hospital of Santa Maria-RS, Brazil, during 12 months (September 2008 to August 2009). Patient's clinical characteristics, clinical diagnosis and death were recorded by reviewing the hospital's medical registry. Serum creatinine was recorded using the laboratory database. GFR was estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. The primary outcome assessed was all-cause in-hospital mortality.
Results: The mean age of the cohort was 62.2 ±12.4 years and 63 (61.2%) were male. The mean estimated GFR was 86.3± 36.8 mL/min/1.73m². The patients were categorized into 2 groups according to the estimated GFR: GFR ≥ 90 mL/min/1.73m² (n=60) and GFR <90mL mL/min/1.73 m² (n=43). In-hospital mortality rates were approximately 3 times higher in patients with GFR < 90 mL/min/1.73m² (10.6% vs. 3.8%, log rank 4.917, P=0.027). Estimated GFR < 90 mL/min/1.73m² was identified as a powerful independent predictor of in-hospital mortality (hazard ratio=2.603, 95% confidence interval 1.078-6.289; P=0.033).
Conclusions: These findings allow concluding that even mildly decrease in kidney function is associated with in-hospital mortality in patients with AMI. Thus, the GFR evaluation can be useful to identify higher risk patients, improving risk stratification in the setting of AMI.

W121
METABOLIC INDICES IN NIGERIAN PREGNANT WOMEN WITHIN THE FIRST TRIMESTER

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Background: Metabolic alterations occur in pregnancy and measures of adiposity are thought to contribute to pre-eclampsia. However, the contribution of adiposity to the disease is poorly understood. The relationship among metabolic indices in first trimester of pregnancy was investigated.
Methods: Two hundred and fifty six pregnant women aged 30.72±268 (S.E.M) were recruited in their first trimester from antenatal clinics of three major hospitals in South West Nigeria. Weight, body mass index (BMI) and percentage body fat (PBF) were determined using Omron BF400 Body Fat Monitor (Japan). Height, blood pressure –systolic (SBP) and diastolic (DBP), gestational age (GA), fasting plasma glucose (FPG), waist circumference (WC), hip circumference (HC) and waist to hip ratio (WHR) were determined using standard methods. Data were analysed using Pearson Correlation Coefficient and were significant at P <0.05.
Results: Significant correlations were observed among adiposity measures. BMI, weight, HC, and WC correlated positively with SBP, DBP and PBF. WHR correlated positively with DBP and PBF while PBF correlated positively with SBP but negatively with DBP. FPG correlated positively with WC but negatively with GA. Age correlated positively with measures of adiposity (PBF, weight, WC,HC, WHR and BMI) (P<0.05)
Conclusion: Measures of adiposity may contribute to metabolic alterations observed in hypertensive disorders in pregnancy. Early detection in the first trimester might improve pregnancy outcome.

W122
THE ASSOCIATION BETWEEN HSCRP AND TRADITIONAL RISK FACTORS OF CARDIOVASCULAR DISEASE IN NIGERIAN HIV-INFECTED PATIENTS WITH AND WITHOUT ANTIRETROVIRAL THERAPY

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Background: Worldwide, cardiovascular disease (CVD) is a significant cause of death, with atherosclerosis being the pathological process that leads to it. This process involves a combination of vascular endothelial dysfunction, dyslipidaemia and chronic inflammation. High sensitivity C-reactive protein (hsCRP) is routinely monitored as a marker of low grade vascular inflammation. Several studies have demonstrated that increased hsCRP is positively associated with a risk of future coronary heart disease. The chronic inflammatory state of human immunodeficiency virus (HIV) infection predisposes patients to increased risk of atherosclerosis, and therefore CVD. Thus, the aim of this study was to determine the association between hsCRP and traditional risk factors of CVD in HIV-infected patients.
Method: This was a cross-sectional study of 200 participants (119 females and 81 males) consisting of 100 HIV-positive treatment-naïve (HIVnaive) and 100 HIV-positive antiretroviral treated (HIVtreated). Age, blood pressure, body mass index (BMI), hsCRP, fasting plasma glucose, and lipid profile were determined. Pearson's correlation was used to determine association and p ≤0.05 was considered statistically significant.
Results: The mean ± SD hsCRP levels were HIVnaive: 6.3±6.6 mg/L; HIVtreated: 2.5±3.4 mg/L. In the HIVnaive subjects, there was a significant positive correlation between hsCRP and age (r=0.252, P=0.018), BMI (r=0.259, P=0.027), total cholesterol (TC) (0.212, P=0.049); and negative correlation with HDL-cholesterol (HDL-C) (r=-0.398, P=0.000). There was no significant association (P >0.05) with the other risk factors in HIVnaive and all risk factors in HIVtreated subjects.
Conclusion: The HIVnaive subjects had higher hsCRP levels with significant association with increasing age, BMI, TC, and low HDL-C. There was no significant association between hsCRP and the traditional risk factors for CVD in the HIVtreated subjects. The unhindered HIV may be responsible for causing chronic inflammation which may be averted with antiretroviral therapy.

W123

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL) AND ACUTE KIDNEY INJURY IN CARDIAC SURGERY

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Background: Acute Kidney Injury (AKI) occurs frequently after cardiac surgery. It occurs with an incidence of 5 to 20% and its development is related to a prolongation of intensive care unit stay and hospital stay, to an increased risk of use of continuous replacement renal therapy (CRRT) and a worsening of the prognosis. AKI is a potentially reversible condition with early diagnosis. For this purpose, in last years there have been several studies that have identified the neutrophil gelatinase-associated lipocalin granulocyte (NGAL) as a protein which may indicate a kidney damage with greater sensitivity and specificity. The aim of this study is to verify that AKI (defined according to the RIFLE criteria), caused by hemodilution in extracorporeal circulation (ECC), may be identified early by NGAL.
Methods: Serial samples of blood and urine were obtained from 50 patients undergoing cardiac surgery in ECC. Urinary NGAL (uNGAL) and the hemoglobine (Hb) levels were measured before ECC and 30, 60 and 90 min from the beginning of ECC. uNGAL was determined by a chemiluminescent immunoassay (ARCHITECT i1000SR®, Abbott Diagnostics) while Hb levels was determined by blood gas analyzer (Stat Profile pHOx, Nova Biomedical). In order to evaluate the AKI's onset, the postoperative sCreatinine was measured by a colorimetric assay, Jaffé reaction, (Cobas c501, Roche/Hitachi).
Results: 6 patients developed AKI. An inverse correlation between the lowest Hb and the highest uNGAL values intra ECC has been observed (r^2=0.08, P=0.004). Results showed an inverse correlation between the lowest Hb value in ECC and the postoperative creatinine's peak (r^2=0.065, P=0.04) and a linear correlation between the highest uNGAL value in ECC and the postoperative creatinine's peak (r^2=0.2, P=0.001).
Conclusions: Monitoring intraoperative uNGAL and Hb values could be useful to early identify AKI after major cardiac surgery, thus allowing a more timely treatment.

W124

THE HIGH SENSITIVITY C-REACTIVE PROTEIN AND LOW DENSITY LIPOPROTEIN CHOLESTEROL IN RISK PREDICTION OF ISCHEMIC HEART DISEASE

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Background: Atherosclerosis is an inflammatory process and inflammation plays a significant role in the development and progression of Coronary artery disease. Cholesterol screening has been used for decades to identify individuals at risk for heart disease. It had been shown that cholesterol profiles are not elevated in almost 50% of the more than one million people who develop myocardial infarction. It is now recognized that inflammation plays a significant role in the development and progression of Ischemic Heart Disease and strokes. Conventional testing for CRP was not sensitive enough to detect the persistent low levels of inflammation present in people with early CHD. A newer test, the high sensitivity-CRP assay (hs-CRP), is now being used to detect these very small elevations of CRP.
Methods: In this study we have taken 100 Patients of IHD and 50 Apparently healthy Normal subject. Fasting blood samples were taken for investigations like hs-CRP (immunoturbidimetric method), LDL-C (direct method), HDL-C (direct method), Total Cholesterol, Triglyceride, Plasma glucose, Ck-MB and analysis was done on fully auto analyzer- Miura (ISE-Italy) at NABL(ISO 15189:2007) accredited, Clinical Biochemistry Section, Laboratory services Sir T. Hospital, Bhavnagar. The study was approved by IEC/IRB and written informed consent taken.
Results: We observed that the hs-CRP was significantly elevated in Ischemic Heart Disease patients (P <0.01) than the control group. Though LDL-C was also elevated in Ischemic Heart Disease patients significantly (P <0.05) as compare to the control group, 55 patients with normal LDL-C had Ischemic Heart Disease but their hs-CRP level was significantly high. The specificity and sensitivity of hs-CRP were 1.0 (100%) & 0.97 (97%) and same for LDL-C were 0.66 (66%) & 0.44 (44%) respectively.
Conclusions: The study identified that the half of the Ischemic Heart Disease patients with normal LDL-C levels had significantly elevated levels of hs-CRP. This shows that hs-CRP is more sensitive and specific predictor of Ischemic Heart Disease than LDL-C and after further evaluation global guideline may be issued for its application as a better & single predictor of Ischemic Heart Disease than conventional Lipid Profile.

W125

CORRELATION BETWEEN HIGH NT-PROBNP AND RED CELL DISTRIBUTION WIDTH (RDW) IN CLINICAL SETTING

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Background: Several underlying diseases, such as iron or B12/folate deficiency, hemolytic anemia, alcohol abuse, liver and renal diseases, and inflammatory bowel disease, are associated with high RDW. Some of them affect the mean corpuscular volume (MCV) also. Elevated RDW independently predicts adverse outcomes in heart failure, cancer, obesity, pulmonary hypertension, and community acquired pneumonia. We sought to explore the correlation between high NT-proBNP and RDW adjusted by MCV in clinical setting.
Methods: We enrolled 56 consecutive patients (30 males and 26 females; median age 77 years), with acute dyspnea in which the dosage of NT-proBNP was deemed necessary. NT-proBNP was measure in EDTA plasma, using the VITROS NT-proBNP immunometric immunoassay (Ortho-Clinical Diagnostics). We used a cut-off of 125 pg/ml for NT-proBNP, 14.5% for RDW and 81.0–94.0 fl for MCV. Spearman rank correlation coefficient was calculated to study association (not just linear association) between NT-proBNP vs. RDW, and NT-proBNP vs. MCV. Analysis of covariance (ANCOVA) was applied to analyze differences in mean values of NT-proBNP between patients who have RDW less than 14.5% and patients who have RDW values greater than 14.5%, adjusted by MCV and age.
Results: Non significant correlation was found between NT-proBNP and MCV (rs=0.079; p-value=0.5616), but slightly significant correlation was found between NT-proBNP and RDW (rs=0.264; p-value=0.0496). Significant differences (p-value=0.0467) were found between mean values of NT-proBNP for patients with RDW less than 14.5% (2222.17 pg/ml), and patients with RDW greater than 14.5% (4136.56 pg/ml) after controlling by MCV and age, which regression coefficients were not significantly different from 0.
Conclusions: In patients with acute dyspnea high RDW was associated with high NT-proBNP values, and patients with RDW greater than 14.5% had much greater mean values of NT-proBNP than patients with RDW less than 14.5%. This leads to suspect that patients with acute dyspnea with RDW value above 14.5% very probably have a high level of NT-proBNP. These results were not influenced by MCV. Probably, high RDW is useful for clinical decision making in patients with acute dyspnea.

W126

SCREENING OF LIPIDIC PROFILE IN STUDENTS OF UNIVERSITY OF ALGARVE

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Background: The lipid profile analysis evaluates the risk of myocardial infarction or stroke caused by obstruction of blood vessels (atherosclerosis). We aimed to asses the prevalence of dyslipidemia in students of the University of Algarve (UAlg), with ages between 18 and 25 years old, and analyze its relationship with blood pressure, BMI, lifestyle and family history.
Methods: After informed consent 87 students, 68 (78.2%) female and 19 (21.8)% male, from various campi of UAlg, were included in the present study. The students filled out a questionnaire about lifestyle and family history and we performed a collection of a whole blood sample to determine the lipid profile and glucose. We also recorded the blood pressure and BMI. The data were analyzed using SPSS 17.0, we performed descriptive analysis and the possible relationships between the occurrence of dyslipidemia and the other variables were analyzed using Chi-Square test; significant relationship was assumed for $P < 0.05$.
Results: The prevalence of dyslipidemia in students was 47.1%, been 52.6% in males and 19.6% in females. Among the variables studied, the unique relationship observed was the oral contraceptive use and prevalence of dyslipidemia ($P=0.001$).
Conclusions: The prevalence of dyslipidemia in students of University of Algarve is very high and there is no single cause for it. There is an urgent need to promote a healthier lifestyle in students.

W127

25-HYDROXYVITAMIN D LEVEL, BIOMARKERS OF ENDOTHELIAL DYSFUNCTION AND SUBCLINICAL ORGAN DAMAGE IN ADULTS WITH HIGH NORMAL BLOOD PRESSURE/GRADE 1 HYPERTENSION

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Background: Low 25-hydroxyvitamin D has been associated with higher risk of hypertension. The mechanism underlying the association of 25 (OH)D with blood pressure (BP) is not well understood, but besides the regulation of renin-angiotensin-aldosterone system seems to involve influence on endothelial function, insulin sensitivity and cardiac remodeling. We assessed the association of 25 (OH)D3 level with biomarkers of endothelial function, subclinical organ damage and insulin resistance in 122 adults with normal BP or newly diagnosed hypertension without cardiovascular and kidney disease.
Methods: Patients were classified based on ambulatory BP monitoring: in 65 high normal BP/grade 1 hypertension was diagnosed, 57 had normal BP. In all laboratory assays, echocardiography, pulse wave velocity (PWV), intima-media thickness (IMT), left-ventricular mass (LVM) measurements were performed.
Results: Serum 25(OH)D3 was significantly lower in patients with high normal BP/grade 1 hypertension than in the reference group. In the study group 63.6% had 25(OH)D3<20ng/mL, 58.1% increased PWV, 41.8% insulin resistance. Vitamin D in the study group correlated weakly but significantly with systolic BP (r=-0.39), PWV, IMT (r=-0.33) and diastolic BP (r=-0.26). Multiple linear regression analysis in the study group has shown that model 1 with 25(OH)D3, intercellular adhesion molecule (ICAM), left ventricular mass (LVM) as independent variables explained 38% of SBP variation. Two-variable models with 25(OH)D3 and either PWV or IMT explained 21% and 23% of SBP variation. In each model 25(OH)D3 contribution accounted for 15%.
Conclusion: 25-hydroxyvitamin D level may influence on SBP by mechanisms involving endothelial function, cardiac remodeling and arterial stiffness.

W128

DIAGNOSTIC PERFORMANCE OF THE ARCHITECT GALECTIN-3 IMMUNOASSAY

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Background: Galectin-3 is a novel cardiovascular biomarker which has shown to be useful in assessing the prognosis of patients with heart failure. We describe the analytical validation of an automated, microparticle-based chemilumescent immunoassay method designed to measure Galectin-3 in human serum and EDTA plasma.
Methods: Assay performance characteristics such as precision, analytical sensitivity and specifity were measured following the protocols by the Clinical Laboratory Standards Institute (CLSI). Samples from apparently healthy blood donors were included in the normal range study (n=130). Method comparison against Galectin-3 ELISA from BG Medicine (BGM) was evaluated using surplus clinical specimens from patients with normal and elevated BNP levels.
Results: The detection limit for this assay was 0.58 ng/mL, the limit of quantitation was <6 ng/mL. In the precision study using the assay controls total coeficient of variation was below 6%. The assay was linear upon dilution. In apparently normal blood donors 95 percentile was 17.5 ng/mL with women having higher values than men (95 percentile: 17.9 ng/mL versus 15.4 ng/mL). Comparison with the BGM Galectin-3 assay yielded a linear correlation coefficient of 0.96. Passing Bablok fit was found to be y=1.23x+0.95, n=151.
Conclusions: The ARCHITECT Galectin-3 assay measures Galectin-3 rapidly, accurately, and precisely in human serum and EDTA plasma. It can provide useful improvements in assessing the prognosis from patients with chronic heart failure.

W129
TROPONIN T IN PATIENTS WITH CHEST PAIN

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Introduction: New high sensitivity cardiac troponin (hscTnT) assays which can measure the 99th percentile of the normal reference population are being introduced. Lowering the diagnostic threshold may be beneficial but will increase the classification of myocardial infarction and provide additional challenges in the interpretation of results. Our study is unusual in that we investigated the impact of the introduction of the hscTnT assay (Roche diagnostics, UK) in the emergency department of a non-University hospital ie in an unselected cohort of patients.
Methods: The distribution of hscTnT was determined within 205 community patients, not being investigated for acute coronary syndrome. Two hundred consecutive patients admitted with suspected acute coronary syndrome were stratified by the hscTnT assay into 4 groups: ≤5 ng/ (n= 63),>5 to ≤14 ng/L (n=39), >14 to <60 ng/L (n= 59), ≥60 ng/L (n= 39). HscTnT was measured at 8-12 h following admission. Clinical characteristics, cardiovascular risk factors, drugs on admission, TIMI risk scores, ECG, and management during admission was assessed. The diagnosis was made by clinicians blinded to the results of hscTnT values <60 ng/L.
Results: This study suggests that in the local random population the 99th percentile was 18.8 ng/L. Adoption of the manufacturer's lower cut-off level of 14 ng/L will increase the number of patients with a possible diagnosis of myocardial infarction by 100%. Patients with hscTnT ≤ 5 ng/L had a lower TIMI risk score, were younger and less likely to have a previous history of ischemic heart disease (IHD), or cardiac risk factors. In this population 61% of patients with hscTnT values >14 to <60 ng/L were diagnosed with noncardiac causes, 19% with angina but with a cut-off value of 60 ng/L, were less likely to be referred for further cardiac assessment or treatment for acute coronary syndrome.
Conclusions: A hscTnT value in the range >14 to <60 ng/L was not in itself diagnostic of dynamic cardiac damage and clinical decisions may depend on serial measurements. In an unselected group of patients, lowering the threshold for hscTnT can potentially identify 19% of patients with hscTnT values >14 to <60 ng/L who would be referred for further cardiac assessment.

W130
PRODUCT OF SERUM CALCIUM AND PHOSPHORUS (CA X PO4) AS PREDICTOR OF CARDIOVASCULAR RISK IN PREDIALYSIS PATIENTS.

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Background: Chronic Kidney Disease (CKD) has become a global epidemic and 10.2% prevalence has been reported in Nepal. Cardiovasular diseases (CVD) are major cause of death in CKD patients. Apart from traditional and new CVD markers, in CKD patient, there has been increasing concern about vascular ossification. Many studies suggest product of serum calcium and phosphorus (ca x po4) as its marker. So, aim of this study was to assess the utility of ca x po4 in prediction of CVD in predialysis CKD patients.
Methods: This cross-sectional study, conducted in TU Teaching Hospital, Nepal included 150 pre-dialysis CKD patients and 150 healthy controls (75 male and 75 female both), with mean±SD estimated GFR 18.1±7.9 & 91.2±16.2 mL/min respectively. CKD was defined as per NKF-KDOQI guideline and GFR was estimated by revised MDRD formula. We measured various biochemical analytes in fasting blood, corrected calcium for albumin & performed electrocardiogram. CVD risk was measured by traditional and CKD related CVD risk factors, presence of multiple risk factors (NCEP-ATP III) and Framingham risk score. Data were analyzed using Chi-square test, t-test, ANOVA and logistic regression. P-value of <0.05 was considered significant.
Results: CKD cases had higher Ca x po4 than controls- 52.7 vs 30.6 mg2/dl2 respectively (P=0.013). Ca x po4 had positive correlation with total cholesterol (P=0.007), triglyceride (P=0.01), LDLc (P=0.001), non-HDLc (P <0.001), oxidized LDL (P <0.001), lipoprotein a (0.002), parathyroid hormone (P=0.001) and negative correlation with HDLc (P=0.04). Similarly, ca x po4 had positive association with presence of hypertension (P=0.023), multiple risk factors (P <0.001), hyperhomocysteinemia (P=0.017), systemic inflammation (p <0.001), dyslipidemia (P=0.031), anemia (P=0.033), metabolic syndrome (P=0.019) and left ventricular hypertrophy (P=0.021). After adjustment for age, gender, Diabetes, smoking and hypertension, cases in highest quartile of ca x po4 had 1.52 & 2.01 times higher risk for general CVD (P=0.031) & stroke (P=0.009) than those in lowest quartile as predicted by Framingham risk score.
Conclusion: CKD patients had higher ca x po4 than controls. And increased ca x po4 is independent predictor of presence of CVD risk factors and future CVD in CKD.

W131
INDUCIBLE NITRIC OXIDE SYNTHASE GENE POLYMORPHISM AND ENDOTHELIAL DYSFUNCTION IN CORONARY ARTERY DISEASE

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Background: Patho-physiological processes in coronary artery disease are influenced by genetic factors. Vascular function is affected by endothelial derived factors which lead to the activation of Inducible Nitric Oxide Synthase (iNOS). The aim of this study was to look for a relationship between the C150T polymorphisms of the iNOS and endothelial dysfunction in Coronary Artery Disease (CAD).
Methods: A total of 300 subjects (150 patients with stable CAD and 150 healthy volunteer) were included in this case-control study. Their blood samples were analyzed for Nitric Oxide, Endothelin and iNOS gene polymorphism.
Results: Mean (±SEM) NO levels in study group were significantly lower compared with controls (16.5±2.9 vs 26.8± 3.6, P <0.001) and Endothelin levels were significantly higher compared with controls (29.1±5.08 vs 12.5±3.1, P <0.001). Genotype/allele distribution was significantly different among groups (CC, 118 (78.6%) vs 141 (94%); CT, 32 (21.3%) vs 9 (6%); respectively in study and control groups). Additionally, NO levels in different genotypes were significantly different in Study group (CC, 13.4±0.12; CT, 9.21±0.02; P <0.001).
Conclusion: Endothelial dysfunction plays a major role in Coronary Artery Disease. The presence of T allele might be associated with increased risk of Coronary artery disease (CAD).

W132
COULD DETERMINATION OF BCL-2 AND CASPASE-3 ACTIVITY INDICATE PLAQUE EVOLUTION IN ISCHEMIC HEART DISEASE PATIENTS?

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Background: Apoptotic cell death may play a critical role in a variety of cardiovascular diseases, especially in those developing on the basis of atherosclerosis. The goal of this study was to compare the activity of caspase-and values of Bcl-2 protein in sera in patients with various forms of ischemic heart disease, and to correlate these markers with inflammatory and lipid parameters.
Methods: We studied 30 patients with chronic stable angina pectoris (SAP), 27 with unstable angina pectoris (USAP), 39 with acute ST-elevation myocardial infarction (STEMI) and 27 age-matched healthy volunteers (Control group). Caspase-3 activity was determined by a colorimetric commercially available method while serum Bcl-2 concentrations were determined using commercially available immunoassays (ELISA).
Results: Caspase-3 was significantly higher only in the USAP group (0.122±0.062 μmol/mg protein, P <0.05) in comparison with the control group (0.092±0.022 μmol/mg protein). Concentrations of Bcl-2 were significantly higher in patients with SAP (0.310±0.075 ng/mL) and USAP (0.329±0.102 ng/mL) compared to healthy (0.250±0.069 ng/mL, P <0.01) and the STEMI (0.266±0.041 ng/mL, P <0.01) groups. ROC curve analysis showed that Bcl-2 had the best characteristics in patients with SAP and USAP and represents the best indicator of atherosclerotic plaque activity. However, Bcl-2 could not be a marker of patients' stratification because there was no significant difference between areas of Bcl-2 curves of these two patient groups. These results suggest that simultaneous determination of caspase-3 activity and Bcl-2 can indicate plaque evolution from stable to unstable one.
Conclusions. The studied markers of apoptosis present valuable parameters in evaluation of atherosclerotic plaque acivity and a new targets for drug therapy.

W133
BIOMARKERS OF APOPTOSIS AND THEIR SIGNIFICANCE IN ISCHEMIC HEART DISEASE PATIENTS

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Background: Apoptotic cell death may play a critical role in a variety of cardiovascular diseases, especially in those developing on the basis of atherosclerosis. The goal of this study was to compare the activity of caspase-3, the values of soluble forms of Fas/APO1 and FasL and Bcl-2 protein in sera in patients with various forms of ischemic heart disease, and to correlate these markers with inflammatory and lipid parameters.
Methods: We studied 30 patients with chronic stable angina pectoris (SAP), 27 with unstable angina pectoris (USAP), 39 with acute ST-elevation myocardial infarction (STEMI) and 27 age-matched healthy volunteers (Control group). Caspase-3 activity was determined by a colorimetric commercially available method, while serum Fas/APO1, FasL and Bcl-2 concentrations were determined using commercially available immunoassays (ELISA).
Results: Caspase-3 was significantly higher only in the USAP group (0.122±0.062 μmol/mg protein, P <0.05) in comparison with the control group (0.092±0.022 μmol/mg protein). Fas/APO-1 values were significantly higher in the STEMI group (6.981±2.689 ng/mL, P <0.01) than in USAP (5.627±2.270 ng/mL) and healthy (5.092±1.252 ng/mL). Concentrations of Bcl-2 were significantly higher in patients with SAP (0.310±0.075 ng/mL) and USAP (0.329±0.102 ng/mL) compared to healthy (0.250±0.069 ng/mL, P <0.01) and the STEMI (0.266±0.041 ng/mL, P <0.01) groups. ROC curves analysis showed that Bcl-2 was the best marker an atherosclerotic plaque acivity in SAP and USAP patients.
Conclusions. The studied markers of apoptosis present valuable parameters in evaluation of atherosclerotic plaque acivity and a new targets for therapy.

W134
EFFECTS OF VITAMIN D SUPPLEMENTATION ON COUNTERREGULATORY HORMONES REGULATING PHOSPHOCALCIC METABOLISM IN ELDERLY PATIENTS WITH CHRONIC HEART DISEASE: INVESTIGATING AROUND THE REGULATION OF FGF23. FIRST EVIDENCES AFTER 3 MONTHS OF TREATMENT

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Background: One effect of 25-hydroxyvitamin D (25OHD) supplementation in patient with chronic heart failure (HF) is the reduction of secondary hyperparathyroidism, the excess of PTH underlies cardiomyocytes hypertrophy; several study suggest that the early increase of FGF23 would cause the reduction of 25OHD levels and hyperparathyroidism.
Objective: To investigate the short-term effects of 25OHD administration to patients with HF on physical performance at 6mwt test, on PTH and FGF23 serum levels.
Methods: Randomized, double-blind, placebo-controlled trial conducted among 30 elderly (mean age = 78+7) with HF between March and July 2012 in Modena, Italy. Participants were randomly assigned to receive an initial dose of 30000IU oral vitamin D3 then 50000IU/month (n=16) or placebo administered in an identical dosing regimen (n=14). The primary end point was distance walked at the 6mwt, secondary end points were the hormonal levels.
Results: The baseline mean performance at 6mwt was 237+/-70 m; the mean baseline hormonal levels were: 25OHD= 9,5+/-6 ng/mL, PTH= 81+/-53 pg/ml, FGF23= 35,3+/-31 pg/mL. After 3 month a statistically significant difference in the physical performance was not observed (treated= 227+/-97 m, placebo= 259+/-73 m), vitamin D administration increased serum level to 36+/-6 ng/mL; an increase in the 25OHD level in the placebo group (13+/-7 ng/mL) due to the sun effect in the last month of the trial was observed, but the difference was statistically significant only in the treated group. PTH mean level lowered to 51+/-21 pg/mL in the treated, remained unchanged in placebo group (80+/-63 pg/mL) with a statistically significant difference between groups. FGF23 mean level was 44,3+/-26 pg/mL in the treated and 58,4+/-39 pg/ml in the placebo group, the increase was statistically significant within each group, but the difference between groups was not so.
Conclusions: Vitamin D supplementation has corrected the secondary hyperparatyroidism and has produced an increase of FGF23 levels in patients with HF, confirming the the presence of a stimulatory effect of vitamin D on the synthesis of FGF23. Is non yet clarified the clinical utility of FGF23 altereations but this trials confirms the presence of a positive feedback.

W135

DIAGNOSTIC ACCURACY OF AQT 90 FLEX CARDIAC TROPONIN T IN THE ASSESSMENT OF ACUTE MYOCARDIAL INFARCTION

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Background: New sensitive cardiac troponin(cTn) assays allow early detection and exclusion of acute myocardial infarction (AMI). Improved diagnostic sensitivity is accompanied by a loss of specificity since many other acute and chronic causes of myocardial damage can result in increased cTn levels. The aim of the present study was to examine the diagnostic accuracy of a new sensitive cTnT assay (TnT AQT 90 Flex) for the diagnosis of AMI and to compare the results with our current routine cTnT assay (Roche hs-TnT).
Methods: Prospective multicentre study conducted in four hospitals of Spain during a two-month period. Patients were included if they presented to the ED with symptoms of AMI and the onset occurred within 8 h before presentation. Patients presenting with evident alternative diagnosis and those with chronic kidney disease stage 5 were excluded. AQT 90 cTnT (cTnTAQT) concentrations were determined in EDTA whole-blood samples obtained at presentation (T1) and at 3-6 h (T2) after the onset of symptoms. The reported analytical sensitivity cTnTAQT assay is 10 ng/L and the 99th percentile 17 ng/L (CV 15,2%). Sensitivity, specificity, NPV and PPV of the 99th percentile for diagnosis of AMI was calculated at T1 and T2 and the diagnostic accuracy of cTnTAQT at T1, T2 and its d value for the outcome of AMI was assessed by ROC curve analysis.
Results: A total of 180 patients were finally enrolled, with an adjudicated final diagnosis of AMI in 48 patients (27%), unstable angina in 29 (16%), cardiac noncoronary disease in 42 (23%) and noncardiac chest pain in 61 (34%). Of these patients, 123 (68%) had detectable cTnTAQT baseline levels. On admission, cTnTAQT clinical sensitivity and specificity for the diagnosis of AMI was 85% and 63% respectively with a NPV of 92%. The diagnostic accuracy (AUC) of baseline cTnTAQT concentration for the identification of AMI, was similar to that obtained with hs-cTnT (0,88 vs. 0,90; p=0,28). Both assays showed better diagnostic accuracy in women than in men: cTnTAQT (0,94 vs. 0,86), hs-cTnT (0,96 vs. 0,90).
Conclusions: Using this cTnT assay with improved sensitivity we were able to detect concentrations above the 99th in 73% of patients with a final diagnosis of AMI thus reducing time to diagnosis and initiation of therapy.

W136

ASSOCIATION OF SERIAL CHANGES IN GALECTIN-3 WITH ADVERSE CARDIOVASCULAR EVENTS IN AMBULATORY CHRONIC HEART FAILURE PATIENTS

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Background: Heart failure (HF) progresses substantially through adverse cardiac remodeling and fibrosis in response to cardiac injury/stress. Serial values of cardiac troponin (cTn) and natriuretic peptides are predictive of cardiovascular events in ambulatory HF patients, although further refinement and definition of prognosis is needed. Galectin-3 (gal-3) is involved in myofibroblast proliferation, tissue repair/fibrosis, and cardiac remodeling. Accordingly, we evaluated changes in gal-3 in conjunction with cTnT and BNP over time in chronic HF patients.
Methods: A cohort of 191 NYHA class III-IV HF patients was prospectively evaluated with serial specimens collected every 3 months over a 2-year period. Primary endpoints were death or cardiac transplantation; secondary endpoints were combined death/transplantation and HF-related hospitalization. Plasma galectin-3 (BG Medicine) was evaluated against contemporary cTnT (Roche 4th generation) and BNP (Shinogi) assays. Cut-points utilizing a 95th percentile (≥ 22.1 ng/mL) and a clinically defined optimal high-risk cut-point for gal-3 (>25.9 ng/mL) were evaluated.
Results: Time-dependent analyses (dichotomous and continuous variable) demonstrated a gal-3 cut-point of 25.9 ng/mL was strongly associated with an increased risk of death or transplantation (HR 2.9, 95% CI 1.7 to 5.0, P <0.0001) and combined death/transplantation/HF-hospitalization (HR 1.8, 95% CI 1.5 to 2.2, P <0.0001). In multivariate analyses adjusted for cTnT and BNP, galectin-3 no longer remained an independent predictor of primary (HR 1.7, 95% CI 0.96 to 3.0, P=0.07) endpoints compared to cTnT (HR 3.7, 95% CI 2.0 to 6.7, P <0.0001). Gal-3 retained significance in prediction of secondary endpoints (HR 1.6, 95% CI 1.3 to 1.9, P <0.0001), adding to the predictive accuracy of cTnT (HR 1.3, 95% CI 1.1 to 1.6, P=0.01) and BNP (HR 1.5, 95% CI 1.2 to 1.8, P=0.0003).
Conclusions: Serial monitoring of gal-3 adds to the diagnostic ability of cTnT and BNP in predicting mortality, transplantation and HF-related hospitalization in ambulatory chronic HF patients. A serial monitoring strategy including gal-3, cTnT, and BNP may identify patients at increased risk for events, allowing for further risk attenuation and reduction in mortality.

W137
HIGH-SENSITIVITY CARDIAC TROPONIN I IMMUNOASSAY CONDUCTED ON ANTIBODY-COATED SPOTS

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Background: Cardiac troponins (cTn) are the recommended biomarkers of myocardial infarction. A new generation of high-sensitivity assays can measure cTn also in apparently healthy individuals. Commonly occurring autoantibodies can interfere with the cTn detection. Therefore, our objective was to develop a high-sensitivity cTnI immunoassay based on an assay configuration previously reported not to suffer from cTnI-specific autoantibodies.
Methods: The developed immunoassay used spot coating (Ø 1.8 mm) containing three capture antibodies/antibody fragments directed against the N-terminus, midfragment and C-terminus of cTnI and an europium chelate-labeled tracer antibody against the C-terminus. Following a 3-h sample incubation, cTnI was quantified directly from the washed well surface.
Results: The limit of blank (LoB) and dynamic range of the developed cTnI assay were 0.0015 and 0.0015-50 ng/L, respectively. Intra-assay imprecision was <10%. The measured cTnI concentrations were above the LoB in 25% of serum samples obtained from apparently healthy volunteers (n=32, median age 30 years).
Conclusions: We were able to create a highly sensitive cTnI assay by utilizing antibody-coated spots. This assay, designed to be minimally affected by cTnI-specific autoantibodies, can serve as an important tool for exploring the possible relationship between circulating autoantibodies and elevated cTnI.

W138
EVALUATION OF AUTOANTIBODY PREVALENCE IN PATIENTS WITH SUSPECTED MYOCARDIAL INFARCTION WITH IMPROVED IMMUNOASSAY FOR DETECTING AUTOANTIBODIES TO CARDIAC TROPONIN

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Background: Autoantibodies to cardiac troponins (cTnI and cTnT) have been found in 5%-20% of healthy individuals and cardiac patients. These autoantibodies can interfere with the measurement of cTnI by immunoassays used for the diagnosis of myocardial infarction (MI). In the present study, an improved version of a previous autoantibody assay was used to evaluate the autoantibody prevalence in patients presenting to an emergency department with suspected MI.
Methods: We analyzed 510 admission samples collected from suspected MI patients with two sandwich-type immunoassays. Autoantibodies were first bound to added troponin complex (ITC) and the formed complexes were subsequently captured on a streptavidin coated microtiter well by biotinylated cTnI-specific antibodies. Capture epitopes 23-29 and 190-196 were used in the old assay, and epitopes 26-35 and 137-148 in the new assay. Finally, bound autoantibodies were detected with europium labeled anti-human antibody. Autoantibody positivity was defined as ≥100 counts after the background correction (no ITC added) when the T-test gave a P value <0.05.
Results: Sample specific backgrounds were lower with the new assay (median 1225 counts, 25-75 percentile 973-1635 counts) than with the old assay (median 2693 counts, 25-75 percentile 2104-4043 counts) (P <0.001). In addition, net signals of autoantibody positive samples were higher for the new assay (median 5076 counts, 25-75 percentile1953-17754 counts) than for the old assay (median 3921 counts, 25-75 percentile1326-11909 counts) (P <0.001). From all patients, 9.2% were autoantibody positive with the new assay and 7.3% with the old assay.
Conclusions: More autoantibody positive patients were detected with our new, more sensitive autoantibody assay. Autoantibodies are common in patients evaluated for suspected MI and can interfere in state-of-art cTn assays. This fact should be acknowledged by clinical chemists, physicians and kit manufacturers.

W139

IMPORTANCE OF OXIDATIVE STRESS IN PATIENT WITH DEEP VEIN THROMBOSIS

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Background: Venous thromboembolism (VTE) is a complex serious vascular disease with multifactorial pathogenesis. The first and more common manifestation of VTE is deep venous thrombosis (DVT). The second and more serious manifestation, pulmonary embolism (PE), occurs as a complication of DVT. We aimed to investigate the role of oxidative stress and asymmetric dimethylarginine (ADMA) on the development of DVT. The most important difference of the current study from the previous studies is the investigation of the contribution of antioxidant enzymes glutathione peroxidase and catalase.
Methods: 35 patients with DVT and 34 healthy subjects were studied. The two groups were similar characteristics in terms of age, body weight index and gender. Diagnosis of DVT was confirmed by physical examination, d-dimer and doppler ultrasonography. DVT was eliminated at levels lower than 500 μmol/L of d-dimer. In the study group, there were 21 female and 14 male patients ranging in age from 20 to 64 years. The control group who were not diagnosed with DVT and cardiovascular disease comprised 34 apparently healthy volunteers (20 female and 14 male), ranging in age from 22 and 73 years.
Results: Although serum MDA levels were significantly higher in DVT group than control group (P=0.005), vitamin B6 levels were lower in study group (P=0.009). However, there were no differences between study and control subjects with regard to serum ADMA, catalase, GSH-Px, vitamin B12, and folic acid (P >0.01). No correlations between parameters related with control group were observed. On the other hand, correlations between parameters related with DVT patients were observed.
Conclusions: This study reveals that patients with DVT have increased oxidative stress compared to healthy controls; however, DVT does not show any effect on serum concentrations of ADMA. Thus, ADMA does not seem to represent a risk factor in the development of venous thrombosis. Though GSH-Px deficiency increases risk of arterial thrombosis, its deficiency may not seem to represent a risk factor for a prothrombotic state in the development of venous thrombosis.

W140

PRESENCE OF PREFORMED ANTI-HLA ANTIBODIES ENHANCES PREDICTIVE VALUE OF PREGNANCY ASSOCIATED PLASMA PROTEIN A IN HEART TRANSPLANT RECIPIENTS

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Background: Preformed anti-HLA antibodies (anti-HLA) are associated with risk of graft rejection after solid organs transplantation. High pre-transplant pregnancy-associated plasma protein A (PAPP-A) level, a modulator of IGF-I bioavailability, is a predictor of adverse outcome in patients with ischemic heart disease. The aim of the study was to evaluate the association between presence of preformed anti-HLA, pre-transplant PAPP-A and outcomes in pts after heart transplantation (HTx).
Methods: The study included 65 heart recipients, 48 male, aged 39.3±7.0 years which were monitored on cardiovascular complications (cardiac allograft vasculopathy, acute cellular and antibody-mediated rejection) development. Presence of anti-HLA class I and II and PAPP-A level were detected by ELISA prior to HTx.
Results: During follow-up (108 months after HTx) cardiovascular complications developed in 26 (40%) pts. Preformed anti-HLA class I and/or II were detected in 17 (65.4%) pts with complications and only in 1 (2.6%) patient without those. 30 pts (46.2%) with high pre-transplant PAPP-A level (above median; ≥11 mIU/l) had >4-fold higher risk cardiovascular complications than pts with low PAPP-A (RR 4.1; 95% CI: 1.18 to 25.9, P <0.01). Detection of both high PAPP-A and anti-HLA in 26 (40%) pts before HTx significantly increased the risk of cardiovascular complications (RR: 9.64, 95% CI: 1.39; 66.64, P=0.001).
Conclusion: Preformed anti-HLA and PAPP-A are associated with development of cardiovascular complications after HTx. Presence of anti-HLA enhances predictive value of PAPP-A for outcomes in heart recipients.

W141

DIFFERENT PREDICTIVE SIGNIFICANCE OF PLACENTAL GROWTH FACTOR, SOLUBLE CD40 LIGAND, AND PREGNANCY-ASSOCIATED PLASMA PROTEIN A FOR CARDIOVASCULAR COMPLICATION DEVELOPMENT AFTER HEART TRANSPLANTATION

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Background: Biomarkers of neoangiogenesis (PlGF), thrombosis (sCD40L), and a modulator of IGF-1 bioavailability (PAPP-A) are significant risk factors of cardiovascular complications in patients with ischemic heart disease. The study was aimed to determine the role of PlGF, sCD40L, and PAPP-A in cardiovascular complication development in patients after heart transplantation (HTx) using a multivariate analysis.
Methods: 76 heart transplant recipients (67 men, aged 34.4±12.3 years) were followed up to 120 months after HTx. Endpoints of the study were acute cellular (ACR), antibody-mediated rejection (AMR), diagnosed by endomyocardial biopsy, as well as cardiac allograft vasculopathy (CAV), verified by coronary angiography. Pretrasplant concentrations of PlGF, sCD40L, and PAPP-A were measured using ELISA.
Results: During follow-up, episodes of ACR, AMR, and CAV occurred in 24 patients (31.58%) at 7.5±10.2 months after HTx, and were detected more often in recipients with pretransplant PlGF, sCD40L, and PAPP-A levels above median: in 18 recipients with PlGF >12 pg/mL; in 16 recipients with sCD40L >1.6 ng/mL, and in 20 recipients with PAPP-A >11 mIU/l (log-rank test P=0.01, P=0.02, P=0.0006 resp.). Cox regression analysis revealed that an independent, statistically significant risk factor was PAPP-A level (HR 4.2, 95% CI:1.33;13.24, Chi-square 14.97, P=0.002).
Conclusion. A measurement of pretransplant PAPP-A concentrations as an independent factor might be useful to identify patients at high risk of CAV development, as well as severe episodes of ACR and AMR after HTx.

W142

VALUE OF STUDYING THE COMPLEMENT SYSTEM IN CHRONIC HEART FAILURE PATIENTS

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Background: Heart failure (HF) is a worldwide growing health problem with significant morbidity, mortality, and cost to the health care systems. As the pathogenesis of this condition remains incompletely understood we thought worthwhile to study the role of the complement system in providing information that is independent of conventional biomarkers.
Methods: A group of 165 patients (81 male and 84 female), aged 75 +/-13 years, diagnosed with chronic heart failure (CHF) were studied in two moments: upon arrival to our hospital and at discharge. Biometric data and other information were collected by medical interview upon the collection of a venous blood sample. In this population other comorbidities were detected: 56.0% had also dyslipidemia, 38.4% had type 2 diabetes mellitus and 6.1% had hypertension. A complete blood count, components of the complement system (C1q, C1i, C3, C4 and C5), rheumatoid factor, C reactive protein (CRP), interleukin 6 (IL6) and B-type-natriuretic peptide (BNP) were determined according to our hospital routine. Differences between moments were evaluated by paired t-test. Spearman´s correlation coefficient between all the measurements and discharge BNP and acute BNP were calculated. Differences between these groups were evaluated by a ONE-way ANOVA test. Data was stored and analysed in SPSS v20.0 software. Statistical differences were accepted with a p value <0.05.
Results: With the exception of C5, all the other components of the complement system (C1q, C1i, C3 and C4), showed a significant difference between the acute state and time of discharge (P <0.0001). CRP and BNP (P <0.0001), but not IL6 (P =0.060), also showed a difference between both moments of the study, whereas neutrophils counts, but not monocytes, also significantly changed. Both C4 and IL6 correlated with BNP at the moment of hospital discharge, whereas none of the other measured parameters showed any significative correlation. However, when categorized by New York Heart Association (NYHA), for both moments of the study, there were no differences.
Conclusions: The results presented show a good evidence of changes in the complement system status in patients with CHF. Its ususefulness as prognostic markers in these patients still has to be better evaluated.

W143

EFFECT OF NATURAL DIETARY SUPPLEMENTS ON ASYMMETRIC DIMETHYLARGININE UNDER PHYSIOLOGICAL CONDITIONS AND IN DISEASE

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Background: Asymmetric ω-N,N-dimethylarginine (ADMA) is a naturally occurring product of posttranslationally methylated protein breakdown. Although the exact mechanism governing this process remains unclear, ADMA is known for its inhibitory effect on nitric oxide synthase. Therefore, ADMA has been extensively studied in relation to several cardiovascular-associated diseases (CVD), such as atherosclerosis, hypertension, hypercholesterolemia, diabetes mellitus or renal disease. Indeed, ADMA concentrations have been proved to correlate with certain parameters of antioxidant capacity and lipid profile markers. In general elevated ADMA levels are being considered as an independent risk factor of CVD. In view of the present knowledge, we evaluated the effect of natural lipid-altering dietary supplements with antioxidative properties on ADMA levels in plasma.
Methods: Adult male Wistar rats were included as study models in all experiments. Healthy and streptozotocin-induced diabetic animals were administered with oils containing predominant concentrations of omega-3 (ω3) or omega-6 (ω6) fatty acids for two months. Another group of healthy animals was treated with polyphenolic extract (PE). ADMA has been estimated in plasma by the ELISA method. Standard biochemical laboratory tests were used for measurements of biomarkers characterizing the lipid profile and antioxidant status.
Results: PE and ω3 oil treatment resulted in significantly decreased ADMA levels in healthy rats (0.34±0.30 and 0.55±0.09 μmol/L, respectively; P <0.05) compared to control (0.77±0.23 μmol/L) accompanied in the ω3 group by a decrease of cholesterol concentrations. The same effect could not be observed in the concurrent diabetic branch. On the contrary, ω6 oil administration did not lead to ADMA concentration changes in healthy animals, but caused marked ADMA increase (0.89±0.21 vs. 0.70±0.12 μmol/L; p 0.05) under diabetic conditions. However this effect was associated only with a nonsignificant cholesterol rise.
Conclusions: ω3 oil and PE may lead in healthy subjects to decrease of ADMA levels and thus lower the risk of CVD. ω6 oil intake is rather counterproductive and may contribute by means of elevated ADMA levels to increased CVD risk.
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W144

ALTERATIONS IN BLOOD CARNITINE AND ACYLCARNITINE LEVELS IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION

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Carnitine plays an essential role in fatty acids metabolism and therefore actively involved in cardiovascular pathology. Fatty acid oxidation is the major energy providing pathway of the myocardium and its inhibition impairs myocardial function. Earlier studies have demonstrated the malfunction of heart due to myocardial as well as systemic deficiencies of carnitine. This study was aimed to compare the blood levels of carnitine and acylcarnitines between normal subjects and patients with acute myocardial infarction (AMI). We recruited 50 AMI male patients (aged 58.24 (+\-) 11.63 y) (21 STEMI and 29 NSTEMI) and 144 age-matched male normal controls. The levels of carnitine and short-chain (C2+C3), medium-chain (C6+C8:1+C10:1+ C10:2+C12:1) and long-chain (C14:2+C16:1+C18+C18:1+ C18:2) acylcarnitines in the blood spots were determined using LC-MS/MS. Total carnitine levels were significantly higher in STEMI (57.26 (+\-) 4.51 μmol/L) and NSTEMI (54.05 (+\-) 3.52 μmol/L) patients as compared to control subjects (35.84 (+\-) 1.15 μmol/L) (ANOVA F=29.76, P <0.001). Similar trends were observed for free carnitine levels (ANOVA F=22.72, P <0.001). There was a significant increase in short-chain acylcarnitine levels in STEMI (12.38 (+\-) 1.01 μmol/L) and NSTEMI (11.90 (+\-) 1.04 μmol/L) patients than the control group (6.51 (+\-) 0.22 μmol/L) (ANOVA F =47.10, P <0.001). Medium - chain acylcarnitines were also significantly increased in STEMI (0.39 (+\-) 0.02 μmol/L) and NSTEMI (0.37 (+\-) 0.02 μmol/L) as compared with normal subjects (0.22 (+\-) 0.01 μmol/L). There was no difference in long-chain acylcarnitines among the three groups. These findings indicate poor uptake and/or increased leakage of carnitine through the ischemic myocardium. The clinical implications of these findings for the risk screening or diagnosis and prognosis of AMI require additional follow-up studies. (This study was supported by National Plan for Science and Technology Program by King Saud University Project Number 08-BIO571-02).

W145

PATHFAST CTNI MEETS THE CRITERIA FOR HIGH-SENSITIVITY TROPONIN ASSAYS AND IS COMPARABLE TO HIGH-SENSITIVITY TROPONIN T (HS CTNT) FOR DETECTION OF MYOCARDIAL INFARCTION

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Background: The PATHFAST cTnI assay revealed an imprecision CV <10% at the 99th percentile cutoff and therefore has been classified as “guideline acceptable”. We assessed if the PATHFAST cTnI assay is comparable to hs cTnT and meets the criteria of high-sensitivity cTn assays.
Methods: We examined 117 healthy individuals in whom cardiac disorders were excluded to check the manufacturer recommended 99th percentile cutoff-value of the PATHFAST cTnI assay and determined an imprecision profile. Additionally, PATHFAST cTnI and cobas® hs cTnT assay were measured in 193 consecutive emergency patients admitted to the chest pain unit with symptoms of acute coronary syndrome at presentation, 3 and 6 hours after admission. The results were related to the discharge diagnoses.
Results: The cTnI determination of the healthy control group revealed a mean of 0.0021 (95% CI: 0.0016-0.0026) μg/L, 0.018 μg/L as highest value, and a 95th percentile of 0.0087 (95% CI: 0.0047-0.014) μg/L. Quantification of cTnI between 0.001 and 0.018 μg/L was possible in 49 samples. The imprecision profile according to NCCLS demonstrated 20%, 10% and 5% CVs at cTnI concentrations of 0.002, 0.003 and 0.02 μg/L, respectively. To evaluate the diagnostic validity for detection of NSTEMI the results of cobas® hs cTnT and PATHFAST cTnI were compared by ROC analysis. AUC values obtained from the ROC analysis using the manufacturer recommended 99th percentile cut-off values at admission, after 3 hours and after 6 hours were 0.926, 0.963 and 0.958 for hs cTnT and 0.910, 0.958 and 0.949 for cTnI, respectively. The corresponding sensitivity/specificity relations were 85.5/82.9 %, 94.3/75.5 % and 95.7/72.4 % for hs cTnT and 77.0/96.3 %, 91.9/96.3 % and 90.3/93.6 % for cTnI, respectively.
Conclusions: The PATHFAST cTnI met the criteria of high sensitivity cTn assays and was comparable to hs cTnT for highly sensitive detection of NSTEMI with increasing sensitivity already at admission and after 3 hours, not going along with decreased specificity. Moreover, the PATHFAST cTnI assay allows reliable determination of cTnI within 17 min from whole blood samples. Therefore, the method might be useful at the point-of-care for early diagnosis of NSTEMI in patients admitted to the emergency ro

W146

COMPARISON OF FOUR COMMERCIAL LDL-CHOLESTEROL METHODS USED IN SMALL DENSE LDL-CHOLESTEROL CALCULATION EQUATION

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Background: Small, dense low density lipoprotein (sdLDL) particles are a powerful predictor of atherogenesis. However, most sdLDL methodologies are expensive, time consuming and technically demanding, making them too laborious for routine clinical practice. In our previous study, we developed the equation for estimating the sdLDL-cholesterol (sdLDL-C) concentration from the classic lipid measures of non high-density lipoprotein cholesterol (nonHDL-C) and both calculated (cLDL-C) and direct LDL-cholesterol (dLDL-C) levels as sdLDL-C (in mg/dL) = 0.580 (nonHDL-C) +0.407 (dLDL-C) -0.719 (cLDL-C) -12.05. However, the dLDL-C results may have contributed to the variation found in the calculated sdLDL-C. The aim of this study was to evaluate the performance of four different commercial LDL-C methods for used in the equation to estimate sdLDL-C.
Methods: We measured total cholesterol (TC), triglycerides (TG), and HDL-C using standardized methods, in 263 sera from the outpatient clinics of Ramathibodi Hospital. For sdLDL-C, a novel homogenous enzymatic assay (Denka Seiken, Japan) was used. Four different dLDL-C assays, liquid selective detergent (Seimens: dLDL-CS and Abbott: dLDL-CA), elimination (Denka Seiken: dLDL-CD), and selective micellulary solubilization methods (Roche: dLDL-CR) were performed. The cLDL-C (in mg/dL) was calculated using the Friedewald formula: cLDL-C = TC – HDL-C – TG/5.
Results: The least-squares regression statistics obtained between the calculated (y) and the measured (x) sdLDL-C were y=0.910x+5.43, R2=0.852; y=0.919x+9.73, R2= 0.812; y=0.996x+6.253, R2=0.862 and y=0.939x+7.98, R2=0.831, for the dLDL-CS, dLDL-CA, dLDL-CD and dLDL-CR respectively. There were no significant differences in the slope or intercept for the regression equations for any of the dLDL-C assay (P >0.23). The mean bias between measured and calculated sdLDL-C values was small, ranging from 2.4 to 7.0mg/dL.
Conclusions: The results demonstrated that the sdLDL-C calculation equation can provide the potential application with the direct LDL-C methods based on a wide range of different analytical concepts.

W147

USEFULNESS OF INTERLEUKIN-6 FOR PREDICTING ADVANCED HEART FAILURE IN PATIENTS WITH THE FIRST ANTERIOR ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION TREATED BY PRIMARY PERCUTANEOUS CORONARY INTERVENTION

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Background: Interleukin 6 (IL-6) is a pleiotropic cytokine and Its effects largely depend on the concentration of the soluble receptors (sIL-6R and sgp130). It is known that the raised levels of IL-6 correlate with decreased cardiac functional class, progression of heart failure and poor prognosis.There is no data about the association between IL-6 levels and the risk of advanced heart failure (HF) in patients with ST-segment elevation myocardial infarction (STEMI) treated by primary percutaneous coronary intervention (PCI). The aim of our study was to evaluate predictive value of plasma interleukin-6 (IL-6) level with respect to risk of advanced HF in patients with STEMI treated by primary PCI.
Methods: This study consisted of 75 consecutive patients with first anterior STEMI underwent primary PCI within 6 hours of the symptom onset. Interleukin-6 and soluble level IL-6 receptor was measured on admission using biochip array analyzer, Evidence® (Randox, UK). The Receiver Operating Characteristic analysis was performed to identify the most useful IL-6 cut-off level for the prediction of advanced HF (Killip class>2).
Results: Plasma IL-6 level >=8,95 pg/mL measured at admission had a 83.3% sensitivity and 62.3% specificity in predicting advanced HF. The area under the curve was 0.816 (P <0.011). The patients were divided into two groups according to the cut-off IL-6 level: high IL-6 group (>= 8.95 g/mL, n=31) and low IL-6 group (< 8.95 pg/mL, n=44). Patients in high IL-6 group had significantly higher CRP level (P <0.01) compared with low IL-6 group. The incidence of advanced HF was 8%. The incidence of advanced HF was 16.1% (5/31) in high IL-6 group, and 2.3% (1/44) in the low IL-6 group (P <0.05). Multiple logistic regression analysis identified plasma IL-6 level as an independent predictor of advanced HF (OR 1.073, 95% CI 1.007-1.143, P=0.030).
Conclusions: In patients with first anterior STEMI treated by primary PCI, plasma IL-6 level is an independent predictor of advanced HF.

W148

COPEPTIN AS BIOMARKER IN EARLY DIAGNOSIS OF PATIENTS WITH ACUTE CORONARY SYNDROME

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Background: Diagnosis of acute coronary syndrome (ACS) is based on clinical symptoms, findings of electrocardiogram and cardiac biomarkers as reflected by cardiac troponin. Emerging biomarkers such as copeptin may be helpful in early diagnosis and risk stratification in patients with ACS.
Methods: In this evaluation study we measured 155 samples of patients with ACS admitted to the coronary care unit. Copeptin was determined using an automated immunofluorescent assay on a Kryptor instrument (B·R·A·H·M·S GmbH, Hennigsdorf, Germany). Levels of high-sensitivity troponin T (hsTnT) were measured with an electrochemiluminescence immunoassay on an Elecsys system (Roche Diagnostics, Mannheim, Germany).
Results: One hundred and thirteen male and forty two female patients with a mean age of 64±13 years presented with chest pain. Of these, 100 patients (64%) were classified as having a ST-elevation myocardial infarction (STEMI), 51 (33%) a non-ST-elevation MI (NSTEMI) and 4 (3%) an unstable angina pectoris (UAP). Copeptin levels were higher in UAP patients compared to those having STEMI and NSTEMI (median 48.6 pmol/L vs 25.2 pmol/L vs 19.8 pmol/L). Thirteen patients had hsTnT levels between 14 and 50 pg/mL. Of interest, 9 of these 13 patients had copeptin levels above the cutoff point whereas in 4 out of 13 patients copeptin levels were within the normal range (blood sampling 1 hour and 2-6 days after onset of chest pain in 1 patient and 3 patients, respectively).
Conclusions: The results of our study suggest an additional diagnostic value of copeptin measurements in patients with hsTnT levels below 50 pg/mL. However, the clinical value of copeptin in the management of ACS should be further evaluated.

W149
CETP, LCAT, HDL AND LDL ISOFORMS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background: Reverse cholesterol transport is a multi-step process resulting in the net movement of cholesterol from peripheral tissues back to the liver via the plasma. Lecithin-cholesterol acyltransferase (LCAT) is an enzyme that converts free cholesterol into cholesteryl esters. The enzyme is bound to high-density lipoproteins (HDLs) in the blood plasma. Cholesteryl ester transfer protein (CETP) promotes an equimolar exchange of cholesteryl esters (CE) and triglyceride between lipoproteins.
Material and methods: For the first time in R. Macedonia, CETP and LCAT concentrations were determined by ELISA method in 100 healthy subjects as well in 100 patients with coronary artery disease (CAD). HDL and LDL subclass phenotyping was done using 3-31% gradient polyacrilamide gel electrophoresis.
Results: Small LDL subclasses were dominant in 60% of patients with predominance of LDL3 subclasses. The incidence of small HDL subclasses was significantly higher in patients with CAD compared with healthy subjects. The CETP concentration was higher in patients compared to controls group ($P < 0.01$). In patients with CAD monodysperse profile is characterised with higher CETP concentration compared to polydisperse profile. There was no difference(in LCAT concentration between healthy subjects and patients with CAD ($P > 0.05$). Age, diastolic blood pressure, CETP concentration and LDL particle size were independent factors for determing IMT by multiple linear regression analysis. They accounted for 35 % of the observed variability in IMT.
Conclusions: The present study shows that increasing CETP levels are associated with an increased risk of future CAD. Those in the highest CETP quartile had an OR of 1.44 (95% CI, 1.06 to 2.00, P=0.03) compared with those in the lowest quartile.

W150
ASSOCIATION BETWEEN SERUM CYSTATIN C LEVELS AND CARDIOVASCULAR DISEASE IN TYPE 2 DIABETIC PATIENTS

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Background: Serum cystatin C concentration was recently reported as a marker of cardiovascular disease (CVD). In the present study, we evaluate the association between the increase of serum cystatin C levels and the risk of CVD in type 2 diabete.
Methods: 42 patients with type 2 diabetes were included in the present study. 27 of them have CVD. The control group consisted of 30 healthy adults. Cystatin C, creatinine, microalbuminuria and CRP were measured on Cobas 6000TM.
Results: Cystatine C level was significantly higher in patients with CVD. A significant difference in serum cystatin C was found in patients with and without CVD among albuminuria. No differences of serum cystatin C level were found according to number of vessels affected. A cystatin C level >1.10mg/L was associated with increase of risk of CVD with significant difference (OR=42.52 and P=0.029).
Conclusion: Our results suggested that the increase of serum cystatin C concentrations is a potential marker for CVD.

W151

GLYCOGEN PHOSPHORYLASE BB IS ASSOCIATED WITH HEMODYNAMIC PARAMETERS IN CIRRHOTIC PATIENTS DURING TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNTS INSERTION

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Background: The aim of our study was to test glycogen phosphorylase BB isoenzyme (GPBB) as cardiac ischemia or necrosis marker in relation to echocardiographic and hemodynamic parameters in cirrhotic patients before and after transjugular intrahepatic portosystemic shunts (TIPS) insertion. The device decompresses portal preasure by redirecting portal blood volume into the systemic venous circulation. This fact results in a marked increase in ventricular preload could potencially precipitate cardiac dysfunction.
Methods: The study population consisted of 55 patients (38 men and 17 women, aged 55.6±8.9 years, range 37-74) with liver cirrhosis treated with transjugular portosystemic shunting. GPBB, echocardiographic and hemodynamic parameters were measured before and 24 hours after TIPS. Determination of GPBB concentration was performed by protein biochip system Evidence Investigator (Randox Laboratories, UK).
Results: Serum post-procedural GPBB concentrations were increased in comparison with baseline (2.67 vs. 5.58 μg/L, P <0.001). GPBB concentration after TIPS was associated with hemodynamic parameters - baseline systemic vascular resistence (SVR) and cardiac index (CI). Spearman's coefficients were: GPBB vs. SVR: r=0.330 (P=0.017); GPBB vs. CI: r=0.313 (P=0.025).
Conclusions: The GPBB may be a useful for monitoring myocardial ischemic during TIPS procedure.

W152

HIGH SENSITIVITY CARDIAC TROPONIN T IN CIRRHOTIC PATIENTS DURING TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNTS INSERTION

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Background: It has been known for more than four decades that liver cirrhosis is associated with cardiovascular abnormalities. The aim of our study was to test high-sensitivity troponin T as cardiac necrosis marker in relation to echocardiographic and hemodynamic parameters in patients before and after transjugular intrahepatic portosystemic shunts (TIPS) insertion.
Methods: The study population consisted of 55 patients (38 men and 17 women, aged 55.6±8.9 years) with liver cirrhosis treated with TIPS. Cardiac troponin T was measured by high-sensitivity electrochemiluminiscence immunoassay for Elecsys analyzator (Roche Diagnostics, Germany) before and 24 hours after TIPS. Eechocardiographic and hemodynamic parameters were measured in the same time.
Results: During baseline measurement in patients before TIPS insertion, hs-cTnT was increased above the 99th percentil of healthy reference population (0.014 μg/L) in 39.2% patients. We have found no significant changes in hs-cTnT after TIPS in comparison with pre-procedural level. Serum hs-cTnT concentration correlated with peak late atrial filling velocity (A) and left atrium diameter (LA) measured before TIPS insertion. Spearman's coefficients of rank correlation (r) were as follows: pre-procedural hs-cTnT vs. A: r=0.470 (P=0.001); vs. LA: r=0.313 (P=0.025); post-procedural hs-cTnT vs. A: r=0.380 (P=0.011); vs. LA: r=0.292 (P=0.037).
Conclusions: A minor significant increase of hs-cTnT before and after TIPS was associated with peak late atrial filling velocity and left atrium diameter. The hs-cTnT and GPBB may be a useful for monitoring myocardial ischemic during TIPS procedure.

W153
PERFORMANCE EVALUATION OF VIDAS GALECTIN-3 ASSAY

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bioMérieux R&D ImmunoAssay

Background: Cardiac fibrosis plays a causal role in the development and progression of heart failure (HF). Macrophage-derived galectin-3, a member of the family of beta-galactoside-binding lectins, has been implicated as an important mediator in this process because it induces cardiac fibroblast proliferation and collagen deposition. Elevated blood levels of galectin-3 have been found to be significantly associated with a higher risk of death or hospital readmission in patients diagnosed with HF, both in the acute decompensated as well as the stable chronic phase. This prognostic information is independent from B-type natriuretic peptides (NT-proBNP) and may be helpful to optimize patient care decisions. We evaluated the analytical performance of the Galectin-3 assay on the VIDAS instrument.
Methods: Limit of blank (LoB), Limit of detection (LoD), Limit of Quantification (LoQ), linearity, precision (repeatability, reproducibility), interference were assessed following CLSI® procedures. Method comparison testing (Passing and Bablok regression analysis) was performed using BG Medicine Galectin-3 ELISA as reference assay on a cohort of 133 frozen EDTA plasma samples (range 4.3 to 90.4 ng/mL).
Results:LoB was determined to be 2.2 ng/mL, LoD was determined to be 2.4 ng/mL. LoQ was determined to be 3.3 ng/mL. VIDAS Galectin-3 assay is linear over 3.3 to 100 ng/mL. Total CVs ranged from 5.0 to 6.5% and within run CV's from 1.1 to 1.6%. An interference was found with clearly hemolyzed samples and not with purified hemoglobin (5 g/L). Method comparison results show a correlation coefficient of 0.98 and an equation Y = 0.958 X + 0.471 (with Y=VIDAS Galectin-3 and X=BGM Galectin-3). At HF risk stratification cut-off levels of 17.8 ng/mL and 25.9 ng/mL, previously defined for the BG Medicine Galectin-3 assay, this presents a bias of -1.55% and -2.38%, respectively.
Conclusion: VIDAS Galectin-3 demonstrates acceptable analytical performance for quantifying galectin-3 in human plasma.

W154
ROLE OF BETA-TRACE PROTEIN IMPROVING THE PREDICTION OF ADVERSE EVENTS IN ATRIAL FIBRILLATION

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia in the general population, with an overall prevalence of around 1%, and affects up to 10% in elderly population. Chronic kidney disease (CKD) carries an increased cardiovascular risk, including AF. The objective was to evaluate the association of β-trace protein (BTP) (recently described as an accurate biomarker of kidney disease) with cardiovascular risk, bleeding events and mortality in a population of AF patients stable under oral anticoagulation.
Methods: We collected nonvalvular AF patients with stable anticoagulant treatment. BTP was determined by nephelometry in a BN ProSpec analyzer (Siemens Healthcare). We calculated the score of CHA 2 DS 2 -VASc and HAS-BLED schemes and we recorded the cardiovascular events (stroke, acute coronary syndrome and acute pulmonary edema), major bleeding events (criteria ISTH 2005), and mortality. Glomerular filtration rate (eGFR) was calculated. The cutoff for BTP was calculated using ROC curves (cardiovascular events = 0.572 mg/L, major bleeding= 0.548 mg/L and mortality= 0.609 mg/L).
Results: We included 914 patients with nonvalvular AF with stable anticoagulant therapy (INR in 6 months previously: 2.0-3.0), 50% male, age: 75 (p25-75: 70-85) years. The median follow-up was 956 days (p25-75: 783-1084). Median BTP was 0.61 (p25-75:0,47-0, 81) mg/L, which significantly correlated with eGFR (r=-0.34, P <0.001). During the follow-up were recorded 111 cardiovascular events (annual rate: 4.67%), 75 bleeding events (annual rate: 3.15%) and the annual rate of total mortality was 4.04% (96 events). BTP concentration above the cutoff was significantly associated after adjusting by CHA2DS2-VASc and eGFR, both major cardiovascular events [Hazard ratio (HR) 1.98 (1.27 to 3.10), P=0.003] as mortality [HR 2.11 (1.32 to 3.38), P=0.002]. Also associated with major bleeding scale adjusted HAS-BLED with [HR 2.01 (1.14 to 3.52), P=0.015].
Conclusions: The prevalence of CKD in AF patients, as well as numerous clinical implications that it brings in the management of this anticoagulated patients suggests the possible use of kidney damage biomarker as BTP in the prediction of severe events in patients with AF.

W155

SERUM ADIPONECTIN LEVELS AS A BIOMARKER FOR CORONARY ARTERY CALCIFICATION AND CORONARY LESIONS SEVERITY

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Background: Coronary artery calcification (CAC) is an independent risk factor for coronary atherosclerosis and predicts cardiovascular outcomes, but only know how concern the underlying mechanisms of plaque calcification. Plasma adiponectin may be cardioprotective and varies depending on gender category and pathological conditions. Low adiponectin levels might be associated with CAC progression and prone plaque rupture.
Methods: We included 139 steady patients undergoing CT for coronary stenosis and CAC assessment. Serum biomarkers levels were analyzed: electrochemiluminescence, Roche Diagnostics for testosterone and enzymelinked immunosorbent assay, R&D Systems for adiponectin. Calcium content was explored using 64-slice CT to calculate the calcium score according to Agatston score (high when ≥400 Hounsfield units). Severe coronary lesion severity was confirmed by coronariography (stenosis ≥70%).
Results: The 46.8% of patients were males, 58.5±11.4 aged. 17.3% had high CAC and 18.7% had severe coronary lesions. Higher calcium score was associated with lower adiponectin levels (AUC=0.65, p=0.021). The best cut-off for adiponectin was 8418 μg/mL (31% and 93% positive and negative predictive values, respectively). Univariate analysis showed age, low adiponectin and diabetes mellitus associated with CAC. Likewise, multivariate analysis found age (p=0.004), low adiponectin (p=0.004), and high testosterone (p=0.045) independently associated with CAC. Besides, adiponectin modestly predicted coronary lesion severity (AUC0.68, p=0.004), with the best cut-off 8005 μg/mL (32% and 90% of positive and negative predictive values, respectively). Smoking and diabetes mellitus (well-recognized atherosclerosis risk factors), low adiponectin, high testosterone, male gender and high CAC significantly were associated with severe coronary lesions in univariate analysis. Only low adiponectin (p0.004), high CAC (p 0.001) and high testosterone (p0.002) remained as independent predictors of severe coronary lesions in multivariate analysis.
Conclusions: Adiponectin values predict high CAC. Low adiponectin, high CAC and elevated testosterone were associated with the presence of severe coronary lesions in patients undergoing CT, suggesting their role in atherosclerosis and calcification.

W156

BIOMARKERS ASSOCIATED WITH RENAL FUNCTION IMPAIRMENT IN ATRIAL FIBRILLATION

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Background: There is a clear relationship between atrial fibrillation (AF) and chronic kidney disease (CKD), possibly because they share common pathophysiological mechanisms that are easily related to atherosclerotic disease. So, the AF risk is increased in patients with CKD and moreover increases the risk of stroke. Nowadays there is an effort to find biomarkers that could predict the risk of thrombosis in AF patients. The aim of our study was to correlate adiponectin and interleukin 6 (IL6), two biomarkers previously associated with atherosclerosis, with impaired renal function in a cohort of stabled AF patients.
Methods: We recruited 835 consecutive patients with nonvalvular AF under stable anticoagulation therapy (50% male, median age 75 years [70-81]). The glomerular filtration rate (eGFR) was calculated by the MDRD equation at inclusion and after 2 years of follow up (monitoring renal function in 656 patients) and we recorded the changes in renal function. Adiponectin was determined by enzyme immunoassay (ELISA Quantikine, R & D Systems) and IL6 by an automated electrochemiluminescent immunoassay (Cobas, Roche Diagnostics). ROC curves were calculated to search the optimal cutoff point for the biomarkers (IL6 =4.42 pg/mL, adiponectin =3066 mcg/mL)
Results: The median baseline eGFR was 70.25 (54.41-83.69) mL/min/1.73m^2. 29 patients showed severe CKD (eGFR <30mL/min/1.73m^2) at baseline and after excluding them, we observed how 182 patients (28%) reduced their eGFR in over 10 mL/min/1.73m^2 during follow-up and 14 (2%) dropped to <30 mL/min/1.73m^2. In the univariate analysis the variables associated with the development of CKD were severe heart failure, baseline eGFR, ischemic heart disease, adiponectin and IL6. In multivariate analysis continued statistical significance: baseline eGFR [OR: 3.03 (1.07-8.56), P=0.036], heart failure [OR: 4.29 (1.12-16.46), P=0.034], adiponectin <3066 mcg/mL [OR: 4.53 (1.43-14.37) P=0.010], while IL6 [OR =11.03 (0.90-10.81) P=0.074] lost their statistical significance.
Conclusions: The renal function impairment in AF patients is common. Adiponectin predicts the development of severe CKD, which could be considered as an adverse event over AF patients with subclinical atherosclerosis.

W157

NOVEL BIOMARKERS HFABP, COPEPTIN, GP-BB AND MRP 8/14 IN THE VERY EARLY DIAGNOSIS OF ACUTE MYOCARDIAL INFARCTIONÙ

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Introduction: Differentiating ischemic from non-ischemic chest pain during the first few hours after onset of pain remains challenging as symptoms and ECG are often unspecific. This study aims to test the diagnostic validity of novel biomarkers in an all-comers population of patients with acute chest pain in a tertiary care hospital.
Methods: 344 patients presenting with chest pain for less than 6 h were included. Venous blood was drawn at admission (0), 3 and at 6 hours later. The classic markers (hs Troponin T (hs-T), myoglobin, CK-MB) and four novel parameters were measured: glycogen phosphorylase (GPBB), myloid-related protein 8/14 (MRP8/14), Copeptin and heart fatty-acid-binding-protein (hFABP). GP-BB and MRP8/14 were measured semi-automatically (ELISA, Bio-Rad), hFABP immunologically (Concile). All other markers on Cobas 6000 (Roche Diagnostics). Patients underwent a clinical assessment on entry and coronary angiography if indicated. The clinical diagnosis was defined as ischemic (ST-elevation myocardial infarction (MI), non-ST-elevation MI and unstable angina) or non-ischemic as well as non-cardiac. Receiver operating characteristics (ROC) curves were used to assess the diagnostic validity and area under the curve (AUC) was compared between the tested markers.
Results: At admission AUC were 0.80 for hFABP and 0.81 for hs-T. At 3 hours 0.86 for hFABP and 0.88 for hs-T. At 6 hours 0.86 (hFABP) and 0.91 (hs-T). The performance of the other markers was moderate in comparison to hs-T and hFABP. Early dynamic changes (rise and fall) of hFABP was much more pronounced as compared to hs-T. In 20% of patients with MI hFABP was elevated at admission, while hs-T was not.
Conclusion: In this study hFABP and hs-T demonstrated similar characteristics, but hFABP often rose earlier. The dynamic characteristics of hFABP makes it a clinically useful and relevant parameter. hFABP in combination with hs-T enhances sensitivity to rule in MI early after onset of pain. Copeptin, GP-BB and MRP8/14 yielded little additional information to the classic markers. Commercially available rapid tests for hFABP can be a useful tool in clinical decision making in an ambulatory stetting such as hospitals, doctors offices and (air)ambulances.

W158

SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR AS A RISK STRATIFICATION BIOMARKER IN PATIENTS AFTER MYOCARDIAL INFARCTION - PILOT STUDY.

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Background: The aim of the study was to assess the risk connected with cardiovascular events in patients with first acute myocardial infarction (AMI) treated with percutaneous coronary intervention (PCI) procedure. Serum level of soluble urokinase plasminogen activator receptor (suPAR) was assessed as a risk biomarker in comparison to inflammatory marker high sensitivity C-rective protein (hsCRP) and plaque destabilization biomarker pregnancy associated plasma protein-A (PAPP-A).
Methods: We enrolled 126 patients with first AMI, confirmed clinically and in laboratory tests, with negative history of heart failure. PCI procedure was applied as a treatment. In all patients following parameters' serum levels were measured before discharge and also in follow up studies after 6 months: suPAR (ELISA, Virogates); hsCRP (immunotubidimetricassay, AU680 Beckman Coulter); PAPP-A (ECLIA, cobas e411, ROCHE)
Results: The ROC analysis for serum suPAR indicated 89% sensitivity for predicting cardiovascular events in 6 moths time (cutoff 3.46 ng/mL, AUC 0.62, P=0.019) with 87,5% specificity for a cutoff value declared by a producer as a high-risk-assosiated (5,54 ng/mL). suPAR level correlated with hsCRP and PAPP-A levels ($P < 0.05$, $0.25 < r < 0.35$). There was a statistically relevant difference in suPAR (4.56 ng/mL and 5.77 ng/mL, $P < 0.0001$) and PAPP-A levels (4.0 mIU/L and 7.7mIU/L, $P < 0.0001$) in patients' serum between discharge and a follow up point after 6 months.
Conclusion: The results indicate that suPAR may be a useful marker in cardiovascular events prediction after first AMI. Additionally differences in PAPP-A levels indicate acceleration of atherogenesis process during follow up studies in patients after AMI. Also growth in suPAR level may correspond to disease intensification and general health deterioration. Further studies are neaded.

W159

GALECTIN-3 LEVELS IN PATIENTS WITH FIRST ACUTE MYOCARDIAL INFARCTION WITHOUT PRIOR HEART FAILURE – THE CORRELATION WITH CLINICAL AND OTHER BIOCHEMICAL FACTORS

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Background: Galectin-3 is emerging biomarker of inflammation and fibrosis which has been studied in heart failure (HF) cohorts. Myocardial infarction (MI) is one of the major cause of HF. There is no studies evaluating galectin-3 levels in patients with first acute MI without history of prior HF.
Methods: We studied 145 consecutive patients with first myocardial infarction treated with primary coronary angioplasty. The exclusion criteria were: history of prior HF, cancer, liver or kidney fibrosis. Serum levels of galectin-3 (FPIA, Vidas, Biomerieux), NT-proBNP (ECLIA, Cobas e411, Roche Diadnostics) and hs CRP (immunoturbidimetricassay, AU680, Beckman Coulter) were measured on the 3-5th day after acute MI (on discharge).
Results: Galectin-3 levels were higher (>13.3 ng/mL, median value) in female (63% vs 43%, P <0.05), elderly (65.3 ± 8.9 vs 58.5±10.7 years, P <0.001) and diabetic patients (68% vs 44%, P <0.05). There was statistically significant positively correlation between galectin-3 and NT-proBNP (r=0.392, P <0.001), galectin-3 and hs CRP levels (r=0.273, P <0.05) measured on discharge. In addition, we observed higher glucose levels on admission (8.86 ± 4.0 vs 8.02 ± 4.0 mmol/L, P <0.05) and lower eGFR (94.3±38.9 vs 113.6 ± 37.0 mL/min, P <0.01) in group with elevated galectin-3 concentration. There was no difference in concentration of troponin T, fibrinogen, LDL, HDL, total cholesterol, triglycerides in patients with galectin-3 levels > 13.3 ng/mL or ≤13.3 ng/mL.
Conclusions: Only week or moderate relationship between galectin-3 and clinical risk factors (age, diabetes, kidney function), hs CRP and NT-proBNP levels confirms that galectin-3 reflects another pathways in pathology of heart diseases. Measuring galectin-3 can provide additional information to optimize patient theraphy after acute MI.

W160

SERUM LEVELS OF CERULOPLASMIN AND MYELOPEROXIDASE IN PATIENTS WITH STABLE CORONARY ARTERY DISEASE PRESENTING HIGH SENSITIVE C-REACTIVE PROTEIN BELOW 3 MG/L

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Background: Ceruloplasmin (CP)is increased in acute coronary syndromes (ACS). Myeloperoxidase (MPO) has been listed as a potentially useful risk marker in ACS. Inflammation is essential for atherogenesis, and many inflammatory markers have been analyzed for their association with short- and long-term outcome in patients with manifestations of coronary artery disease (CAD). C-reactive protein (CRP) plasma levels increase in patients with ACS and it is an important prognostic marker in ACS. Although CRP will remain over time a useful marker, the role and implications of increased plasma concentrations of other acute phase proteins, such as CP in patients with CAD is still not completely defined. The aim of this study was to evaluate the CP activity, MPO enzyme activity and MPO concentration and analize the association with other parameters.
Methods: We studied 20 patients with stable coronary disease and CRP below 3 mg/L, and 20 healthy subjects. CP activity was determined by evaluating ferroxidase activity. MPO concentration and MPO enzyme activity were measured with the ELISA method. Interleukine 6 (IL-6), glucose, HDL-cholesterol, cholesterol, triglyceride were measured by immunometric assay and standardized methods, respectively.
Results: CP activity and MPO concentration were significantly higher in CAD patients than in healthy subjects (868±45 vs 596±23 IU/L, P <0.0001); (403±75 vs 153±23 ng/mL, P=0.0049), respectively. There were not differences in MPO activity values in any of the groups (34.2±5.1vs35.0±6.6 ng/mL, P=0.9202). Significant associations were found between MPO concentration and CP activity (r =0.4790, P=0.0443), and there weren´t association with: glucose (r=-0.2288, P=0.3611); HDL-cholesterol (r=-0.2375, P=0.3427); total cholesterol(r=-0,3061, P=0.2167) and triglyceride (r=0.09013, P=0.7221). There was not significant correlation between CP activity and IL-6 (r= 0.2433, P=0.3307).
Conclusions: CP and MPO were elevated in patients with stable CAD. MPO activity did not discriminate these cohorts of patients. MPO levels were not correlated with cardiovascular risk factors. We did not observe significant associations between plasma CP levels and inflammatory marker, known to indicate risk for CAD including IL

W161

PROGNOSTIC ACCURACY OF OLD AND NEW BIOMARKERS FOR ALL-CAUSE MORTALITY IN ELDERLY PATIENTS HOSPITALIZED FOR SUSPECTED LOWER RESPIRATORY TRACT INFECTION

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Background: The aim of the study was to assess the prognostic accuracy for mortality of a panel of biomarkers in a population of older subjects hospitalized for suspected lower respiratory tract infection (LRTI).
Methods: From 20/2/12 to 23/7/12, 50 patients (pts) were hospitalized to the Geriatrics Unit [24 females (F), 26 males (M); age: range, median=67-102, 86 years (yy)]. At the follow-up monitoring (3/10/12), 28 pts (56%) survived (A) (14 F, 14 M), and 22 pts (44%) died (B) (10 M, 12 F).The mean hospital stay was 9 days (dd) (range, median=1-30, 7). Death occurred on average 26 dd after discharge (range, median=0-162, 12). We evaluated, at discharge: 1-Primary diagnosis; 2-Concomitant multiple diagnosis. At hospitalization: 3–Routine parameters: WBC, CRP, ESR, CRE, CF, O2SAT, Fever (>37°C); 4-New biomarkers: mid regional pro-adrenomedullin (MR-proADM), procalcitonin (PCT), Presepsin; 5-Multidimensional prognostic index, MPI: a frailty instrument, significantly correlated with mortality in hospitalized older pts (grade of risk: low, L, ≤0.33; moderate, M, 0.34-0.65; severe, S, ≥0.66).
Results: 1-Primary diagnoses (n pts, %): LRTI (26, 52%), cardiac diseases (CD) (12, 24%), respiratory diseases other than LRTI (4, 8%), other (8, 16%). 2-Concomitant diagnosis (n pts, %): LRTI (13, 26%), cardiac disease (29, 58%), neurologic disorders (18, 36%), muscle disorders (16, 32%), gastroenteric disease (8, 16%), pulmonary insufficiency and BPCO (6, 12%), other (20, 40%). 3–Fever: A vs B=61 vs. 32%; Mann Whitney test (MW) (p): n.s. for all parameters. 4-A vs B, MW (p): MR-proADM (0.01), PCT (0.07), Presepsin (0.23). 5-A vs B (MW, P=0.08): L+M=54%, S=46% (A); L+M=32%, S=68% (B).
Conclusions: The most frequent primary and concomitant discharge diagnosis was LRTI and CD, respectively. In the routine markers' panel, only Fever differed significantly between A and B pts. Among the new biomarkers, MPI levels as well as MR-proADM and PCT concentrations showed a different distribution between A and B pts, even if not always statistically significant.

W162

THE EFFECT OF METFORMIN TREATMENT ON LIPOXIN LEVELS AND INFLAMMATORY MARKERS IN DIABETIC PATIENTS

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Background: Diabetes Mellitus (DM) is characterized with lipid, carbonhydrate and metabolism disorders which caused by lack or resistance of insulin. Metformin is an antihyperglycemic drug used to treat non-insulin dependent diabetes mellitus (Type 2 DM). Cardiovascular complications of obesity and Type 2 DM are associated with endothelial dysfunction, elevated plasma vascular inflammation markers and a prothrombotic state. This study aimed to determine whether serum levels of inflammation markers were changed due to metformin treatment in Type 2 DM patients.
Methods: The study included twenty patients who were diagnosed as Type 2 DM and treated with metformin. Control group was twenty healthy individuals. Blood samples were collected in to heparinized tubes after overnight fast. High-sensitivity C-reactive protein (hsCRP) levels were determined by Roche Diagnostics Modular System using standard kits. Myeloperoxidase (MPO) activity was measured by spectrophotometric method. 15-epi-lipoxin A4 and lipoxin A4 levels measured by commercially available enzyme-linked immunosorbent assay kit.
Results: Control and metformin groups median (25%-75%) plasma MPO concentrations were 0.87U/L (0.76-0.92), 1.43 U/L (1.33-1.47) respectively and significantly different (P <0.05). hsCRP levels were not significantly different between control 1,50 mg/L (1.00-4.00) and metformin 2.21 mg/L (1.05-4.75) groups. 15-epi-lipoxin A4 and lipoxin A4 median (25%-75%) levels of control and metformin groups were 62, 83 g/mL (28.94-118.67), 28.3 pg/mL (13.3-109.98) and 84.23 pg/mL (67.43-133.31), 67.03 pg/mL (55.82-84.78) respectively. The decreased median levels of lipoxins in metformin group were not statistically significant when compared to controls.
Conclusions: Low grade chronic inflammation has an important role in atherogenesis. We examined the effects of metformin treatment on inflammation in Type 2 DM patients. Besides decreased MPO activity in metformin group, only a slight but not statistically significant difference was observed for 15-epi-lipoxin A4 and lipoxin A4 levels. Therefore, metformin treatment has no or limited effect on inflammatory markers especially lipoxin levels.

W163

GLYCATED ALBUMIN TO HAEMOGLOBIN A1C RATIO: COULD IT BE A CLINICAL MARKER FOR HEPATIC FUNCTION IN PATIENTS WITH CHRONIC LIVER DISEASES?

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Background: Glucose metabolic abnormalities is a frequent complication in patients with chronic liver diseases (CLD) such as hepatitis and liver cirrhosis .In patients with CLD neither HbA1c nor glycated albumin (GA) reflects glycaemic control. We investigate the association of HbA1c, GA and GA/HbA1c ratio with hepatic function tests and the possibility of these glycaemic control indices to be a clinical marker of hepatic function.
Methods: This study was conducted on 50 hospitalized patients with CLD and 80 type 2 diabetic patients without CLD as a control group. Fluctuation in plasma glucose levels were examined for all patients and mean plasma glucose was calculated. At the same time liver function tests as: Cholinesterase, serum albumin, total bilirubin, direct bilirubin and platelets count were measured. Glycated Albumin was also measured. Estimated HbA1c values were calculated from the mean plasma glucose. The G/HbA1c ratio was obtained by dividing GA over HbA1c .
Results: The statistical comparison between patients and control group shows that the correlation of glycated haemoglobin (HbA1c) levels with glycated albumin (GA) levels was found to be higher in patients with CLD than in type 2 diabetic patients without CLD (y=2.6x + 1-6 versus y=2.7x + 8.7). The multivariate analysis showed a significant association of G/H ratio with cholinesterase and direct bilirubin (P: 0.012 and P: 0.025) respectively. The G/H ratio was not associated with the mean plasma glucose.
Conclusion: Our result showed that GA/HbA1c ratio reflects the hepatic function independently of plasma glucose levels, so GA/HbA1c ratio can be used to monitor diabetic patients for the development of CLD.

W164

THE EFFECT OF HAPTOGLOBIN POLYMORPHISM ON THE STATUS ANTIOXIDATIVE ENZYMES IN TYPE 2 DIABETES MELLITUS

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Background: Increased oxidative stress and impaired antioxidative defenses is well documented in diabetes. Here, we investigated the bearing of Haptoglobin (Hp) polymorphism on the status of antioxidative enzymes in type 2 diabetes mellitus.
Methods: Study population consisted of 165 type 2 diabetics and 94 healthy controls. Fasting blood glucose, HbA1c, the activity of eryrthrocytic enzymes superoxide dismutase, SOD; glutathione peroxidase, GPx; catalase, CAT; and the activity of plasmatic ceruloplasmin ferroxidase (Cp) were determined by spectrophotometric procedures. Hp phenotypes (1-1, 2-1, or 2-2) were determined by gel electrophoresis.
Results: Irrespective of Hp phenotype, while the levels of Cp (U/L) and GPx (U/g Hb) were significantly higher in patients than in controls (136.4+42.7 vs. 103.7+32.5; 41.3+11.6 vs 37.1 + 12.2; P=0.000 and 0.004, respectively), those of SOD (U/g Hb) were lower (567+198 vs. 882+304, P=0.000). No significant differences observed for CAT levels. When Hp polymorphism is taken into account, significant Hp-phenotype differences in the activity of these enzymes were noted in patients vs controls. Within the diabetic group however, significant Hp-dependent differences were also observed. In that, Cp levels within Hp2-2 (145.6+38.0) were significantly higher than that in Hp1-1 (133.1+49.0; P=0.043) or Hp2-1 patients (126.7+44.3; P=0.004). In contrast, levels of SOD and GPx in Hp2-2 patients (514+141 and 38.7+8.0) were significantly lower than in that in Hp1-1 (677+288 and 46.7 + 13.1) and Hp2-1 (599+207 and 43.1+13.9). CAT levels were similar among the Hp-dependent patient groups. Interestingly, when patients are treated by Spearman's univariant analysis as a single group, significant yet weak correlations between enzymes and HbA1c were observed. Cp correlated positively while GPx and SOD correlated negatively. However, when patients are treated as separate Hp-dependent groups, stronger correlations between these enzymes and HbA1c were only noted among Hp2-2 patients.
Conclusions: These findings suggest that Hp polymorphism has some bearing on the activity of antioxidative enzymes in diabetics and that Hp2-2 diabetics are under increased oxidative stress as compared with those expressin

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EXTENDED RELEASE NICOTINIC ACID: INDUCER OF INSULIN RESISTANCE BY INHIBITION OF DIACYLGLYCEROL ACYLTRANSFERASE (DGAT)

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Background: In a previous randomized cross-over study, we showed that nicotinic acid (NA) induced hepatic insulin resistance measured by hyperinsulinemic euglycemic clamp and decreased triglycerides content in VLDL subfraction, in men. It seems necessary to explore the mechanism of action of NA on a hepatic cellular model to link hepatic insulin resistance to the decrease in TG-VLDL subfraction.
Methods: After incubating HuH7 cells with or without nicotinic acid (NA) (16 hours, 3 mM), we studied both, insulin action by measuring protein kinase B (PKb) phosphorylation in presence of insulin and the impact of NA on de novo lipogenesis, in particular on DGAT inhibition, a key enzyme implicated in TG synthesis. We measured indirectly TG synthesis by measuring flow synthesis of TG in presence of oleate and TG and diacylglycerol (DAG) hepatic concentrations by appreciating fatty acid contents in these neutral lipids subfractions after lipid extraction, thin layer chromatography separation and analysis of fatty acid composition in DAG and TG subfractions by gas chromatography. To investigate the link between inhibition of lipogenesis and insulin resistance, we explored the insulin-signalling pathway, which could be activated by DAG by measuring protein kinase C epsilon (PKcε) phosphorylation and reversion of insulin resistance by using PKcε inhibitor peptide and measuring PKb phosphorylation in presence of both insulin and NA.
Results: We confirmed the decrease in insulin action via the decrease in PKb phosphorylation in presence of insulin and NA (5.0±1.0 vs 2.8±0.6 AU, P=0.02). We also demonstrated that NA decreased the flow synthesis of TG, induced significantly DAG accumulation and decreased TG hepatic concentration suggesting inhibition of DGAT. In connection with DAG accumulation, we showed a slight increase in PKcε phosphorylation after NA exposition without reversion of decrease in insulin action in presence of PKcε peptide inhibitor, insulin and NA.
Conclusions: NA could inhibit DGAT enzyme implicated in de novo TG synthesis and induce lipid intermediates accumulation that could decrease insulin action and induce hepatic insulin resistance. Beneficial effect of NA on TG concentration seems correlated to its deleterious effect on insulin sensitivity.

W166

ACCURACY OF THE NOVA STATSTRIP POCT GLUCOSE ANALYZER FOR CLASSIFICATION OF FASTING HYPERGLYCAEMIA IN HIGH-RISK INDIVIDUALS

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Background and aim: Preanalytical sample handling is critical in obtaining accurate plasma glucose values, influenced substantially by in vitro glycolysis. Any delay in sample processing may result in reduced plasma glucose values and miss-classification of asymptomatic patients with diabetes and intermediate hyperglycaemia. Potential use of point-of-care glucose meters for diagnostic purposes has been considered to be unreliable due to insufficient accuracy. The aim of this study was to assess the performance of a POCT glucose analyzer specifically designed for hospital use (StatStrip Glucose, Nova Biomedical, USA), for classification of fasting hyperglycaemia in high-risk individuals.
Methods: Fasting blood samples were taken from consenting subjects, referred to our clinic with a working diagnosis of hyperglycaemia. Venous and capillary sample collection and testing was performed within 5 min for the reference laboratory procedure (hexokinase, Olympus AU400, Beckman Coulter, USA) and StatStrip glucose measurement, respectively. WHO criteria for fasting plasma glucose were used to classify patients into diagnostic categories of glycaemia.
Results: A total of 187 subjects (M/F: 85/102; age range 18-89, median 56 years) were included in this study. There were no differences between capillary and venous fasting plasma glucose values, as measured by the StatStrip glucose meter (7,75±1,86 vs. 7,57±1,88mmol/L, P=0,336). Passing-Bablok regression analysis revealed no difference between the laboratory (venous plasma) and StatStrip glucose values (capillary plasma) [regression equation: y=0,261+0,98x; intercept A=0,2610, 95% CI=0,1-0,62; Slope B=0,98; 95% CI=0,933-1,000), and cusum test showed no significant deviation from linearity (P >0,10). Inter-rater agreement analysis showed very good agreement (weighted kappa=0,883) between the methods when classifying subjects into diagnostic categories of normoglycaemia, impaired fasting glucose and diabetes mellitus by the fasting plasma glucose classification criteria.
Conclusion: StatStrip POCT glucose analyzer could serve as an accurate and reliable tool for classification of fasting hyperglycaemia in high-risk individuals.

W167

ASSOCIATION BETWEEN TYPE 2 DIABETES AND INFLAMMATION MARKERS IN PATIENTS WITH CORONARY ARTERY DISEASE

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Background: Diabetes mellitus has been recognized as an independent major cardiovascular risk factor, coronary artery disease being the result of the synergistic effects of diabetes and inflammation in promoting atherothrombosis. The aim of this study was to evaluate the association between plasma levels of high sensitivity C reactive protein (hs-CRP) and interleukin-6 (IL-6) in diabetic patients with angiographically confirmed coronary artery disease.
Methods: This prospective study comprised 201 consecutive patients with angiographically confirmed coronary artery disease (CAD) that were divided in two groups considering the presence of type 2 diabetes: 64 patients with coronary artery disease and type 2 diabetes (group A) and 137 patients with coronary artery disease without type 2 diabetes (group B). We measured serum lipid profile, high sensitivity C reactive protein (hs-CRP) and serum interleukin-6 levels (IL-6) in all patients. Serum hs-CRP levels were assessed by ELISA method.
Results: Patients from group A had significantly higher levels of total cholesterol, triglycerides and LDL-cholesterol compared with patients from group B ($P < 0.001$). We observed statistically significant differences between mean values of hs-CRP between group A and group B ($P < 0.001$). We also observed that group A had significantly higher levels of plasma IL-6 ($P < 0.001$).
Conclusion: The study showed that increased levels of high sensitivity C reactive protein and serum interleukin-6 are associated with type 2 diabetes in patients with coronary artery disease.

W168

A NEW GLYCATION GAP BASED ON GLYCATED HAEMOGLOBINE AND GLYCATED ALBUMIN VALUES IN PATIENTS WITH DIABETES

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Background: The glycated albumin(GA) assay has shown several advantages over other glycemic control parameters. It seems to reflect shorter term changes in glycaemia and better glycemic variability than glycated hemoglobin (A1c), and. It is even more precise than fructosamine (FA). In addition, GA is expressed in the same units as A1c do, thus its clinical meaning can be more easily understood by doctors and patients. On the other hand, it has been reported that the glycation gap (GG), computed on A1c and fructosamine values, lets assign a glycation phenotype and is a predictor of the progression of nephropathy. The main goal of this investigations is to compute a glycation GA based glycation gap.
Methods: We have enroled 414 patients with diabetes. All of them were caucasian and satisfied some inclusion criteria such as their glycemic status has been requested by A1c and FA and they have no known haemoglobinopathy or erythrocyte disorders. Fructosamine was assayed using Genzyme GlyPro kits. A1c was determined by Menarine HPLC method. All A1C values were converted to DCCT-aligned units. The glycated albumin were determined using Lucica GA-LTM assay from Asahi Kasei Pharma Corporation. Pearson's correlation coefficient, relationships for each checked couple and Cohen's Kappa coefficient have been obtained with SPSS (ver.16).
Results: In all patients, a huge scatter between A1c and fructosamine was found, as it happened with A1c and GA as well. The r-coefficient between A1C and FA was 0.852 and its value between A1c and GA was pretty similar (0.848). Meanwhile the r-coefficent between FA and GA was better (0.936). The frutosamine-based glycation gap was computed as GGF(%)= A1c(%)-[0.011 * FA (μM)+3.816] ; meanwhile glycated albumin-based glycation gap was computed as GGGA(%)= A1c(%)–[0.199 * GA(%)+3.424]. In both cases, we have divided the population into tertiles which would correspond to low, medium and high glycator phenotypes, respectively. Cohen's Kappa coeffcient value for inter agreement was 0.675 which means a good concordance through the all cathegories.
Conclusion: It is possible to compute a GGGA gap and there is a good inter-rate agreement among its glycation phenotypes and those assigned by GGF.

W169

SERUM LIPID PEROXIDATION, URIC ACID AND PROLONGED PHYSICAL ACTIVITY IN PATIENTS WITH TYPE 1 DIABETES

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Background: Physical activity is widely encouraged to the type 1 diabetes (T1DM) patients to reduce cardiovascular risk. Oxidative stress, including lipid peroxidation is a widely accepted component in the development of cardiovascular disease. Inconsistent results have been reported in patients with T1DM for all the commonly measured markers of oxidative stress. We aimed at investigating the levels of serum lipid peroxidation in a group of T1DM patients and a group of well-matched healthy controls and the impact of prolonged moderate exercise on oxidative stress levels.
Methods: Nine patients (47±10 years, 73±15 kg, 170±10 cm; Hba1c 7.1±1.1%) and 15 healthy controls (46±10 years, 75±16 kg, 174±10 cm) performed a 3-hrs constant intensity walk at 30% of the heart rate reserve. Patients were administered appropriate amounts of carbohydrates to avoid an excessive fall of glycemia according to Francescato et al. (MSSE, 2011). Capillary blood samples (n=240) for lipid peroxidation evaluation were taken in duplicate at the start and subsequently every 30 min throughout the exercise to perform the Free Oxygen Radicals Test (FORT, CR-2000 Callegari1930, Italy). Venous blood samples were obtained before and at the end of the trials for determination of glucose by means of a hexokinase based methodology (Olympus Diagnostic Systems AU2700) and insulin levels, which included the exogenous administered insulin by Immunoassay system (Beckman Coulter, Fullerton, CA).
Results: Exercise-induced hypoglycemia was avoided in all patients. Lipid peroxidation remained constant in both T1DM patients and controls throughout the whole exercise (p=NS). However, type 1 DM patients showed higher values as compared to healthy controls (380.1±14.7 vs 293.1±9.6 arbitrary units; P <0.05). In addition, a linear relationship was detected between lipid peroxidation and serum uric acid (R=0.61; P <0.05).
Conclusions: Our study showed higher lipid peroxidation values in type 1 diabetic patients as compared to healthy people. Prolonged moderate exercise, however, did not exacerbate this potentially harmful condition. Interestingly, an inverse association was found between lipid peroxidation and serum uric acid levels.

W170

EFFECTS OF CETP AND TRIB1 GENE VARIATIONS ON MARKERS OF INSULIN RESISTANCE AND DYSLIPIDEMIA IN BOSNIAN POPULATION.

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Background: The primary defect underlying lipoprotein abnormalities in Type 2 diabetes (T2D) is insulin resistance (IR), which leads to increased levels of triglycerides (TG), dominance of small, dense LDL and low HDL levels.Number of genes are associated with blood lipids levels, including CETP and TRIB1.Interestingly, variations of CETP gene, coding cholesteryl ester transfer protein (CETP), have been associated with fluctuations in HDL levels mainly, while TRIB1 polymorphisms appeared to be associated with TG levels and T2D development.In this study, we analyzed the association of CETP (rs1800775) and TRIB1 (rs17321515) variations with the development, as well as anthropometric and biochemical parameters of T2D.
Methods: A total of 86 subjects were enrolled in the study, including 47 T2D patients and 49 control nondiabetic subjects, who were about 40-55 years old. Diabetic patients were recruited at the Department of Endocrinology,Clinical Center University of Sarajevo, and General Hospital in Tesanj,Bosnia and Herzegovina.Genotyping analysis was performed on MassArray Sequenom iPLEX platform.All biochemical analyses were performed by standard IFCC methods.
Results: Our results showed that TRIB1 and CETP genotype frequencies in T2D patients and control subjects were in accordance with Hardy-Weinberg equilibrium.The genotype distributions for both, TRIB1 and CETP, were not significantly different between diabetic and control subjects.However, our data showed a significant association of CETP variant (-629A>C) with IR markers.The control individuals, carriers of -629A>C, showed increased insulin levels (P=0.008) and HOMA-IR (P=0.014) as compared to heterozygotes and wild-type homozygotes.Interestingly, our data also demonstrated an association of CETP variation with HDL levels (P=0.01) in control subjects.
Conclusions: Our data demonstrated an association of TRIB1 polymorphism (rs17321515) with lower LDL (P=0.012) and total cholesterol levels (P=0.007), suggesting a protective role of TRIB1 variant in diabetic patients.However, CETP variation appeared to be associated with the increased markers of insulin resistance (HOMA-IR, insulin levels), as well as decreased HDL levels in control subjects and thus, could be considered as a risk allele for T2D.

W171

SERUM ASYMMETRIC DIMETHYLARGININE LEVELS OF PREGNANT WOMEN WITH GESTATIONAL DIABETES

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Background: For diabetic patients, increased ADMA levels and endothelium dysfunction has been established. Our aim was to measure plasma concentrations of ADMA and arginine in patients with gestational diabetes and find a relationship between serum ADMA levels and endothelial dysfunction.
Methods: Three groups were included in this study. 64 patients with normal 50 gr glucose loading test result (group 1, NGT test normal), patients whose 50 gr test result was high and 100 gr test normal, namely those with impaired glucose tolerance (grup 2, IGT) and in 8 patients diagnosed with gestational diabetes (group 3,GDM). ADMA and arginine levels were investigated.
Results: Arginine levels were significantly higher in IGT than those in NGT (P=0.03). ADMA levels were higher in GDM compared to control group (P=0.66). There was statistically significant relation between arginine levels and ADMA level and ADMA level and diabetes history. Correlation was also found with BMI, age and the history of DM.
Conclusions: In conclusion, in IGT group, increasing ADMA levels may have been masked by the effect of high arginine levels. ADMA tends to increase in patients with IGT and GDM. In addition, serum ADMA and arginine levels do not seem to influence the weight of the infant.

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CARROT JUICE CAN INHIBIT POSTPRANDIAL PLASMA GLUCOSE PEAK IN HEALTHY HUMAN

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Background: Diabetes is one of chronic diseases which are main public health problems world wide. Carrot (Daucus carota), a vegetable widely used as food, is known for not only its health nourishing effect, but also its hypoglycemic effect. But no scientific evidence on the hypoglycemic effect of carrot juice has ever been reported in human.
Methods: In this study, the effect of carrot juice consumption on peak plasma glucose concentrations in healthy subjects was investigated. Carrot juice was prepared from peeled raw carrot roots extracted with a juice maker. Healthy subjects (n=20) aged between 19-23 years of both sexes were used, and standard oral glucose tolerance test (OGTT) was used as a tool to demonstrate glucose absorption pattern. The study was designed as a before/after experiment. The before or 'baseline' experiment was study in the subjects without carrot juice consumption and the after or 'treated' experiment was the same group of subjects who consumed 300ml of carrot juice 15 minutes after the oral glucose load in standard OGTT. Peaks glucose absorption of both experiments were compared.
Results: The results showed that the mean peak glucose concentration in the 'treated' group is significantly lower than the 'baseline' group (132±27 vs. 125±26 mg/dL; P=0.003).
Conclusions: Carrot juice may have potential use for decreasing plasma glucose in diabetes mellitus patients. However, a bigger group of diabetic patients is needed to confirm those results.

W173
THE IMPACT OF OBESITY TO THE LIVER ENZYME ACTIVITIES IN A STUDENT'S POPULATION

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Background: It has been reported that obesity was associated with metabolic syndrome, insulin resistance, cardiovascular risk but also with non-alcoholic fatty liver disease (NAFLD). The prevalence of obesity in children and adolescents is increasing rapidly all over the world. The aim of this study was to analyze the value of liver enzymes: AST, ALT and γGT in a group of obese students in order to establish their correlation to anthropometric parameters such as: BMI (body mass index), WC (waist circimference), HC (hip circumference), and WHR (waist to hip ratio) comparing to non-obese students who comprised the control group (CG).
Methods: In this study, 238 students from the University of Novi Sad, both sexes (126 men and 112 women), mean age of 22.32 ± 1.85 years were included. They were divided into 2 subgroups according to their anthropometric parameter levels: BMI <25 kg/m^2>, WC <94 cm > for males, and WC <80 cm >for females, HC <108 cm >for males and HC <111 cm >for females, and WHR <0.90 >for males and WHR <0.80 >for females.
Results: Statistical processing data revealed significantly higher values of AST, ALT and γGT in the group of students with BMI >25 kg/m^2, WC >94 cm for males and WC >80 cm for females, HC >108 cm for males and HC >111 cm for females, and WHR >0.90 for males and WHR >0.80 for females (P <0.001). Significant association betwen anthropometric parameters and liver enzyme levels was established (P <0.0001).
Conclusion: According to obtained results it can be concluded that obese students with higher BMI, WC, HC and WHR values had higher liver enzymes activites and higher chance to develop NAFLD in the future.

W174
EVALUATION OF PROTEIN OXIDATION PRODUCTS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Aim: This study was aimed to investigate AGE (advanced glycosylation end products) formation, oxidative stress and carbonyl stress in a diabetic rat model induced by streptozotocin (STZ) which seem to be responsible for diabetic complications and the possible role of melatonin (MEL) which is a powerful antioxidant drug in this mechanism.
Materials and methods: Four study groups, each containing ten Sprague Dawley rats, were defined as control, MEL, STZ and STZ-MEL. STZ and STZ-MEL groups were given a single 50 mg/kg dose of STZ. to induce diabetes. MEL, 25 mg/kg was given intraperitoneally to MEL and STZ-MEL groups on a daily basis for 42 days. During the study, the rats were weighed weekly. Blood and 24 h urine samples were collected at the begining and at the end of study, and also once in two weeks and weekly, respectively. Glucose and Hb A1c were measured in the blood samples. Also the levels of methylglyoxal (MGO) which is one of the AGE precursor, and the activities of GLO I (glyoxalase I) and GLO II enzymes that are members of glyoxalase detoxification system were also determined in tissue samples.
Results: Blood and urine glucose levels were found to be high in rats. Rats which were excreting gradually more urine lost weight during the study. Although STZ group had been shown to have higher tissue MGO levels and lower GLO I and GLO II activities, MEL treatment had suppressed high levels of MGO and increased enzymatic activities in STZ-MEL group.
Conclusion: It has been shown that STZ induced diabetic rats had high MGO levels and the supression of GLO detoxification system indicates that AGE formation in diabetes is inevitable. Therefore the use of MEL which is an antioxidant may be suggested to prevent diabetic complications in a manner.

W175
EFFECTS OF OMEGA-3 ON LIPID PROFILE AND HAEMATOLOGICAL PARAMETERS IN HYPERLIPIDEMIC RATS

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Background: The present study was designed to evaluate and compare the effects of different doses of omega-3, gemfibrozil and atorvastatin on lipid profile and haematological parameters in hyperlipidemic rats.
Methods: Forty eight rats were divided into two groups. The first groups included 18 rats; they were subdivided into three subgroups each of 6 rats. The first subgroup served as a control. The second and third subgroups received omega-3 (15 mg/kg) and (30 mg/kg) orally (PO) daily respectively. The second group included 30 hyperlipidemic rats and was subdivided into five subgroups of six rats each. The first subgroup served as a positive control. The second and third subgroups received omega-3 (15 mg/kg) and (30 mg/kg) PO daily respectively. The fourth and fifth subgroups received gemfibrozil (3.5 mg/kg) PO daily and atorvastatin (2 mg/kg) PO daily respectively.
Results: After four weeks of therapy, (30 mg/kg) of omega-3 showed a significant reduction in the level of triglyceride (TG), total cholesterol (TC) and low density lipoprotein (LDL-C) in control rats. Whereas (15 mg/kg) omega-3 could only reduce the level of TC and LDL-C significantly. Four weeks of daily administration of both doses of omega-3 produced significant reduction in serum (TC, TG and LDL-C) of hyperlipidemic rats. However neither (15 mg/kg) of omega-3 nor omega-3 (30 mg/kg) could increase the level of high density lipoprotein HDL-C in the treated and non treated hyperlipidemic rats.
Both doses of omega-3 produced a significant increase in the level of HB, RBC and MCH in normal rats. The same doses of omega-3 showed a significant increase in the levels of hemoglobin (HB), red blood cell (RBC), hematocrit (HTC) and mean corpuscular hemoglobin (MCH) in hyperlipidemic rats after 4 weeks of therapy. Following four weeks treatment with both gemfibrozile and atorvastatin there was a significant reduction in serum (TC, TG and LDL-C) and a significant raise in serum HDL-C in hyperlipidemic rats.
Conclusion: No significant differences were found between the effects of both doses of omega-3 and gemfibrozile or atorvastatin on TC, TG, and LDL-C of hyperlipidemic rats. In contrast to omega-3, gemfibrozile and atorvastatin induced a significant raise in the level of HDL-C.

W176
BIOCHEMICAL PARAMETERS IN CONTROL OF EFFICIENCY OF ORAL THERAPY IN DIABETES MELLITUS TYPE 2

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Introduction: Diabetes mellitus is a serious medical problem around the world. The aim of this work is to use biochemical analyzes as control of effectively of oral therapy in diabetes mellitus type 2.
Methods: The study included 75 patients with diabetes mellitus type 2, on the oral therapy, 30-60 years of age, and a control group of 28 healthy individuals. The patient group was divided into three subgroups taking the values of blood sugar as a criterion: first group from 3.85 to 5 mmol/L, second group from 5.2 to 7.2 mmol/L and the third from 7.3 to 13.8 mmol/L. Using standard methods, were performed: glucose, triglycerides, total cholesterol, HDL, LDL, HbA1c, C-peptide, BMI and proteinuria.
Results: The results of the evaluated parameters of the control group are: glucose =4.25±0.46 mmol/L, HbA1c =28.7±7.69 mmol/mol, BMI =22.4±1.2 kg/m2, triglycerides =1.09±0.37 mmol/L, total cholesterol =4.18±0.72 mmol/L, HDL =1.45±0.43 mmol/L, LDL =2.24±0.66 mmol/L, C-peptide = 1.76 ± 0.59 ng/ml, proteinuria =0.08±0.04 g/L; for first group: glucose =4.34±0.46 mmol/L, HbA1c =38.75±7.57 mmol/mol, BMI =27.8±2.8 kg/m^2, triglycerides =1.71±1.35mmol/L, total cholesterol =5.34±0.99 mmol/L, HDL =1.13±0.34 mmol/L, LDL =3.86±1.04 mmol/L, C-peptide =2.77±1.35 ng/mL, proteinuria =0.14±0.07 g/L; for second group are: glucose =6.167± 0.616 mmol/l, HbA1c =48.83±10.09 mmol/mol, BMI =28.2±4.3 kg/m2, triglycerides =2.43±1.47 mmol/L, total cholesterol =5.69±1.098 mmol/L, HDL =1.17 ± 0.39 mmol/L, LDL =3.52 ±1.17 mmol/L, C-peptide 2.91 ± 2.04ng/mL, proteinuria =0.14±0.11 g/L and for third group are: glucose =9.32±1.87 mmol/l, HbA1c =62.09±21.36 mmol/mol, BMI =29.8±5.7 kg/m2, triglycerides =2.38±1.33 mmol/L, total cholesterol =5.49±1.09 mmol/L, HDL =1.16±0.30 mmol/L, LDL =3.0±1.06 mmol/L, C- peptide = 2.27 ± 1.48 ng/mL, proteinuria =0.17±0.16 g/L. The results of biochemical parametres of the control group and the patients were compered with OriginPro 6.0 statistical program. The t-test had shown significant differences between the concentrations the all parameters for the control group and the three groups of patients, with exception of value of HDL cholesterol.
Conclusion: The biochemical analyzes, including glucose, triglycerides, total cholesterol, HDL, LDL, HbA1c, C-peptide, BMI and proteinuria, are necessary to control the benefit of oral therapy for diabetes mellitus type 2.

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MARKERS OF INFLAMMATION IN TYPE 2 DIABETES PATIENTS WITH AND WITHOUT CHRONIC COMPLICATIONS

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Background: Type 2 diabetes is considered as disease associated with low-grade inflammation. On the other hand, inflammation is involved in the pathogenesis of chronic diabetic complications. The aim of this study was to evaluate the relationship between inflammatory markers C-reactive protein (CRP) and serum amyloid A protein (SAA) and the presence of diabetic complications.
Methods: 80 patients with type 2 diabetes were enrolled into the study. The patients were divided into group 1 (40 patients with chronic diabetic complications such as retinopathy, nephropathy, neuropathy and diabetic foot) and group 2 (40 diabetic patients without any complications). Serum CRP and SAA levels were measured using immunonephelometry. The result were not normally distributed and are presented as ranges, medians and quartiles Q1-Q3.
Results: There were no significant differences in CRP and SAA level between patients with and without diabetic complications. However, significant differences were observed in CRP level (P=0,045) between diabetic patients with at least 3 different coexisting complications (CRP=8,66[4,42-91,4] mg/L) and without complications (CRP=2,68[1,08-9,88] mg/L). Similar relationship was found for SAA concentration (SAA level in diabetic patients with at least 3 different coexisting complications: 17,5 [15,7-867,0] mg/L and without complications: SAA 7,7 [5,1-15,2] mg/L; P=0,025). Differences between CRP and SAA levels in groups with increasing number of complications (from 0 to 4) was confirmed using ANOVA test (CRP: P=0,013; SAA: P=0,012). Higher levels of both CRP and SAA (P=0,014 and P <0,001, respectively) were observed in obese patients with BMI >30 kg/m2 (CRP: 2,95[1,09-10,26] mg/L; SAA: 9,05[6,15-16,1] mg/L) compared to those with normal BMI (CRP:1,19[0,51-2,61] mg/L; SAA:4,55[2,7-7,6] mg/L). In the group of diabetic patients the correlation between CRP and SAA was statistically significant (P <0,001, r=0,696).
Conclusions. Both studied markers of inflammatory response were significantly associated with the advancement of chronic diabetic complications. Higher levels of both markers in obese patients confirmed the chronic low-grade inflammation resulting from the secretory activity of fat tissue.

W178

EFFECTS OF HYPOCALORIC DIET ON LABORATORY PARAMETERS IN OBESE PATIENTS

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Background: Obese individuals have a higher risk to develop chronic diseases. Little is known about the impact of diet on changes in laboratory markers during the follow-up of these patients. In this study, we evaluated selected laboratory parameters in obese individuals before and 1, 4 and 6 months after the treatment with a hypocaloric diet.
Methods: 23 patients (17 ♀ and 6 ♂) with obesity, selected by Clinical Nutrition Unit were enrolled. The patients were subjected to dietary counseling and a Mediterranean, hypocaloric diet (1400-1600 Kcal/die) for 6 months. In addition to weight and BMI, we analyzed the following laboratory parameters: glucose, insulin, γGT, cystatin C, adipokines (leptin, adiponectin, resistin), hsCRP, TNFα, IL-6, IL-8, fibrinogen, PAI-1, tryglicerides, total cholesterol, HDL and LDL cholesterol. Statistical analysis was performed using SPSS 20.0. All variables studied were treated as continuous variables. P ≤0.05 was considered statistically significant. The analysis of variance (ANOVA for repeated measures and paired sample tTest) was used to evaluate intra-group differences between anthropometric variables and laboratory parameters.
Results: The hypocaloric diet determines a significant decrement of body weight and BMI (ANOVA P <0.0001). An average weight loss of 3% , 5%, and 7% was observed, respectively, after 1, 4 and 6 months of treatment. A positive correlation is shown between weight loss and reduction of fibrinogen (P <0.032), after 1 month, leptin (P <0.029) and insulin (P <0.020) after 4 month, and leptin (P <0.020) and resistin (P <0.045) after 6 months from treatment. Leptin is the biomarker that varies significantly throughout the whole time period (ANOVA P <0.0001). Insulin, fibrinogen, PAI-1, glucose, total cholesterol, LDL-cholesterol, cystatin C, γGT show a short-term variation - one month - whereas adiponectin, HDL-cholesterol, resistin, GOT, GPT, tryglicerides, hsCRP, IL-6, IL-8, VEGF, TNF-α, MCP1 vary in a long-term fashion - after 6 months of diet.
Conclusion: Weight loss induced by hypocaloric diet in obese patients is associated with early improvement of insulin sensitivity and pro-thrombotic risk biomarkers. Serum leptin level is reduced significantly and throughout the whole time of the study.

W179

THE INFLUENCE OF PRESENCE ESCHERICHIA COLI IN URINE SAMPLES FROM DIABETIC PATIENTS ON GLUCOSE, MICROALBUMINURIA AND CREATININE CONCENTRATIONS

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Background: The most common bacterial pathogens are often present in different concentrations in the urine samples intended for the determination of glucose, creatinine and microalbuminuria in diabetic patients. The aim of the study is to determine the influence of the presence of most common patogen Escherichia coli in urine samples on the concentration of these parameters or at what concentration of bacteria is influenced on the test result.
Methods: To determine the effect of the presence and concentration of bacteria in urine samples was used native urine with pathological high levels of glucose, creatinine, and microalbuminuria. Each of the samples was inoculated with one of the most common urinary tract pathogen - Escherichia coli in various volumes of inoculum (105–8 CFU/ml) for different intervals (6,12,24,48 hour). It was intended to change the concentration of glucose, creatinine, and microalbuminuria in urine. Glucose, creatinine and microalbuminuria were measured on Roche Modular system. Colony forming units were prepared by McFarland standards.
Results: For glycosuria (10 mmol/L-100 mmol/L) was demonstrated dependence on the size of the inoculum and the time interval. For the interval of 6 hours were measured changes in concentrations glycosuria from 33mmol/L. Glycosuria levels significantly decreased with increasing time interval and concentration changes were significant ($P < 0.001$) in the inoculum greater than 106 CFU/mL. For creatinine (2 mmol/L - 20 mmol/L) was significantly reduced only at inoculum concentrations greater than 107 CFU/mL and after 24 and 48 hours. A different situation was in microalbuminuria (20 mg/L - 250 mg/L) when decline set as measured after 48 hours from inoculum 108 CFU/mL. For the statistical eavaluation was used paired t-test and Statistica software by StatSoft.
Conclusion: The study showed that urine samples with high concentrations of glucose, creatinine and microalbumin, which are typical especially for diabetes can lead to bias in the case of contamination uropatogenic strains of E. coli.

W180

BIOCHEMICAL AND PHENOTYPE IMPROVEMENT IN SEVERELY OBESE PATIENTS AFTER BARIATRIC SURGERY

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Background: Adipocyte and adipose tissue dysfunctions are the primary defects in obesity and may link obesity to such disorders as increased risk of insulin resistance, type 2 diabetes, fatty liver disease, hypertension, dyslipidemia, atherosclerosis and cancer. Bariatric procedures have been shown to be more effective in the management of morbid obesity than lifestyle interventions and pharmacotherapy. The aim of this study was to investigate if the significant and sustained weight loss after laparoscopic adjustable gastric banding (LAGB) resulted in an improvement in the metabolism of obese subjects in terms of serum biochemical parameters and phenotypic characteristics (cell size and number) of subcutaneous adipose tissue (SAT).
Methods: We evaluated 19 severely obese subjects before LAGB (T0, mean body mass index [BMI] 45 kg/m2) and after the loss of >30% excess weight (T1, mean BMI 32 kg/m2). We also evaluated 10 normal weight subjects. We collected SAT and serum samples from all subjects. Conventional biochemical parameters were measured by routine laboratory procedures, and leptin and adiponectin by Luminex xMAP technology. Five-micron sections were prepared from all paraffin-embedded SAT blocks. Slides were then stained with hematoxylin & eosin.
Results: Levels of insulin, homeostasis model assessment-insulin resistance, triglycerides and liver markers as well as the leptin/adiponectin ratio were significantly lower at T1 vs T0 ($P < 0.05$). The number of SAT adipocytes was greater and their size smaller at T1 than at T0 ($P < 0.05$). Moreover, the morphological characteristics of SAT adipocytes at T1 did not differ from those of control adipocytes ($P = 0.89$).
Conclusions: LAGB induces an improvement in the obese metabolic status, which could result in a decreased risk of obese-associated diseases. Moreover, the normalization of adipocyte features at T1 vs T0 suggests a regression of SAT inflammation.
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W181
ASSESSMENT OF TRACE ELEMENTS; IRON, COPPER, SELENIUM AND LEAD IN OBESE DIABETES (TYPE 2 DIABETES) PATIENTS

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Background: It is evident that the metabolism of some trace metals is altered in diabetes mellitus and these micronutrients have specific roles in the pathogenesis and progression of the disease. The aim of this study was to investigate the level of trace elements: iron, copper, selenium and lead in obese diabetic patients.
Method: The study was carried out on two hundred and two (202) subjects, aged 20-68 yrs which comprises of fifty nine (59) obese diabetic subjects and forty five (45) obese non-diabetic patients, thirty eight (38) non-obese diabetic patients and sixty (60) non-obese non-diabetics (apparent healthy volunteers) as control group. The trace elements were analyzed using atomic absorption spectrophotometer and it is expressed in µg/dL.
Results: The mean ± standard deviation of iron (56.20±17.65), copper (55.57±15.27), selenium (25.10±S6.586), and lead (7.32±1.95) in obese diabetics. The mean ± standard deviation of iron (61.55±11.01) copper (59.03±12.89) selenium (28.78±5.821), and lead (7.92±1.46) in obese non diabetics. The mean ± standard deviation of iron (62.85±19.10) copper (29.05±9.886), selenium (60.58±17.56) and lead (8.71±3.69) in non obese diabetics. The mean ± standard deviation of iron (60.25±13.79) copper (58.40±14.61), selenium (28.91±8.382), and lead (8.76±4.37) in non obese non diabetics. Although the analysis of variance did not show any significant difference between groups (except selenium and lead) all trace element studied were decreased when compared with control (NONDH) group. Also the levels of these trace elements negatively correlate with BMI and worsen with diabetes complicating obesity.
Conclusion: This study showed that trace elements are useful in monitoring progression of obesity and obesity - induced complication-Dm.

W182
LIPID PROFILE IN OBESE DIABETIC PATIENTS

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Background: Obesity has been showed to be associated with varying degrees of dyslipidaemia hence this study was designed.
Method: The study (lipid profile) was carried out on two hundred and two (202) subjects, aged 20-68 yrs which comprises of fifty nine (59) obese diabetic subjects and forty five (45) obese non-diabetic patients, thirty eight (38) non-obese diabetic patients and sixty (60) non-obese non-diabetics (apparent healthy volunteers) as control group. Venous blood samples were collected from all subjects to determine FBS, plasma lipid profile which includes: total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), High density lipoprotein (HDL) and very low density lipoprotein (VLDL). TC, TG and HDL were estimated using enzymatic method (standard method) while LDL and VLDL were calculate using Frieldward formula.
Results: It was observed that in the mean ± SD of plasma TC (4.78±1.0), TG (0.63±0.63), HDL (2.25±0.98), LDL (2.22±1.15) and VLDL (0.40±0.35) of obese diabetic subjects, TC (5.03±1.30), TG (0.86±0.47), HDL (1.40±0.28), LDL (3.14±1.25) and VLDL (0.53±0.53) of non- obese, non- diabetic (control group), TC (4.49±1.30), TG (0.92±1.00), HDL (1.48±0.37), LDL (2.59±0.99) and VLDL (0.45±0.46) of obese non-diabetic, TC (4.73±1.08), TG (0.89±0.72), HDL (1.60±0.56), LDL (2.71±1.15) and VLDL (0.55±0.51) of non- obese non- diabetic (control group) respectively; when compared HDL and LDL in obese diabetic subjects were significantly higher ($P < 0.05$) than those of their control groups while the TC, TG and VLDL were not significant ($P > 0.05$), in correlation of age and BMI with lipid profile of obese diabetic patients and obese non-diabetic, the LDL has significant ($P < 0.05$) correlation. Mean±SD of plasma lipid profile of obese diabetic subjects on the basis of age groups and sex were not significant ($P > 0.05$).
Conclusion: These findings are proof that coronary heart disease, arteriosclerosis, dyslipidemia and diabetes mellitus are associated complications of obesity.

W183
OXIDATIVE STATUS IN THE LIVER OF DIABETIC RATS

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Background: Diabetes-related oxidative stress conditions lead to progressive tissue damage and disfunctionality. Mechanisms underlying liver pathophysiology during diabetes are not fully understood. The aim of this study was to examine oxidative status parameters in diabetic rats' liver.
Methods: Oxidative status parameters (plasma level of reactive oxygen species, liver antioxidant capacity) were determined in 20 rats that received saline (mL/kg) (control group) and 40 diabetic rats. Effect of diabetes was analyzed two (development stage) and eight weeks (stable diabetes) after single intraperitoneal injection of streptozotocin (STZ).
Results: Measuring the super oxide anions level in the plasma of diabetic rats showed almost linear increase starting from the 2nd, until the 8th week after STZ induction. The concentration of hydrogen peroxide was significantly increased in the development stage of diabetes, when it reached its maximum value (2.8 fold than in the controls). In stable diabetes, the concentration of hydrogen peroxide decreased but remained increased compared to control samples. In the development stage of diabetes, activity of super oxide dismutase (MnSOD), catalase (CAT) and glutatione S transferase (GST) in the liver was significantly lower ($P < 0.05$) compared to control. Their activity returned to the control values in the stable diabetes. The decline of activity in the development stage of diabetes among all analyzed enzymes was noticed, but most extensively in catalase activity which was associated with a significant increase in hydrogen peroxide concentration.
Conclusion: The elevated level of reactive oxygen species, especially hydrogen peroxide, was accompanied by reduced activity of CAT, GST and MnSOD during the development stage of diabetes. The obtained findings support the view that the tissue antioxidant status may be an important factor in the etiology of diabetes and its complications.

W184
RELATIONSHIP BETWEEN ALDOSTERONE, AND EACH COMPONENT OF METABOLIC SYNDROME IN ADULT MEN WITH CENTRAL OBESITY

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Introduction: Visceral Obesity is related with low grade chronic inflammation, and is the main component of metabolic syndrome (MS). MS is associated with increased cardiovascular disease. Several studies have reported a strong correlation between Renin Angiotensin Aldosterone System with cardiovascular disease, but the relationship between Aldosterone and each component of MS has not been fully elucidated. The aim of this study is to investigate the relationship between Aldosterone and each component of metabolic syndrome in adult men with central obesity.
Methods: This study is a cross sectional observation. After an overnight fast, the blood pressure and concentration of Aldosterone, fasting glucose, HDL Cholesterol, Triglyceride, Aldosterone, were analysed from 80 subject men with central obesity (MS=29 and non MS=51)
Results: Aldosterone had positive correlation with triglycerides ($r=0.234$; $P=0.036$), but not with other component of metabolic syndrome such as waist circumference ($r=0.014$), systolic blood pressure ($r=-0.047$), diastolic blood pressure ($r=0.083$), HDL-Cholesterol ($r=-0.165$), and Fasting Glucose ($r=-0.173$)
Conclusions: There were significant correlation between Aldosterone and Trygliceride. Since Trygliceride is one of the metabolic syndrome component means that increasing of aldosterone can affect the increasing Metabolic syndrome.

W185

OPTIMIZATION OF A WESTERN BLOT ASSAY TO DETECT SOLUBLE RAGE ISOFORMS IN HUMAN SERUM

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Background: The receptor for advanced glycation end products (RAGE) is a member of the immunoglobulin superfamily, multiligand and is associated with diabetes complications. RAGE has several isoforms, two predominant in plasma. The isoforms of soluble RAGE (sRAGE) resulting from cleavage by metalloproteinase (cRAGE) or by alternative splicing (esRAGE) and has been reported as biomarkers for inflammatory processes, diabetes and coronary artery disease. The aim of this study was to develop and optimize Western blot (WB) assay for soluble RAGE isoforms characterization in human serum.
Methods: Serum samples (n=8) from healthy subjects and diabetic patients were subjected to polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred to WB PVDF membrane (Amersham Hybond-P; GE Healthcare) using ECL Plus Western Blotting Detection System (GE Healthcare). Four different concentrations of primary antibody (rabbit IgG anti-RAGE H-300, Santa Cruz) and a secondary (anti-rabbit IgG conjugated with peroxidase; NA-934, GE Healthcare) were tested for detection of isoforms of sRAGE. The electrophoretic migration pattern was compared with the molecular mass marker (SpectraTM Multicolor Broad Range Protein Ladder, Fermentas). The high concentration of serum albumin can impair the sRAGE detection. To remove this interference, the samples were treated with sodium sulfite 24% in the presence and absence of caproic alcohol, a globulin aggregating agent.
Results: We identified three isoforms with molecular mass of approximately 50 kDa, 45kDa and 25 kDa. The serum albumin did not affect the recognition of sRAGE isoforms by primary antibody. Pre-treatment of the samples by precipitation with alcohol globulins caproic provides greater sensitivity in identification bands sRAGE.
Conclusions: The conditions were optimized: (1) gel electrophoresis in 10% polyacrylamide 19:1 (1X Laemmli buffer, 200 V, 53 minutes) with serum diluted 1:20 in saline, (2) WB: primary antibody diluted 1:20,000; secondary antibody diluted 1:4000.

W186

CSI: LIPOPROTEINS - CAN NANOTECHNOLOGY HELP?

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Background: Lipoproteins are among the most studied structures in biology. Despite intensive research efforts and the constantly increasing knowledge in the field, there are questions that remain to be answered. Nanotechnology is an emerging and exciting field aiming to a better and in depth understanding and manipulation of atoms and molecules and it has already started yielding medical benefits. The use of modern nanotechnology techniques in the study of some of the most interesting nanoparticles met in humans, lipoproteins, remains a scientific challenge. The aim of the study was to image low density lipoprotein (LDL) particle with the use of atomic force microscope (AFM) and investigate its interaction with surfaces.
Methods: LDL fraction was isolated by ultracentrifugation from healthy subjects. Experiments were performed on smooth and clean substrates of different hydrophobicity (HOPG,c-Si), with varying LDL concentrations (5,15,50 μg/mL), at different incubation times (1h, 2h, 12h). AFM measurements were performed with tapping mode in air.
Results: Images of LDL on HOPG were obtained. Various LDL aggregates were discernible and dimensions attributed to single particles were determined. The particles demonstrated preferential accumulation at the HOPG hydrophilic steps. The adsorption increased with the increase of the solution concentration. Full c-Si surface coverage from the LDL layer was observed. Lipoproteins formed clusters without being aggregated on special morphology features. LDL concentration was positively correlated with grain size. In all cases, lipoproteins were displaced by the tip, which may be a manifestation of the weak LDL-substrate interaction.
Conclusions: With the use of AFM images allowing identification of LDL particles can be obtained. The adsorption is dependent on the substrate wetting properties. It is also affected by LDL concentration and surface morphology. Further research is required in order to determine the effect of incubation time, AFM parameters (tip geometry, applied force, ambient/liquid environment) and pathological conditions on LDL shapes, sizes and biological behavior. The study highlights the potential of the AFM to extend our understanding on nanometer-sized biological particles, such as lipoproteins.

W187

GAMMA GLUTAMYLTRANSFERASE AND INSULIN RESISTANCE IN A MIDDLE-AGED AFRICAN POPULATION

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Background: In recent years, γ-glutamyltransferase (GGT) has been linked with incident cardiovascular diseases (CVD), diabetes and other components of the metabolic syndrome (MetS). A convergence point between liver function and diabetes may be via insulin resistance (IR) and nonalcoholic fatty liver disease, the hepatic manifestation of MetS. The objective of this analysis, therefore, was to assess the association of GGT levels with insulin sensitivity in a South African urban cohort and to assess whether the enzyme adds to existing metabolic risk profiles in identifying subjects with IR.
Methods: 1198 participants aged >15 years were drawn from the Bellville South suburb of Cape Town. The homeostatic model assessment of insulin (HOMA-IR), β-cells function (HOMA-B%), fasting insulin resistance index (FIRI) and the quantitative insulin-sensitivity check index (QUICKI) were calculated. The association of GGT levels with baseline covariates was assessed on a continuous scale and as categorical variables defined by sex-specific quarters of GGT levels using Spearman's correlation. Generalized linear and logistic regressions models were used to adjust for potential confounding factors.
Results: Levels of GGT were related to markers of IR and glycaemia. As GGT levels increased, so too did the indicators of IR (HOMA-IR, FIRI and fasting insulin) while those of IS (Sib and QUICKI) diminished significantly across the GGT quartiles. Subjects in the highest GGT quartiles also had the highest levels of fasting glucose and HbA1c. When analyzed as a continuous variable, serum GGT was significantly associated with HOMA-IR and most of the other markers of IR and glycaemia except β-cell function. In multivariable-adjusted models adjustment for sex, age, BMI, cigarette smoking and alcohol intake yielded the strongest, significant associations between GGT and all markers of IR/IS and glycaemia excluding glucose insulin ratio.
Conclusion: The association of GGT with insulin levels and sensitivity has immense relevance to public health. As they are inexpensive and are routinely obtained in clinical settings, they potentially offer an attractive possibility of substituting, or supplementing existing methods as simple and reliable tools for screening IR.

W188

RELATIONSHIP CARDIAC OXIDATIVE STRESS AND METABOLIC SYNDROME IN RATS WITH HIGH FRUCTOSE FEEDING

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Background: Rats fed a high fructose diet show the symptom of metabolic syndrome. It has been shown that oxidative stress is induced in the heart of rats with high fructose feeding. However, it is still unclear how cardiac oxidative stress is related with metabolic syndrome in rats with high fructose feeding. Therefore, we examined the relationship between cardiac oxidative stress and metabolic syndrome in rats with high fructose feeding.
Material and methods: Five-week-old male Wistar rats were fed MF diet containing 60% fructose (HFD) and MF diet containing 60% dextrose (control diet, CD) for 2, 4, 6, 8 or 10 weeks. Rats fed HFD or CD were killed at each feeding period. Serum separated from the collected blood was used for assays of insulin, glucose, triglyceride (TG), uric acid, and lipid peroxide (LPO). The heart isolated from each rat was used for assays of LPO, ascorbic acid, reduced glutathione, vitamin E, superoxide dismutase, catalase, and glutathione peroxidase.
Results: Serum insulin level was higher in HFD-fed rats than in CD-fed rats at all feeding periods except 10-week feeding and the peak of the level was found at 6-week feeding. Serum triglyceride and uric acid levels were higher in HFD-fed rats than in CD-fed rats at all feeding periods and the peak of both levels was found at 6-week feeding. Serum glucose level did not change at all feeding periods. Serum and cardiac LPO levels were higher in HFD-fed rats than in CD-fed rats at all feeding periods except 2- and 10-week feeding and the peak of the serum and heart LPO levels was found at 6-week feeding. Cardiac ascorbic acid, reduced glutathione, and vitamin E levels in HFD-fed rats showed different changes during feeding period but the level of each component was the lowest at 6-week feeding. Cardiac superoxide dismutase and glutathione peroxidase activities were lower in HFD-fed rats than in CD-fed rats at 6- and 8-week feeding but both activities were higher in the former group than in the latter group at 10-week feeding. Cardiac catalase activity was lower in HFD-fed rats than in CD-fed rats at all feeding periods except for 10-week feeding.
Conclusion: These findings indicate that cardiac oxidative stress is closely related to metabolic syndrome in rats with high fructose fee.

W189

MARKERS OF PLATELET ACTIVATION (SCD40 L, SP-SELECTIN, MPV) AND PLATELET COUNT IN TYPE 2 DIABETES PATIENTS DEPENDING ON THE PERCENTAGE OF GLYCOSYLATED HEMOGLOBIN (HBA1C)

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Background: Platelet hyperactivity with the elevated concentration of soluble CD40 ligand (sCD40L), soluble P-selectin (sP-selectin) and mean platelet volume (MPV) have been observed in type 2 diabetic patients. The aim of this study was to investigate the aforementioned parameters and platelet count (PLT) depending on HbA1C in diabetic cases.
Methods: The study group consisted of 84 patients suffering from type 2 diabetes, divided into: 1st group with the HbA1C ≤7.0% (32 patients,mean age 70.1) and the 2nd group with the HbA1C >7.0% (52 patients,mean age 67.1. The control group consisted of 30 healthy subjects (mean age 66.1). The sP-selectin and sCD40L concentrations were determined in the serum with the use of ELISA method. MPV and PLT were determined with the use of hematological analyzer and HbA1C with the use of biochemical analyzer in whole blood. Mann-Whitney's test was used in order to compare significant differences between two groups of patients and a control group. Differences were considered statistically significant for P ≤0.05.
Results: The sCD40L and sP-selectin medians were statistically higher in the 1st group (115.5 pg/mL and 84 ng/mL respectively) as well as in the 2nd group (139 pg/mL;101 ng/mL) than in healthy subjects (90.5 pg/mL; 68ng/mL) (P <0.05). The differences between the groups of patients were also significant (P <0.05). MPV medians were significantly higher in both groups of patients (9 fl) than in a control group (8 fl). The difference between the groups of patients was not significant (P ≥0.05). Platelet count median was a little bit higher in the 2nd group (241.5x103/μL) compared to the 1st group (233x103/μL) and healthy subjects (211.5x103/μL), but these values were not statistically significant (P ≥0.05).
Conclusions: Type 2 diabetes is associated with platelet hyperactivity (increased MPV, sP-selectin and sCD40L concentration), which is connected with the percentage of HbA1C. Improved glycaemic control might lead to correct abnormal platelet activation and thus may decrease the risk of cardiovascular and thrombotic complications in type 2 diabetes patients. OM. Koper and J. Kamińska were supported by a scholarship Studying, researching, commercialization-PhD students of the Medical University of Bialystok support program.

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LIPID PROFILE IN DIABETIC PATIENTS ACCORDING TO THEIR GLYCAEMIC CONTROL

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Background: Diabetes mellitus is associated with abnormalities in lipid profile which can indicate the risk for development of diabetic complications. The aim of this study was to investigate the lipids in diabetic patients with good and poor glycaemic control.
Materials and Methods: This study included 76 patients with diabetes mellitus type 2 (average age 52±3.2 years, average duration of disease 8, 5±2.3 years) and 30 healthy persons (average age 49±4.7 years) as control group. The patients were divided into two groups depending on their level of glycosylated haemoglobin (HbA1c): patients with good glycaemic control (GGC) with HbA1c ≤8% (n=42) and patients with poor glycaemic control (PGC) with HbA1c ≥8% (n=34). K3EDTA plasma samples were used for measurment of: HbA1c, triglycerides, total cholesterol, HDL and LDL. HbA1c was measured by ion exchanging chromatography and the other parametars were measured photometrically.
Results: The levels of triglycerides and total cholesterol in PGC group were significantly increased (P <0,01) compared to GGC and control groups. LDL was significantly increased (P <0,01) in both diabetic groups compared to the control group. HDL was significantly decreased (P <0,001) in both diabetic groups compared to control group.
Conclusion: Lipid abnormalities are present in both diabetic groups but they are lower in patients with good glycaemic control. Good glycaemic control is important to decrease the risk of development of diabetic vascular complications.

W191

CHARACTERIZATION OF MC4R, SIRT1 AND FTO GENE POLYMORPHISMS IN SEVERELY OBESE ITALIAN SUBJECTS: SNP RS9939609 IS ASSOCIATED WITH METABOLIC SYNDROME

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Background: Human obesity results from a complex interaction among environmental, behavioral, developmental and genetic factors, the latter accounting for 30-40% of the obese phenotype. Genome-wide associated studies have shown that many single nucleotide polymorphisms (SNPs) in several genes are involved in common forms of obesity or in obese-associated diseases. The aim of this study was to look for associations between SNPs in the melanocortin receptor 4 (MC4R rs12970134, rs477181, rs502933), silent mating type information regulation 2 homolog 1 (SIRT1 rs3818292, rs7069102, rs730821, rs2273773, rs12413112) and fat mass and obesity (FTO rs1421085, rs9939609, rs9930506, rs1121980) genes and obesity in a Southern Italy population.
Methods: One-thousand unrelated non diabetic severely obese patients (62% females, mean body mass index [BMI] 46.9 kg/m^2, mean age 32.8 years) and 100 controls (33% females, mean BMI 23.2 kg/m^2, mean age 29.9 years) entered the study. MC4R, SIRT1 and FTO were genotyped by Real Time Taqman assay. Anthropometric, clinical and biochemical data were collected for all enrolled subjects. Metabolic syndrome was diagnosed according to the American Heart Association criteria.
Results: Metabolic syndrome was diagnosed in 37.2% of patients. The four FTO SNPs were significantly associated with the obese phenotype (0.0001< P <0.007). At binomial logistic regression analysis, only SNP rs9930609 was significantly associated to obesity after correction for sex and age (OR/95%CI: 2.5/1.4-4.4 and 3.8/1.9-7.5, for the heterozygous and the homozygous mutated genotypes, respectively). The heterozygous mutated genotype in rs9930609 was found to be a predisposing factor for insurgence of metabolic syndrome (OR/95%CI: 2.5/1.1-5.5).
Conclusions: This study confirms that FTO is a susceptible gene for obesity risk, and patients bearing the polymorphic allele in the rs9939609 SNP could be at high risk of obese-associated diseases, such as metabolic syndrome. Consequently, once identified, these individuals can be enrolled in preventive programs.
Work supported by grants PRIN 2008.

W192

AUTOMATIC CALCULATION REVEALS HB TACOMA AS A COMMON CAUSE OF FALSELY LOW HBA1C RESULTS IN THE STOCKHOLM REGION - A NEW MODEL FOR HBA1C CHROMATOGRAM EVALUATION

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Background: Hb Tacoma [beta cd 30 Arg→Ser, AAG→AGT] is a clinically silent hemoglobin (Hb) variant which was found during HbA1c (A1c) analysis in a Swedish patient of Finnish descent already in 1993. Later, Hb Tacoma was found in several samples showing unexpectedly low A1c results when analyzed by Bio-Rad A1c methods. The corresponding immunological A1c results were significantly higher. The presence of Hb Tacoma was easily overlooked since no major variant peak was separated in the A1c chromatograms. Therefore we aimed at finding an algorithm by which suspected chromatograms could be identified even in circumstances with high workflow.
Methods: A1c was routinely quantified by chromatographic ion-exchange methods (Bio-Rad). Results were compared with immunological methods (Roche Tina-quant®, Siemens DCA Vantage®). Hb Tacoma was identified by ES-MS/MS or cleavage of amplified DNA with Bsl I.
Results: Using the BioRad Variant II A1c an increased LA1c/CHb peak was noted in Hb Tacoma samples. The ratio of the LA1c/CHb value (Area %) divided by the A1c value (NGSP Calibrated Area %) was 0.25-0.30 in normal samples and 0.40-0.60 in samples from uremic patients. For 16 patients heterozygous for Hb Tacoma the ratio was higher (mean 0.91, range 0.73-1.13). Automatic calculation of the ratio was initiated and results with a ratio >0.70 were highlighted to the operator during technical evaluation. During one year 262 out of 140,000 A1c results showed a ratio >0.70. 108 of these results were found to be due to Hb Tacoma. After introducing the Variant II Turbo 2.0 system as well as the IFCC A1c calibration a new cut-off of >0.075 (LA1c (Area %)/A1c (IFCC mmol/mmol) was etablished. During a 6 months period 75,000 HbA1c results were delivered and 80 different patients heterozygous for Hb Tacoma were identified. The chromatographic A1c results obtained in this group were on average 41% lower than the immunological A1c results, the latter being well in line with what was clinically expected.
Conclusions: Certain Hb variants, although globally rare, might be of significant importance in particular populations. The evaluation of A1c chromatograms, especially in high-throughput laboratories, can be significantly improved by relevant automatic calculations.

W193

ASSOCIATION BETWEEN HEMOGLOBIN A1C, LDL CHOLESTEROL AND OXIDIZED LDL CHOLESTEROL IN NON-DIABETIC INDIVIDUALS

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Background: It has been shown an increase in glycation in non-diabetic patients, which is maybe due to lipid peroxidation, consequently, the levels of malondialdehyde (MDA) increase and there is modification in the apolipoprotein B (apoB) of low-density cholesterol (LDL). The oxidative modification of LDL confers specific pro-atherogenic properties. The presence of oxidized LDL (oxiLDL) and an increased tendency to LDL peroxidation may contribute to increase A1C levels and might be associated with higher risk of cardiovascular complications in diabetic patients. The aim of this study was to investigate the association between A1C levels and the levels of LDL and oxiLDL in subjects without diabetes.
Methods: This is an observational cross-sectional study. A total of 196 individuals without diabetes were analyzed and divided into three groups according to A1C and fasting plasma glucose (FPG) values: Group 1 (n=64) - A1C <39 mmol/mol (5.7%) and FPG <5.6 mmol/L; Group 2 (n=69) - A1C ≥39 mmol/mol and ≤46 mmol/mol (6.4%) and FPG <5.6 mmol/L, Group 3 (n=63) – A1C ≥39 mmol/mol and ≤46 mmol/mol and FPG ≥5.6 and <7.0 mmol/L. OxiLDL was measured by enzyme immunoassay (Mercodia®), ApoB by immunoturbidimetry, total cholesterol (TC), triglycerides and high density cholesterol (HDL) by automated enzymatic assays and LDL was calculated by Friedewald equation.
Results: There were significant difference in oxiLDL (P <0.001) and Apo B (p=0.026) between the three groups. A1C values showed positive association with oxiLDL (r=0.431, P <0.001), LDL (r=0.148, P=0.039), non-HDL (r=0.192, P=0.007) and Apo B (r=0.171, P <0.001). These positive associations remained significant even after adjustment for multiple linear regression analysis for variables such as alcohol, drugs, body mass index (BMI) and age. TC (r=0.142, P=0.048), triglycerides (r=0.155, P=0.030) and BMI (r=0.263, P <0.001) also showed positive correlations with A1C values.
Conclusions: There is association between A1C levels and the atherogenic lipid particles LDL, Apo B, non-HDL cholesterol and oxiLDL. Individuals classified with high risk of developing diabetes have higher levels of oxiLDL. Our data showed that oxiLDL levels are significantly associated with A1C in non-diabetics individuals.

W194

DYNAMIC VARIATION OF PLASMA GLUCOSE DURING ORAL GLUCOSE TOLERANCE TEST AMONG PATIENTS WITH NORMAL FASTING GLUCOSE

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Background: Oral glucose tolerance test (OGTT), a laboratory test for diabetes mellitus, is performed by measuring plasma glucose concentration two-hour after an oral glucose load (75g of anhydrous glucose). This study examines the variation in plasma glucose during OGTT among patients with normal fasting glucose.
Method: All anonymized OGTT results that included fasting, one- and two-hour plasma glucose concentrations after oral glucose load, and measured in the same setting, were extracted from our laboratory database from 01/01/2009–01/11/2012. Oral glucose tolerance tests were administered per World Health Organization guidelines. Patients with fasting plasma glucose of >6.0mmol/L were excluded from this analysis. Plasma glucose concentration was measured by Siemens Advia 2400 at the National University Hospital, Singapore. Ethics approval was not required for study of this nature.
Results: Overall, 114 patients were studied. Of these, 71 (62%) had normal OGTT (<7.8 mmol/L), 30 (26%) had impaired glucose tolerance (≥7.8 and <11.1 mmol/L) and 13 (11%) had diabetes (≥11.1 mmol/L). Fasting, one- and two-hour post-OGTT average (SD) glucose concentrations were 4.8 (0.5), 7.9 (1.9) and 5.7 (1.1) mmol/L among patients with normal glucose tolerance; 5.3 (0.5), 10.3 (2.4) and 9.0 (0.9) mmol/L among patients with impaired glucose tolerance; 5.7 (0.3), 13.1 (1.7) and 13.7 (1.6) mmol/L among patients with diabetes mellitus. The percentage changes in glucose concentrations at one- and two-hour, relative to fasting plasma glucose, were +64% (33) and +20% (25) for normal patients; +95% (40) and +74% (25) for patients with impaired glucose tolerance; +129% (33) and +140% (32) for patients with diabetes.
Conclusions: The small sample size in this study was due to the infrequent OGTT requests that included a 60-minute plasma glucose measurement at our institution. Single fasting plasma glucose failed to identify 11% of individuals with diabetes. Plasma glucose variation was lowest during fasting, followed by two-hour post-OGTT. Generally, plasma glucose variation was lowest among normal individuals, followed by those with impaired glucose tolerance and diabetes. Two-hour plasma glucose remained elevated only among patients with diabetes.

W195

ASSOCIATION OF LPIN1 GENE VARIANTS WITH FATTY ACIDS IN BOSNIAN POPULATION WITH METABOLIC SYNDROME

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Background: Metabolic syndrome (MS) is one of the most prevalent health problems in modern population and it is estimated that about 20-30% of the world's adult population have MS. The pathogenesis of the metabolic syndrome is thought to involve a complex interaction of multiple factors, including obesity and abnormal fat distribution, insulin resistance, hepatic, vascular, and immunologic factors, as well as lifestyle and genetic contributions. A newly discovered gene for lipin 1 (LPIN1) resides in the 2p25 region. Lipin-1 is multifunctional protein that participates in the metabolism of lipids in different ways. Numerous studies implicate the role of fatty acids in development of obesity and MS. As LPIN1 gene variants could influence on lipin 1 activity, we studied whether gene variants (rs11693809 and rs2716610) are associated with fatty acid (FA) concentrations in patients with MS and control subjects.
Methods: This study included 57 patients from General Hospital Tesanj diagnosed with MS, and 41 control subjects. LPIN1 gene variants (rs11693809, rs2716610) were analyzed by using hydrolysis probes. Preparation of FA samples was done by using a modified protocol by Lepage and Roy. After transesterification, detection and quantification of FAs levels was performed by gas chromatography/mass spectrometry.
Results: Our data showed a tendency of association of LPIN1 gene variants (rs11693809, rs2716610) with concentrations of C14:1 and 20:3 FAs in patients, and C14:0 FA in controls.
Conclusion: Our results suggest that LPIN1 gene variants (rs11693809 and rs2716610) could have an important effect on FA concentrations, further implicating the role of LPIN1 gene in FA metabolism, and pathogenesis of obesity and MS.

W196

CORRELATION OF GLUCOSE AND GLYCOSYLATED HEMOGLOBIN WITH PALMITIC, STEARIC AND OLEIC ACID CONCENTRATIONS IN PATIENTS WITH TYPE 2 DIABETES

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Background: It has long been recognized that elevations of plasma fatty acids, in particular free fatty acids, disturb the normal glucose homeostasis and influence the function of pancreatic β-cells. Acute elevation of plasma fatty acids stimulates secretion of insulin, while chronic elevation of plasma fatty acids alteres insulin secretion. Although, emerging evidence suggest a strong association of glucose, glycosylated hemoglobin and fatty acids levels with diabetes mellitus type 2 (T2DM), limited number of studies have examined the association of different types of fatty acids with progression of the disease.
Methods: The study included 42 patients of General Hospital Tesanj, diagnosed with Type2 diabetes mellitus, and 40 healthy subjects. All subjects included in this study were free of evidence of hepatitis, viral infection, or active liver and kidney damage. We analyzed levels of glucose and glycosylated hemoglobin by standard methods (IFCC) with the use of autoanalyzer Alcyon analyzer while concentracions of palmitic, stearic and oleic acid were determined by gas chromatography analysis.
Results: As espected, the results showed a significant positive correlation of glucose and concentrations of palmitic, stearic, oleic acid in Type 2 diabetic patients ($P < 0.01$; $P < 0.05$, respectively). Interestingly, significant correlation was observed between palmitic, oleic acid and glycosylated hemoglobin in diabetic patients with T2DM ($P < 0.01$; $P < 0.05$, respectively).
Conclusions: Our data suggest relevance of sinchronous monitoring of glucose, glycosylated hemoglobin and concentrations of palmitic, stearic and oleic acid in progression of diabetes.

W197
LIPID PROFILE AMONG DIABETICS AND NON-DIABETICS IN CERTAIN LOCALITY IN NEPAL

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Backgroung: Diabetes is one of the leading causes of death worldwide. Its association with dyslipidemia has been seen pronounced and is the main reason for the increased mortality due to other induced metabolic syndrome. A region specific study of dyslipidemia pattern may help for effective implication of plan and policies.
Methods: A total 200 subjects from the Kathmandu and Patan were enrolled in our study, among which 100 were non-diabetic (controls) and 100 diabetic subjects. Serum glucose and lipid profile was determined in all fasting samples.
Result: Higher values of cholesterol (P <0.001), triglycerides (P <0.01), LDL-C (P <0.05) and TC: HDL ratio (P <0.001) whereas lower values of HDL cholesterol (P <0.001) were found in diabetic subjects as compared to healthy control. The significant difference of higher triglycerides suggests hypertriglyceridaemia. Similar lipid profile values were found in both diabetic male and female. However, the triglycerides in diabetic females were slightly higher than that of diabetic males. Increased TG and decreased HDL-C were seen in all age groups of diabetic subjects as compared to control. Moreover, no significant difference was observed between hypertensive and non-hypertensive in both diabetic and control.
Conclusion: Diabetes have direct relationship with dyslipidaemia mostly hypertriglyceridaemia. The condition is found ascending with increase in age but have no marked relationship with gender and hypertension.

W198
DERIVATION AND VALIDATION OF A WAIST CIRCUMFERENCE OPTIMAL CUTOFF FOR DIAGNOSING METABOLIC SYNDROME IN A SOUTH AFRICAN MIXED ANCESTRY POPULATION

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Background: Waist circumference (WC) thresholds for defining high risk status vary across populations, but no validated cut-offs exist for populations in Africa. We derived and validated WC cut-offs for predicting metabolic syndrome (MS) in mixed ancestry populations in South Africa.
Methods: Participants were adults drawn from an urban community of Bellville South, Cape Town. Oral glucose tolerance test and metabolic syndrome components were measured. The optimal WC cut-offs were determined by applying the Youden's index and the closest top left point approaches to the derivation sample (215 men, 705 women). The performance of the derived and recommended thresholds was then tested in a validation sample (82 men, 205 women) comprising participants recruited 3 years later.
Results: The two approaches identified a similar cut-off in men, 91.7 cm (sensitivity 69.2%, specificity 76.6%), but with two different values in women 88.4 (46.1%, 79.6%) and 94.7 (60.2%, 64.6%) cm. The derived cut-offs performed better than the usually recommended cut-off of 102 in men and 80 cm or 88 cm in women. In both males and females a cut-off of 90 cm was always among the best performing in the overall validation sample.
Conclusion: Although different WC cut-offs were evident in men and women, a threshold of 90 cm for both genders in this population is simple, practical and efficient and will misclassify only few participants as compared with the sex-specific optimal cut-offs. Further studies are needed to confirm the applicability of this cutoff both in mixed-ancestry and other African populations.

W199

RELATION BETWEEN FAMILY HISTORY OF COMMON CHRONIC DISEASES, NUTRITIONAL HABITS AND BLOOD BIOCHEMICAL PARAMETERS

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Background: Cardiovascular diseases, diabetes, osteoporosis are the most common chronic diseases (CCD) in Lithuanian population. The goal of the study was to compare lifestyle, dietary habits and biochemical phenotype between subjects with or without family history (FH+/-) of CCD.
Methods: 199 healthy subjects, aged 2– 30y (77 subjects with FH(-) and 122 subjects with FH(+)) were randomly selected from 33 Lithuanian cities. Subjects were interviewed using of validated questionnaire consisting of nutritional habits, family history and lifestyle factors. 72-hour recall method was used to evaluate the consumption of lycopene. Serum cholesterol, HDL-Ch, LDL-Ch, triglyceride, apo A-1, apo B, Lp (a), CRP and glucose concentration were measured.
Results: Lifestyle factors. Subjects in FH (-) and FH (+) groups were 81% and 74% normal body weight, 18% and 20% - overweight, and 1% and 6% - obese, respectively. Smoking cases were rare - 5,6% persons in FH (-) and 6,6% in FH(+) smoke regularly. Both groups of individuals characterized their physical activity as average. Nutritional habits. 39.5% FH(-) and 49.2% FH(+) subjects were using grain products every day. More FH(-) subjects (18.4%) were eating fresh vegetables every day to compare with 5% in FH(+) (P=0.02). The consumption of dairy, meat and fish products were not different in both groups. However, sweets 3-5 times per week or every day were used by 21.4% FH(-) and 37.6% of FH(+) (P=0.05). Median lycopene intake was higher in FH(-) (median=3.31 (IQR 0.77-12.38) mg/d) than in FH(+) (median=1.24 (IQR 0.15-3.33) mg/d) (P=0.01). Biochemical parameters. There was no statistically significant difference in cholesterol, trigliceryde, HDL-Ch, LDL-Ch, CRP, Apo B, Apo A-1, Lp(a), glucose concentration between study groups.
Conclusions: The increased incidence of obesity, decreased physical activity, and dietary habits with very low consumption of vegetables and fish, and high consumption of sweets and lower intake of lycopene are more common in group of children and young adults with positive FH of CCD.
The study was supported by LITGEN Project (VP1-3.1-ŠMM-07-K-01-013).

W200

ASSOCIATION STUDY OF POLYMORPHISMS -2548A/G LEP GENE AND -233A/G LEPR GENE WITH OBESITY IN AN ADULT POPULATION OF COLOMBIA

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Background: Obesity is a serious public health problem becoming the fifth leading cause of death worldwide. This has led to a search for candidate genes implicated in disease. Most of these genes encode components of molecular physiological systems involved in the regulation of energy balance. Thus, we evaluated the relationship of leptin gene (LEP) and leptin receptor (LEPR) with obesity and its cardiometabolic complications. However, conflicting results have been reported around the world, indicating the need for further investigations. Objective. Analyzing the polymorphisms LEP 2548-A/G and LEPR 233-A/G associated with obesity in a sample of adults in the city of Valledupar - Colombia.
Methods: We studied 203 individuals: 103 overweight or obese and 100 normal weight. The polymorphisms of LEP 2548-A/G and LEPR 233-A/G were determined by PCR-RFLP. Anthropometric measures were also evaluated, lipoprotein profile and fasting glucose.
Results: There was no statistical difference in allele frequency and genotype polymorphism LEP 2548-A/G between the study groups. On the contrary, it was observed that the mutated allele of the polymorphism LEPR 233-A/G homozygote genotype were significantly more prevalent in individuals with a BMI ≥ 25 kg/m2. Thus, carriers of the A allele and AA genotype were 3.4 and 2.9 times the risk of developing the disease [OR of A = 3.4 (95% CI = 2.072- 5.743)], [OR A/A = 2.9 (95% CI = 1.079 - 7.696)]. Regarding clinical variables studied, the polymorphism LEP 2548-A/G was associated with increased fasting plasma glucose and hip circumference, and LEPR 233-A/G with increased triglycerides and LDL. For both polymorphisms could establish significant association with low HDL.
Conclusions: Polymorphism 233-A/G LEPR gene is associated with the regulation of body weight in the study population. Both LEP 2548-A/G as LEPR 233-A/G have some influence on cardiometabolic variables associated with obesity.

W201
SHORT TERM EFFECT OF TRANDOLAPRIL ON INSULIN SENSITIVITY

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Aim: This study was design to investigate the effect of short term ACE inhibitor treatment on insulin sensitivity and to examine possible underlying metabolic effect on insulin-resistant subjects.
Methods: A randomized, double-blind placebo controlled trial was performed in 25 insulin-resistant women (age 40±5 years, homeostasis model assessment of insulin resistance, 5.6±0.7), who were free of any medications. The aim was to examine the effects of two weeks of ACE inhibitors treatment (trandolapril, 1mg/day) on insulin sensitivity.
Results: Trandolapril treatment decrease ACE activity compared with placebo (-22± 1.4 vs. 0.3±1.3U/l, respectively, P<0.001), resulting in a significant reduced blood pressure. Trandolapril treatment had no effect on whole body insulin mediated glucose disposal (before 17.9±2.0 after: 18.2±1.5μ mol kg body weight -1 min-1, P <0.03)
Conclusion: Short term trandolapril treatment adequately reduced ACE activity and blood pressure , but had no significant effects on insulin sensitivity on insulin resistant patients.

W202
MEASUREMENT OF CHOLESTEROL PRECURSORS AND CHOLESTEROL IN HUMAN HEPATOMA CELLS AND EFFECTS OF FLAVANONES AND ISOFLAVONES ON CHOLESTEROL BIOSYNTHETIC PATHWAY

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Background: Many studies show that an increased cholesterol level in blood is one of the major risk factors for atherosclerosis or coronary heart diseases. Lowering cholesterol level in blood is, therefore, one of the approaches for the prevention of these diseases. Several studies using animal models, cell cultures or biochemical tools suggest that polyphenols have a hypocholesterolaemic effect. The studies of mechanisms of lowering plasma cholesterol have mainly focused on the inhibition of cholesterol biosynthesis. For cholesterol biosynthesis, there are two alternative pathways, i.e., via lathosterol and via desmosterol, from lanosterol to cholesterol. In this study, effects of polyphenols on cholesterol biosynthetic pathways were evaluated by measuring the level of cholesterol precursors and cholesterol in human hepatoma cells by gas chromatography-mass spectrometry (GC-MS).
Methods: HepG2 cells, a human liver carcinoma cell line, were cultured in DMEM containing 10% FBS. After 10 h cultivation, medium were changed to DMEM containing 10% lipoprotein deficient bovine serum (LPDS) to promote cholesterol biosynthesis, and then added polyphenols to the cell culture medium. After 36 or 72 h cultivation, the cells were collected, and then pretreated by saponification with potassium hydroxide. After neutralization with phosphoric acid, cholesterol precursors and cholesterol were extracted with n-hexane and then they were derivatized with trimethylsilyl (TMS) reagent. The TMS-derivatives of cholesterol precursors and cholesterol were determined by GC-MS with selective ion monitoring (SIM).
Results: Hesperetin, naringenin and eriodictyol of citrus flavonoids decreased the level of cholesterol, however, increased that of lathosterol and 7-dehydrocholesterol with a dose dependent manner, suggesting they inhibit 7-dehydrocholesterol reductase (DHCR7). Daidzein and genisitein of soybean isoflavones slightly decreased the level of cholesterol, but increased that of desmosterol suggesting that it inhibits 24-dehydrocholesterol reductase (DHCR24).
Conclusions : These results it was recognized that polyphenols (hesperetin, naringenin, eriodictyol, daidzein and genistein) have a new inhibition site (DHCR7 or DHCR24) on cholesterol biosynthesis pathway.

W203
METABOLIC SYNDROME AND ATHEROSCLEROTIC RISK IN OBESE SUBJECTS

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Background: Obesity plays a role in cardiovascular disease development often because accompanied by metabolic syndrome (MS), a condition characterized by dyslipidemia, hyperglycemia and hypertension. Atherosclerosis is a manifestations of vascular damage in MS. It's well known that inflammation, hyperhomocysteinemia and oxidized LDL (oxLDL), a marker of lipid peroxidation, are involved in the atheromatous plaque formation.
Methods: In order to evaluate atherosclerotic risk, 149 consecutive healthy occupational obese subjects (36M/113F; aged 52.3±12.5; BMI 34.1±5.7 kg/m2; waist circumference, M: 111.3±10.9 cm, F: 98.4±10.9 cm) without previous cardiovascular events were enrolled at the "Obesity and Work" outpatient clinic from the Clinica del Lavoro "L. Devoto" of Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan. Subjects were evaluated for the presence of MS (NCEP-ATP III criteria) and for carotid plaque by ultrasonographic imaging (MyLabÔ70, Esaote). Total Hcy (tHcy) and high sensitive CRP (hsCRP) concentrations were assessed by routine Modular Analytics (Roche, Swiss), oxLDL levels by ELISA method (Mercodia, Sweden).
Results: Subjects were divided in plaque group (P; 7M/34F, aged 61.3±9.7) and no plaque group (NP 29M/79F, aged 48.9±11.8). About 33% of subjects showed MS; a positive association between MS and the presence of plaque was found (Fisher's exact Test: P=0.007; OR=2.82). No significant differences between P and NP were found for hsCRP (0.51±0.78 vs 0.46±0.48 mg/dL), tHcy (10.9±4.2 vs 11.3±5.1 μM), oxLDL (68.0±28.2 vs 71.6±28.5U/L). Multiple logistic regression analysis showed a significant association between age and presence of plaque (P <0.0001) and a trend toward the significance for MS and plaque development (P=0.08).
Conclusions: No association between presence of plaque and markers of endothelial damage was highlighted probably because almost all the subjects showed mean values within reference interval and all the plaques were characterized by abundant fibro-calcified tissue. Although multiple logistic regression analysis showed age as the main factor involved in plaque formation, risk of developing plaque was more than doubled by MS presence suggesting the importance of cardiometabolic prevention in obese subjects.

W204
CRP AND MICRO-ALBUMIN AS NEW MARKERS OF DEGENERATIVE COMPLICATIONS IN TYPE 2 DIABETES

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Background: Diabetes mellitus, especially type 2 diabetes, is a public health problem that has reached the range of epidemic due to the rapidly increasing rates of the disease throughout the world. In this study we intend to determine the correlation between urine albumin excretion rate, CRP levels and type of vascular complications in type 2 diabetes.
Methods: study subjects comprised 42 patients with type 2 diabetes subdivided into three groups according to the type of vascular complications. Urine albumin excretion rate, HsCRP was determined.
Results: we found a significant correlation between HsCRP and micro-albumin levels and a significant elevated levels of HsCRP and micro-albumin (P <0.05) when we compared diabetics with vascular complications to those without any complication. Diabetics with macrovascular complications have the highest levels of Hs CRP and Micro-albumin.
Conclusion: The determination of Hs CRP and micro-albumin levels represents an interest in risk stratification of vascular complications in type 2 diabetics.

W205

FASTING PLASMA GLUCOSE (FPG) ASSAY: THE EXPERIENCE OF OUR LABORATORY THROUGH PHASES OF IMPROVEMENT

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Background: The fasting plasma glucose (FGP) assay is fundamental in the diagnosis of diseases related to glucose metabolism. Its adequate and standardized measurement facilitates an early diagnosis of diabetes in the general population, optimizing the time of prevention and intervention reducing the dangerous complications of this disease. The guidelines' recommendations suggest the use of an inhibitor of glycolysis in parallel to a rapid preanalytic management. There are however many organizational obstacles to a proper compliance with the recommendations, especially in centralized and highly automated laboratories. This retrospective study describes the experience of our laboratory for the determination of FPG, and the influence that some organizational improvement actions have had on the FPG level measurement, taking into account the seasonal average temperature.
Methods: We compared the distributions of the FPG measurement in blood samples drawn from outpatients that reached the General Laboratory in the years 2009, 2010 and 2011. In 2009, 33128 FPG were measured in serum samples centrifuged after an average of two hours from collection. In 2010, after reducing the centrifugation time within one hour from blood draw, 32154 FPG results were collected. In 2011, 30431 FPG levels, assayed in samples drawn in tubes containing sodium fluoride and centrifuged within 1 hour, were measured. The data extracted from the laboratory database were analyzed to check the FPG levels in relation to the improvement actions implemented.
Results: The analysis of the distributions of FPG values obtained in different years in relation to organizational changes constantly showed an average level in every month, except during the summer months, significantly lower in 2009 compared to 2010 and to 2011 ($P < 0.05$). In all the years and regardless of the proposed model the seasonal temperature affects the values of FPG. The percentage of samples with blood glucose levels >126mg/dl in 2009, 2010 and 2011 were respectively 5.8%, 7.1% and 7.4%.
Conclusions: Adhesion to the recommendations of international guidelines has allowed an improvement of the FPG assay. The adoption of sodium floride did not inhibit the influence of seasonal temperature on FPG measurement.

W206

THE ASSOCIATION BETWEEN BODY MASS INDEX AND WAIST CIRCUMFERENCE AMONG NIGERIANS: VALIDATING THE NEW INTERNATIONAL DIABETES FEDERATION CRITERION FOR CENTRAL OBESITY

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Background: According to the 2006 International Diabetes Federation (IDF) consensus worldwide definition for metabolic syndrome, a person must have central (abdominal) obesity, which is defined as waist circumference (WC) with ethnicity-specific values, for which sub-Saharan Africa presently uses europid cut-points: male ≥94 cm,female ≥80 cm. However, central obesity is to be assumed if the body mass index (BMI) is >30 kg/m^2 and WC need not be measured. These alternatives in defining central obesity make it more feasible in clinical practice and community-based screening programs, but needs to be validated in specific ethnicities. The aim of this study is to determine the association between BMI and WC among Nigerians, in a bid to validate the interchangeable use of the parameters in the diagnosis of metabolic syndrome.
Method: This was a cross-sectional study of outpatients from various departments at the Lagos University Teaching Hospital, Nigeria. A total of 337 subjects (226 females, 111 males) were voluntarily recruited. Their body-weight was measured with a calibrated, portable, digital weighing scale to the nearest 0.1 kg, height was measured with a vertical rule to the nearest 0.01 m and BMI was calculated as body-weight/(height2).The WC was taken midway between the inferior margin of the ribs and the superior border of the iliac crest with a non-elastic tape rule to the nearest 0.1 cm. Pearson's correlation was used to determine association and $P \leq 0.01$ (2-tailed) was considered statistically significant.
Results: The total mean±SD were: female BMI 30.5 kg/m^2 (8.7), WC 94.0 cm (17.6); male BMI 27.1 kg/m^2 (5.9), WC 91.3 cm (14.3). One hundred and thirty-one subjects (101 females, 30 males) had BMI >30 kg/m^2 with mean±SD female BMI 37.9 kg/m^2 (6.9), WC 107.2 cm (14.4); male BMI 34.5 kg/m^2 (3.0), WC 108.63 cm (7.8). There was a strong positive correlation between all BMI and all WC which was very significant ($r=0.799$, $P < 0.0005$). Also, BMI >30 kg/m^2 versus WC ≥80 cm in females ($r=0.722$, $P < 0.0005$), and BMI >30 kg/m^2 versus WC ≥94 cm in males ($r=0.746$, $P < 0.0005$) showed positive correlations that were very significant.
Conclusion: The association between BMI and WC has been demonstrated in Nigerians especially at cut-points recommended by IDF for defining central obesity.

W207
BIOLOGICAL VARIABILITY OF GLYCATED ALBUMIN

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Background: The assessment of biological variability of a laboratory parameter is of pivotal significance for defining the analytical goals as well as the optimal number of samples to be analyzed for establishing the homeostatic point relevant to the clinical use of the test. No information is available so far on the biological variability in healthy subjects of Glycated albumin (GA), a protein increasingly used to assess glycemic control in diabetic patients.
Methods: Five specimens from each of 18 apparently healthy subjects (9 men and 9 women, ages 26–52 years), were collected on the same day, every two weeks for two months, following a well-standardized protocol. Samples were stored at −80 °C until analysis and tested in duplicate within a single run. GA was measured using an enzymatic assay (Lucica® GA-L, Asahi Kasei Pharma, AKP, Tokyo, Japan) on a Modular P Roche system (Roche Roche Diagnostics GmbH, Mannheim, Germany). Significance of data was analyzed with ANOVA.
Results: The analytical coefficient of variation (CVA) for GA was 1.7%, and within-subject (CVw) and between-subject (CVG) coefficients of variation were 2.1% and 10.6%, respectively. No difference was observed in values of GA between genders. The estimated critical difference (CD) was 7.5%. Two samples may hence be sufficient to estimate the individual homeostatic point of this biomarker.
Conclusions: The good quality achieved by the analytical method of GA assessment along with the modest within-subject biological variation suggest that this test may be actually recommended in clinical practice for evaluation of glycemic control in diabetics. Moreover, GA has marked individuality, thus limiting the use of population-based reference intervals for test interpretation.

W208
REFERENCE INTERVALS FOR ACETYLATED HBF IN HEALTHY NEWBORNS

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Background: Acetylated fetal hemoglobin (AcHbF) derives from a post-translational modification on the N-terminal glycine residue of γ-chains and its presence is well documented in newborn blood. Some discordant evidences have been reported on a possible relationship between AcHbF levels and maternal glycemic control during pregnancy. However, no established data are available on reference intervals for AcHbF in newborns.
Methods: Ninety-two healthy infants (48 males and 44 females), born at Del Ponte Hospital, Varese, Italy, from October 2011 to July 2012, were recruited in the study. All of them were of Caucasian origin, had a gestational age ranging from 37 to 41 weeks, an adequate birth weight for gestational age and presented negative neonatal, as well as gestational anamnesis. Newborns of mothers with diabetes, impaired fasting glucose or impaired glucose tolerance (both pre-existing and gestational) were not included. Blood samples (5 μL) were collected between the 2nd and 4th day of life by heel pricking. Hemoglobin pattern was analyzed by HPLC (Variant II, Dual Kit, Bio-Rad Laboratories).
Results: AcHbF results were normalized for HbF content in order to account for differences in hemoglobin switch. No difference was found in AcHbF values between genders (P=0.858). AcHbF values were found to have a normal distribution (Shapiro-Wilk test, P=0.737). AcHbF results were as follow: 12.8±0.8% (mean±SD), reference interval: 11.3-14.3%. No relevant correlation was found between AcHbF and newborn birth weight or length.
Conclusion: The reference intervals for AcHbF have been defined in a well characterized population of healthy newborns of not diabetic mothers. This finding could facilitate further studies aimed to assess the possible use of AcHbF as an index of fetal exposure to glucose during pregnancy.

W209

EVALUATION OF THE REQUEST OF GLYCATED HAEMOGLOBIN (HBA1C) IN THE POPULATION OF GRANADA (SPAIN)

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Background: Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action. Chronic hyperglycemia is associated with different pathologies but the major health problem is the increased risk of coronary diseases.Glycated haemoglobin (HbA1c) is the most important marker to the control of glycemic status in diabetic disease.The level of HbA1c in a blood sample provides the glycemic status of the 120 days previous.It is important to establish an early diagnosis of diabetes and an effective control of the disease to significantly reduce the risk of developing complications.The objective of this study is to know the amount and periodicity of glycated haemoglobin requests of adult population in Granada (Spain) and the incidence of the most common pathologies associated with the disease.
Methods: It was made a retrospective study of all requests of HbA1c from adult patients made at the San Cecilio hospital in 2011.The HbA1c was measured by ion exchange HPLC.The data base was obtained from the informatic system of laboratory. Data of% HbA1c were grouped according control level of diabetic in <6.5;[6.5-8]; >8. In the last group,data were regrouped in four intervals:[8-10];(10-12];(12-14]; >14%.It was calculated the periodicity of the request for each patient. Patients with values above 14% were chosen to study the risk factors and complications associated with this disease Results:41386 were the total requests with a diary average of 113. The 50% had HbA1c <6.5% and 18.5% above 8%.The 1% of this group had a value above the 14%.More than 90% of HbA1c requests were made within the first 3 months. Patients did not show differences about sex and age.According risk factors and complications: 32.8% were overweight, 46.6% had hypertension, 36.2% dislipemia and 17.2% all factors simultaneously. 13.8% of the patients had diabetic neuropathy and the 12.1% diabetic retinopathy. The 17.2% had cardiovascular disease and 12.1% respiratory disease.
Conclusions: Given the prevalence of high HbA1c values and the severity of complications associated with this increasingly common disease,we need a re-education and awareness of the diabetic population. The use of informatic warning rules can improve efficiency of the test

W210

CORRELATION BETWEEN CONCENTRATIONS OF MG AND HDL CHOLESTEROL IN PATIENTS WITH TYPE 2-DIABETES MELLITUS

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Background: Magnesium (мg) is needed for insulin synthesis, secretion, for insulin sensitivity of cells , for the transport of glucose into the cells and for the functioning of the key enzymes in the glucose metabolism. Controversial reports are available regarding the effect of mg on lipid profile and glycemic control in patients with type 2 diabetes mellitus (type 2dm). Magnesium deficiency in patients with type 2dm may have a negative impact on glucose homeostasis and insulin resistance.
Methods: The aim of our study was to analyze the concentration of serum mg, ldl and hdl cholesterol as well as fasting blood glucose in order to explore the role of those parameters as risk factors for the development of complications of diabetes mellitus. The study included 12o patients ; 60 with dm type 2 (the mean duration of disease was 7±2 years) and 60 healthy, age, sex and bmi matched controls. Those parameters we analyzed using commercially test on clinical chemistry analyzer Olympus au 420.
Results: Glucose, total and ldl cholesterol levels were significantly higher in diabetics than in control subjects (P < 0.001). In contrast the serum mg and hdl cholesterol levels were significantly lower in diabetics than in controls (P <0.001). A significant correlation of hdl-c concentration adjusted by serum glucose levels with serum mg levels (n=0.72; P <0.001) was found in patients with type 2 dm.
Conclusion: The results of this study suggest that a significant hypo-magnesemia was observed in diabetic patients type 2 compared to controls and it may be involved to decreased hdl-c.

W211
INCIDENCE OF HYPERGLYCAEMIA IN THE ELDERLY IN A TERTIARY HOSPITAL SETTING

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Background: Hyperglycaemia has been recognized as one of the important diseases of the elderly. The mortality and morbidity from associated conditions is higher in elderly diabetic subjects than in non diabetic people of the same age. This study was designed to assess the incidence of the different types of hyperglycemia in the Nigerian elderly.
Subjects and Methods: The glucose result records of 489 elderly individuals, aged 60 years and above, done in chemical pathology laboratory, University College Hospital, Ibadan, Nigeria were categorized as normal, impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and diabetes (DM). The plasma glucose concentration was determined using glucose oxidase method. Impaired fasting glucose was defined as fasting plasma glucose between 100 and 125 mg/dL, impaired glucose tolerance as two hours post prandial between 140 and 199 mg/dL and diabetes as fasting plasma glucose > 126 mg/dL or two hours post prandial >200 mg/dL. Subjects excluded from this study include patients on diabetic treatment and those with missing data on age and hospital diagnosis.
Results: Sixty-one subjects had impaired fasting glucose, eighteen had impaired glucose tolerance, thirty three had these two combined while sixty-eight had plasma glucose values characteristic of diabetes. Thus, accounting for 12.5, 3.68, 6.7 and 13.9% of the population studied respectively. The overall hyperglycaemic subjects in total is one hundred and eighty (36.8%).
Conclusion: Our results suggest that a significant proportion of the elderly who visit the hospital are prone to hyperglycaemic disorders. The diabetic elderly is most times of concern however this study shows that a significant proportion are in the prediabetic range. In view of the high incidence of hyperglycaemia in this population it is recommended that screening for hyperglycaemia be done in the elderly that have cause to visit the hospital.

W212
FRUCTOSE, URIC ACID AND NON-ESTERIFIED FATTY ACID PLASMA CONCENTRATIONS IN MEN WITH METABOLIC SYNDROME: DIAGNOSTIC VALUE

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Background: An increase in the consumption of fructose to be considered as a probable cause of metabolic syndrome (MS) epidemic. Fructose metabolism contributes to non-esterified fatty acids (NEFA) synthesis, and purine degradation with uric acid (UA) formation. The aim of the study was to determine fructose, NEFA, and UA plasma concentrations; to estimate diagnostic value of elevated fructose, UA and NEFA concentrations in men with MS and without MS.
Methods: The study included 314 men with cardiovascular diseases (mean age 58,7±9,1 years), divided into 2 groups: without MS (n=69) and with MS (n=245) according to the criteria proposed by the Russian Society of Cardiology (2009). Fasting plasma fructose, UA, and NEFA concentration were measured using a reagent kit «Fructose Assay Kit» by BioVision, a reagent kit for UA by Beckman Coulter International, and a reagent kit «NEFA» by Randox, respectively.
Results: Men with the MS, compared to men without MS, have higher levels of UA ((390.0 ± 81.2) vs (349.1 ± 67.7) μmol/L, P <0.0001), fructose ((0.228 ± 0.263) vs. (0.105 ± 0.152) mmol/L, P <0.05) and NEFA ((0.54 ± 0.25) vs. (0.46 ± 0.22) mmol/L, P <0.005) in plasma. Detection of hyperfructosemia >0.03 mmol/L in MS has low sensitivity (79.4%) and low specificity (32.0%). Using data about fasting plasma fructose concentration in healthy volunteers a novel reference interval was calculated. It amounted to 0.010–0.245 mmol/L. Detection of hyperfructosemia >0.245 mmol/L in MS has high sensitivity (92.0%) and positive predictive value (93.8%), but low specificity (30.9%). Detection of UA and elevated NEFA concentrations in plasma for MS has rather high specifity (88.4% and 95.7%, respectively), and high positive predictive value (93.8% and 88.5%, respectively), but low specifity (34.4% and 9.7%, respectively). Hyperuricemia in combination with hyperfructosemia has high specificity (96.8%) and positive predictive value (96.8%). Sensitivity of this test was 30.2%.
Conclusions: Fructose, UA, and NEFA levels in plasma are significantly higher in the presence of MS than in the absence MS. Increased fasting NEFA, UA and fructose concentration in plasma, and their combination may be considered as additional laboratory markers of MS.

W213
STUDY OF VITAMIN D STATUS IN CHILDREN AND ADOLESCENTS WITH NEWLY DIAGNOSED TYPE 1 DIABETES MELLITUS

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Background. Recent evidence has reported that vitamin D deficiency predisposes individuals to T1DM and T2DM. Vitamin D deficiency is becoming a global public health problem although it is largely unrecognized. The study aimed at assessing the prevalence of Vitamin D deficiency in recently diagnosed type 1 diabetes children and to define factors which may possibly influence Vitamin D levels.
Methods. Children were evaluated clinically; ALT, serum urea, ionized calcium, phosphorus, ALP, PTH and 25-OH Vitamin D (chemiluminescent immunoassay (CLIA) technology) were measured. HbA1c was measured in cases only.
Results . Fifty children and adolescents with recently diagnosed type 1 diabetes were prospectively recruited for this study from patients attending the diabetes clinic at the Alexandria University Children's Hospital. Forty healthy children served as control. All children were evaluated clinically ; There was a significant difference between cases and controls as regards history of delayed gross motor development (P=0.02) that might point to suspected vitamin D deficiency during infancy.A larger number of diabetic children had high alkaline phosphatase levels compared to controls with a statistically significant difference between both groups (P=0.05).Diabetic cases had significantly lower levels (10-30 ng/mL) of 25-OH Vitamin D (68.0%) than control (22.5%) group (P <0.0001). A significantly higher percentage of Vitamin D insufficient diabetic cases had DKA and/or polyuria at presentation (P=0.05).
Conolusions. Children and adolescents with type 1 diabetes showed lower 25-(OH)-D levels soon after their diagnosis compared with control subjects.As serum 25-(OH)-D is lower in diabetic cases, vitamin D concentrations may contribute to the development of type 1 diabetes.

W214
DOES IT WORTH TO REPEAT 100G GLUCOSE CHALLENGE TEST IF ONLY ONE MEASUREMENT IS HIGHER THAN THE CUT-OFF POINTS?

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Background: Our Public Health Area is applying National Diabetes Data Group (NDDG) criteria (100-g 3-hours glucose challenge test (GCT) deciding not to adopt new American Diabetes Association (ADA) 2000 criteria in diagnosis of gestational diabetes mellitus (GDM). Our GDM diagnostic protocol is based on screening 50-g glucose challenge at 24–28 weeks of gestation, where a result >140 mg/dL (>7.8 mmol/l) determines the performance of a diagnostic 100-g GCT. GDM is defined when more than two plasma glucose measurements were equal to or higher than the cut-off points. Actually to avoid loosing border line cases, we repeat GCT in the interval of four weeks, when only one of plasma glucose measurements is higher than the cut off points. At the moment, we know we are not having problems with the appropriate diagnosis and treatment of adverse complications for mother and child related with GDM. GCT is an uncomfortable test for patients. Would it worth to avoid repetition in cases with only one pathological result? An evaluation group was formed that consisted of laboratory doctors, endocrinologists and obstetricians.
Methods: 1.5 years 100g 3h GCT results obtained from Laboratory Information System: Servolab (Siemens).
Results: Along 1.5 years 1917 GCTs were performed to 1599 patients. Data were classified as: normal; two or more values over the cut-off; challenges not performed or finished due to absence, vomiting or indisposition. GCT is not performed if fasting result is higher than 123 mg/dL (>6.79 mmol/L). 978 GCT were pathological (corresponding to 698 patients). We focused on 303 GCT that had only one measurement higher of the cut-off. Cut-offs: fasting: >105 mg/dL (>5.8 mmol/L), 1 h: >190 mg/dL (>10.6 mmol/L), 2 h: >165 mg/dL (>9.2 mmol/L), 3 h: >145 mg/dL (>8.1 mmol/L).
Results of 303 GCT: Fasting result higher than 123 mg/dL: 6; Not repeated (different causes):149; Normal: 60; One point over cut-off: 40; Two points over cut-off: 34; Three points over cut-off: 14. That means: 60 normal and 88 pathological over 148 repeated GCT.
Conclusion: To repeat GCT, despite discomfort for the pregnant patient, improves diagnosis of 32% cases with one point higher than the cut off that would be lost in case of lack of second CGT.

W215
SERUM MELATONIN LEVELS IN PATIENTS WITH METABOLIC SYNDROME

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Background: The metabolic syndrome (MS) is characterized by central obesity, insulin resistance, atherogenic dyslipidaemia. It is also frequently associated with prothrombotic and proinflammatory state and hormonal changes. Melatonin is secreted mainly at night by the pineal gland and seems to be involved in a variety of physiologic and metabolic processes. Previous studies have suggested that reduction in melatonin secretion may alter energy regulation, resulting in elevated body weight and adiposity.
Aim: The objective of this study was to determine serum melatonin levels in patients with MS.
Methods: In the study 40 patients with MS (mean age 34.17± 1.36) and 40 healthy controls (mean age 34.17±1.41) were included. The diagnosis of MS was made according to the IDF criteria. For melatonin determination blood samples were taken at 3:00 and 8:00 AM. Serum melatonin concentrations were calculated in the "Sirio S microplate reader" (SEAC, Italy) using ELISA kit (IBL, Hamburg, Germany). Anthropometric measurements included weight, body mass index (BMI), waist and hip circumferences. Serum levels of glucose, HDL-cholesterol, triglycerides (Konelab 60i) and insulin (Axsym ™ system) were measured. Statistical analysis of data was made with SPSS v. 17 (P <0.05).
Results: Comparing the statistical variables of both groups we found in the MS group increased levels (mean±SEM) of weight (P=0.0001), BMI (P=0.0001), waist and hip circumferences (P=0.0001), glucose and insulin (P <0.01)and decreased HDL-cholesterol (P <0.01). There were no significant differences in triglycerides. The mean melatonin did not significantly differ between study groups at 3:00 AM (89.79±7.42 pg/mL in MS group vs 87.46 ± 6.68 pg/mL in the control group, t=0.233, P=0.816). Serum melatonin levels significantly increased in MS patients compared to controls at 8:00 AM (55.19±5.41 pg/mL vs 30.69±3.78 pg/mL, t=3.715, P=0.0001 respectively). The mean night-day melatonin difference was significantly lower in MS patients compared to controls (34.60 ± 6.31 pg/mL vs 56.77± 5.47 pg/mL, t=2.653, P=0.010).
Conclusions: Our data suggests that serum melatonin levels could be of help to clarify the role of melatonin in the pathological processes.

W216
EFFECT OF MORINGA OLEIFERA ON LIPID METABOLISM GENE EXPRESSION IN HEPG2 CELLS

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Background: Moringa oleifera (MO) leaves are known to treat hyperlipedemia in Thai traditional medicine but the mechanism is unclear. Oxidative stress plays a major role in the pathogenesis of many degenerative diseases induced by free radicals, such as hyperlipidemia, diabetes mellitus and cardiovascular disease. The aim of this study was to determine the antioxidant activity and the effect of MO on lipid metabolism gene expression in HepG2 cells such as HMG-CoAR, LDLR, PPARs and LXRα.
Methods: We evaluated the antioxidant activity of MO using ORAC assay, Folin Cioculteau Phenol assay and total flavonoid assay, respectively. To determine the effect of MO on HepG2 cells viability using MTT assay, oxidative stress using DCFH-DA assay and lipid metabolism gene expression using RT-PCR assay.
Results: We found that lyophilized form of MO extract in 80% ethanol possessed total antioxidant, polyphenolics and flavonoids contents within the range 9306.7±364 TEmM/kg dry mass, 218.3±1GEmM/kg dry mass and 285.7±12 QmM/kg dry mass, respectively. MO extract at high dose (2,000-3,000 mg/L) induced cytotoxicity. However, MO extract 100-3,000 mg/L could reduce intracellular oxi¬dative stress in a dose-dependent manner (P <0.05). MO extract significantly down regulated the mRNA expression of HMG-CoAR, PPARα1 and PPARg (P <0.05).
Conclusions: Moringa oleifera could be beneficial for health promotion by reducing oxidative stress and reducing cholesterol and lipid synthesis by suppression of HMG-CoAR, PPARα1 and PPARgamma gene expression, thereby maintaining lipid homeostasis.

W217

CORRELATION OF BLOOD SUGAR AND GLYCATED HEMOGLOBIN WITH LIPID PROFILE IN DIABETIC PATIENTS ATTENDING TERTIARY CARE HOSPITAL IN EASTERN NEPAL

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Background: Measurement of blood sugar level and glycated hemoglobin (HbA1c) are widely used laboratory tests done for the long term management of diabetes and its complications. Dyslipidemia is frequently associated with diabetes as a strong risk factor for developing cardiovascular disease, and its control can prevent cardiovascular complications. There is growing evidence that control of blood sugar level and HbA1c within the control limits can also control dyslipidemia although the results are controversial which necessitates further study to establish the evidence. So, we sought to determine the status of lipid profile in diabetic patients attending a tertiary care hospital of Eastern Nepal and to determine the correlation between blood sugar, HbA1c, estimated average glucose (eAG) with lipid profiles specially Total Cholesterol (TC) and Triglycerides (TAG).
Methods: In this retrospective cross sectional study, fasting blood sugar (FBS), postprandial blood sugar (PPBS), HbA1c and the lipid profile of 726 diabetic patients attending B.P. Koirala Institute of Health Science, Dharan were obtained from the Biochemistry Laboratory during January to December, 2011. Blood glucose, TC, TAG and HbA1c were measured by Glucose oxidase-peroxidase, Cholesterol oxidase-peroxidase, Glycerol-phosphate oxidase-peroxidase method and Nycocard Reader respectively. eAG was calculated from the data of HbA1c. The correlation coefficient was determined and P value <0.05 was considered statistically significant.
Results: We obtained 4816 samples with lipid profile and out of them we analyzed only 726 samples whose blood glucose level was in diabetic range according to American Diabetes Association (ADA) criteria. The median values for FBS, PPBS, TC, TAG and HbA1c were 152.5 mg/dL, 287.0 mg/dL, 172.0 mg/dL, 154.0 mg/dL and 7.4% respectively. There was a significant correlation between FBS and TC, TAG, HbA1c and eAG. Females were found to have more median total cholesterol level than males (178 mg/dL in females and 168 mg/dL in males) which was statistically significant.
Conclusion: Diabetic patients experience dyslipidemia due to deranged glucose and lipid metabolism. Proper control of blood lipid level is necessary to prevent from cardiovascular diseases.

W218

ELUCIDATION OF ANTIDIABETIC EFFECT OF WATER EXTRACT OF FRUIT OF WITHANIA COAGULANS ON INSULIN RELEASE FROM ISOLATED PANCREATIC ISLETS

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Background: Withania coagulans fruit is widely used for its anti-hyperglycemic effect in traditional health care system in India. Aim of this study, was to evaluate the glucose lowering and insulinotropic effect of aqueous extract of Withania coagulans (aqWC) in vitro pancreatic β cell islets isolated from nicotinamide-streptozotocin induced type 2 diabetes mellitus.
Methods: Oral glucose tolerance test was performed to standardize the effective dose of aqWC. Diabetic animals were treated with aqWC extract (50mg/incubation mixture) for 30 days. The pancreatic islets were isolated, suspended in culture medium and insulinotropic effect of aqWC has been studied. The isolated pancreatic islets were treated with glucose at 3 mM (non-stimulus concentration) and 11mM (stimulus concentration).
Results: Treatment of diabetic animals with aqWC at a dose of 250 mg/kg for 30 days produced significant decrease in glucose tolerance test and increase in circulating insulin levels as compared to diabetic-untreated animals. Similarly, in vitro release of insulin from isolated pancreatic β-cells of healthy control and diabetic-untreated animals showed increased insulin levels after 11 mM of glucose load. Whereas, the simultaneous addition of glucose at 3 mM and 11 mM followed by addition of aqWC showed significantly increased insulin release from pancreatic islets ($P < 0.05$). The pancreatic islets from diabetic treated with aqWC for 30 days was further treated in vitro with glucose (3 and 11 mM concentration) + aqWC also showed significant elevation in insulin release. ($P < 0.05$). The release of insulin from diabetic-treated with aqWC for 30 days showed higher concentration of insulin release as compared to diabetic-glucose treated animals.
Conclusion: These results suggest that aqWC has effective glucose lowering potential might be due to modulation of pancreatic β-cells and produced insulinotropic effect from pancreatic β cells.

W219

PERFORMANCE EVALUATION OF THE CAPILLARYS 2 FLEX PIERCING ANALYZER FOR HBA1C

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Background: Hemoglobin A1c (HbA1c) levels are widely used to monitor glycemic control in diabetes mellitus patients, and various methods are used for the determination of HbA1c levels. The capillarys 2 flex piercing (Sebia, Norcross, GA) is a fully automated, high-throughput glycohemoglobin (HbA1c) analyzer based on capillary electrophoresis. We evaluated the performance of the analyzer.
Methods: The analytical performance of the capillarys 2 flex piercing analyzer was evaluated by its precision, linearity, correlation with the Variant II Turbo (Bio-Rad Laboratories, Inc., USA), and the interference of carbamylated hemoglobin. We also investigated agreement with National Glycohemoglobin Standardization Program (NGSP) targets. All evaluations were performed according to CLSI guidelines EP05, EP06, and EP09.
Results: Coefficients of variation (CVs) for within-run and total imprecision were 1.7% and 1.8% at low concentrations and 1.2% and 1.3% at high concentrations, respectively. The linearity was excellent with R^2=0.9882 in the range of 5.13–13.83%. No significant interference of carbamylated hemoglobin was noted. It was well correlated with Variant II Turbo (R^2 =0.9978) in the range of 4/7-13.5% (capillarys 2 flex piercing=1.000 x Variant II turbo + 0.05 by Passing-Bablok regression analysis). The mean and 95% confident interval of differences to NGSP target were -0.0138 and -0.3618–0.3343%.
Conclusions: The capillarys 2 flex piercing showed excellent precision, linearity, correlation with Variant II Turbo and agreement with NGSP target. Therefore, its analytical performance is satisfactory for diagnosis and monitoring the treatment of diabetes.

W220

PROTON NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (1H-NMR) AS MEDICAL INSTRUMENT FOR URINE BIOCHEMISTRY IN TYPE 2 DIABETES MELLITUS

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In the first study, Proton Nuclear Magnetic Resonance Spectroscopy (1H-NMR) was applied to investigate the urinary patterns of type 2 diabetes mellitus (T2DM) patients, to obtain information about the mechanisms involved in the metabolite excretion and to identify possible biochemical changes that may accompany T2DM. In the second study, we analyzed T2DM patients according to estimated glomerular filtration rate (eGFR), gender, age, body mass index (BMI) and the 1H-NMR metabolite concentrations established. Serial urine samples of 118 healthy subjects and 145 T2DM patients were investigated by 1H-NMR. The 1H-NMR spectra have been recorded on a Bruker Avance DRX 400 MHz spectrometer. To 0.9 mL urine, 0.1 mL of a stock solution of 5 mM sodium 3-(trimethylsilyl)-[2, 2, 3, 3-d4]-1-propionate (TSP) in 10% D2O has been added. The results were evaluated in mmol/mol of creatinine. $p < 0.05$ was taken as significant. A significant difference between the urinary excretion of valine, 3-hydroxyisolateric acid, alanine, gamma-aminobuthyrate, betaine, citrate, trimethylamine-N-oxide and glycine in T2DM patients vs. healthy individuals was found. There are significant differences between the excretion of lactate, pyruvate, citrate, hippurate, glycine and trimethylamine-N-oxide in T2DM patients with values of glycosuria less than 3mmol/L vs. healthy subjects. We obtained that the concentrations of valine, lactate, alanine, 3-hydroxyisovaleric acid, gamma-aminobuthyrate, citrate, dimethylamine, trimethylamine and trimethylamine-N-oxide increased in T2DM patients with values of glycosuria up then 3 mmol/L in comparison with control group. Dimethylamine, gamma-aminobuthyrate and pyruvate were significantly related to the eGFR (ml/min/1.73m^2) in T2DM patients. Highly significant differences were seen for lactate, gamma-aminobuthyrate and hippurate in T2DM women than the T2DM men patients. The T2DM patients above 55 years old tended to have higher urinary concentrations of lactate than patients below 55 years old. Our analysis revealed significant decreased concentrations for citrate, dimethylamine and glycine in T2DM patients with the increase of BMI. 1H-NMR could explore urinary metabolite as markers for early detection of associated diseases and complications in diabetes.

W221

COMPARISON BETWEEN THE INDICES FOR THE EVALUATION OF INSULIN RESISTANCE IN THE HEALTH SURVEILLANCE OF WORKERS

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Background: Night shift workers present a high risk to develop metabolic and cardiovascular disorders for alterations that involve effects on circadian rhythms at the level of insulin resistance (IR). Therefore monitoring such parameter is a crucial step in health surveillance. To this aim, the currently in use test consists in the calculation of the HOMA-IR index . In these study it was compared to other two calculated index and cost/performance was evaluated.
Methods: This study was carried out on 217 workers at Centre for Obesity and Occupational Medicine of the Occupational Medicine Clinic of Milan and it were calculated IR as a)HOMA index [basal insulin (μU/mL) x basal glycemy (mmol/L)/22.5], b) TyG index as Ln [Triglycerids (mg/dL) x Glucose (mg/dL)/2] and c) triglycerids/HDL-cholesterol ratio. Biochemical parameters were assayed by routine Modular Analyzer (Roche, Switzerland).
Results: The statistical analysis was based on the calculation of the Pearson coefficient (r) between the different indexes. This analysis showed a weak correlation between HOMA-IR and the ratio triglycerides / HDL (r=0.29) while it was possible to show a good correlation between HOMA-IR index and TYG (r=0.43). The determination of HOMA-IR has a quite considerable cost of about 13 Euros, meanwhile other indexes cost altogether about 5 Euros.
Conclusions: The study confirmed the correlation between HOMA-IR and TyG and identified the TyG as the index with the best cost/performance ratio. Our future goal is to establish cut-off values, necessary to adopt the TyG as first choice index.

W222

NON HDL-C AND TC/HDL RATIO IN TYPE-2 DIABETES MELLITUS DYSLIPIDEMIA

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Background: Diabetic dyslipidemia is characterised by elevated triglycerides and low levels of HDL cholesterol, with a predominant small dense LDL particles amidst relatively normal LDL cholesterol levels. Thus looking at LDL cholesterol (LDL -C) alone may not be sufficient to assess the dyslipidemic features in diabetes. Non HDL Cholesterol (Non HDL-C) calculated as total cholesterol minus HDL-C provides a measure of all atherogenic Apolipoprotein B containing lipoproteins. The predictive value of Total cholesterol/HDL cholesterol ratio (TC/HDL-C) is greater than the isolated parameters. Thus the objective of the study is to evaluate the utility of NonHDL-C and TC/HDL-C to assess the dyslipidemia in diabetes.
Methods: The study was conducted with 60 diabetic diagnosed by ADA criteria and 30 age & sex matched non diabetic. Anthropometric measurements were done. Fasting blood samples were collected. The glucose,Triglycerides(TGL),Total Cholesterol(TC),HDL -C were measured. The LDL-C was calculated by Friedewald formula: LDL-C =TC –(HDL-C + TGL/5). Non HDL-C and TC/HDL-C were calculated employing their respective formula. Datas were analyzed using SPSS 16 version. The Students t test, ANOVA, correlation & regression were used.
Results: Non HDL-C,TC/HDL–C ratio,TGL,TC,HDL-C,LDL-C, were significantly different (P <0.001) between diabetic & non diabetic. In diabetics both NonHDL-C and TC/HDL-C ratio were significantly correlated with TGL (r=0.530, P <0.001; r=0.575, P <0.001) TC (r=0.821, P <0.001 ;r=0.969, P <0.001),HDL-C (r= -0.708, P <0.00; r=-0.444, P <0.001), LDL-C (r=0.918, P <0.001; r=0.985, P <0.001) , fasting glucose (r=0.260, P=0.013; r=0.283, P=0.007) & duration of diabetes (r=0.346, P <0.001; r=0.408, P <0.000) respectively. Based on TC/HDL-C ratio diabetic subjects were classified into two groups (High risk group TC/HDL-C >4, low risk group TC/HDL-C<4). There was significant difference between the groups in TC,TGL,HDL-C, LDL-C&NON HDL-C (P <0.001). The diabetics were categorised into three groups according to NON HDL-C levels. Non HDL-C <130 mg/dL (low risk group), Non HDL-C 130-160mg/dL (moderate risk), Non HDL-C >160 mg/dL (high risk group).There was significant difference between the groups in TC (P <0.001), TGL (P <0.001), HDL-C (P=0.011), LDL-C (P <0.001) & TC/HDL-C ratio (P <0.001). Diabetics were stratified into tertiles according to TGL levels.TGL tertile 1 (<130 mg/dL), TGL tertile 2 (130-190 mg/dL), TGL tertile 3(>190 mg/dL).Thus in all the 3 tertiles significant correlation were found between (TC/HDL-C) ratio and LDL-C (r=0.813, r=0.807, r=0.789 respectively, P <0.001). For each tertiles equation of linear regression of TC/HDL-C(y) and LDL-C(x) were found. The intercept of the equation is higher in tertile 3 compared to other two tertile. Tertile 1(y=0.778+0.024*x,) Tertile 2 (y=0.778 + 0.024*x) Tertile 3 (y=1.323+0.022*x). Thus diabetics in TGL tertile3 (>190 mg/dL), has higher TC/HDL ratio for any given value of LDL- C value than the daibetics in other two tertiles. Similarly linear regression equation were found between NON- HDL-C(y) and LDL-C(x) in all the 3 tertiles. Tertile 1 (y=28.152+ 0.972*x) tertile 2 (y=31.972+1.019*x) Tertile 3 (y=39.876+1.031*x). In Diabetics of tertile 3 (TGL>190 mg/dL) for a given value of LDL-C(x), NON HDL-C(y) is higher. Thus in case of hypertriglyceridemia , TC/HDL-C & NON HDL-C levels were higher for a given value of LDL-C.
Conclusion: In diabetics when TGL are higher, LDL-C levels from Friedewald formula underestimates the dyslipidemic status. TC/HDL-C ratio and non HDL-C levels can project the dyslipidemic status of diabetes in a better way.

W223

DETECTION OF PREDICTIVE URINARY BIOMARKERS OF NEPHROPATHY IN TYPE 1 AND TYPE 2 DIABETES BY PROTEOMIC ANALYSIS

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Background: Nephropathy associated with diabetes is a severe complication that cause slow kidneys deterioration, leading to end-stage renal disease. Renal involvement during diabetes mellitus may affect all the structural components of the kidneys, causing functional and organic alterations frequently associated with inflammatory processes, that give rise to multiple clinical manifestations. Currently, despite rapid research progress, predictors able to assess prospectively and with high precision the risk to develop diabetic nephropathy (DN) are still lacking.
Methods: The aim of this project was to identify differences in urinary protein excretion, both in type 1 (T1D) and type 2 diabetic (T2D) patients, in comparison with healthy control subjects. Ninety diabetic patients were recruited and divided in 3 groups (for each diabetes type), according to the level of albuminuria: normoalbuminuric, with microalbuminuria (MA) and with overt proteinuria. Second void morning urine samples were collected and centrifuged to remove cell debris and contaminations. Urinary proteins were separated by two-dimensional electrophoresis (2-DE) and identified by mass spectrometry analysis (MS).
Results: Comparing the patients proteomic profiles with those of normal subjects, firstly we noted a significant increase of alpha-1-antitrypsin and albumin, also in the form of numerous fragments, in urine of diabetic subjects. Particularly, statistical analysis and spot quantification by PDQuest image software revealed several proteins differentially expressed in diabetes condition. Some proteins resulted increased in urine of both T1D and T2D patients with MA, such as transthyretin, apolipoprotein-A1 and transferrin, while the majority of the over-excreted proteins were found in T2D patients with proteinuria, e.g. vitamin-D-binding protein, protein AMBP, zinc-alpha-2-glycoprotein, fetuin-A and ganglioside GM2 activator.
Conclusions: This protein pattern might represent a potential tool for a better understanding of DN and could help to identify patients at increased risk of renal disease progression. Therefore, in diagnostic field, 2-DE and MS proteomic analysis could be a suitable approach to discover early and predictive biomarkers of DN in urine of diabetic patients.

W224

STATUS OF TRIGYCERIDES AND URIC ACID LEVEL IN HEALTHY WORKING NEPALESE POPULATION

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Background: Increased serum level of Triglycerides [Tg] and uric acid [UA] have been associated with morbidity conditions like coronary syndrome, diabetes mellitus type II, hypertension and obesity. Non-vegetarian diet and more sedentary lifestyle affect the metabolism of Tg and UA. Thus, the objective of this study is to identify the status of Tg and UA level in healthy Nepalese working population of capital city in the scenario of non-vegetarian fast food eating habit and sedentary working lifestyle.
Methods: A total of 116 subjects [68 males and 48 females] in the age of 20-50 years were investigated who had come for general health check up as part of their job requirement in Tribuwan University Teaching Hospital during the period of Jan –Sept 2012. Tg level was analyzed by GPO –PAP method and UA level by PAP–Uricase method.
Results: The level of Tg was 1.7±0.9 mmoles/L in males and1.5±0.8 mmoles/L in females respectively .The level of uric acid was 339±86 micromoles/L in males and 272±77 micromoles/L in females. Significant difference in uric acid level between males and females [P=0.01] was observed which was not seen with triglycerides . .The serum Tg level significantly correlated with serum uric acid level [r=0.317,P=0.01]. Both Tg and UA positively correlated with BMI [r=0.2, P=0.05] but no significant difference between the triglycerides and uric acid level between underweight [n=20], normal [49] , and overweight [n=47] groups.
Conclusions: The male healthy working Nepalese need to be cautious of their health and food habit than the females for rewarding professional and personal life as they fall in the borderline risk region of Tg level and since high Tg level increases UA level too.

W225
EVALUATION OF ADAMS ™ A1C MENARINI HA-8180 VARIANT MODE HPLC ANALYZER FOR HBA1C DETERMINATION

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Background: ADAMS A1c HA-8180 is a HPLC system for the measurement of HbA1c. The analysis time is 90 seconds per sample in variant mode, Hb variants can be detected. The analytical performance was evaluated to verify quality of results according to the documents of consensus.
Methods: Precision and linearity studies were performed according to CLSI guidelines. Effect of the total Hb concentration was studied by sequential dilutions and coexistent interfering substances was studied adding increasing concentrations of Glucose, Sodium Cyanide solutions or cord blood. Correlation with ADAMS ™ A1c HA-8180 fast mode; 170 samples were analyzed with both modes of operation and Passing - Bablok regression applied to the couples of results Samples wtih Hb variants were also analyzed.
Results: Precision mean 42 mmol/mol within run CV 0.47%; between run CV 0%; between day CV 0.39%; Total CV 0.61 %. Mean 102 mmol/mol within run CV 0.25%; between run 0%; between day CV 0.24%; Total CV 0.35%. Linearity: y=1.0 x - 0.1, r=0.999; analytical range 14 - 113 mmol/mol. A value of 37 mmol/mol HbA1c is not affected by a concentration of Hb in the range 16 - 4.8 mmol/L. Concentrations of Hb A1c 36 mmol/mol and 86 mmol/mol are not affected by the presence of a labile fraction of 4.7 %, nor by carbamylated Hb of 5.1%, nor 15% HbF. Correlation between ADAMS A1c HA-8180 fast and Variant modes (range 30 – 133 mmol/mol). y=1.0 x - 0.1, r = 0.999. Intercept 95% CI -0.1- 0.1; Slope 1.0-1.0, deviation from linearity P <0.01. The presence of Hb variants were detected, Hb S, Hb C, Hb D and Hb E were correctly identified. The presence of Hb S and Hb C don´t interfere with HbA1C measurement (target values were assigned with HA-8160 analyzer).
Conclusions: The drastic reduction of the analysis time does not impair the overall analytical quality of results. Given the short time of the analysis this is a suitable system for the control of diabetic patients in laboratories with high workflow.

W226
HBA1C AS A DIAGNOSTIC TOOL IN SUBJECTS AT HIGH RISK FOR DEVELOPING TYPE 2 DIABETES

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Background and aim: Recently proposed use of HbA1c for the diagnosis of type 2 diabetes had been challenged for poor diagnostic accuracy in comparison to the conventional plasma glucose-based diagnostic tests. While it is obvious that different diagnostic criteria may identify different patient populations, the opportunity to detect undiagnosed diabetes and pre-diabetes with a specific measure of glycaemia, unaffected by high biological variability and preanalytical interferences, remains the major assumed benefit for the use of HbA1c as a diagnostic tool. The aim of this study was to evaluate diagnostic accuracy of HbA1c in subjects at high risk for developing type 2 diabetes.
Methods: Blood samples were taken from the subjects with a previous history of hyperglycaemia, reffered to our clinic for diagnosis of type 2 diabetes. After blood sampling for fasting plasma glucose (FPG) and HbA1c, a 75 g oGTT was performed, followed by a second blood sampling for plasma glucose at 2h (2hPG). Glucose was measured by enzymatic (hexokinase, Olympus AU400, Beckman Coulter, USA), and HbA1c by immunoturbidimetric procedure (TinaQuant HbA1c, Cobas Integra Plus, Roche Diagnostics, USA), respectively. WHO criteria were used to classify patients into diagnostic categories of glycaemia with FPG and 2hPG values. WHO- and ADA-recommended HbA1c values ≥6.5% (48 mmol/mol), and 5.7-6.4%(39-47 mmol/mol) were used as diagnostic for diabetes and pre-diabetes, respectively.
Results: A total of 317 subjects (M/F: 139/178; age range 21-85 years) were included in this study. Multiple regression analysis identified FPG, 2hPG, and age as significant determinants of HbA1c values (P <0.0001; P=0.0015 and P=0.0020, respectively). Plasma glucose results classified 70 (22.1%), 146 (46%) and 101 (31.9%) of the subjects into categories of normoglycaemia, pre-diabetes and diabetes, respectively. Inter-rater agreement analysis showed fair agreement (κ=0.350) between HbA1c- and glucose-classification systems, which improved to moderate (κ=0.431) when two diagnostic categories (normoglycaemia and any hyperglycaemia) were used.
Conclusion: Our study revealed a moderate agreement between HbA1c- and glucose-based diagnostic criteria in subjects at high risk for developing type 2 diabetes.

W227

MEASUREMENT OF 3-HYDROXY-3-METHYLGLUTARYL-COENZYME A (HMG-COA) LYASE ACTIVITY IN HUMAN HEPATOMA HEPG2 CELL EXTRACTS AND EFFECTS OF POLYPHENOLS ON ITS ACTIVITY

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Background: Fatty acid metabolism is activated when carbohydrate metabolism is impaired. Excess acetyl-CoA is generated from fatty acids by β-oxidation and is used for the formation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) and subsequently acetoacetate. High levels of secreted ketone bodies (acetoacetate and 3β-hydroxybutyrate) lower the pH of blood and urine, resulting in ketoacidosis. HMG-CoA lyase in hepatic cells is a rate-limiting enzyme catalyzing the cleavage of HMG-CoA to acetoacetate, and thus, inhibition of this enzyme results in reduced acetoacetate production–in other words–impaired ketogenesis. Based on the notion that inhibition of HMG-CoA lyase possibly prevents ketoacidosis and the identification of therapeutic targets, we examined the inhibitory effects of polyphenols on HMG-CoA lyase in cellular extracts of human hepatoma HepG2 cells.
Methods: Human HepG2 cells were disrupted with zirconia beads, and then the resulting homogenate was centrifuged. Then, 25 microL aliquots of the supernatant were mixed with a cocktail solution containing HMG-CoA as a substrate. After the enzymatic reaction, the supernatant was collected to determine the level of acetoacetate formed by HMG-CoA lyase-catalyzed reaction. Acetoacetate was determined by the HPLC method developed by us. Ten polyphenols, that is, daidzein, hesperetin, naringenin, catechin, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG), gallic acid, and pyrogallol were tested as candidates of HMG-CoA lyase inhibitors.
Results: Daidzein, hesperetin, naringenin, catechin, and EC caused only a negligible change in activity. On the other hand, ECG, EGC, EGCG, gallic acid, and pyrogallol significantly decreased the activity of HMG-CoA lyase. All of these compounds inhibited HMG-CoA lyase in a concentration-dependent manner. Type of inhibition was determined by Lineweaver–Burk plot. ECG, EGC, EGCG, and pyrogallol showed mixed non-competitive inhibition, while gallic acid showed a non-competitive inhibition.
Conclusions: Our results indicate that ECG, EGC, EGCG and gallic acid are useful for preventing ketoacidosis or as adjuncts to the treatment of ketoacidosis.

W228

AN ASSESSMENT OF CORRELATION BETWEEN ASYMMETRIC DIMETHYLARGININE AND C-REACTIVE PROTEIN IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Background: Possible association between asymmetric dimethylarginine (ADMA), as a marker of endothelial dysfunction, and C-reactive protein (CRP), as a marker of subclinical inflammation, in type 2 diabetes mellitus (T2DM) is far from being entirely investigated. The aim of this study was to determine and compare serum ADMA and CRP concentration in patients with T2DM and healthy controls, and to assess correlation between ADMA and CRP in patients with T2DM.
Methods: Serum ADMA concentration was determined by ELISA method (DLD Diagnostics, Hamburg, Germany) and CRP level was determined by particle-enhanced immunonephelometry (BN Systems, Dade Behring, Marburg, Germany) in 60 patients with T2DM and 60 healthy individuals matched for age and sex.
Results: Results have shown that serum ADMA concentration was significantly higher in T2DM patients (1.54±0.06 μmol/L) compared to serum ADMA concentration (0.62±0.02 μmol/L; P <0.0001) in healthy subjects. Serum CRP concentration in patients with T2DM (2.15;1.03-5.45 mg/L) was significantly higher then serum CRP concentration determined in healthy controls (1.30;0.60-2.30; P <0.01). A significant, positive, correlation between serum ADMA concentration and CRP levels was observed (rho=0.442; P <0.001) in T2DM patients.
Conclusions: Our results suggest that there is an association between endothelial dysfunction and chronic low-grade inflammation in type 2 diabetes mellitus. Possible explanation for obtained results may be decreased bioavailability of nitric oxide and increased oxidative stress in the conditions of T2DM. Larger, longitudinal studies are required that will fully evaluate observed relation between increased ADMA and CRP levels in patients with T2DM.

W229

ANTI-LEUKAEMIC EFFECT OF AQUEOUS EXTRACT OF ANDROGRAPHIS PANICULATA LEAVES AND 5 FLUOROURACIL ON BENZENE - INDUCED LEUKAEMIA IN WISTER RATS

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Background: The menace of cancer and leukaemia has greatly affected the whole world. Cancers have defiled all the available means of treatment as virtually all classical pharmacological treatment options have limitations due to undesirable side effects such as toxicity. The purpose of this study is to provide the much desired new natural products, which are a natural option for immunopotentiators, immunoinitiators and biological response modulators (BRM) that act to prevent cancer progression and induce carcinostasis. The anti-leukaemic effect of aqueous extract of Andrographis paniculata leaves on benzene - induced leukaemia in Wister rats was studied. Method: Leukaemia was successfully induced in Wister rats by intravenous injection (0.2 mL) of a benzene solution every 2 days for 5 consecutive weeks. The aqueous solution of Andrographis paniculata leaves (10 mg/mL) produced by water extraction was orally administered (10 mg/mL) into the rats before, during and after leukemia induction. Leukaemia burden was assessed by comparing the haematological parameters of the control rat groups (baseline) and after leukaemia induction from blood samples of the experimental rats. A rat group treated with standard anti-leukaemic drug 5 Fluorouracil was also set up in like manner with the extract. Results: Leukaemia induction resulted in significant anaemia indices and leukocytosis ($P < 0.05$) in the experimental rats. Results show that the extract demonstrated anti-leukemic activities by ameliorating the induced leukemia condition in the affected rat groups. There is no significant difference ($P > 0.05$) between the antileukemic activities of Andrographis paniculata leaves extract and the standard anti-leukaemic drug 5 Fluorouracil. Conclusion: The aqueous extract of Andrographis paniculata exhibited profound non-toxic anti-leukaemic potential on animals after oral administration in various experimental groups.

W230

SERUM AND URINE FREE LIGHT CHAINS IN MONITORING OF PATIENTS AFFECTED BY LIGHT CHAIN MULTIPLE MYELOMA

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Background: The role of serum free light chains (FLCs) measurement in the management of patients with monoclonal plasma proliferative disorders is well documented. The behaviour of FLCs in patients affected by light chain multiple myeloma (LCMM) was evaluated in respect to the information given by Bence Jones protein analysis, which, in addition to immunofixation, involves the measurement of urinary kappa and lambda free light chains.
Materials and methods: 48 serum samples from 4 patients affected by kappa LCMM were tested for FLC assay in therapeutic monitoring and the results were compared to those obtained from urinary FLCs. Serum FLCs were determined using FREELITE reagents from The Binding Site Ltd applied on Siemens BNII nephelometer; urinary FLC were measured on 24 hours collected samples using The New Scientific Company reagents on BNII nephelometer. Urinary immunofixation was carried out on concentrated samples, performed on Hidrasys1.
Results In all LCMM patients monitored from 10 to 13 samples available for a ranging time between 27 and 42 months, serum and urine FLC evidenced a good correlation, Pearson correlation index ranging form 0.77 ($P=0.014$) to 1.00 ($P < 0.001$). The best correlations were found in patients with partial or complete remission (n=3), where the greatest decrease in FLCs values was observed. Such correlations were irrespective of previous autologous transplant (2 out of 4 patients), pharmacological therapy (3 out of 4 patients), renal disease (2 out of 4 patients). Disappearance of Bence Jones proteinuria was indeed observed only in the patient with complete remission. In the other 3 subjects, despite of the decrease of kFLCs concentrations and in some samples FLC ratio <1.20, Bence Jones protein was always present.
Conclusions: monitoring patients affected by LCMM serum and urine FLC evidenced the same behaviour, irrespective of the therapeutic approach and renal disease, and in agreement with the compliance to the therapy. Indeed, only in one patient with complete remission, the Bence Jones proteinuria became negative, confirming that the information provided from quantitative FLCs measurements and qualitative immunofixation are complementary.

W231
PREVALENCE OF SICKLE CELL DISEASE IN MAROONS

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Background: Sickle cell anemia is an hereditary hematological disease. So from the heterozygous for sickle cell anemia asymptomatic (AS) parents crossing there is the possibility of forming the homozygous profile (SS), which gives the aggravating symptomatic form disease. Sickle cell anemia has its origin in the African continent from where it was disseminated for the world through the processes of racial miscegenation, potentially favored in the slavery period, which forced the negro migration. This disease has a heterogeneous distribution in the world, in higher prevalence into regions that were more influenced historically by immigration of slaves. But there are still remnants of these historical populations, as the maroons. Thus, making this study relevant regarding the data collection that enable control over the appearance of sickle cell disease homozygous cases (through genetic counseling) and improved quality of life for maroons. The main objective of this study was based in investigating the presence of variant hemoglobin S in the maroon from the Kalunga Community Ema Teresina de Goiás Farm, in Goiás, Brazil, in 2011.
Methods: This study was performed by the hemoglobin electrophoresis test, in 103 maroons - 57 females and 46 males.
Results: No individuals were found with homozygous (Hb SS) for sickle cell anemia profiles. However it was noted the prevalence of sickle cell trait in 16.5% of female participants and 14.6% in male one. Totalizing a prevalence of 31% of sickle cell trait in this population.
Conclusions: A high sickle cell anemia trait prevalence, indicating the immediate need of a closer evaluation in this population and their customs to project ways to enhance their quality of life. And also removing of the possibility of the disease in homozygous. Fact this that must be monitored and analyzed at the governmental sphere, since the quilombos are historical and cultural patrimony of humanity, besides being a population of a few members. What could lead them to extinction.

W232
EXTERNAL QUALITY ASSURANCE OF IMMUNOFIXATION AND SERUM FREE LIGHT CHAIN IN LABORATORIES PARTICIPATING TO THE PIEDMONT AND AOSTA VALLEY CONSORTIUM FOR SYSTEMIC AMYLOIDOSIS

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Background: The diagnosis of AL amyloidosis is based on histologic finding as well as on some laboratory tests. According to the most recent guidelines serum and urine immunofixation, and serum free light chain measure are of paramount importance. With the present work we evaluated the performance of the above mentioned tests in 9 laboratories belonging to the Piedmont and Aosta Valley consortium for systemic amyloidosis through an External Quality Assurance (EQA).
Methods: In 2010 all laboratories of Piedmont and Valle d'Aosta were invited to participate to an EQA in order to evaluate their performance in the identification of monoclonal components and the measure of free light chains in frozen serum samples. 9 laboratories joined the evaluation. Protein electrophoresis and immunofixation were performed in 3 laboratories by using capillary electrophoresis and in the remaining 6 by an automated gel electrophoresis. Sebia reagents (Evry, France) were used. Measure of free light chains was performed in 5 laboratories: 4 with the Binding Site Freelite method (Birmingham, UK) and 1 with New Scientific Company method (Fonegrò, Italy).
Results: The assessment of monoclonal component by serum proteins electrophoresis and immunofixation showed an overall sensitivity of 100% and an overall specificity of 61%. The quantification of the monoclonal component showed a good agreement in the results even though a laboratory did not performed quantification and 3 more laboratories showed only a percentage and not quantification in g/l. Assessment of free light chains showed variations coefficient greater than 40% between laboratories.
Conclusions: While improved as compared to a previous EQA performed in 2009, the present EQA emphasized critical issues, especially in the determination of free light chains. The adoption of the same analytical method by all laboratories is probably needed in order to compare data from different laboratories and improve diagnostic sensitivity.

W233
ASSESSMENT OF IGA HEAVY/LIGHT CHAIN IMMUNOASSAYS UTILITY IN MULTIPLE MYELOMA PATIENTS

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Background: International guidelines recommend serum protein electrophoresis (SPE) for quantification of monoclonal immunoglobulins (M-Ig) in multiple myeloma (MM) patients. However, co-migration of the monoclonal IgA with other serum proteins can make quantification by SPE inaccurate. In such instances total IgA measurements are recommended. However, these measurements inherently include a proportion of normal polyclonal IgA. Heavy/light chain (HLC) assays have been developed that quantify IgAκ and IgAl. Here we compare the utility of IgA HLC, SPE and total IgA to identify and monitor IgA M-Ig. Methods: HLC levels were measured nephelometrically on serum samples from 53 IgA (22 IgAκ; 31 IgAl) MM patients at various stages of treatment at St Helier Hospital, London, UK. HLC IgAκ/IgAl normal range: 0.80-2.04. Results were compared to retrospective total IgA (normal range: 0.8-5 g/L) and SPE. Results: At presentation all samples had abnormal HLC ratios; 11/53 (21%) samples (8 IgAκ; 3 IgAl) were quantifiable by SPE (median 6 g/L, range 1-57g/L). In the remaining 42 samples (23 IgAκ; 19 IgAl) total IgA measurements were used to quantify the M-Ig (median 5g/L, range 1-20g/L) and compared to IgA HLC measurements. There was good correlation between total IgA and summated HLC (IgAκ+ IgAl; Passing-Bablok slope: 1.07; 95% CI: 0.87-1.19). In 14/42 samples total IgA levels were <5 g/L; 8 of these had an abnormal IgA HLC ratio. In 23 patients with sequential samples, 2/23 patients were concordantly assessed using SPE and HLC measurements. Total IgA was used in the remaining 21 patients, of whom 10/21 had total IgA values <5 g/L. HLC ratios were abnormal in 21/21 patients and in 3/21 patients evaluation of HLC ratios provided additional information regarding the patients disease. The HLC ratio was an earlier marker of relapse in 2/3 patients and in an additional patient where FLC values had previously been the only indication of clonality, HLC ratio identified a subtle intact immunoglobulin clone. The later result suggests a role for HLC measurements in identification of clonal changes in MM patients. Conclusion: IgA HLC ratios are able to sensitively detect clonality even when SPE is not quantifiable or when total IgA is within the normal range.

W234
HEPCIDIN LEVELS AND THEIR DETERMINANTS IN DIFFERENT CHRONIC DISEASE

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Background: Anemia of chronic disease (ACD) is a cytokine mediated anemia, most frequent anemia among hospitalized patients and characterized by hypoferremia with adequate reticuloendotelial iron stores, normal to elevated ferritin concentrations and it is a frequent complication of chronic inflammatory conditions. Hecidin as new hormone iron, play a central role in the disturbance of iron metabolism in ACD.
Methods: In our study are included 187 subjects, 156 patients and 31 control group. Anemia was defined as hemoglobin <12 g/dL in females, <13 g/dL in males. Anemic patients were further evaluated for the type of anemia with iron profile, peripheral blood smear, retikulocit count, prohepcidin, IL6, TNFα, Hs-CRP levels.DRG ELISA kits were used for sTfR and prohepcidine determinations. SPSS 15 was used for statistical analysis.
Results: Anemia was observed in 51.9% patients, 54.3% have ACD, 23.4 % IDA, 16% ACD+IDA. Sideremia, ferritinemia, Tfs are significantly lower in IDA vs ACD vs ACD+IDA, onlyTf is significantly higher in IDA vs ACD (P <0.001). TfR, sTfR/log ferritin is significantly higher in IDA and ACD+IDA vs ACD. Hepcidin, IL6, TNFα, Hs-CRP are significantly higher in ACD and ACD+IDA (P <0.001).
Conclusions: ACD is the most frequent anemia among hospitalized patients. Panel iron (especially ferritina) together with hepcidin and cytokins are the best indicator to find ACD. STfR, sTfR/logferritin is good indicators to distinguish from ACD especially mixed type ACD+IDA.

W235
THE ROLE OF THE CELL-DYN RUBY FLAG ATYP DEP IN THE EARLY DIAGNOSIS OF IMPORTED MALARIA IN A NON-ENDEMIC REGION

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Background: Although malaria is one of the most important parasitic diseases worlwide,it is sometimes not easy for the physician to suspect it in a non-endemic area,due to the unfamiliarity with most of tropical diseases.
Methods: The Cell-Dyn Ruby (Abbott Diagnostics) haematology Analiser uses the MAPPS (multiple-angle polarized scatter separation) technology for White Blood Cell analysis.The light depolarizing properties of the eosinophils granules is used to distinguish eosinophiles from neutrophils but it is also useful to detect haemozoin-containig monocytes wich can be found in malaria cases.The haemozoin-containing monocytes are shown in a scatter-plot labelled as lobularity-granularity and the Complete Blood Count (CBC) is flagged with atyp dep.We use thick and thin smear and Field stain to detect malaria.
Results: We had 11 cases of malaria between june 2008 and october 2012. All of them were people who had recently travelled to Africa and had high fever when they asked for assistance in our hospital.A blood test was ordered,including a CBC wich was performed with Cell-Dyn Ruby.The CBC was flagged with atyp dep in all cases and several purple dots indicating haemozoing-containing monocites above the threshold line in the granularity-lobularity scatter plot were found.In 7 of these cases, the clinical suspicion of malaria was a first choice and a malaria smear was ordered in the first blood test.We found plasmodium falciparum in 6 cases with a 0.5 – 4% parasitemia.The other case was a mixed infection of plasmodium falciparum and plasmodium malariae with a 5% parasitemia. In the other 4 cases, no malaria smear was ordered.The main symptoms and clinical suspicions were:Case 1: fever and vomiting.Case 2: fever and abdominal pain in an ulcerative colitis under inmunosupressive therapy oriented as an outbreak of the illness.Case 3:fever and headache under antibiotica treatment. Case 4:fever and urinary symptoms oriented as an urinary tract infection.We found plasmodium falciparum in all cases with a 0.5 – 2% parasitemia.
Conclusions: The Cell-Dyn Ruby atyp dep flag is very useful for an early diagnosis of malaria specially when there is a lack of clinical suspicion, avoiding misdiagnosis, several reconsultations and therefore improving morbidity and mortality.

W236
PROGNOSTIC MARKERS FOR MULTIPLE MYELOMA

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Background: Multiple Myeloma (MM) is a malignant disease characterized by infiltration of the bone marrow by cancerous plasma cells. Numerous prognostic markers have been identified however there was no consensus about the best prognostic indicators or the proper staging systems. Serum albumin concentration is part of the recent International Staging System (ISS) for MM, which stratifies prognosis based on albumin and serum β2-microglobulin level. The aim of our study is to evaluate the prognostic role of various biochemical markers in patients with MM.
Methods: The study enrolled 35 patients, both sexes, mean age 67±11.3. According to stage of the disease all patients was divided in two groups; group 1– patients in stage I and II and group 2– patients in terminal stage of disease. Laboratory analyses included determination of albumin, β2-microglobulin, creatinin, fibrinogen, sedimentation rate (SE), Hemoglobin (Hb) and Leukocytes (Le).
Results: Patients in group II had higher levels of SE, creatinin ($P < 0.05$) as well as β2-microglobulin ($P < 0.01$), and lower Hb ($P < 0.05$). In total patient group, we found significant correlation between survival and β2 microglobulin level ($r = -0.463$, $P < 0.01$) as well as survival and SE ($r = -0.413$, $P < 0.05$), while correlation with albumin vas borderline. After entering these parameters into multilinear regression analysis only β2 microglobulin remain independent association with survival (standized $\beta = 0.476$, $P < 0.5$) Also, in patients with compromised renal function (increased creatinin level) only β2 microglobulin correlated with survival ($\rho = -0578$, $P < 0.05$). Additionally, binary logistic regression showed that only β2 microglobulin had significant power to predict death in patients with MM (OR=0.537(95th CI (0.312-0.898)). Interestingly in group II survival correlated only with RBC Sedimentation rate ($r = 0.601$, $P < 0.01$) and Hb level ($r = 0.536$, $P < 0.01$).
Conclusions: Our results showed that prognostic power of mentioned parameters is depend on stage of disease and renal function.We consider that β2 microglobulin can be used as a reliable prognostic parameter in patients with MM. and that the decrease of albumin appears in context of kidney malfunction.

W237
HEMATOLOGY FIELD APPLICATION OF A SYSTEM OF REMOTE IMAGE CAPTURE

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Background: The development and progress in technology have made possible to manage small routine and emergencies with innovative ways maintaining the centrality of the service and the expertise of central laboratories. We wished to point out the actual possibility of carrying out remote diagnosis for those samples handled in peripheral centers in the presence of technical personnel only, leaving the reporting to graduates in force at the central laboratory of reference wich is also a center of excellence in this field.
Methods: It is possible to handle the slides in peripheral hospital facilities by technicians and to report the cases by hematologists in central corelab. For this purpose, using modern scanners D-SIGHT (Menarini Diagnostics) that digitize the blood slide making it available in Jpeg format, we undertook a study to investigate the possibility of setting up a remote validation at a distance. The scanning unit installed in the area are equipped with a D-SIGHT 05 system with a special scanning module. The overall workflow generated by the introduction of the D-SIGHT involves three distinct phases of work: Acquisition, Upload, View Image for consultation and/or reporting. For six months the peripheral hospital routine (400 slides\samples) was doubly assessed: on the one hand microscopy classic slide, subject to the physical transport from the periphery to the central laboratory of reference, and, on the other hand, digital virtual microscopy for evaluating a file at the central laboratory acquired remotely by the scan of the slide (controlled by the central laboratory) and then made available on the dedicated web platform.
Results: The diagnosis of 400 cases / slides led to the same results: 337 samples negative / normal samples and 63 positive/ pathological. The timing of reporting (classical microscopy vs. virtual microscopy) falls from 2.5 hours to 20 minutes. The costs are extraordinarily reduced: the routine can be managed without the need for active guards with university graduates in the device, it eliminates the cost of logistics for the physical transportation of the sample between the two hospitals. Improving risk management.
Conclusions: Possible applications are those intended for the diagnosis, counselling, teaching, quality control, decentralization of validation.

W239
ANALYTICAL COMPARISON OF POLYCLONAL VERSUS MONOCLONAL ANTIBODY BASED FREE LIGHT CHAIN (FLC) ASSAYS IN MULTIPLE MYELOMA PATIENTS

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Background: Monitoring serum free light chains (FLC) in patients with multiple myeloma (MM) helps guide therapy and results in a better understanding of the patient's disease. To date FLC have been measured by the polyclonal antibody based Freelite assay; lately a monoclonal antibody based method has become available. Here we compare the clinical utility of both assays.
Methods: FLC were measured using Freelite (Binding Site, UK) and N Latex FLC (Siemens, Germany) assays in sequential serum samples from 64 MM (40κ, 20λ, 2 biclonal, 2 non-secretory (NS)) patients enrolled onto a multicentre UK trial. Results were compared to SPE and IFE. Measurable disease was classified as levels of involved FLC (iFLC) >100 mg/L.
Results: In 42/64 (66%) patients the two assays provided broadly similar clinical information, with 29/42 patients having measurable disease in both. However, FLC measurements were highly discordant between the assays, and correlation poor (FLCκ R2=0.734, FLCλ R2=0.465). 11/64 (17%) patients had disease measurable by Freelite (median iFLC=153.25 mg/L, range=114.65-637) but not N Latex FLC (median iFLC=24.98 mg/L, range=12.22-49.71). Importantly, in 11/64(7κ, 3λ, 1NS, 17%) patients the N Latex FLC assay was clinically insensitive and gave discordant information. In 4/11(3κ, 1NS) patients an abnormal Freelite ratio and positive IFE (except for the NS patient) at presentation confirmed clonality, which was not detected by N Latex FLC. In sequential samples of 5/11(4κ, 1λ) patients with IFE positive and abnormal Freelite ratio, the N Latex FLC ratio normalised; incorrectly indicating response. In 1λ patient the two assays showed response; however while Freelite ratio normalised, N Latex FLC ratio remained abnormal despite no evidence of FLC production. Finally, in 1λ patient with stable disease by SPE and Freelite, fluctuating FLC levels with N Latex FLC indicated relapse and subsequent response.
Conclusion: In a moderate number of patients the two assays offered similar clinical information, although absolute values were discordant. The data presented suggests the two assays may not be recognising the same analyte, as indicated by the failure of the N Latex FLC assay to identify 3κ clones and the variable response pattern in a patient with stable disease.

W240
RETICULATED PLATELETS AS DIAGNOSTIC TOOL IN DIFFERENTIATION OF THROMBOCYTOPENIA

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Introduction: Platelets play fundamental role in haemostasis. They are formed by fragmentation of megakaryocytes, during maturation of this progenitor cells. Nascent platelets contain mitochondria and ribosomal RNA and all the components necessary for their function in haemostasis, but do not contain nuclear material. This group of cells is called reticulated platelets and represent the youngest form of circulated platelets. The aim of this study was to look into the utility of reticulated platelets as additional diagnostic device in thrombocytopenia of different origin.
Material and methods: Results were obtained from 28 patients, divided in two groups according to the cause of low platelet count: hiperdestuctive (N=16) and hypoplastic (N=12) thrombocytopenia and 20 healthy volunteers, as control group. Total platelet count and percentage of reticulated platelets were measured on Cell Dyn Sapphire, Abbott Diagnostics, by flow cytometry. In statistical analysis t-test was used, with significance level of $P < 0.05$.
Results: In control group mean values for total platelet count, mean platelet volume and percentage of reticulated platelets were 232.0 x 109/L, 8.06 fL and 1.776%, in group of patients with hypoplastic thrombocytopenia were 33.67 x 109/L, 10.55 fL and 6.022%, as in group of hiperdestuctive thrombocytopenia were 14.37 x 109/L, 12.32 fL and 18.780 %, respectively. Statistically significant difference in the value of tested parameters was stated between the groups.
Conclusion. Determination of reticulated platelets can be considered as part of diagnostic algorithm in interpretation of low platelet count.

W241
14Q32 REARRANGEMENTS IN MULTIPLE MYELOMA

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Introduction: Multiple myloma (MM) is a malignant disorder characterized by clonal expansion of plasmocytes in bone marrow. One of the most common abnormalities in MM are the translocations that involved immunoglobulin havy-chain (IgH) locus. Cytogenetic studies indicate that IgH translocations are found in nearly 50-70% of MM and are associated with hypodiploid karyotype. The most frequent partners of IgH are: BCL1 in t (11;14); FGFR3 in t (4;14), MAF in t (14;16), MAFB in t (14;20) and cyclin D in t (6;14). The aim of our study is to determine the frequency of 14q32 rearrangements and its cytogenetic variants t (11;14), t (4;14) and t (14;16) in cases with MM.
Methods: Interphase FISH studies of bone marrow cells of 37 MM patients were carried out. In all MM patients dual-color, split assay with DNA specific probes for IgH region at 14q32 was performed. The cases with positive split probe were tested for t(11;14), t(4;14) and t(14;16). Dual-color, dual fusion assay using DNA specific probe for FGFR3/IgH, MYEOV/IgH and MAF/IgH were used (Kreatech).
Results: Molecular cytogenetic aberrations affecting 14q32 were found in 22 (59%) of the 37 MM patients. Four cases (31%) were with t(4;14), three cases (23%) were with t(14;16) and one case (7%) was with t(11;14). In 9 (24%) patients various patterns of IgH deletion was identified: 5 cases (56%) were with deletion of the variable (V) segment (1F/1R), 2 cases (22%) were with deletion of both Joining (J) and constant (C) segments (1F/1G), 1 case (11%) was with bialelic deletion of V segment (2R) and 1 case (11%) was with deletion of V,D, J and C segments.
Conclusion: IgH translocations are nonrandom genetic event in the pathogenesis of MM. The most common IgH translocation is t(4;14). Considerable part of 14q32 rearrangements included various patterns of IgH deletion.

W242

SENSITIVITY FOR NON-TUMOR IMMUNOGLOBULIN SUPPRESSION IN IFE AND SPE POSITIVE MULTIPLE MYELOMA MONITORING SAMPLES BY HEVYLITE™ AND TOTAL IMMUNOGLOBULIN

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Background: Measuring monoclonal protein (M-protein) concentrations in myeloma patients as monitoring tool can be challenging, especially at low concentrations, due to imprecsion inherent in electrophoresis, and the presence of interfering proteins. Immunoparesis, as measured by suppression of the non-tumor immunoglobulin proteins (Ig), has recently been reported as a clinically useful and prognostic tool for evaluation of myeloma patient outlook and treatment. We compared non-tumor Ig by Hevylite™ (HLC) and Total Ig assays in IgG and IgA myeloma monitoring patient samples. Methods: The HLC and Total Ig assays were run on a BNII™ nephelometer and published reference ranges were used. Only IFE positive samples (Sebia Hydrasys) with measured monoclonal protein (Sebia Capillarys) of less than 20 g/L (30 IgGκ, 9 IgGλ, 20 IgAκ, 15 IgAλ) were evaluated in this study. Results: The mean and range concentrations by HLC (g/L) and Total (g/L), respectively, for each sample set were; IgGκ 9.04 (3.81-24.3) and 12.4 (4.82-39.3), IgGλ 6.17(3.03-18.9) and 9.85 (4.66-24.5), IgAκ 10.44 (2.78-22.7) and 8.26 (2.32-22.0). IgAλ 5.63 (1.34-18.30) and 5.88 (1.41-18.30). By HLC, 31/39 (80%) of the IgG MM samples and 24/35 (69%) IgA MM samples were below normal concentrations of the uninvolved Ig (i.e. Ig'λ in Ig'κ MM samples and Ig'κ in Igλ MM samples). When measuring Ig suppression by the Total Ig assay, 25/39 (64%) of IgG MM samples were below normal for IgA concentrations and 20/39 (52%) were low for IgM. In the 35 IgA MM samples, 29 (83%) were below normal for IgG concentrations and 28 (80%) were low for IgM. Conclusions: HLC appears to be a relatively sensitive assay for measuring suppression of the non-tumor Ig, especially in IgG MM patient samples.

W243

COMPARISON BETWEEN AUTOMATED AND MICROSCOPIC ANALYSIS IN BODY FLUIDS CYTOLOGY

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Background: Conventional methods for the cytological analysis of body fluids samples require a manual chamber counting of red and white blood cells and leukocytes differentiation using a cytocentrifuged and stained preparation. It is a time-consuming procedure, which is also subjective and prone to interoperator variability. The aim of this study was to evaluate the performance of the Sysmex XE-5000 body fluid application as an alternative to the microscopic analysis of body fluids.
Methods: We studied 200 pleural and peritoneal fluids. All samples were sent in an anticoagulant-treated tube and analyzed up to 2 hours after collection. The laboratory routine included manual erythrocytes (RBC) and leukocytes (WBC) and differential counts (cytocentrifuged air-dried hematological staining of May-Grunwald) and automated total and differential cell counts (Sysmex XE-5000). Validation protocol also included precision, carryover and linearity studies.
Results: Our results met all the requirements for analytical quality regarding precision (CVs < desirable specifications for imprecision) and linearity (r >0.99). Carryover effect was minimal. The automated WBC and RBC counts were highly correlated with that of the microscopic reference method (r >0.95 in both cases). A good agreement between both methods was also observed for mononuclear cells (r=0.85) and polimorphonuclear cells (r=0.86). Eosinophils are reported separately as a research parameter and demonstrated a good correlation with microscopy (r=0.85). The presence of high fluorescence cells >2.0/100 WBC is visible at the upper border of the scattergram and indicates the presence of macrophages, mesothelial cells or malignant cells.
Conclusion: Automated RBC, WBC and differential leukocytes counts show good correlation with the manual method. Considering that body fluids are generally sent for urgent analysis, its laboratory routine requires a skilled personal and microscopic analysis may not be available 24h/day in most laboratories, the use of this automated analyzer has the potential of reducing the time to report a preliminary result to the clinician.

W244
BIOLOGICAL VARIATION OF FREE LIGHT CHAINS (FLC) IN SERUM

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Background: The International Myeloma Working Group recommends the measurement of serum immunoglobulin k and λ FLC and FLC ratio calculation for screening, prognostic evaluation and monitoring of multiple myeloma and related plasma cell disorders. However available studies evaluating FLC biological variation (BV) suffer of important limitations, e.g. analyses carried out in singlicate on only two individual samples or enrolment of diseased subjects. Here we assessed BV components of FLCs and FLC ratio by an accurately designed experimental and statistical protocol.
Methods: We collected five blood specimens from each of 21 healthy volunteers (9 men and 12 women; age range, 23–54 years) on the same day, every two weeks for two months. Serum specimens were stored at –80 °C until analysis and analyzed in a single run in duplicate. FLC were determined using a SPAplus immunoturbidimetric platform and Freelite reagents (The Binding Site). Cochran's test and Reed's criterion were performed for outlier identification, while a Shapiro-Wilk test was used to check data distribution. Data were analyzed by ANOVA.
Results: For all parameters the normality hypothesis was accepted for ≥94% of subjects. Serum λ FLC concentrations were significantly (P <0.01) higher in men, whereas no differences were found for κ FLC and FLC ratio. Intra-individual variances were not different between genders, while the inter-individual variance of λ FLC was higher than that of κ FLC. Within- and between-subject CVs were 8.1% and 14.1% for κ FLC, 7.0% and 27.5% for λ FLC, and 4.5% and 15.3% for FLC ratio. All parameters had a low index of individuality showing that classical reference intervals have little use in the interpretation of FLC results. The average reference change value was ~20%. Desirable analytical goals for imprecision (CV), bias and total error were <4.0%, ±4.1% and ±10.7% for κ FLC, <3.5%, ±7.1% and ±12.9% for λ FLC, and <2.3%, ±4.0% and ±7.7% for FLC ratio.
Conclusions: We defined BV components of serum κ and λ FLC and FLC ratio and derived indices that may improve their clinical use. Particularly the smaller intra-individual BV of FLC ratio when compared with FLCs is in agreement with its use directed to minimize FLC variations due to hydration status and hematocrit value.

W245
POLYMORPHONUCLEAR LEUKOCYTE AGGLUTINATION AND HAEMATOLOGY ANALYSERS: KEEP YOUR EYES PEELED!

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Introduction: Leukoagglutination is an uncommon finding believed to be EDTA-dependent and temperature-dependent, though probably the trigger is the presence of agglutinins in the serum of patients affected by this phenomenon. Haematology analysers provide quick and accurate results even if sometimes they yield spurious counts for some of the many measured parameters. Scattergrams of a patient showed a double polymorphonuclear (PMN) population: a population with normal mean cellular volume and the other with a higher one, nearly twofold. The case have been revised by microscopic examination.
Methods: The patient's blood was collected in Beckton-Dickinson tubes with EDTA -the anticoagulant of choice for these analyses- and processed on Beckman-Coulter LH-780 which applies classical VCS Coulter principle (Volume, Conductivity and Scatter) that integrates details from impedance, radiofrequency and laser beam scattering measures. Smear examination was performed in bright field microscopy (1000X) after May-Grünwald-Giemsa staining.
Results: Differential count scattergram showed a double PMN signal on volume axis suggesting the presence of an heterogeneous population. On the smear we found many PMN agglutinates formed by 4-7 cells exclusively made of PMN: there were no other leukocytes classes cells, nor red blood cells nor platelets. As expected the agglutinates were found in smear lateral and tail edges.
Conclusions. The infrequent finding of leukoagglutination can explain the poor knowledge of this phenomenon. The lack of instrumental alarms suggests that probably the bulky cell clusters cannot pass through the count apertures giving rise to total leukocyte and PMN underestimation though somewhat modest in the present case as proven by leukocyte count after about 24 h when leukoagglutination was no more present (leukocytes with agglutination 16.3x109/L, leukocytes without agglutination 11.9x109/L). Automated haematology analysers, even if they provide excellent qualitative and quantitative performances, indeed always need supervision by qualified and trained staff in order to avoid potential instruments' software errors caught, in our case, only by instrumental flags "Immature NE 1 and 2" – however commonly found in inpatients routine laboratory controls.

W246
PSEUDO-GAUCHER CELLS IN MYCOBACTERIAL INFECTION: A REPORT OF ONE CASE

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Background: We report a case of a 28 years-old female with a previously known HIV1/AIDS CDC-C3 diagnosis, who had recently started highly active anti-retroviral therapy. The patient presented asthenia, unintentional weight loss, sweating and abdominal pain. The physical examination revealed a remarkable massive splenomegaly and the laboratory workup showed pancytopenia.
Methods: Our methodology was based on the following laboratory techniques: hemogram, myelogram (May-Grünwald-Giemsa,, Periodic Acid Schiff, Sudan Black, Ziehl-Neelsen and auramine stains) and microbiological examination of medular blood.
Results: After an intensive clinical and laboratory investigation, Mycobacterium Avium Complex (MAC) was identified in the bone marrow aspirate. This diagnosis was only made possible by the high degree of suspicion raised by the presence of Pseudo-Gaucher cells in the bone marrow, positive Ziehl-Neelsen and Auramin stains. The microbiological examination of medular blood with probes Accuprob confirmed MAC. After initiation of adequate therapy the patient gradually recovered although there was still a need for regular transfusional support as pancitopenia persisted.
Conclusions: This case reinforces the importance of suspecting a mycobacterial infection in immunocompromised patients with bone marrow infiltration Pseudo-Gaucher cells in the bone marrow, allowing an early and timely initiation of appropriate therapeutic.

W247
COMPARISON OF TWO DIFFERENT IMMUNOASSAYS FOR THE DETECTION OF IMMUNOGLOBULIN'S SERUM FREE LIGHT CHAINS

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Introduction: A new free lights chains assay which uses monoclonal antibodies rather than polyclonal antibodies, has become commercially available. Serum free light chain (sFLC) measurements with polyclonal antibodies are recommended by international guidelines for screening, prognosis and monitoring monoclonal gammopathies. This study aims to compare the performance of the two sFLC assays.
Methods: sFLC were measured by the N-Latex FLC assay based on monoclonal antibodies on a BNII nephelometer (Siemens Healthcare Diagnostics), and compared with sFLC measurements by the Freelite assay based on polyclonal antibodies on a SPA+ turbidemeter (Binding Site). 69 samples from patients with known B cell dyscrasias were studied and sFLC quantifications were contrasted with clinical data. Concordance between the two assays was assessed by correlation analysis. R2 >0.95 were considered significant as recommended by The Clinical and Laboratory Standards Institutes guide to Method Comparison and Bias Estimation Using Patient Samples (EP09-A2-IR).
Results: 20 MGUS, 1 SMM, 42 MM, 2 LNH, 2 AL and 2 plasmacytoma samples were analyzed by both assays. 65/69 presented altered ratios by Freelite vs 51/69 by N Latex. The 13 samples missed by N Latex were 6 MGUS, 6 MM and 1 plasmocytoma. Assay correlations for all patients (N=69) were: k-sFLC (slope:0,6467; R^2=0,5532); L-sFLC (slope:0,9077; R^2=0,6557); k/L sFLC ratio (slope: 0,0601; R^2=0,2447). When correlations were separately established among patients presenting monoclonal k-sFLC, monoclonal L-sFLC, polyclonal k-sFLC and policlonal L-sFLC, the correlations were: pts with monoclonal k (slope: 0,6118; R^2=0,4804) vs patients with polyclonal k (slope: 1,2711; R^2=0,9622); and pts with monoclonal L (slope: 0,8944; R^2=0,5885) vs patients with polyclonal L (slope: 1,8249; R^2=0,8718).
Conclusions: No correlation was found between the two assays. The 13 samples missed by N LATEX demonstrate that this assay may not be valuable for MGUS risk stratification, monitoring and strict complete response evaluation, in MM patients, possibly due to the limited range of recognizable epitopes typical of a monoclonal antibody based assay.

W248
SERUM VERSUS URINE FREE LIGHT CHAINS IN MONOCLONAL GAMMOPATHIES

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Introduction: Bence Jones proteins are the oldest tumour marker used for diagnosing and monitoring monoclonal gammopathies (MG). serum Free Light Chains (sFLC) assay has been proved to increase the diagnostic sensitivity for screening monoclonal proteins, whereas routine nephelometric determination of urinary FLC (uFLC) is not recommended by international guidelines since it has not demonstrated its benefits. The aim of this study is to validate the sensitivity of the sFLC over the uFLC determination, and alongside validate the need for a specific sFLC renal reference range in patients with compromised renal function.
Methods: 1997 samples were retrospectively compared, for which uFLC were quantified (NSC, on a Siemens BNII) and confirmed by IFE when uFLC values were between 0.1-3mg/dL; sFLC measured by Freelite on a SPAplus (Binding Site; normal range (NR) 0.26-1.65, renal range (RR) 0.37-3.1); and the renal function assessed by MDRD (NR >60 mL/min/1,73 m^2). SPSS 16.0 was used for the statistic analysis.
Results: 1127 (56. 43%) samples had an abnormal sFLC ratio, of which 365 (18.28%) were uFLC positive and 762 negative. From the 870 samples with normal sFLC ratio, 23 (1.2%) had uFLC, corresponding to: 6 MGUS, 6 MM, 1 WM-K. If sFLC RR was applied when MDRD <60 mL/min/1,73 m^2, 13 samples from 10 patients with λ disease changed from normal to abnormal sFLC ratio: 4 AL, 2 MM, 4 MGUS (1 MGUS corresponding to a previously normal sFLC and uFLC positive with chronic renal failure and MDRD=27). On the other hand, 56 samples from 40 patients became normal within the RR: 1 chronic hepatitis, 26 MGUS + 1 bi-clonal MGUS, 3 WM, 1 AML, and 8 MM (of which 2 in CR, 1 in PR, and 1 not susceptible to treatment). These data indicates that 94.1% of the cases with uFLC also had an abnormal sFLC ratio and would therefore be identified without the need for the uFLC determination. The clinical significance of the discrepant results remains to be elucidated.
Conclusions: sFLC abnormal ratio was more frequent than uFLC alterations, confirming its high sensitivity for the identification of MG on this sample set. The proposed RR may provide more sensitivity and specificity for patients with altered renal function, although follow-up studies are needed to confirm it.

W249
DIFFICULTIES IDENTIFYING A MONOCLONAL COMPONENT IN A BENCE-JONES MULTIPLE MYELOMA, A CLINICAL CASE

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Introduction: Clinical manifestations such as bone pain, pathological fractures, renal failure and anemia alert the diagnosis of multiple myeloma (MM). The presence of free light chains (FLC) in the absence of intact monoclonal immunoglobulin supports the diagnosis of Light Chain MM (LCMM). Objective: Identification of a monoclonal component (MC) in a case of MM with discordant results between different laboratory methods.
Methods: A 49-years old patient presented to Rheumatology with low back bilateral rib pain unresponsive to analgesic and anti-inflammatory treatment. A low serum protein level (6,3mg/dL) and strong hypoglobulinemia triggered the study protocol. serum FLC (sFLC; Freelite, Binding Site) and urinary FLC (uFLC; New Scientific Company) were measured on a BNII (Siemens), and serum (sIFE) and urine (uIFE) immunofixations were done (Sebia Hispania).
Results: The initial serum sample showed: low gamma fraction on SPE (0,22 g/dL); low immunoglobulin levels (IgG 192,5 mg/dL; IgA 6,5 mg/dL; IgM 6,1 mg/dL); 1500 mg/L of κ-sFLC (NR 3,3-19,4) and 1,06 mg/L of λ-sFLC (NR 5,71-26,3), resulting in an abnormal κ/λ ratio of 1415,09 (NR 0,26-1,65). IFE on undiluted serum sample revealed a very weak free-κ band. 24 h-uIFE was negative, κ -uFLC was 4.86 mg/L (NR 0-10) and λ-uFLC under the detection limit. Bone marrow biopsy confirmed 51,6% of plasmatic cells, and TAC showed multiple lytic lesions, with a final diagnosis of k-LCMM IPI I, stage III. κ-sFLC levels decreased during treatment until 530 mg/L, while sIFE, uIFE and uFLC remained negative. Surprisingly, using Freelite to detect uFLC revealed 50 mg/L of κ-uFLC and 1,1 mg/L of λ-uFLC (abnormal uFLC κ/λ ratio of 45,4 (NR 2,04-10,37)). Immunoelectroforesis with Freelite antibodies confirmed the presence of a specific free-kappa band in serum and urine.
Conclusions: In this case the Freelite reagents were the only with specificity to detect and quantify the MC both in serum and urine, allowing treatment monitoring (initial sFLC ratio 1415,09; last sFLC ratio 446,28). The presented case exposes how the inter-individual FLC variability may affect the ability of different commercial reagents on detecting the MC. The combined use of clinical and laboratory data allowed to determine this special case of k-LCMM.

W250

MENINGOENCEPHALITIS BY LISTERIA MONOCYTOGENES AS DEBUT OF ACUTE MYELOMONOCYTIC LEUKEMIA WITH EOSINOPHILIA (M4EO) SECONDARY TO CHEMOTHERAPY FOR FOLLICULAR NON-HODGKIN´S LYMPHOMA: A CASE STUDY

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Medical Background: A 44-year-old woman came to emergency derived from a secondary hospital by hydrocephalus, fever, headache and disorientation. She was diagnosed as Follicular Lymphoma (FL) stage IV-B grade 3-A one year ago. The patient received six cycles of R-CHOP chemotherapy: Rituximab (anti-CD20 monoclonal antibody), cyclophosphamide, adriamycin, vincristine and prednisone achieving a partial response. She followed with Rituximab as maintenance treatment. Currently she had received three cycles of maintenance.
Methods: Hemogram: Hemoglobin 123g/L; hematocrit 32.3%; platelet 28x109/L; leukocyte 9x109/L (neutrophils 1%, lymphocytes 13%, monocytes 86%). Peripheral Blood Morphology: Severe anisocytosis with 3 erythroblasts per 100 white blood cells count. Leukocytes: 53% atypical monocytes with irregular nuclei, visible nucleoli, 27% promonocytes, 7% lymphocytes and 3% hypogranular neutrophils. Severe thrombocytopenia.
Biochemistry: Within normal except Glucose 287 mg/dL, GGT 114 U/L, LDH 758 U/L and PCR 8.38 mg/L. Blood and Urine cultures: Negatives. Cerebrospinal Fluid: Turbid color, leukocyte 109/mm3 (81% polymorphonuclear, 19% mononuclear), protein level 91 mg/dL and glucose level 69 mg/dL. Flow cytometry was performed and were detected 2.3% lymphocytes, 40% neutrophils, 10% monocytes and 47.7% atypical eosinophils. No organisms were seen on Gram staining but after 72 h the culture yielded L. monocytogenes.
Clinical Course: The patient was admitted in Neurosurgery and her initial diagnosis was Meningoencephalitis in Immunosuppressed by atypical Germ. During admission, due to the worsening of pancytopenia and the findings in peripheral blood a bone marrow aspirate was performed. Myelogram: Mature granulopoiesis (metamyelocytes, band neutrophils and segmented): 19%; Erythropoiesis: 5%; Monocytic lineage: 35% atypical monocytes, 25% promonocytes and 8% monoblasts; Eosinophilic lineage: 34% atypical eosinophils; Myeloblasts: 3%; Lymphopoiesis: 3%. Fluorescent in Situ Hybridization (FISH): Investment chromosome 16 (69%). Conventional karyotype: Investment chromosome 16 (70%).
Results: The patient was diagnosed as meningoencephalitis by Listeria monocytogenes as debut of Acute Myeloid Leukemia with inv (16) (p13.1q22) according to the WHO 2008 classification secondary to chemotherapy for FL.
Conclusions: Secondary leukemia should be considered in the differential diagnosis of severe pancytopenia in patients who received chemotherapy o radiotherapy due a other neoplasms.

W251

HEAVY/LIGHT CHAIN (HLC) AND FREE LIGHT CHAIN (FLC) ANALYSIS ALLOW SENSITIVE MONITORING OF MULTIPLE MYELOMA PATIENTS AND AID DETECTION OF CLONAL CHANGES

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Background: Traditional tests to monitor monoclonal intact immunoglobulin (M-Ig) components in multiple myeloma (MM) include SPE and IFE. Whilst these adequately identify gross changes, they may miss subtle changes in M-Ig concentrations. Serum free light chains (FLC) assessments have improved detection of subtle plasma cell changes. A previous report stated that in 66% of patients at relapse there is a change in plasma cell clone, which changes the M-Ig/FLC expression profile. Sensitive assessments of M-Ig may add value when monitoring these patients. Nephelometric assays have been developed that measure Ig'κ/Ig'λ (heavy/light chain; HLC) and FLC (Freelite®). Here, we present two case studies showing HLC/FLC analysis aids detection of clonal changes at relapse.
Methods: In 2 patients HLC IgAκ/IgAλ (normal range: 0.80-2.04) and FLCκ/FLCλ (normal range: 0.26-1.65) were measured nephelometrically and compared to SPE, IFE and total IgA (TIgA).
Results: An IgAλ MM patient presented with 31 g/L IgAλ (measured by TIgA) an abnormal HLC IgAκ/λ ratio (0.33) and abnormal FLC κ/λ ratio (0.14) with 93mg/L involved FLC (iFLC). The patient achieved a VGPR after 137 days (97% reduction in TIgA), which was reflected by a HLC ratio that approached normal (0.67 ratio). In contrast the patient's iFLC concentration and FLC ratio became increasingly abnormal during this time (433 mg/L and 0.03, respectively), indicating an unresponsive FLC clone. After 277 days of treatment the iFLC levels dropped to 74 mg/L (FLC ratio 0.05), however increases in TIgA by 28 g/L and HLC IgAλ (29 g/L) indicated the re-emergence of an IgAλ expressing clone. An IgAκ MM patient presented with 15 g/L IgAκ (SPE), an abnormal IgAκ/λ HLC ratio (75) iFLC (1184 mg/L) and an abnormal FLC ratio (203). Following 4 cycles of VAD therapy and ASCT, the patient achieved a stringent complete response; HLC ratio normalised at this point. The patient response was maintained after 574 days, after which a monoclonal band by SPE and HLC ratio both indicated re-emergence of disease, but failed to meet relapse criteria. However, an increase in FLCκ (1150 mg/L) and FLC ratio (124) indicated FLC relapse.
Conclusions: Measuring HLC and FLC ratios in MM patients offers a simple way of detecting clonal changes at relapse.

W252
IDENTIFICATION OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA (PNH) CLONES WITH FLOW CYTOMETRY

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Background: PNH is a clonal disorder of hematopoietic stem cells, caused by a somatic mutation in the X-linked PIG-A gene, resulting in a deficiency of glycosylphosphatidylinositol anchored proteins (GPI-APs) on the cell surface. Flow cytometric (FCM) analysis for PNH, generally, involves GPI-AP expression of CD59, CD55, CD16 and/or fluorescent aerolysin (FLAER) staining. FCM routine and high sensitivity assays were evaluated for PNH detection.
Methods: 100 peripheral blood patient samples were assessed with white blood cell (WBC) routine analysis (CD64/CD16/CD45/CD14 & CD55 or CD59 combined with CD15/CD45/CD33) and FLAER/CD33/CD14/CD45 WBC high sensitivity analysis. Furthermore, to improve red blood cell (RBC) analysis sensitivity, a CD235a/CD59 protocol was developed; cocktails of the reagents in several clones and fluorochromes and various concentrations, were used, in order to define the best clone and best concentration for each antibody and each combination, presenting no RBC aggregation and retaining a good signal, as well.
Results: FLAER-based assay detected a $FLAER^-$ (PNH) granulocyte and/or monocyte clone (sensitivity 0.28% & 0.09%, respectively) in 42 (42%) and 58 (58%) samples, respectively, detecting a total of 64 (64%) $FLAER^-$ samples, while WBC routine assay showed a lower efficiency in PNH clone detection, especially with CD59. RBC analysis of CD235a-FITC (clone 11E4B76 KC16, Beckman Coulter)/CD59-PE (clone MEM43, Invitrogen), with antibody concentrations in the sample 3:140 and 1:140, respectively, showed a higher sensitivity (0.02%) and a very good efficiency, compared to the FLAER-based assay; applied in 28 $FLAER^-$ samples, it could detect a RBC $CD59^-$ clone in all those samples, with RBC clone size always smaller. Moreover, it provided a good discrimination between PNH type III, II and I (normal) subtypes in all the 28 samples, while FLAER-based assay achieved subtype discrimination only in 4 (14.3%) of those 28 samples.
Conclusions: FLAER-based assay is a more robust primary screening assay for detecting PNH clones in clinical samples. CD235a/CD59 RBC assay shows a higher sensitivity, a more accurate discrimination between PNH subtypes, a similar efficiency, but a lower quantification of the PNH clone, compared to the FLAER-based assay

W253
QUANTITATIVE JAK2 V617F ESTIMATION: CLINICAL RELEVANCE OF VALUES IN THE LOW LEVEL AREA IN THE DIAGNOSTICS OF MYELOPROLIFERATIVE DISEASES

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Background: The mutation in the Janus Kinase 2 gene (JAK2) causing valine in position 617 to be replaced by phenylalanine occurs in myeloproliferative disorders and is confined to the clonal hematopoetic cells of the patients. More than 90% of patients with polycythemia vera (PV) harbor this mutation, while it occurs in lower frequency in essential thrombocythemia (ET) and myelofibrosis. The proportion of mutated gene copies in relation to the number of total JAK2 gene copies is subjected to large variations in the patients. As part of the diagnostic process the occurrence and fractional rate of mutated gene copies is determined in the leukocytes of peripheral blood. We report patient samples with a detected frequency of ≥0.5% as positive for the mutation while specimens >0.015% are considered negative. The clinical significance of samples containing mutated gene copies between 0.015 and 0.5% is uncertain.
Aim: To establish the diagnostic accuracy of the quantitative JAK2 mutation assay in those patients having mutated gene copies in the interval >0.02- <0.5%.
Methods: Quantitation of the V617 F mutation in JAK2 was determined in peripheral blood leukocytes using the JAK2 MutaQuantTM kit (Ipsogen SA, Marseille). Patient samples with low levels of JAK2 mutated gene copies (0.015- <0.5%) were selected, and their clinical diagnosis determined. Results. Of 62 analysed patient samples, 11 were found to have mutated gene copies in the interval 0.2-0.31%. Of the latter, 4 patients were diagnosed as PV, 2 as ET and 5 were considered not to have any myeloproliferative disorder. In the interval 0.03- <0.2% (51 samples), 1 patient was diagnosed as PV, 2 as ET and 2 as myelofibrosis. In the patients from whom the remaining 46 samples were drawn, no signs of myeloproliferative diseases were detected. 20 samples were analysed more than once, and of these 6 became negative (none clinically with MPD), 1 positive, and the others yielded similar results.
Conclusions: Patients with JAK2 mutation ratios between 0.2 and 0.5% should be carefully evaluated with regard to other parameters used to diagnose MPD, as the quantitation of JAK2 mutation in this region seems to be of lesser significance when establishing the correct diagnosis.

W254

THE RELIABILITY OF BLAST FLAGGING ON THE SYSMEX XE-5000 IS QUESTIONABLE

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Background: Automated hematology instruments produce flags to indicate the presence of pathological cells and the flags are used as a criterion for microscopic examination of cell morphologic features. The utility of the flags is dependent on their diagnostic sensitivity and specificity. Laboratory productivity is related to the number of manual film reviews; the higher manual rates, the lower the productivity. The aim of this study was to assess the inter-instrumental concordance of the blast flag by three Sysmex XE-5000 instruments integrated onto a track based automation system.
Methods: A total of 408 samples were selected from the routine clinical case flow based on reports of flags indicating the presence of pathological cells including blasts. All samples were collected on Greiner tubes containing K2EDTA and within four hours analysed on three Sysmex XE-5000 instruments calibrated and harmonized by the manufacturer. The factory setting threshold for blast flagging, pre-set to an arbitrary unit of 100, was used. The inter-instrument reproducibility for both blast q-values and the blast flag was observed. SPSS was used for the statistical analyses.
Results: The three instruments (XE1, XE2 and XE3) showed a significant difference in the number of blast flags (p=0.006) and in the median q-value (P <0.001). Blast flags were generated for 191, 164 and 146 samples by XE1, XE2 and XE3, respectively. The median q-values (quartiles) reported for the 408 samples were 70 (0, 300), 40 (0, 280), and 30 (0, 250), respectively. A low level of inter-instrumental concordance for the q-values (ICC 0.85) and the blast flag (kappa value 0.73) was found.
Conclusions: This study showed poor inter-instrumental reproducibility in blast flagging by the Sysmex XE-5000s. The observed low performance questions the utility of the q-value as a predictor of blasts and whether a blast flag reported by the XE-5000 is sufficient as a criterion for performing a microscopic review.

W255

RELATION BETWEEN MORPHOLOGICAL AND HEMORHEOLOGICAL ALTERATIONS IN HEREDITARY SPHEROCYTOSIS AND ELLIPTOCYTOSIS

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Background: Study of relation between the alterations in the hemorheologic parameters and morphology of red blood cells (RBCs) could be a useful tool in certain clinical situations because they can produce serious disturbances in the microcirculation. Particularly, the study of this alterations in hemolytic anemias like hereditary spherocytosis (SH) and hereditary elliptocytosis (EH) is very interesting and can provide useful information for evaluation of these pathologies. The SH is characterized by the presence of spherocytes, jaundice, reticulocytosis and splenomegaly and the EH for the presence of elliptocytes in peripheral. The Scanning Electron Microscopy (SEM) offers greater advantages for the study of the cellular morphology because it presents greater to be able of resolution and greater depth of field. The purpose of this work was study the morphologic alterations in SH and EH by SEM and compare the results with the alterations in the viscoelastic properties of the RBCs from these pathologies. Methods: Fresh blood samples were collected from 2 SH and 3 EH patients with and 10 healthy donors by venipuncture in sterile vials containing EDTA as anticoagulant. Washed RBCs were fixed with glutaraldehyde, mounted on specimen stub, dried with air at room temperature and coated with gold for the examination in Scanning Electron Microscope at 10 keV. The Erythrodeformeter based in laser diffractometry technique was used to determine the RBC elastic modulus (μ), surface viscosity (hm), deformability index (ID) and dynamic viscoelastic parameters of RBCs. Results: Results show a significantly decrease in ID from SH, the samples from EH showed alterations in the hematological phenotype, height degree of hemolysis and very significant alterations in the hemorheologic parameters. The SEM microscopic images obtained of RBCs in these pathologies show different alterations depending of the disease specific degree and reveal several surface holes not observable by light microscopy, which can be related with loss of portions of membranes during the circulation. Conclusions: The relation between rheological alterations and SEM images of RBCs in SH and EH can be useful to describe the specific alteration in the membrane structure and evaluate more accurately the evolution of these pathologies.

W256

THE QUALITY OF THE WBC DIFFERENTIAL COUNT IN LEUKOPENIC SAMPLES ON CELLAVISION DM96 IS COMPARABLE TO MANUAL MICROSCOPY

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Backgroud: Low leukocytes are related to an increased risk of infections with high morbidity. Automated hematology instruments are well validated for counting low leukocytes as well as for differential count of leukopenic samples. The reference method for the differential count of leukocytes is the manual blood-smear count. The time-consuming manual count is still required in the follow up of pathological findings reported by the automated hematology instruments. During recent years pattern-recognition systems, such as CellaVision DM96 (DM96), are increasingly used in order to improve the quality and the efficiency of the differential count. The aim of the study was to investigate the quality of differential count in leukopenic samples on DM96 compared to Sysmex XE-2100 (Sys) and manual count (MC).
Methods: Differential counts in 71 blood samples with mean leukocyte counts 1.95 x 109/L (range 0.13- 3.96 x 109/L) were performed on DM96, Sys and by MC. The manual counts as well as the reclassification of cells on DM6 were performed by the same trained person. Two smears from each sample were evaluated by one investigator and the mean of these two was used. The relationship between differential counts by DM96, Sys and MC was investigated using Spearman's correlation. The precision (reproducibility) for the counting of each cell population was based on two parallel results from each sample.
Results: The correlation coefficients between differential counts on DM96, Sys and MC for all cell populations were found to range between 0.91 and 0.98 except for basophils on DM96 versus MC (r=0.59). For DM96 and MC the reproducibility for neutrophils was 15% and 13%, lymphocytes 12% and 8%, monocyte 31% and 23%, eosinophils 80% and 40% and basofils 148% and 96% respectively.
Conclusions: The study shows good correlations between DM96, Sys and MC for neutrophils, lymphocytes, monocytes and eosinophils but not for basophils. The precision of the differential count on DM96 is comparable to manual count for neutrophils, lymphocytes and monocytes. DM96 is a reliable tool for differential counting in samples with low leukocyte counts and can thus be applied in routine setting to reduce the time consumed for performing manual differential count.

W257

EVALUATION OF THE MINDRAY BC 6800 AUTOMATED HEMATOLOGY ANALYSER: COMPARISON WITH ABX PENTRA 120

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Background: The Mindray BC 6800 analyzer (Mindray Bio-Medical Electronics Co, Shenzhen, China) is a new developed hematology analyser with high throughput rate. The aim of this study was to evaluate the Complete Blood Count (CBC) and Differential Leukocyte Count (DLC) from the Mindray BC6800 in comparison with the ABX Pentra DX120 (Horiba ABX, Montpellier, France).
Methods: The BC6800 technology used for the red and platelet series is the impedance generating RBC and platelets counts and volume measurement. The technologies used for the DLC include fluorescence and laser light scatter. The evaluation of the instrument was performed by analyzing 1025 blood specimens from routine samples. All samples were analyzed in parallel on the BC-6800 and ABX Pentra 120 analyzers. Due to non-normal data distribution, non parametric statistics were used: the Wilcoxon test was used to test the differences between the data sets for significance. All correlations were tested for significance by calculation of Spearman´s rank correlation (rS) and the equations of the corresponding regression lines were calculated according to the method of Passing and Bablok. Bland-Altman analysis was also performed for evaluating intermethod bias.
Results: BC6800 demonstrated no significant carryover and good reproducibility. Although significant differences between BC6800 and ABX Pentra results ($P < 0.0001$) were observed, the correlation coefficients revealed that BC6800 gave CBC results that correlate with those obtained by ABX Pentra ($rS \geq 0.919$); a slightly lower correlation was observed between BC6800 and ABX Pentra monocyte ($rS=0.854$), eosinophil ($rS=0.809$) and basophil count ($rS= 0.583$). Although significant deviation from linearity was observed comparing BC6800 and ABX Pentra CBC and DLC data, BC 6800 results compared well with those obtained by ABX Pentra and these data were confirmed by Bland-Altman analysis: significant bias was observed only for platelet count (mean of difference 32.3).
Conclusions: our evaluation data show that the BC-6800 is a precise screening device and is comparable to ABX Pentra 120 except for platelet counts. The ease of use contributes to the performance of the BC-6800, enhancing its application in the clinical laboratory.

W258
DIAGNOSTIC IMPORTANCE OF LABORATORY EXAMINATIONS IN MDS

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Introduction: The myelodysplastic syndromes are all disorders of the stem cell in the bone marrow. In MDS, hematopoiesis (blood production) is disorderly and ineffective. The number and quality of blood-forming cells decline irreversibly, further impairing blood production.
Background: The myelodysplastic syndromes are all disorders of the stem cell in the bone marrow. In MDS, hematopoiesis (blood production) is disorderly and ineffective. The number and quality of blood-forming cells decline irreversibly, further impairing blood production.
Methods: We are presenting a case report of a 45 years old female patient, which came in our clinic complaining of intermittent asthenia and anemia after having undergone an hysterectomy operation. In the laboratory examinations was noticed: anemia (MCV 108), vitamin B level was within normal values, Coombs test was negative, ferritine 74, LDH 336, erythropoietin 7.5, haptoglobine 58, serology for hepatitis was negative. Blood morphology showed moderate anisocytosis and moderate hipochromia. We made further examinations: bone biopsy where was seen a nonhomogenous celularity, mieloeritroid proportion 1:1, granulopoesis with a delayed maturation, erythropoesis in different maturation stages, whereas megakariocytosis was normal. The immunohistochemistry for the CD 34 count showed a raise of CD 34 and of the immature elements.
Result: We concluded with a final diagnosis: mielodispasia.
Conclusion: MDS patients are often asymptomatic, and the diagnosis is made at the time of routine laboratory screening tests that reveal cytopenias in one or more lines or dysplasia on the blood smear. Typical disease manifestations include fatigue and weakness from anemia, infections from neutropenia, or bleeding due to thrombocytopenia or platelet dysfunction.

W259
HIGH SERUM VITAMIN B12 CONCENTRATION DUE TO HIGH MOLECULAR WEIGHT PROTEIN COMPLEXES

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Background: High serum vitamin B12 concentrations have been reported in several pathologies such as hepatic disease, myeloproliferative disorders, hypereosinophylic syndrome and disseminated neoplasia as well as in some patients under vitamin B12 treatement. In this work, we report two cases of high serum vitamin B12 concentration: a patient without any of above mentioned conditions (patient A) and a patient with high dose B12 treatment (patient B).
Methods: Serum vitamin B12 concentration was analyzed by a commercially available immunoassay in an automated platform (Architect, Abbot Diagnostics). Heterophile antibody interference was tested by incubating serum in heterophile blocking tubes (Scantibodies Laboratory, Inc.) previously to reanalysis. Poliethilenglycol 6000 (PEG 6000) precipitation and size exclusion HPLC were used to investigate the presence of high molecular weight forms of B12 protein complexes.
Results: Serum vitamin B12 concentrations were 3659 pmol/L and 3865 pmol/L for patient A and B respectively. None of both serum samples showed heterophile antibody interference. PEG 6000 precipitation showed a serum vitamin B12 concentration in supernatant of 210 pmol/L and 3840 pmol/L respectively. Size exclusion chromatography confirmed the presence of high molecular weight protein complexes (MW > 150KDa) containing immunoreactive vitamin B12 in patient A and normal immunoreactive vitamin B12 corresponding to serum vitamin B12 binding proteins molecular weight in patient B.
Conclusions: These cases illustrate the utility of laboratory tests (PEG precipitation and size exclusion chromatography) to identify patients with high molecular weight complexes of vitamin B12. These complexes falsely increase serum vitamin B12 concentrations and could mask vitamin B12 deficiency.

W260

AN EVALUATION OF IMMATURE GRANULOCYTE COUNT AS A BIOMARKER FOR POSTOPERATIVE INFECTION

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Background: During infection and other causes initiating inflammation, an increased production of leukocytes cause immature cells to appear in peripheral blood. The increase of rod neutrophils are referred to as “left shift” and has been considered as useful in the diagnosis of infections. In some automated hematology systems, such as Sysmex XE5000, the total number of myeloid precursor cells are characterised and reliably counted as immature granulocytes (IG). The aim of this study was to evaluate the usefulness of IG compared to leukocyte count (WBC), neutrophils (Neutro) and C-reactive protein (CRP) in differentiating inflammation due to surgical trauma from postoperative infection.
Material and Methods: Thirty patients admitted to the Department of Gastroenterological Surgery, Akershus University Hospital, for elective bowel surgery, were included in the study. Venous blood samples analysed for IG, WBC, Neutro and CRP were drawn preoperatively and on five consecutive days postoperatively. The patients were divided into two groups; normal postoperative course (NORM) and clinical signs of infection (INF). WBC, Neutro and IG were measured on a Sysmex XE5000 instrument. The reproducibility of the IG measurement is 17 % based on two measurements of each of 404 samples.
Results: All biomarkers were affected by the surgical intervention. Higher values were found in patients with infection. A significant increase in IG was observed both in the NORM-group ($P < 0.01$) and in the INF-group ($P < 0.01$) on Day 1 after surgery. The mean value of IG was in the INF-group 0,04*109/L and in the NORM-group 0,02*109/L ($P < 0.05$). This difference remained almost the same the consecutive two days. For WBC and Neutro only small and not statistically differences were found between the groups at Day 1. At Day 1 CRP was 25 % higher in the INF-group compared to NORM ($P < 0.05$). On day 2 however significant differences were observed for these three parameters.
Conclusions: This study indicates that IG is a better marker for early detection of postoperative infections than WBC and Neutro. A larger study is now in progress in order to verify this conclusion.

W261

IDENTIFYING SPECIFIC PRODUCTION CLONAL IN A GAMMOPATHY USING NEW DIAGNOSTIC MARKERS OF MONOCLONAL COMPONENT

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Background: Analisys of specific pairs heavy/Light chain (HLC) is a quantitative alternative to serum immunofixation electrophoresis (IFE). Recent evidence indicates the existence of a progressive clonal evolution in multiple myeloma (MM), showing the prognostic value of the determination of HLC along with the ratio of free light chains (rsFLC).
Case: A 73 year old woman diagnosed with IgA λ MM “quiescent” in 1996, progressing to MM in November 2004, monoclonal protein (MP) 3.49 g/dL. In September 2005 she was treated with a VMCP/VBAD+6 cycles of pamidronate achieved partial remission (PR), MP: 0.6 g/dL. June 2006 is subject to stem cell transplant with melphalan, achieving very good partial remission (VGPR) MP 0.37 g/dL. In October 2006 starts with interferon maintenance until August 2010 which revealed a relapse MP 2.92 g/dL, IFE: IgA λ polymers, with 40% bone marrow plasma cells. VMP treatment starts reaching complete remission but with positive IFE, maintained until November 2011 that begins to be seen again progressively increasing monoclonal component, found in April 2012 CM 1.97 g/dl. At moment she was in 3rd progression.
Results: The patient had the 1st, 2nd and 3rd relapse in August 2010, November 2011 and July 2012. Throughout the evolution of rsFLC patient has remained within the reference range, the IFE was always positive. At this time the patient is in relapse 3rd, MP: 4.16 g/dL. The result of the IgAκ HLC uninvolved appeared immunosuppressed, IgAκ: 0.131 g/L from July 2012 with patological rHLC: 0.17 until now. HLC detect the second relapse 45 days before the usual methods SPEP while total IgA: 451 mg/dL (70 – 400) was only a slight increase from the reference value.
Conclusion: The model rsFLC / rHLC contributed in this case more information than conventional tests. Given that the patient’s FLC ratios were consistently normal, pathological ratios were very helpful to the diagnosis, abnormal IgA HLC pair ratios indicated a relapse 45 days before the appearance of MP. Suppression uninvolved HLC could help in identifying patients with worse outcomes, however more studies would be needed.
The joint determination and rHLC rsHLC could indicate the election of a particular treatment within the options.

W262

NEW SERUM MARKERS OF MONOCLONAL GAMMOPATHIES: SERUM FREE LIGHT CHAINS AND IMMUNOGLOBULIN HEAVY CHAIN/LIGHT CHAIN PAIRS. A CLINICAL CASE OF MGUS CONVERSION TO MM

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Introduction: The current IMWG guidelines for managing monoclonal gammopathies of undetermined significance (MGUS) recommend the determination of the progression risk factor at the time of diagnosis based on the size of the monoclonal protein (MP) < or >1.5 mg/dL, the type of the MC IgG vs IgA/IgM, and the serum free light chains ratio 0.26<rFLC<1.65. Recently it has been shown that the suppression of the uninvolved Hevylite pair of the same immunoglobulin class also has a prognostic value for MGUS,and we have confirmed that this phenomenon occurs with a relative frequency in our MGUS population.However, for monitoring MGUS patients the recommendations are to follow the MP size by protein electrophoresis. In the presented case, we raise the question whether the new techniques for the sFLC detection and the Hevylite specific suppression should be considered also for MP follow-up.
Methods: sFLC and HLC (FreeliteTM, Hevylite Binding Site) were measured on a BNII (Siemens), the MP quantified by serum electrophoresis (SPE) and typified by immunofixation (IFE) (Sebia)
Results: A 74 year old man was diagnosed in 2006 with an IgGλ MP of 0,7 mg/dL and an abnormal rFLC (0.15, corresponding to a low-intermediate risk of progression case. In 2011 there was a markedly increase of the MP (1.7 mg/dL), with concomitant IgA and IgM immunoparesia and a more abnormal rFLC (0.03). Retrospective analysis as shown a normal HLC IgG-κ, IgG-λ and the IgG-κ/IgG-λ ratio at the time of diagnosis, however in late 2009 the HLC ratio become abnormal (0.59) with a small suppression of the uninvolved IgG-λ HLC pair, 1 year prior to the serum MP increase. In 2012 the patient presented bone lytic lesions, 21% infiltration of plasmatic cells on bone marrow, with 5.5% of aberrant plasmatic cells determined by flow cytometry, with a final diagnosis of MMIIA, IPI 1. The patient remains asymptomatic and untreated.
Conclusions: In this MGUS case, rFLC was a pertinent indicator of risk of conversion to a more malignant stage of the disease, and HLC follow-up of the uninvolved pair was the first factor indicator of this progression. The use of the new rFLC and HLC markers may be relevant for monitoring the clonal evolution on MGUS patients and allowing the early identification of progression

W263

DIFFERENTIAL EXPRESSION OF MULTIDRUG RESISTANCE PROTEINS IN ACUTE MYELOID LEUKEMIA TYPES WITH AND WITHOUT MONOCYTIC INVOLVEMENT

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Background: Identification of drug resistance is of interest in several malignancies. We used a functional test, the MultiDrugQuant assay kit, to evaluate the activity of three proteins that are involved in the development of multidrug resistance (MDR).
Methods: The activity of P-glycoprotein (ABCB1, Pgp), multidrug resistance related protein-1 (MRP1, ABCC1) and breast cancer resistance protein (BCRP, ABCG2) were quantitated in relevant cell lines, monocytes of 12 healthy volunteers and in the mononucelar cells of 24 bone marrow samples, derived from de novo acute myeloid leukemia patients before treatment. Fourteen samples were obtained from de novo monocytic/monoblastic (CD64+/CD4+/cyFXIII+) cases and 10 samples were derived from de novo myeloblastic (CD33+/CD117+/CD33+) cases. Phenotyping of cases was carried out by 8 color analysis using FacsCanto II. MDR protein activities expressed in multidrug resistance activity factor (MAF) were measured by the MultiDrugQuant assay kit (Solvo Biotechnology, Hungary) using selective inhibitors.
Results: Positive (KB-V1, GLC4/ADR and MDCK-G2) and negative (KB-31, GLC4, MDCK) cell lines provided suitable discriminitaion and accuracy of all three transporters by the MultiDrugQuant assay. This resulted in MAF values, 64-84 in positive cell lines and 5-22 in negative cell lines. MAF values for Pgp and MRP1, in myeloblastic leukemia samples were significantly higher than that of normal monocytes; 24.5 versus 11.2 for Pgp and 15.0 versus 3.6 for MRP1, P <0.01 for both markers. Monoblastic AML cases also expressed higher values in Pgp activity compared to normal monocytes 15.7 (P <0.04) but no difference was observed for MRP1 activities. In case of BCRP, normal monocytes and monoblastic as well as myeloblastic leukemia samples showed very similar results, MAF values being, 6.8, 8.5 and 8.9 respectively (P=non significant).
Conclusions: These data suggest, that MDR proteins are expressed differentially in AML subtypes and suggest that the role of Pgp and MRP1 is of pivotal importance particularly in myeloblastic forms.

W264

HAIRY CELL LEUKEMIA WITH DISCREPANT RESULTS OF BRAF V600E MUTATION AMONG SPECIMENS

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Background: Very recently, BRAF V600E mutation has been described as a molecular marker of classic hairy cell leukemia. However, detection of BRAF V600E mutation has been reported to be variable according to the detection method, specimen type or percentage of malignant cells in specimen. We describe a case of hairy cell leukemia with discrepant results of BRAF V600E mutation among specimens.
Case & result: A 52-year-old-woman presented with pancytopenia (severe leukopenia with monocytopenia). She had splenomegaly. In her bone marrow aspiration, increased abnormal lymphocytes (82.7% of all nucleated cells) showed inconspicuous nucleoli, frayed cytoplasm, and hairy cytoplasmic projections. Bone marrow biopsy was packed with abnormal lymphoid cells showing diffuse solid infiltration and showed massive fibrosis. Flow cytometry analysis revealed B cell population expressing strong CD11c, CD19, CD20, HLA-DR, cCD79a and ectopic CD2. The BRAF V600E mutation analysis was performed by mutation-specific real-time PCR kit (Real QTM BRAF V600E detection Kit, BioSewoom Inc., Seoul, Korea). The BRAF V600E mutation was not detected in bone marrow aspiration specimen whereas it was detected in right and left bone marrow biopsy specimens. She was diagnosed as having classic hairy cell leukemia.
Conclusion: BRAF V600E mutation can be a new diagnostic tool for distinguishing HCL from other small B-cell lymphomas. This case underscores that the type of specimen, especially in fibrotic specimens, should be considered for the interpretation of this molecular results.

W265

ACUTE LEUKEMIA WITH B/MONOCYTIC MIXED PHENOTYPES: A CASE REPORT AND REVIEW OF THE LITERATURE

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Background: Mixed phenotype acute leukemia (MPAL) with B/myeloid phenotype is rare, probably accounting for 1% of newly diagnosed leukemia. We describe a patient who had MPAL with B/monocytic bilineage differentiation.
Case & result: A 64-year-old female presented with fever and abdominal pain. Her complete blood cell counts were as follows: white blood cell counts, 4.8 x 109/L with blasts (2%); hemoglobin 11.6 g/dL; platelet counts, 84 x 109/L. Increased blasts (60.5% of all nucleated cells) in her bone marrow, showed dimorphic populations, one resembling lymphoblasts and the other resembling monoblasts. Flow cytometry analysis revealed two distinct populations of leukemic cells expressing monocytic (CD11c/CD14) and B-cell (strong CD19/CD20/CD79a) phenotype. Cytogenetic analysis showed complex structural and numerical abnormalities. She expired 18 days after induction chemotherapy due to multiple organ failure.
Conclusion: While the B/myeloid or T/myeloid MPAL has been reported, MPAL with B/monocytic mixed phenotypes that fulfills the WHO 2008 classification has never been reported so far. To our knowledge, this is the first case report of B/monocytic MPAL that fulfills the diagnostic criteria by WHO 2008 classification.

W266

THE OPTIMIZATION OF LABORATORY MONITORING OF PRIMARY HEMOSTASIS DURING THE CHRONIC MYELOPROLIFERATIVE DISORDERS

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Background: The aim of our research was the optimization of laboratory monitoring of primary hemostasis during the chronic myeloproliferative disorders (MPDs) on the basis of exposure of the features of platelet hemostasis for opportunely prevention of hemorrhagic and thrombotic risks.
Methods. Platelet aggregation was estimated by a photometric method using a turbidimetric aggregometer AP-2110 Solar (Belarus). Platelet aggregation was induced with the following inductors: adenosine5'-diphosphate (ADP) in final concentrations 0,5; 1,5; 2,5 and 5,0 micromole; epinephrine (Adr) -5,0 micromole and solution of a collagen 2,0 mg/mL. The platelet quantity was adduced to standard value: in thrombocytosis the dilution was made, in thrombocytopenia - concentration.
Results: The research of a primary hemostasis was conducted for 153 patients with MPDs: chronic myeloid leukemia (n=50), polycythemia vera (n=53), essential thrombocytemia (n=38) and idiopathic myelofibrosis (n=12). The control group consisted of the healthy donors of both sexes comparable by age. It has been established that the changes of platelet hemostasis during MPDs have unilateral nature and do not always determine thrombotic risk. It has been demonstrated that Adr-induced aggregation of platelets was decreased in 89±2,5% (n=136) cases among all examined patients with MPDs. Moreover, the depression of platelet response on 1,5 micromole ADP-stimulation in 92±2,2% (n=140) of patients with MPDs was detected. It should be noted that collagen-induced aggregation of platelets was preserved for all patients with MPDs under study.
Conclusions: The established changes of the platelet hemostasis were typical for all patients with different MPDs nosology under study. It has been demonstrated that the changes of functions of platelet hemostasis during MPDs depend not on the degree of thrombocytosis, but on platelet quality changes, determined by platelet origin from the neoplastic megakaryocytes clone. Our conclusions are confirmed by absence of similar changes during secondary thrombocytosis, and more importantly by normalization of a platelet function after allogeneic marrow transplantation.

W267

THE USEFULNESS OF RETICULOCYTE PARAMETERS IN DIAGNOSTICS OF HEREDITARY SPHEROCYTOSIS IN CHILDREN

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Background: Innovations in laboratory equipment allow to widen the spectrum of hematological parameters obtained from single measurement of peripheral blood samples. The meaningful progress is observed in analysis of reticulocyte parameters, including measurement of different reticulocyte fractions volume or the hemoglobin concentration in immature fractions of red blood cells. However, the novel markers are rarely used in diagnostics of pediatric anemia and they are not widely applied by physicians. The aim of the study was to analyse the results of novel hematological parameters in different types of anemia in children and to find a useful markers to support the diagnosis of hereditary spherocytosis.
Methods: Altogether 116 children, aged 1 month – 17 years with anemia defined as decreased hemoglobin concentration were enrolled to the study. Thirteen of them suffered from hereditary spherocytosis (HS) confirmed by clinical and laboratory investigations. Complete blood count extended with analysis of reticulocyte parameters as mean reticulocyte volume (MRV), mean sphere cell volume (MSCV), immature reticulocyte fraction (IRF) and high-light scatter reticulocyte (HLR) were measured with use of Beckman Coulter LH 750.
Results: The MSCV in the group of children with HS was 66.11±7.58 fL whereas in other anemic patients MSCV were 88.32±10.84 fL, $P < 0.05$. Mean reticulocyte volume was 80.01±7.78 fL in HS patients, whereas in the group of children with other types of anemia MRV was 110.15±14.86 fL, $P < 0.05$. In HS children average mean corpuscular volume of red blood cells (MCV) was higher than MSCV value (MCV-MSCV was 13.85±3.78 fL), inverse correlation was observed in group of children with other anemias (MCV-MSCV was -0.24±6.55 fL), $P < 0.05$. No differences in IRF was observed between both analyzed groups, however significant difference was found between ratio of absolute reticulocyte count and IRF fraction (Ret#/IRF) - 0.67±0.3 in HS group and 0.25±0.18 in non-HS anemic group, respectively.
Conclusion: Presented results suggest that analysis of reticulocyte parameters as Ret#/IRF or MSCV may be useful in diagnostics of hereditary spherocytosis and should be brought in the routine CBC analysis in anemic children.

W268
IGD MULTIPLE MYELOMA: PRESENTING CLINICAL AND BIOLOGICAL FEATURES. REVIEW OF 17 CASES

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Background: IgD multiple myeloma (MM) is a rare subtype of myeloma which affects less than 2% of all patients with MM. It's characterized by younger age, more aggressive course and poorer outcome than other MM.
Patients and methods: In the present study, from 2002 to 2012, we obtained 1250 monoclonal gammapathies included 590 multiple myeloma. 17 patients among these cases had IgD MM.
Results: Patients with IgD multiple myeloma are present in 2,9% of myelomas. Men predominate with a mean age at diagnosis: 59 ± 12 years. The commonest presenting symptom was bone pain (75%). The other presenting features included: lymphadenopathy (16%), hepatomegaly (25%), splenomegaly (8%) and associated amyloidosis (6%). Renal impairment function and infections were found in 82% and 47% of patients, respectively. Severe anemia and cytopenia are common. Hypercalcemia was present in 37% of our patients. 3 patients developed plasma cell leukemia (17,6%). The serum electrophoretic pattern showed an M-spike in all our patients associated to a hypogammaglobulinemia. IgD M-components are usually not high (Mean =13,22±10 g/L). Bence Jones proteinuria was identified in 71% of cases. The type of light chain was Lambda in 65%. We had accelerated ESR in all patients and high B2microglobulins in 91% of cases. The survival of our patients was short, approximately 9 months. We also measured FLC (Free Light Chain) in serum, which is an automated immunoassay useful for prognosis and monitoring of IgD multiple myeloma. Serum FLC concentrations were abnormal in 93% of patients. It showed great concordance with serum b2microglobulin concentrations and the survival. In addition, because of their short serum half-life, changes in serum FLC concentrations provide a rapid indication of the response to treatment and we noticed that in 2 patients.
Conclusion: IgD MM appear to have a more aggressive disease course, poorer prognosis and shorter survival than other subtypes. The contribution of immunologic analysis is crucial for the diagnosis, prognosis and monitoring of patients wit.

W269
DEVELOPING OF NORMAL HEPCIDIN LEVELS IN BULGARIAN POPULATION

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Background: Hepcidin is a 25-aminoacid iron regulating peptide. Increased hepcidin concentrations lead to iron sequestration in macrophages. It plays major role in the pathogenesis of anemia of chronic disease whereas decreased hepcidin is observed in iron deficiency and primary iron overload diseases such as hereditary hemochromatosis. Hepcidin quantification in human blood or urine may provide further insights for the pathogenesis of disorders of iron homeostasis and might prove a valuable tool for clinicians for the differential diagnosis of anemia.
Methods: Serum Hepcidin levels were measured, using monoclonal sandwich ELISA method in 30 healthy individuals. Including criteria were normal liver (average ASAT and ALAT levels respectively 28.7U/L and 23.1U/L) and kidney (average serum creatinine levels were 89.45 μmol/L, average eGFR level of 80.0) function, no evidence of diabetes (average blood glucose fasting 4.55 mmol/L), without concomitant infection disease (average CRP levels 2.66mg/L). The kit uses recombinant human Hepcidin as a standard.
Results: Measured serum Hepcidin levels in our group healthy individuals were average of 12.36 ng/mL (3.13 ng/mL – 27.3 ng/mL), SD 7.66 ng/mL. Used statistical approach of t-test showed high correlation (t=1.03 – 1.11) between serum Hepcidin levels and indexes of iron metabolism – serum iron levels and TIBC (P=0.05).
Conclusions: The results from our preliminary study are the first step of establishing of reference values for men and women of Bulgarian population and study of clinical significance of this new parameter.

W270

USE OF THE NOVEL MONOCLONAL ASSAY FOR THE MEASUREMENT OF CIRCULATING FREE LIGHT CHAIN IN THE DIAGNOSIS, PROGNOSTICATION OF SURVIVAL AND ASSESSMENT OF RESPONSE TO THERAPY IN AL AMYLOIDOSIS

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Background: Circulating free light chains measurement (FLC) improved diagnosis, prognostication of survival and response assessment in AL amyloidosis. We evaluated a novel method for FLC quantitation based on monoclonal antibodies in 353 consecutive newly diagnosed patients.
Methods: Serum FLC concentration was measured on frozen sera by a polyclonal (Binding Site, BS) and a monoclonal (Siemens, S) immunoassay on a Siemens BN ProSpec nephelometer. Reference ranges are kappa 3.3-19.4 mg/L, lambda 5.7-26.3 mg/L, ratio 0.26-1.65 for the BS assay, and kappa 6.7-22.4 mg/L, lambda 8.3-27.0 mg/L, ratio 0.31-1.56 for the S test.
Results: The concordance correlation coefficient of the two assays was (0.92, 95% confidence interval [CI] 0.87-0.91) for kappa and (0.78, 95%CI 0.73-0.82) for lambda FLC. Diagnostic sensitivity was 82% (95%CI 78-86%) for the BS assay and 84% (95%CI 80-88%) for the S test. The combination of FLC measurement with serum and urine immunofixation increased sensitivity to 98% (95%CI 96-99%) with both assays. We evaluated the prognostic relevance of the difference between involved (amyloidogenic) and uninvolved FLC concentration (dFLC). Median values of dFLC were 180 mg/L by BS and 165 mg/L by S. Patients with dFLC above the median value had a worse outcome (41% vs. 65% surviving 2 years, P=0.001, with both methods). These thresholds were incorporated into a staging system, including the median values of N terminal pro natriuretic peptide type B (1800 ng/L) and troponin I (0.07 ng/mL). The resulting systems identified four groups with decreasing survivals. The discrimination between the groups with worse outcome (stages 3 and 4) was not statistically significant with the BS test (P=0.134), while it reached statistical significance with the S test (P=0.022). We evaluated the applicability of the criteria for hematologic response validated with the BS test to the S assay, and observed relevant discrepancies. In particular, 26% of responders were classified as non-responders by S.
Conclusions: The S assay has a diagnostic sensitivity comparable to that of the BS test and can be used for prognostic stratification. The discrepancies observed in the assessment of response indicate that different criteria may be needed when using the S assay.

W271

TESTING THREE DAY STABILITY OF ERITHROCYTE AND RETICULOCYTE COUNT

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Background: Recommendation for EDTA whole blood analysis is within 6h of collection. Analysis after extended storage for up to 24h is not advisable even though certain situations make prompt analysis impossible. The aim of this study was to evaluate stability of erythrocyte and reticulocyte count during the period of prolonged storage at 4 °C.
Methods: Samples of 101 hospital patients were analyzed for erythrocyte (RBC) and reticulocyte (RETIC) count on ADVIA 2120i (Siemens, Dublin, Ireland) hematology analyzer. Blood was collected for routine laboratory analysis in BD Vacutainerä tube with K3-EDTA anticoagulant. Analysis was performed upon arrival into laboratory after which samples were stored at 4°C for further analysis (24, 48 and 72 h). Statistical analysis was performed using MedCalc statistical software (Mariakerke, Belgium).
Results: Repeated measures ANOVA showed significant difference between measurements of RBC as well as RETIC during three day period, P <0.001 and P=0.016 respectively. RBC count (1012/L) expressed as mean±SD at 0, 24, 48 and 72h was: 4.03±0.09, 4.06±0.09, 4.06±0.09 and 4.04±0.09 respectively. RETIC count (109/L) expressed as mean±SD at 0, 24, 48 and 72h was: 84.0±5.7, 84.0±5.6, 86.2±5.6 and 86.7±5.8 respectively. Pairwise comparison tested measurements to each other and calculated mean difference, 95% confidence interval (95% CI) for difference and P value after Bonferroni correction. Mean difference and 95% CI between RBC measurements at time 0 and 24h was -0.024 (-0.039-(-0.008); P <0.001), therefore further analysis was not taken into consideration. Pairwise comparison for RETIC measurements did not show significant difference between measurements. Mean differences and 95% CI between RETIC measurements at time 0 and 24, 48 and 72h were: 0.003 (-2.476-2.482; P=1.000), -2.131 (-4.982-0.721; P=0.282) and -2.625 (-6.007-0.757; P=0.236) respectively.
Conclusions: Our results imply that whole blood samples should not be used for RBC and RETIC analysis after prolonged storage. However, large sample could bias statistical analysis. Relatively small mean differences between sequential measurements have to be judged against clinically significant changes in order to evaluate their reliability for analysis after prolonged storage.

W272

ARE IGM HEVYLITE® IMMUNOGLOBULIN HEAVY CHAIN/LIGHT CHAIN ANALYSIS A USEFUL TOOL TO DIFFERENTIATE IGM MGUS FROM WALDENSTRÖM MACROGLOBULINEMIA?

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Background: Monoclonal IgM is the biomarker that characterize to Waldenstrom's macroglobulinemia (WM),even serum IgM component also is presented in MGUS. New determinations of heavy chain/light chain immunoglobulins pairs (HLC) have been developed as biomarkers to apply to every day clinical practice. The aim of this study is to present our experience in the use of free light chain assay (sFLC) and HLC as biomarkers at diagnostic in order to discriminate between MGUS and WM, and to evaluate their potential prognostic value during disease follow-up.
Patients and Methods: A total of 48 patients were included as carrier a serum monoclonal IgM diagnosed in the Hematology Department of MSUH. Pts were classified as MGUS or WM according to the current diagnosis criteria. Pts were examined every 3-6 months following our clinical protocol in order to detect progression or transformation. Serum samples were collected prior and during treatment and were kept frozen at -70ºC since collection and incorporate to our regional Biobank. Analysis of IgM were performed with the sFLC, (Freelite® test, and the HLC (Hevylite® immunoassay the Binding Site) according the recommendations of manofacturer. Results: A series of 28 WM, 20 IgM-MGUS. Median age: 67.1 y(13-85); IgM HLCR: 114.68 (1.02–353) in WM symptomatic, 71.55 (1.02–286.43) in WM asymptomatic and 9.5 (0.45–50.74)in IgM MGUS (P=0.003). HLCR was higher in WM patients requiring treatment (n=15) at diagnosis than in pts (n=33) not requiring treatment (113 v 15.77 P=0.019) and also HLCR was significantly higher at relapse/refractory vs not relapse (113 v 17.17 P=0.012) uHLC was significantly higher in IgM-MGUS than WM to IgMkappa and IgMlambda iHLC: 0.37 g/L (0.11-1.25) a v 0.1 g/L (0.02-4.03), P=0.022; and 0.69 g/L (0.08-4.09) v 0.32g/L (0.22-0.63), P=0.05 respectively. sFLC level was 64 mg/L (10.88–993) in WM and 31.7 mg/L (6.08–141) in IgM MGUS (p=0.05). sFLC level was higher in WM requiring treatment at diagnosis than asymptomatic pts (73.7 v 36.85 P=0.039). sFLC level was not significative in relapse.
Conclusion: The HLCR and sFLC could be good biomarkers to differentiate between IgM-MGUS and WM at diagnostic. High levels of HLC and sFLC were also seen in pts requiring treatment.HLCR discriminates symptomatic MW vs asymptomatic.

W273

CLINICAL UTILITY OF CASE MANAGER SOFTWARE PROVIDED BY THE SYSMEX XE-5000 ANALYZER FOR THE SCREENING OF MICROCYTIC ANEMIA

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Background: The core function of the XE-5000 Case Manager (CM) is rapid recognition of parameter combinations that are characteristic of certain diseases, in order to reduce the growing complexity involved in diagnostics and as such assist the lab physician in reporting and interpreting the relevant clinical information about a patient's condition, thereby supporting the clinician in quick, efficient diagnosis and therapy monitoring. Anemia is frequently associated with the pathology of patients visiting the emergency department. It leads to longer stays in hospital and in some cases is associated with a worse prognosis. The correct classification of this pathology is essential for both the patient and the institution. Microcytic hypochromic anemias (MHA), characterized by decreased Haemoglobin (Hb) production per cell results from deficiency of either haem or globin chains. Iron deficiency anemia (IDA) is the most frequent acquired MHA. Thalassaemia syndromes lead to decreased Hb per cell because of the deficiency of one of the two types of globin chains. In previous work we established diagnostic algorithms for IDA and β-thalassemia that joined the Sysmex XE 5000 analyzers in the CM software. The aim of this study was to determine the clinical efficacy of these algorithms.
Methods: Between April and October 2012, 63.448 cell blood counts (CBC) were processed (Sysmex XE 5000, Roche Diagnostics) in the Emergency Laboratory. Randomized patients with Hb <10 mg/dL were given a CBC with reticulocyte (N=131) and analyzed by CM software V. 3.10.0.10 . The final diagnosis was obtained with the patient's history.
Results: Prevalence of severe anemia (Hb <10 g/dL): 21%, 13.324 patients. Of the 131 patients studied by CM, 103 were classified as presenting severe anemia, 34 as MHA (29 IDA, 5 β –talassemia). The CM correctly classified 96% of the IDA and 80% of the thalassemias, so the use of this tool could have provided a precise diagnosis of these diseases in 4397 cases.
Conclusions: The clinical performance of the CM for diagnosis and classification of MHA is high. It provides relevant and reliable clinical information that leads clinicians to better patient management and clinical decisions, avoiding other diagnostic tests and assisting in resource optimization

W274
COMPARISON OF THE ANALYTICAL PERFORMANCE OF THE POLYCLONAL ANTIBODY BASED FREELITE AND MONOCLONAL ANTIBODY BASED N LATEX FLC ASSAYS IN SAMPLES FROM PATIENTS WITH MONOCLONAL GAMMOPATHIES

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Background: Clinical laboratory standards institute (CLSI) guidelines advise that a linear regression R2 value >0.95 provides confidence that two assays are measuring the same analyte. To date there has been only 1 commonly employed method for quantifying serum free light chains (Freelite®). Here we compare the performance of a new assay (N Latex FLC) to the established tests and comment on their agreement when analysing monoclonal gammopathy (MG) patient samples.
Methods: Freelite and N Latex FLC measurements were compared in 40 randomly selected MG (25 multiple myeloma (13k and 12L; MM) and 15 monoclonal gammopathy of undetermined significance (7k, 1L, 7 unassigned; MGUS) patients, taken through the course of their disease.
Results: Overall Freelite had a greater sensitivity to detect monoclonal FLC compared to N Latex FLC (25/40 v 21/40, respectively). Freelite identified 18/18 MM patients with abnormal N Latex FLC ratios (Freelite: median involved FLC: 223mg/L (1.43-2720 mg/L), median FLCk ratio: 5.1 (1.8-1052), median FLCL ratio 0.04 (0.0004-0.256); N Latex FLC: median involved FLC: 170mg/L (7.36-1130 mg/L), median FLCk ratio: 2.7 (1.85-288), median FLCL ratio 0.03 (0.006-0.235); and identified clonality in an additional 4 patients (2 k: Freelite ratio 1.75 and 2.25, N Latex FLC ratio 0.84 and 1.28; and 2 L: Freelite ratio 0.04 and 0.15, N Latex FLC ratio 0.38 and 0.61). Overall there was poor correlation between the assays (FLCk: Passing-Bablok (PB) slope=0.95 (95% CI: 0.83-1.12), linear regression R2=0.22; FLCL: PB slope=1.36 (95% CI: 0.99-1.88), R2=0.14). Similarly, a subset analysis of the 25 MM patients identified poor correlation between the assays (FLCk: PB slope=1.05 (95% CI: 0.84-1.50), R2=0.21; and FLCL: PB slope=1.06 (95% CI: 0.65-1.81), R2=0.11).
Conclusions: International guidelines recommending FLC analysis in the identification and monitoring of MG patients have to date been based on the Freelite assay. Our results suggest that the N Latex FLC assay is less sensitive in the identification of MG patients. Furthermore, there is poor agreement in values reported by the assays; therefore substantial work is required to establish guidelines for the use of this assay.

W275
EFFECT OF SMOKING ON HAEMATOLOGICAL PARAMETERS

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Background: Smoking has been found to be associated with derangement in health. This study was designed to evaluate the effect of smoking on haematological parameters.
Materials and methods: A total of 120 subjects (60 smokers and 60 non-smokers) were employed for the study. 5ml of whole blood was collected into K3EDTA anticoagulated bottle from both smokers and non-smokers. A smoker was defined as somebody who smoked between 5 – 12 sticks per day. Haemoglobin was measured by cyanmethaemoglobin method. Packed cell volume was read with heamatocrit reader after centrifugation for 5 minutes in a microcentrifuge. Total white cell count was determined under the microscope after lysis of red cells in turk's solution while differential cell count was determined under the microscope after staining with Leishman stain.
Results: The mean cigarette smoking for the smokers is 7 sticks per day. There was no statistical difference in the age of smokers (36.17±5.48) as compared to non-smokers (37.90± 5.58), P >0.05. Packed cell volume was greater in smokers (43.73 % ±4.89) than in non-smokers (40.77% ±2.67), P <0.05. The haemoglobin concentration(14.79 g/dL±1.5 versus 13.6 g/dL ± 0.92, P <0.05) and total white cell count (7.25 x 103±1.5 versus 5.88 103±1.2, P <0.05) were also higher in smokers than in non-smokers however no significant difference was found in the differential white cell of the two groups.
Conclusion: Cigarette smoking appears to be associated with abnormal haematological indices. This may predispose smokers to polycythaemia. It is therefore recommended that smokers be advised on the consequences of smoking on their health.

W276

SERUM FREE LIGHT CHAINS QUANTIFICATION: AN ALTERNATIVE TO BENCE-JONES PROTEINURIA? A COMPARATIVE STUDY

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Introduction: The determination of serum free light chains (sFLC) adds sensitivity to the detection of monoclonal proteins (MP), and is recommended for diagnostic, prognostic and follow-up of different monoclonal gammopathies (MG). Specifically for screening most of the MG, the sFLC quantification is recommended together with serum electrophoresis and immunofixation (SPE, IFE), without the need to study the Bence-Jones proteinuria (BJP). Yet, for monitoring multiple myeloma (MM), 24h-urine IFE (uIFE) and quantitative immunoassays are still required.
Aim: to compare the sFLC results with the BJ determination in urine (by uIFE) in the two following groups: 1) querying for a MG and, 2) MM patients already diagnosed.
Methods: 60 patients with ages comprised between 37-87 years old (average 76) were included, 32 MG queries and 28 already diagnosed MM. uIFE (Hydragel IF, Sebia) and sFLC (Freelite, BindingSite) were preformed for all cases. Qualitative association was determined by the Chi-square test (significant $P < 0.05$). uIFE and sFLC ratio (NR: 0.26-1.65) were reported as positive/negative. Statistic analysis was done on SPSS v15.0.
Results: Querying for MG (n=32), the comparison between the determination of sFLC and uIFE for detecting the presence of a monoclonal component was significant (P=0.012). 23 (72%) cases resulted in either negative or positive for the presence of a monoclonal component by both uIFE and sFLC ratio. 6 cases were positive only for sFLC ratio and 3 only by uIFE, meaning that the sFLC ratio was more sensitive than uIFE when screening for MG. However, the comparison was not significant in MM patients already diagnosed (P=0.074). 20 from the 28 already diagnosed MM patients (71%), 4 were negative and 16 positive by both uIFE and sFLC ratio. While 2 MM cases were positive just by sFLC ratio, 6 cases were positive only by uIFE.
Conclusions: An association was found between sFLC ratio and uIFE in both groups analyzed. However, this association was less significant in the already diagnosed MM subgroup possibly due to the low IFE sensitivity in patients under treatment (7%), and the presence of IFE positive cases with normal sFLC, probably due to the nature of the monoclonal component. The results obtained support the clinic guidelines.

W277

DETECTION OF JAK2 EXON 12 MUTATIONS BY THE HIGH RESOLUTION DNA MELTING CURVE ANALYSIS

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Background: The mutations in the exon 12 of the JAK2 gene have been described in 5 to 10% of the patients with PV and with no JAK2 V617F mutation. The aim of the study was to introduce the High Resolution DNA Melting curve analysis (HRM) method for detection of JAK2 exon 12 mutations in patients with PV or unclear erythrocytosis and with no JAK2 V617F mutation. The results were compared with the previously detected results on patient samples by methods described in Zdrav Vestn 2012; 81 supl 2: II-161–7.
Methods: Granulocytes were isolated from peripheral blood or bone marrow samples by ficoll density centrifugation followed by erythrocytes lysis. DNA was isolated from granulocytes by QIAamp DNA Mini Kit (Qiagen, USA). The patients DNA (20 ng) samples were used to verify the test. 10 patients with PV or unclear erythrocytosis and with no JAK V617F mutation were included in the study (8 with no mutation, one with the p.542_543delAsnGlu, the other with the p.543_544delGluAsp). In addition, a control group of 54 essential thromocythemia patients with no JAK V617F mutation were also analyzed. The PCR was carried out using the MeltDoctor HRM Master Mix (Applied Biosystems, Life technologies, USA) and the primers sets published in Ugo V. et al. (PLoS ONE 2010;5(1): e8893.doi:10.1371/ journal.pone. 0008893). The ViiA7 Real Time PCR instrument (Applied Biosystems, Life technologies, USA) was used for the PCR and HRM curve analyses (in duplicate) according to manufacturer instructions. In order to test for the reproducibility of the method we have analysed 2 positive samples in 3 independent experiments. The quantification cycle (Cq) coefficient of variation for each positive DNA sample was calculated.
Results: The HRM method allowed us to identify every patient known to be mutated, whereas all control patients showed a wild type profile. The reproducibility test results were highly similar in all three experiments. The Cp coefficient of variation for the p.542_543delAsnGlu and p.543_544delGluAsp mutation was 0,84% (mean=26,99; SD=0,228) and 0,47% (mean=28,32; SD=0,132), respectively.
Conclusions: The HRM method tested is reliable and fast in detecting JAK2 exon 12 mutations and is appropriate for the routine detection in PV patients with no JAK2 V617F mutation.

W278
THE GENETIC HETEROGENEITY OF BETA GLOBIN VARIANTS IN THE SICILIAN POPULATION

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In Sicily β-thalassemia is the most common inherited blood disorder as well as in several other Mediterranean countries. It's incidence was estimated about 6% while variant hemoglobin carriers account for 2% of whom Hb S is the most represented. We present a retrospective study performed on 1330 carriers of Hb variants, selected during the last twenty years. The red cell indices were measured on an automated blood cell counter and HbA, HbA2, HbF and Hb variants, during the last 5 years, were identified and measured by HPLC on a TOSOH G7 and G8 system using β-thal mode. DNA was extracted from the buffy-coat using salting out precipitation. Allele-specific oligonucleotide hybridisation (ASO) with radioactive probes, restriction enzyme analysis of amplified product (RE), amplification refractory mutation system (ARMS), reverse dot blot (RDB) analysis were carried out for direct detection of the most common mutations present in the Sicilian population. Radioactive sequencing and successively automatic sequencing were used to screen rare or unknown mutations. Specific primers were used to identify deletional or recombinant defects. We identified 24 variants of the beta globin gene. Most of variants did not show any alteration of hematological and electrophoretic parameters but they were occasionally identified during familiar molecular analysis for prevention of thalassemia. Six were most common: Hb S, Hb Lepore-Boston-Washington, Hb C, Hb D-Los Angeles, Hb G-Copenhagen, Hb G-San José. Hb S, the most representative hemoglobin variant (72.1% of the identified variant hemoglobins). 61 subjects (4.58% of the identified variant hemoglobins) were found to be carriers of Hb C. The hybrid delta-beta globin genes of the Lepore-Boston-Washington was the third most common variant of β-globin gene (4.28% of the identified variant hemoglobins). It is the only type of Hb Lepore found in Sicily. Hb D-Los Angeles and Hb G-Copenhagen were presented respectively in 56 and 47 subjects. The presence of the numerous structural Hb variants identified in the Sicilian population can be considered a further testimony of the presence of many civilizations in the island and contacts with people from Africa, Orient and Mediterranean origin.

W279
MULTIVARIATIVE ANALYSIS AS THE NEW APPROACH TO THE SCREENING FOR B12 AND FOLATE DEFICIENCIES

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Background: The deficiency of Vitamin B12 or folate may lead to the abnormal DNA synthesis. Because this is a nuclear maturation defect, it affect all three blood cell lineages: RBC, WBC and PLT, unlike other anemias that typically involve only RBC. The study aimed to determine the performance of combination of all parameters (related to WBC, RBC and PLT) available on modern haematological instruments in detecting B12 and folate deficiency, thus providing the screening tool for those deficiencies in routine hematologic lab.
Methods: The UniCel DxH 800 Coulter Cellular Analysis System performs measurement of cellular morphometric parameters: volume, cell conductivity in radio-frequency current and 5 angles of light scatter. These measurements (Cell Population Data, @CPD) are reported for every sample. The blood specimens from 243 patients were collected from the routine laboratory samples. For all samples CBC-Diff-Retic parameters, @CPD, and all anemia-related laboratory parameters were analyzed. Our goal was to discriminate patients with anemia, normal CRP, low B12 or low folate (Low group) versus patients with anemia, normal CRP, normal B12, folate and RBC folate (Normal group), thus modelling the clinical situation when the doctor have to suspect or rule out B12/folate deficiency for the anemic patient without inflammation. The originally developed program "EMMA" was used to compute the multi-parametric models to achieve this goal.
Results: From 243 patients 136 were with normal CRP. Our approach allows developing 2 classification models to differentiate Low group (n=39) from Normal group (n=30). The first, 3 parameters signature demonstrated AUC 0.799 in ROC analysis, sensitivity 70%, specificity 83%. The PPV and NPV were of 84% and 68%, whereas the LR+ and LR- were of 4.15 and 0.37. Another 5 parameters-signature, showed AUC 0.839 in ROC analysis, sensitivity 95%, specificity 73%, PPV of 82% and NPV of 92%, whereas the LR+ and LR- were of 3.56 and 0.07.
Conclusions: This study shows the clinical relevance of multivariate analysis for the screening of B12 and folate deficiencies among anemic patients without inflammation to identify specimens that require additional laboratory procedures.
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W280

DECISION RULES WITH MORPHOLOGICAL PARAMETERS ON DXH800 COULTER CELLULAR ANALYSIS SYSTEM HELP TO IMPROVE THE SCREENING FOR B12 AND FOLATE DEFICIENCIES

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Background: Vitamin B12 and folic acid are necessary for nucleic acid synthesis. The deficiency of either causes megaloblastic anemia and production of large cells with nuclear-cytoplasm asynchrony. All three blood cell lineage are affected: RBC, WBC and PLT. The study aimed to develop decision rules on hematology instrument to enable efficient screening for B12/folate deficiencies in routine lab.
Methods: The UniCel DxH 800 Coulter Cellular Analysis System performs measurement of leucocyte morphometric parameters: volume, conductivity in radio-frequency current and light scatter. These measurements (Cell Population Data, @CPD) are reported for every sample and may be included in the laboratory-defined decision rules. The blood specimens from 243 patients were collected from the hospital routine. CBC-Diff-Retic parameters, @CPD, and all anemia-related laboratory parameters were analyzed. Our goal was to classify patients with anemia and normal CRP in 2 groups: patients with low B12 or low folate (Low group) and patients with normal B12, folate and RBC folate (Normal group), thus modeling the situation when the doctor might suspect or rule out B12/folate deficiency for the anemic patient without inflammation.
Results: From 136 patients with normal CRP 69 patients with anemia were analyzed. All parameters, available on DxH800 were used for the development of classification trees which can be implemented as decision rules in the instrument. We developed 2 classification models to differentiate Low group (n=39) from Normal group (n=30). The first, based on @CPD, related to Monocyte and Neutrophil volume and @PDW, demonstrated sensitivity 85%, specificity 80%, efficiency 82%. The second model was supplemented with the @CPD, related to RBC volume in reticulocyte channel, which allowed achieving 100% sensitivity, 63% specificity, 84% efficiency. MCV was not significantly different in 2 groups with mean MCV 93.7fl and 90.1fl in Low and Normal group respectively.
Conclusions: This study shows the clinical relevance of multivariate analysis for the screening of B12 and folate deficiencies using decision rules inside the hematology analyzer to identify specimens that require additional laboratory procedures.
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W281

POIKILOCYTOSIS INDICES CALCULATED FROM RED BLOOD CELL PARAMETERS OF HEMATOLOGY ANALYZER FOR POIKILOCYTOSIS PREDICTION

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Background: A blood cell analyzer has been widely used in hematology laboratory. Red blood cell (RBC) parameters obtained from the analyzer include hemoglobin, hematocrit, RBC indices, RBC distribution width (RDW) are generally used for interpretation of anisocytosis and hypochromic RBC whereas poikilocytosis is still doubtful. Poikilocytosis indices (PI), PI1 (RDW/MCH) and PI2 (MCV×MCH/RDW) was proposed in this study for poikilocytosis prediction.
Methods: A total of 128 blood samples were included in this study. Based on the morphology examined from blood smear using microscopy, the blood samples were divided into 24 blood samples with normal RBC morphology and 104 poikilocytosis (few, 1+, 2+ and≥3+). RBC parameters of all samples were obtained from Coulter LH 500 blood analyzer and poikilocytosis indices; PI1 and PI2 were analyzed using IBM SPSS statistic viewer.
Results: PI1 [mean (95% confidence interval; CI)] of blood samples with normal RBC, poikilocytosis few, 1+, 2+, and ≥3+ were 0.53 (0.49, 0.56), 0.72 (0.63, 0.80), 0.85 (0.74, 0.96), 0.93 (0.82, 1.05) and 0.99 (0.89, 1.10), respectively. The PI1 of blood samples with those with poikilocytosis (1+, 2+ and ≥3+) was significantly different from normal RBC ($P < 0.001$). Accordingly, mean (95% CI) of PI2 were 164.7 (149.0, 180.3), 121.5 (104.1, 138.9), 97.0 (80.7, 113.3), 83.0 (68.7, 97.3) and 74.3 (63.3, 85.2). The significant differences of PI2 between blood samples with normal RBC and poikilocytosis (few, 1+, 2+ and ≥3+) were found ($P < 0.001$). Sensitivity and specificity of PI1 and PI2 were 82.7 vs. 91.7% and 91.7 vs. 75% when cutoff at 0.6 and 116.5 were applied, respectively.
Conclusions: This study shows that both PI1 and PI2 can be used for prediction of poikilocytosis on blood smear. This may indicate that RBC parameters from a blood cell analyzer are useful for making a decision on review slide by microscopy.

W282

BIOLOGICAL PROGNOSTIC FACTORS IN DIFFUSE LARGE B-CELL LYMPHOMA: PRELIMINARY DATA FROM THE MULTICENTRE RANDOMIZED PHASE III CLINICAL TRIAL COMPARING R-HDS VERSUS R-CHOP AS FIRST LINE THERAPY IN HIGH RISK PATIENTS

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Background: Diffuse large B-cell lymphoma (DLBCL) is the most frequent histological subtype of non-Hodgkin lymphoma in western countries. Patients with DLBCL have highly variable clinical outcomes reflecting a heterogeneous group of tumours. The identification of patient subgroups who unlikely will benefit from current therapies is necessary for the development of new therapeutic schedules. Moreover, literature data about the role of biological markers in DLBCL are extremely controversial and debated.
Methods: This study is performed in the frame of a large multicenter phase III randomized clinical trial (R-CHOP versus R-HDS). We analyzed the response to therapy in 129 patients in terms of overall survival (OS) and progression free survival (PFS). Moreover we analyzed the role of the most important biological markers in DLBCL. Bcl2 protein expression and GCB and non-GCB sub classification (Hans'Algorithm) are performed by immunohistochemistry. Analysis of TP53 gene mutation is performed by DNA. Statistical analysis is performed to identify differences between groups (Mann-Withney test and log-rank test) and to estimate OS and PFS (Kaplan-Meier method).
Results: First we evaluated the efficacy of the experimental therapy R-HDS, which resulted effective in the high risk patients population. Subsequently we analyzed the importance of the GCB and non-GCB sub classification. We found a trend of longer OS and PFS in patients belonging to the GCB subgroup treated with R-HDS. Than we analyzed the Bcl2 protein expression pattern and we found that Bcl2 negative patients responded better to therapy compared to the Bcl2 positive patients in the R-HDS treatment group. Finally, we investigated the role of TP53 mutation in our clinical setting. TP53 mutations are detected in 15.4% of DLBCL and we identified a tendency of TP53 mutation to decrease PFS of DLBCL patients.
Conclusions: Despite the intensity of the adopted therapies, some of the analyzed parameters maintained their prognostic role in the identification of high risk patients. The study is still ongoing. The completion of the clinical trial, the analysis of all clinical data, the evaluation of all investigated biological markers will clarify and validate these preliminary results.

W283

EVALUATION OF FLOW CYTOMETRIC CROSSMATCH AND COMPLEMENT-DEPENDENT LYMPHOCYTOTOXICITY CROSSMACH TECHNIQUES FOR DETECTION OF ANTI-HLA ANTIBODIES IN RENAL TRANSPLANT PATIENTS

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New technologies for detection of anti-HLA alloantibodies continue to emerge, so it is necessary to evaluate them and to compare their clinical utility for selection of matching donor-recipient pairs. The goal of this study was to analyze the value of flow cytometric crossmatch (FCXM) and complement-dependent lymphocytotoxicity panel reactive antibody screening (PRA) techniques for identification alloimmunized primary transplant recipients compared to complement-dependent lymphocytotoxicity crossmach (LCXM) technique.
Materials and methods: We studied 197 kidney recipients during the period of January 21, 2009 and November 13, 2011. Lymphocytotoxic antibodies in the patient's sera were screened by PRA (with the panel of 40 cells). Crossmatch testing was performed by FCXM with BD FACScant flow cytometry and LCXM techniques. Statistical package IBM SPSS Statistics Version 20 was used for the data analysis. $\chi 2$ and Fisher's exact test were used for comparison of categorical variables, differences between groups were considered significant if $P < 0.05$.
Results: There was observed agreement between FCXM and LCXM results in 180/197 (91.4%) cases: 169 (85.8%) cases with FCXM-negative and LCXM-negative results; 11 (5.6%) cases with FCXM-positive and LCXM-positive results ($P < 0.001$). Discrepant results were found in 17/197 (8.6%): 4 (2.0%) cases with FCXM-negative and LCXM-positive and 13 (6.6%) cases with FCXM-positive and LCXM-negative results ($P < 0.001$). Analysis of crossmatching results according patients PRA values showed that the FCXM-positive and LCXM-negative cases in non-sensitized patients (PRA≤10%) group were found more frequently than in sensitized patients groups (66.7% and 18.2%, respectively; P=0.016).
Conclusions: Flow cytometric crossmatch technique is more sensitive especially in patient with low PRA group than complement-dependent lymphocytotoxicity crossmatch technique at detecting anti-HLA antibodies. The clinical significance of anti-HLA antibodies detected by FCXM in primary renal transplant patients is unclear.

W284

SERUM FREE LIGHT CHAIN MEASUREMENTS BY FREELITE™ (THE BINDING SITE) AND N LATEX FLC (SIEMENS) METHODS MAY DIFFER IN CLINICAL USE : ABOUT THREE CASE REPORTS

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Background: Serum free light chains (sFLC) as measured by the Freelite™ immunoassay (The Binding Site, UK) have demonstrated high clinical utility in diagnosis, monitoring and prognosis of B-cell dyscrasias. Recently an alternative immunoassay has become available (N Latex FLC, Siemens, Germany). Here we evaluate the performance of this assay, in order to check if the two methods were coherent and whether the results of each technique were interchangeable.
Methods: We conducted a comparison study of 40 samples addressed for biological investigation by a nephrologist or an hematologist in a context of diagnosis or follow up of monoclonal gammapathy. Clinical value of sFLC and K/L ratio were compared using Freelite™ (The Binding Site ; reference range 0.26-1.65) and N Latex FLC (Siemens ; reference range 0.31-1.56). sFLC measurements were performed on a BN ProSpec analyzer (Siemens).
Results: 37/40 samples gave similar ratios between the two assays, however in 3 samples the N Latex FLC assay indicated no clonality when Freelite™ ratios were abnormal. 2/3 patients had Light Chain Multiple Myeloma (LCMM). 1 lambda LCMM patient had osteolytic bone lesions at presentation, Freelite™ FLC ratio was 0.02, in contrast N Latex ratio was normal (0.8). In a second kappa LCMM patient with symptomatic disease (hypogammaglobulinemia), the Freelite™ ratio identified clonal disease (ratio 1.89), whereas the N Latex assay reported a normal ratio (ratio 0.8). Finally, in an intact immunoglobulin Multiple Myeloma patient with symptomatic disease (hypogammaglobulinemia) the two assays were also discordant.
Conclusions: Our results showed discrepancies between Freelite™ (The Binding Site) and N Latex FLC (Siemens) methods, concluding that the two measurements are not interchangeable. On the light of these observations, Freelite™ appears to be in this study the most relevant test regarding to the evaluation of the clinical context.

W285

DETECTION OF KINETICS OF LEUKEMIA BLAST ELIMINATION DURING THE INDUCTION TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA USING MULTICOLOR FLOW CYTOMETRY

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Background: Acute lymphoblastic leukemia is the most common childhood cancer. In order to apply personalized treatment protocols is important to determine the exact phenotype of blasts at diagnosis and to precisely evaluate of minimal residual disease during induction treatment. The study purpose was to compare changes in the level of residual disease at day 15 and 33 of treatment in acute lymphoblastic leukemia of B-cell precursor cells (BCP-ALL) and T-lineage acute lymphoblastic leukemia (T-ALL). We also compared the amount of MRD in patients with BCP-ALL and T-ALL.
Methods: We studied 60 consecutive patients with ALL (30 patients with BCP-ALL and 30 patients with T-ALL), treated at the centers of the Polish Paediatric Group for Leukaemia and Lymphoma. At diagnosis precise leukemia- associated immunophenotype was determined for each patient. Marrow samples were analyzed using a flow cytometer BD FACS CANTO II (8-color) at diagnosis of leukemia, at day 15 and 33 of treatment. We next assessed a percentage of leukemic cells in the bone marrow. The results have been developed using modern Infinicyt software.
Results: At the diagnosis of acute leukemia the median number of blast cells in the bone marrow of patients with BCP-ALL was 80%, at the day 15 of treatment the median MRD was 0.57%, and day 33 of treatment the median value was close to zero. In patients with T-ALL at diagnosis the median number of blast cells was 87%, at 15 day of treatment was 8.9%, and 33 day was equal to 0.31%. The median percentages of blast cells were significantly higher in T-ALL as compared to BCP-ALL both at day 15 and ($P=0.05$).
Conclusions: The level of minimal residual disease in patients with T-ALL at the day 15 and day 33 of treatment are higher than that of patients with BCP-ALL at the same time points.

W286

CLINICAL COMPARISON OF THE FREELITE AND N LATEX SERUM FREE LIGHT CHAIN ASSAYS IN THE DIAGNOSIS AND MONITORING OF AL AMYLOIDOSIS

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Background: Studies with serum free light chain (FLC) assays have demonstrated their clinical usefulness for the screening, prognosis and monitoring of plasma cell dyscrasias. We compared the quantification of FLC based on monoclonal antibodies (N Latex) to the established polyclonal antibody-based assay (Freelite) in AL amyloidosis (AL).
Methods: 62 diagnostic samples from patients with AL were analysed, 32 of which also had a post-treatment sample. Serum FLC concentration was measured by FreeliteTM (The Binding Site, UK) and N Latex (Siemens, Germany) immunoassays on a BNII nephelometer. Serum and urine immunofixation electrophoresis (IFE) were performed on Hydrasys gel systems (Sebia, France).
Results: In the diagnostic samples: for AL of kappa type (n=18), the median involved FLC (iFLC) was significantly lower by N Latex (289 vs 667 mg/L, P=0.0002) whereas in AL of lambda type (n=44) the values were similar (148 vs 161 mg/L, P=0.84). Measurable disease has recently been redefined as a difference between the involved and uninvolved FLC (dFLC) of >50 mg/L. By these criteria, 82% AL would be measurable by N Latex compared to 89% by Freelite. The combination of serum and urine IFE with either FLC assay, however, allowed identification of the amyloidogenic clone in 98% producing comparable sensitivity for both methods. For the 32 patients with monitoring samples the median reduction in dFLC was 40% for N Latex and 61% for Freelite (P=0.03). This led to some differences in assigning response categories. In patients with measurable disease (dFLC >50 mg/L), a partial remission (dFLC reduction >50%) predicted overall survival (OS) by N Latex (n=25, 2year OS 82% vs 27%, P=0.0015) and Freelite (n=29, 2year OS 75% vs 36%, P=0.02).
Conclusions: There are significant differences between iFLC as measured by N Latex and Freelite assays, but overall the two assays have similar diagnostic sensitivity when used in combination with serum and urine IFE. In the monitoring context, preliminary data suggests that disease response as assessed by the N Latex FLC assay predicts overall survival. Because of the likely widespread introduction of this assay and the differences in absolute levels of iFLC measured by these assays, consensus criteria may need to be reconsidered.

W287

SERUM AND DIALYSATE MEASUREMENT OF IMMUNOGLOBULIN FREE-LIGHT CHAIN TO EVALUATE THE EFFECTIVENESS OF THEIR REMOVAL BY HIGH CUT-OFF HEMODIALYSIS IN MYELOMA KIDNEY PATIENTS

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Background: To determine the utility of immunoglobulin free light chain (FLC) measurements in serum and dialysate in monitoring FLC reduction in myeloma kidney patients undergoing high cutoff hemodialysis (HCOHD) as an adjuvant to chemotherapy.
Methods: From July 2010 to June 2012, 3 patients with acute kidney injury (cast nephropathy confirmed by renal biopsy in 2) complicating multiple myeloma (MM) (age 61-77; 2M/1F; 2 $\lambda/1\kappa$), were treated with HCOHD (Theralite 2100 Gambro; HD sessions 6-12; HD duration 6 h). Patients were treated with a chemotherapy regime consisting of Bortezomib/ Dexamethasone /Thalidomide. During HD sessions blood and dialysate samples were collected at 5-180-360 min. Serum and dialysate FLC concentrations were measured by nephelometry on Beckman Immage800 (Freelight BindingSite, UK). A mathematical model was used to calculate the mass of involved FLC removed in each HD session.
Results: The total mass of removed FLC strongly correlated with the basal value of involved FLC (R^2 = 0.908). All patients achieved a very good hemathological response (73-98% reduction of involved FLC). Involved FLC dialysate concentration significantly correlated with serum concentration in two patients (R^2=0.88 and 0.77 respectively; 1 κ and 1 λ), while no correlation was found in the third one (R^2=0.29; λ). Neither total mass of removed FLC, nor hematological response seems to correlate with renal outcome. Dialysis independence was achieved in 2 patients, whereas renal recovery was not complete in the third one. In this patient, who did not achieve dialysis independence, despite a 73% FLC reduction, no correlation was observed between serum and dialysate FLC concentration.
Conclusions: Irreversible renal failure greatly increases the morbidity and mortality of patients with MM. FLC removal by HCOHD is an adjuvant to effective chemotherapy for these patients. Renal recovery is tightly dependent on marked reduction (>50%) of involved FLC, as well as on time to initiating HCOHD. The measurement of involved FLC in serum and dialysate, together with renal function parameters, provide information useful to monitor the efficacy of combined therapy and to establish the appropriate number of HD sessions.

W288

A LABORATORY BASED INTERVENTION TO IMPROVE APPROPRIATENESS OF MONOCLONAL GAMMOPATHIES RELATED TESTING

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Background: The order of laboratory tests related to monoclonal gammopathies (MG) (serum protein electrophoresis, SPE; serum and urine immunofixation, sIFE uIFE; immunoglobulin free light chains, FLC) is often inappropriate. Following the international guidelines and the advices of the clinicians mostly involved in this condition, an ordering work-flow was set in which first and second level tests are tightly controlled by laboratory staff.
Methods: For MG investigation, since February 2012 the laboratory repertoire lists five clinical queries (MG screening , MG diagnostic classification, MGUS follow-up, MM follow-up and Amyloidosis) rather than the single tests (SPE, sIFE, uIFE and FLC). The panel of tests included in each query has been built in collaboration with hematologists. Queries contain only some tests: on the basis of their results and according to shared algorithms, well trained laboratory staff decide if and which other tests are to be added. To make an example, MG screening panel includes only SPE and total protein: if SPE shows a monoclonal component, then sIFE is performed. If sIFE is positive, monoclonal component is quantified, FLC and not involved immunoglobulins are added. Appropriate frequency of each query order is also controlled.
Results: The efficacy of the new ordering approach was evaluated comparing the number of tests performed from March to June 2012 with the same period in 2011. The number of SPE was overall decreased by 44% (inpatients 60%) in 2012 vs 2011 (16534 vs 29543), as well as the number of uIFE (overall decrease 32%; inpatients 75%). On the other hand the number of FLC was almost doubled in 2012 and sIFE was increased by 20%. As far as the ordered query, MG screening accounted for about 95%.
Conclusions: Our primary target was to improve the patient care without wasting human and economic resources. The high level of inappropriateness of the SPE orders was demonstrated by the large observed fall. On the other side, as expected, the number of second level tests showed an overall increase of 14%, demonstrating that MG patients were not properly investigated. Our approach underlines as profitable results may be obtained by the cooperation between clinicians and laboratory professionals.

W289

A NEW HEMOGLOBIN VARIANT DETECTED IN THE MONITORING OF DIABETES THROUGH HBA1C

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Background: Control of Diabetes Mellitus is performed by measuring HbA1c. The HPLC system used for this purpose also perform a screening of hemoglobin variants. Some structural variants of hemoglobin produced by alterations in genes encoding the globin chains are known to cause analytical interference in the measurement of glycated hemoglobin by ion exchange chromatography, and can be mistaken with other variants already known. In this study we characterized a new hemoglobin variant in an 83 year-old Spanish male patient.
Methods: We performed glycosilated hemoglobin analysis by cation exchange HPLC in an automated glycohemoglobin analyser (Tosoh G8). We characterized the new hemoglobin by cation-exchange HPLC Variant II (Bio-Rad), electrophoresis capillary (MiniCap Hemoglobin Sebia) and the study of chains globin by reverse phase HPLC. The molecular study required automatic extraction of the genomic DNA. The most frequent mutations were discarded using an alpha-globin StripAssay and molecular characterization was undertaken using automatic sequencing in an ABI PRISM™ 3100 Genetic Analyzer Sequencer (Applied Biosystems). The alpha-1 gene was specifically amplified; the product of the amplification was sequenced with the commercial ABI PRISM™ BigDye® Kit V1.1 Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems)
Results: A new structural variant of Hemoglobin (Hb Burgos) clinically silent was detected by cation exchange HPLC at measurement of HbA1c with a 1,02 retention time. Abnormal hemoglobin was observed both on electrophoresis capillary (Hb A; Hb X and Hb A2) and by cation-exchange HPLC Variant II (Bio-Rad) a late anomalous Hb (17%) eluted and the study of globin chains by RP-HPLC showed only two peaks (β and α). Selective sequencing of the alpha1 gene showed a GAC>AAC mutation at codon 64 of exon 2. This alters the normally encoded aspartic acid to asparagine, which was identified as Hb Burgos [α164(E13)Asp>Asn; HBA1: c.193G>A]. Alpha-Thalassemia was ruled out.
Conclusion: Structural variants of hemoglobin can be detected during the measurement of HbA1c, and values of glycated hemoglobin may be altered. These cases, though rare, require us, to thoroughly examine the chromatograms, to detect possible interference.

W290
HAPLOIDENTICAL T-ALPHA/BETA AND CD19-DEPLETED HEMATOPOIETIC STEM CELL TRANSPLANTATION IN SCID

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Background: severe combined immunodeficiency (SCID) is a primary immunodeficiency (PID) with incidence <1:100.000 newborns. Neonatal screenings are not implemented in our country. It's characterized by early onset, failure to thrive, diarrhea, infections. Treatment of choice is urgent hematopoietic stem cell transplantation (HSCT). We report the case of a baby referred for P.jiroveci bronchopneumonia.
Methods: 4-month-old female baby, weight 6.3 kg, height 60 cm (both 50° percentile), past medical history: two perforated otitis, oral thrush, bronchopneumonia. Admitted in ICU for severe respiratory failure, P.jiroveci pneumonia, Rotavirus gastroenteritis, requiring assisted ventilation and specific antimicrobic treatment. CBC showed leukopenia with lymphocytopenia (400 lymphocytes/mm^3). Immunophenotype was assessed in the suspect of IDP.
Results: immunophenotype: 8% CD3+CD4+CD8-CD45RA +CD45RO - cells (absence of memory T cells rules out maternal chimerism), 91% CD16/56+, 0% CD19 and CD8; negative CD25, CD38, HLA-DR. Molecular analysis identified RAG1 mutation, supporting SCID T-B-NK+ diagnosis. We performed haploidentical Tα/β-CD19-depleted HSCT from father, after conditioning with Treosulfan, Fludarabine, rabbit antilymphocyte serum, with no other immunosuppressant. PMN and platelets engraftment at day +17 and +15 respectively led to progressive clinical and radiologic improvement, extubation on day +14. 100 days after HSCT CBC is stable at 700 lymphocytes/mm^3; immunophenotype shows 2% CD3 (1% CD4, 1% CD8), 82% CD19, 13% CD16/56. Engraftment is confirmed by 100% donor chimerism, XY chromosome detected by FISH. The baby is well at home, without infections. 9 months after HSCT CBC is stable at 1840 lymphocytes/mm^3; immunophenotype shows 79% CD3 (57% CD4, 15% CD8), 13% CD19, 7% CD16/56. Also the relative distribution of naïve and memory T lymphocytes, the activation pattern, and the B lymphocytes subpopoulations are normal. Engraftment is confirmed by the presence of 54% donor cells in peripheral blood. The baby is well at home, without infections.
Conclusions: Haploidentical Tα/β-CD19-depleted HSCT can be useful in SCID cases with severe active infections.

W291
SCREENING AND PREVENTION OF HIGH FERRITIN LEVELS IN THE MEDICAL LABORATORY ON A ROUTINELY BASIS

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Chronic high ferritin levels are related to organ damage with increased risk of morbidity and mortality [1]. However screening and monitoring is not performed routinely. The aim of the project was to find a structural way to measure high ferritin levels in patients: 1) to screen ferritin levels of polytransfusees, 2) to reduce the total number of ferritin levels above 1000 μg/L, 3) to detect possible hemochromatosis patients, 4) to optimize registration to Dutch authorities (TRIP) [2].
Methods: 1) Every month, a report is generated showing the number of packed cells patients received last year together with ferritin levels. Patients receiving multiple transfusions (>10 packed cells) are checked whether they should be marked as transfusion dependent. If ferritin measurement is missing a code is added which automatically triggers a ferritin measurement at patient's next laboratory visit. 2) Each ferritin level above 1000 μg/L generates a laboratory interpretation. 3) The laboratory specialist runs a query manually showing all increased ferritin levels above reference value in order to screen for hemochromatosis.
Results: 1) The number of polytransfusees with missing ferritin levels is reduced to almost zero; all polytransfusees are registrated, have a recent ferritin level, and treatment is considered in order to normalise ferritin levels. New candidates are registrated monthly as polytransfusee. 2) We expect irregular antibody formation to be reduced compared to last year. 3) The screening is expected to reduce the number of packed cells or iron supplementation or to consider iron chelation. 4) Transferrin saturation is added as reflex test to patients showing elevated ferritin levels in order to determine possible hemochromatosis confirmed by gen mutation research.
Conclusions: Screening for iron overload (high ferritin levels) is routinely integrated in our laboratory and where possible automated. Ferritin screening increases the quality of the laboratory service to doctors. Patients may benefit, when appropriate actions with respect to the reported laboratory interpretations are performed by medical specialists.
References: Shander A et al. Vox Sanguines 97(2009)185-197. CBO Blood Transfusion Guideline 2011.

W292
CAPILLARY ELECTROPHORESIS FOR HBA2 ASSAY

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Background: Capillarys HbA1c kit used on Capillary Electrophoresis analyzer Capillarys 2 (Sebia) is designed for separation and quantification of the HbA1c while detecting Hb variants and HbA2; 8 capillaries in parallel allow a throughput of 40 samples / hour. The reliability of the system for HbA2 measurement was assessed and compared to Hemoglobin mode of the same analyzer, which allows 32 samples/hour throughput.
Methods: Precision within run: 2 samples (low and high levels) were run simultaneously on the 8 capillaries. Between run: 2 samples were analyzed in 3 different series. Interferences: increasing amounts of bilirrubin and triglycerides were added to study the influence. Effect of Hb concentration: a sample was centrifuged for 10 min at 300 rpm. The red cells and plasma obtained were mixed in proportions. Effect of dilution: total blood was diluted in hemolysing solution in proportions (100-20%). Linearity and method comparison studies were performed according to CLSI guidelines (EP6, EP9).
Results: CV were lower than 2.8% : precision within run Mean 2.2% CV 1.9% Mean 4.6% CV 1.0% and precision between run Mean 2.5% CV 2.7 % Mean 5.3% CV 1.9%. Effect of dilution, anemia and interfering endogenous compounds were lower than 0.25% (f Bilirubin 82 µmol/L and Triglycerides 18 mmol <0.1% (2.5%). Effect of Hb concentration (195 – 39 g/L) <0.1% (3.6%) and dilution influence <0.2% (4.0%). Linearity R=0.995 y=0.997x-0.08 (95% CI slope 0.904-1.09; intercept -0.038-0.23), in the range of concentrations 2.4-4.1%.
Method: Comparison HbA1c kit vs Hemoglobin mode (60 normal individuals and 64 beta thalassemia carriers, range 1.4-6.3%) R =0.9766, y =0.9845 x +0.35; 95% CI Intercept -0.68-0.83, Slope 0.75-1.11. The results obtained by the HbA1c kit were systematically lower than those obtained on the Hb program, average bias 0.29%.
Conclusions: the system provides a rapid and reliable separation of HbA2 produce satisfactory results. The measurement is reproducible, which is needed because of the slight difference between normal and pathological values. Nevertheless these samples with pathological results must be analyzed using Hb mode of operation.

W293
STAINABLE BONE MARROW IRON AND MARKERS OF HYPOCHROMIA IN THE DIAGNOSIS OF IRON DEFICIENCY

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Background: The aim was to evaluate the correlation between erythrocyte and reticulocyte parameters and marrow stainable iron to assess the diagnostic efficiency and optimal cut offs to detect the absence of stainable iron in bone marrow, using Perls test as gold standard.
Methods: 66 consecutive anemic patients were evaluated by bone marrow biopsy. Hemograms were obtained with an Advia 120 (Siemens) analyzer. The bone marrow aspirates stained with Prussian blue (Perls test). On basis of the iron content the marrow samples were classified to a semi-quantitative scale from absence to abundant stores. Correlation between parameters was evaluated with the Spearman method and their reliability to assess iron deficiency was evaluated using ROC analysis.
Results: Bone marrow diagnoses were 20 benign iron deficiency and 46 malignancies including patients with acute leukemia chronic myeloproliferative or lymphoproliferative syndrome. Correlation between hematological parameters and iron stores were in all cases less than R=0.5. Parameters with best performance to diagnose lack of iron stores in the marrow were reticulocyte hemoglobin content (CHr), hypochromic erythrocytes (%Hypo) and mean cell hemoglobin (MCH), area under the curve 0.78,0.77, 0.76 respectively. CHr cut-off 28.9 pg, sensitivity 65.5%, specificity 79.9%, positive predictive value (PPV) 76.4%, negative predictive value (NPV) 69.0%. %Hypo cut-off 6.6%, sensitivity 73.1%, specificity 70.5%, PPV 70.8%, NPV 72.9%. MCH cut-off 28.7 pg, sensitivity 69.2%, specificity 76.3%, PPV 74.2%, NPV 71.3%.
Conclusions: Patients with malignancies had functional iron deficiency with abundant iron stores but iron restricted erythropoiesis. Markers of hypochromia and stained bone marrow iron represent complementary aspects of iron metabolism; the former evaluate iron utilization for hemoglobin synthesis represent the erythropoiesis balance, while the latter is a static measure of the presence of iron.

W294
DETECTION OF LATENT IRON DEFICIENCY IN NON-ANEMIC YOUNG WOMEN

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Background: Iron deficiency is the most common cause of anemia in fertile women but a hemoglobin (Hb) level within the reference interval does not exclude Iron deficiency. Deficiency occurs in progressive stages, initially with a negative balance between iron stores and the requirement for erythropoiesis, leading to a progressive depletion of stores and eventually to anemia. The aim was to assess the reliability of Low Hb Density (LHD%), derived from mean cell Hb concentration (MCHC) and reported by LH 780 analyzer (Beckman-Coulter) in the detection of latent iron deficiency (LID), defined as iron depletion without anemia.
Methods: Two hundred and fifty non- anemic women in fertile age (18-40 years), whose analyses had been requested by general practitioners, were included in the study. One hundred and fifty three had ferritin within reference range and Hb >120 g/L; 97 had LID (depletion of iron reserves without anemia) defined by Serum ferritin <20 μg/L and Hb >120 g/L. The diagnostic performance in the discrimination healthy females and those with LID was assessed with Receiver operating characteristic (ROC) curve analysis; soluble transferrin receptor (sTfR) was the gold standard, cut off >2.2 mg/L. The clinical concordance of the different parameters was calculated with Cohen's Kappa Index.
Results: Hb AUC 0.591, cut off 122 g/L Sensitivity 100% Specificity 22.5%; ferritin AUC 0.812, cut off 22 μg/L Sensitivity 75% Specificity 95.5%; LHD% AUC 0.856, cut off 5.1% Sensitivity 84.6% Specificity 81.6%; ferritin AUC /LHD% AUC P=0.112. Applying those cut offs the agreement between ferritin and LHD% was kappa 0.61.
Conclusions: The diagnosis of mild forms of iron deficiency and the early stage of depletion of iron stores presents a great challenge. LDH% emerges as a reliable test for the investigation of LID and could improve the ability to detect iron deficiency before anemia is present, has the advantage can be calculated with no additional cost.

W295
CELL-FREE DNA IN NEUTROPENIC FEVER

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Background: Patients receiving intensive chemotherapy for hematological malignancy often suffer periods of neutropenic fever which may be complicated by development of severe sepsis, septic shock or even death. Early markers are needed to predict the course of neutropenic fever. Elevated cell-free DNA (cfDNA) has been proposed as a novel prognostic biomarker in various diseases including cancer, trauma, stroke, acute myocardial infarct and sepsis. Circulating DNA consists of genomic and mitochondrial DNA, is mostly double-stranded and circulates as fragments in nucleoprotein complexes or in healthy individuals, where present in lower levels, is mainly absorbed to the surface of blood cells. CfDNA can be quantitated by quantitative real-time PCR (qRT-PCR) or with immunoassays.
Methods: We studied 100 patients with neutropenic fever after intensive chemotherapy for hematological malignancy. 32 patients had AML and 68 had received autologous stem cell transplantation (ASCT) for some other hematological malignancy. The patients were treated at the hematological ward at university hospital. Plasma samples were collected right at the beginning of neutropenic fever (day 0) and the following mornings (day 1-day 3). CfDNA was measured with qRT-PCR for beta-globin gene.
Results: Of the 100 patients, 21 patients developed bacteremia, septic shock or needed ICU care (defined as complicated course of neutropenic fever). There was no significant difference in cfDNA levels between complicated and noncomplicated patients on any day. However, on day 0 cfDNA had a tendency to be higher among those who died during the hospital period (n=3) compared to others (P=0.054). CfDNA was lower in AML patients compared to other patients on each day studied but did not predict the course of the disease among them. cfDNA correlated with leukocyte count among AML patients. CfDNA/leukocyte relation on day 0 predicted complicated course of neutropenic fever (P=0.019) among AML but not among ASCT patients.
Conclusions: cfDNA did not predict the development of complications in patients with neutropenic fever. This might be due to neutropenia as neutrophil extracellular traps released by neutrophils are suggested as a source of circulating DNA.

W296

MOLECULAR-CYTOGENETIC ABERRATIONS IN ADULT ACUTE MYELOID LEUKEMIA AS A PREDICTOR OF RESPONSE TO INDUCTION CHEMOTHERAPY

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Backgraund: Acquired genetic alterations such as balanced and unbalanced chromosome aberrations and changes in gene expression are widely recognized as one of the most important prognostic determinants in acute myeloid leukaemia (AML). The aim of the study was to estimate the role of molecular-cytogenetic aberration to predict a response to first line induction chemotherapy.
Methods: We studied 71 adult patients with newly diagnosed AML. First-line therapy consisted of standard-dose cytarabine combined with an anthracycline ±6-mercaptopurine or etoposide. We evaluated complete remission (CR) rate, time to achievement of CR, therapy resistant disease (RD) and early death in AML patients according to cytogenetic, molecular and general laboratory data.
Results: Genetic anomalies were found in 43 (61%) AML patients and a normal karyotype in the remaining 28 (39%) by means of conventional cytogenetics and FISH analysis. Among the 71 treated patients, 29 (40.8%) achieved complete remission (CR) with induction chemotherapy (in a mean period of 2.3 months from therapy initiation). We established higher CR rate (50%) and lower therapy resistance (50%) in hyperdiploidy as compared to hypodiploidy patients. The t(8;21)/AML1/ETO, inv(16)/CBFbeta-MYH11 and t(15;17)/PML-RARA, confer favorable clinical outcome demonstrated the highest frequency of complete remissions (CR rates from 80% to 100%, P=0.03). Patients with t (9;22)/bcr-abl or complex cytogenetic changes, including -5/del (5q), 3q or 7q abnormalities, were therapy-resistant or died within the first three months after AML diagnosis. Low CR rate (33.3%, P=0.01) and high frequency of RD (66.7%) were established in the cases with (+8) as a single aberration. In one case with PML-RARA(+) karyotype, trisomy 8 probably had no influence, since lasting CR was achieved quickly.
Conclusion: Molecular-cytogenetic findings clearly established diagnostic karyotype as one of the most important prognostic factor for the response to treatment in patients with AML. Future randomized clinical trials of adult AML can and should use cytogenetic data to stratify patients into appropriate risk groups so that they may receive the most suitable induction chemotherapy.

W297

DIAGNOSTIC VALUE OF C-REACTIVE PROTEIN AS MARKER OF INFECTION IN ACUTE MYELOID LEUKEMIA PATIENTS WITH NEUTROPENIA

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Background Estimation of C-reactive protein utility (CRP) for diagnostics of infection in patients with acute myeloid leukemia (AML) and neutropenia.
Methods 63 patients with AML were observed (aged 20–77 years, median 50 years). FAB classification variants of AML: M0 – 3, M1 – 9, M2 – 35, M4 – 10, and M5 – 6. All patients had neutropenia associated with chemotherapy (granulocytes count below 0.5x109/L). All patients at different stages of treatment had infectious complications (86 episodes have been included in the study).
Results CRP levels in groups of patients with localized infections (mucositis, abscess, pneumonia etc.) or fever of undetermined origin (FUO) had no statistical differences ($p > 0.05$), but were significant above those in patients without infectious complications ($P < 0.05$). CRP concentrations in patients with systemic inflammatory response syndrome (SIRS) and sepsis did not differ ($P > 0.05$). At the same time, CRP levels at system infections (SIRS, sepsis) was significant above, than at localized infections ($P < 0.001$). Medians of CRP levels in neutropenia patients were: without infectious – 7 mg/L (range 0–37 mg/L), with localized infections or FUO – 56 mg/L (13–104 mg/L), with system infections (SIRS, sepsis) – 168mg/L (103–399 mg/L). CRP concentrations correlated with severity of infectious complications (Spearman $R=0.880$, $P < 0.001$) and body temperature (Spearman $R=0.445$, $P < 0.05$).
Conclusions Thus, CRP is the marker of infectious process severity in AML patients with neutropenia. Increase of its level more than 104 mg/L might be a useful diagnostic tool for the early detection of a systemic infection in such patients.

W298

COMPARISON OF THE ANALYTICAL PERFORMANCE OF THE POLYCLONAL ANTIBODY BASED FREELITE AND MONOCLONAL ANTIBODY BASED N LATEX FLC ASSAYS IN THE DETECTION OF MULTIPLE MYELOMA AND AL AMYLOIDOSIS

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Background: International guidelines for serum free light chain (FLC) measurements relate to the multi-epitope, polyclonal antibody based Freelite® assay (The Binding Site, UK). More recently a monoclonal antibody based assay has been developed N Latex FLC (NLatex, Siemens, Germany). Here we compare the ability of the two assays to measure FLC in monoclonal gammopathy patients.
Methods: Comparison of Freelite and NLatex assays was performed using randomly selected sera taken through the course of treatment from 21 AL amyloidosis (6Kappa, 2Lambda, 13 with no paraprotein) and 36 multiple myeloma (MM) (10Kappa, 26Lambda) patients. Samples were measured using a BN™II nephelometer (Siemens, Germany). Absolute values and ratios were compared; assays' agreement was evaluated in keeping with CLSI guidelines (R2 ≥0.95).
Results: Both assays identified an abnormal Kappa/Lambda ratio in 5/21 AL amyloid (1Kappa, 4Lambda) and 22/36 MM (10Kappa, 12Lambda) patients. In addition Freelite identified 4Lambda AL amyloid and 4Lambda MM patients. Overall Freelite identified 61% of samples tested and N Latex 47%; all samples were confirmed by IFE.
For all samples regression analysis identified good agreement between assays for Kappa measurements (R2=0.92); however median values and ranges differed (Freelite: 307 (22-9100) mg/L; NLatex: 266(32-5910) mg/L; P=0.599). However, there was poor agreement for Lambda values (R2=0.07), which was also reflected by varying medians and ranges (Freelite: 349(2-2980) mg/L;NLatex: 81 (11-1580) mg/L; P=0.024). When disease groups were analysed independently, Lambda values showed better agreement for AL amyloid patients (R2=0.86, median Freelite: 157 (8-613) mg/L; median NLatex: 77 (12-322) mg/L; P=0.793) than for MM patients (R2=0.04, median Freelite: 401 (44-2980) mg/L; median NLatex: 81 (11-1580) mg/L; P=0.019).
Conclusion: By applying CLSI guidelines substantial agreement between Freelite and N Latex assays was not demonstrated. There was a moderate correlation between kappa assays in all patients while lambda values showed better agreement for AL amyloid than for MM patients. Further clinical validations on a larger cohort of plasma cell dyscrasia patients are required for the NLatexassay.

W299

POTENTIAL UTILITY OF HEAVY/LIGHT CHAIN RATIOS IN PATIENTS WITH SYSTEMIC AL AMYLOIDOSIS

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Background: The introduction of the Freelite® assay changed the paradigm of AL amyloidosis patient management and improved outcomes, with intact monoclonal immunoglobulin (M-Ig) measurements being of little use. However, in 5-12% of patients monoclonal free light chain (FLC) abnormalities may not be detectable and alternative methods of monitoring these patients are required. Here we compare the sensitivity of novel polyclonal heavy/light chain (HLC) assays to traditional methods for detecting M-Ig and consider both its potential in monitoring and its prognostic value in patients with normal FLC ratios.
Methods: Presentation sera from 210 AL amyloid patients (selected for normal Freelite ratios, 69/210) were retrospectively analysed with IgG, IgA and IgM HLC immunoassays. Results were compared to historic immunofixation (IFE) and serum protein electrophoresis (SPE) results and clonality identified by comparison to HLC ratio normal ranges: IgGκ/IgGl: 0.98-2.75; IgAk/IgAl: 0.80-2.04; IgMκ/IgMl: 0.96-2.30. HLC ratios outside the 90%ile range in the 210 patients were classed as extreme.
Results: 69/210 (33%) patients had normal FLC ratios, of which 21/69 (30%) had M-Ig identifiable by SPE that was quantifiable in 17/21 patients (median 4 g/L, range 1-24 g/L), 23/69 (33%) had M-Ig by IFE and 37/69 (54%) had M-Ig identifiable by an abnormal HLC ratio. Abnormal HLC ratios were observed in 21/23 (91%) IFE-positive and 20/21 (95%) SPE-positive patient samples (all quantifiable patients had abnormal HLC ratios), whilst 16/37 (43%) and 17/37 (46%) patient samples with abnormal HLC ratios were negative for IFE and SPE, respectively. In addition, 14/69 (20%) patients with extreme HLC ratios had a shorter median overall survival than patients with less extreme ratios (17.2 v 61.6 months, respectively; hazard ratio: 2.68; P=0.031). In contrast, and contrary to a previous report, IFE provided no prognostic value (P=0.329) in this cohort.
Conclusions: HLC ratios are more sensitive than IFE and SPE at detecting intact M-Ig in AL amyloidosis patients with normal FLC ratios. HLC may offer the only method of monitoring these patients and prognostic information that traditional M-Ig assays do not.

W300

ANTI-INTRINSIC FACTOR AUTO-ANTIBODIES ARE NOT A CONCERN FOR THE ROCHE ELECSYS VITAMIN B12 ASSAY IN DIAGNOSIS OF PERNICIOUS ANEMIA

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Background: Low Vitamin B12 serum levels in Pernicious Anemia are often caused by anti-intrinsic factor auto-antibodies which inhibit the uptake of Vitamin B12 by intrinsic factor in the stomach. Two publications in 2012 assert that in-vitro Vitamin B12 competitive-binding luminescence assays (CBLAs) fail in detection of low levels of Vitamin B12 in patients with Pernicious Anemia because of anti-intrinsic factor auto-antibody interference. This interference leads to measurement of a falsely high level of Vitamin B12 and therefore to a medical misclassification of patients with Pernicious Anemia. Therefore, Roche Elecsys Vitamin B12 assay was examined to show that assay specific pretreatment reagent is efficient in in-vitro denaturation and inactivation of potential interferents like anti-intrinsic factor auto-antibodies what leads to measurement of true B12 levels and therefore to a correct medical classification of patients.
Methods: Anti-intrinsic factor antibody was added to native serum samples in augmenting amounts. Afterwards, serum samples were measured with the Roche Elecsys Vitamin B12 assay - “with pretreatment” and “without pretreatment” on Elecsys cobas e601. To sustain Vitamin B12 pipetting scheme, pretreatment was replaced by H2O in measurements “without pretreatment”.
Results: Without pretreatment, an interference caused by addition of anti-intrinsic factor antibody is obvious. Interference is increasing with augmenting concentration of antibody. With pretreatment, interference by anti-intrinsic factor antibody is avoided.
Conclusion: These experiments clearly show that anti-intrinsic factor auto-antibodies are not a concern for the Elecsys Vitamin B12 assay, due to the formulation of the pretreatment reagent, ensuring complete in-vitro denaturation and inactivation of potential interferents like anti-intrinsic factor auto-antibodies.

W301

SERUM HOMOCYSTEINE AND METHYL MALONIC ACID IN THE DIAGNOSIS OF VITAMIN B12 DEFICIENCY

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Background. Low serum vitamin B12 concentration (sVB12) do not always reflect tissue vitamin B12 deficiency. Serum homocysteine (sHCy) and methyl malonic acid (sMMA) are metabolites that accumulate in vitamin B12 deficiency and have been proposed as surrogate markers of such a condition. The aim of this work was to investigate renal function impact on sHCy and sMMA concentration and estimate diagnostic concordance between both markers in patients with low sVB12 concentration.
Methods. We included patients with low sVB12 concentration (<200 pmol/L) and normal erythrocyte folate (ErFo) concentration (> 600 pmol/L). In addition, we included a group of control patients (sVB12 concentration >200 pmol/L, ErFo >600 pmol/L, and normal haemoglobin and mediun corupuscular volume). sVB12, sHCy and ErFo concentrations were measured by commercially available immunoassays inn an automated platform (Architect, Abbot Diagnostics). sMMA was measured by gas chromatography mass spectrometry. Glomerular filtration rate (GFR) was estimated by MDRD-4 equation.
Results. We included 202 patients with low sVB12 concentration (130 with GFR >60 mL/min/1.73m^2 and 72 with GFR<60mL/min/1.73m^2) as well as 118 control patients (70 with GFR>60mL/min/1.73m^2 and 48 with GFR <60 mL/min/1.73m^2). Both, sHCy and sMMA showed higher concentration in control group patients with low GFR (95th percentile: 26.6 μmol/L versus 17.7 μmol/L for sHCy and 0.88 μmol/L versus 0.40 μmol/L for sMMA respectively; p <0.001). For low sVB12 patient’s group analysis, we used the 95th percentiles found in control group as cut-off point to identify vitamin B12 deficiency. Concordance of sHCy and sMMA concentration was 78.5% and 62.5% in patients with normal GFR and low GFR respectively. Regarding discrepancies, 26.5 % of patients with normal GFR and normal sHCy and 32.4 % of patients with low GFR and normal sHCy presented high sMMA concentration.
Conclusions. Renal function must be taken into account to define abnormal sHCy and sMMA concentration. These markers present a good concordance showing vitamin B12 deficiency. As sHCy is analyzed in an easy automated way, we recommend setting up a sequential diagnostic algorithm using sHCy as a first line marker and sMMA as a secondary one.

W302
EVALUATION OF OSTEOPATHY IN THALASSEMIA PATIENTS BY BMD AND BIOCHEMICAL INDICES

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Background: Bone disease in thalassemia and sickle cell disease includes abnormalities of bone metabolism that lead to enlargement of cranial and facial bones, osteopenia or osteoporosis and subsequent spinal deformities, scoliosis and spontaneous fractures. The aim of our study is to evaluate osteopathy in thalassemia and sickle cell disease by bone mineral densitometry (BMD) and biochemical indices.
Methods: A total of 47 patients affecting by thalassemia major and sickle cell disease were examined, 25 male and 22 female, mean age 23.67±10.34 years (range 13-55). Informed written consent has been obtained from all participants enrolled in this study. All patients had started blood transfusion therapy since early childhood and only 38 patients had been undergoing periodical transfusions . As control group, 30 healthy subjects, 10 males and 20 female, were enrolled, mean age 43.5±13.2 years (range 23-62). BMD were measured in all patients and controls by a ultrasound densitometer Sonost 3000. Fasting blood samples were taken for the measurement of biochemical panel and marker of bone turnover. PTH, total Vitamin D, Osteocalcin, Ferritin, and Betta Cross Laps were evaluated by ECLIA method.using. Elexys2010.
Results: Osteoporosis was present in 2.1% (1/47 pts), osteopenia in 17% (8/47 pts). A statistically significant correlation was found between PTH and BMD (P <0.001). The mean concentration of PTH in the control group was 64.9±25. ng/mL (range 34-100.5), vs 172±89.29 ng/mL (range 7.9-1051) in the thalassemic and sickle cell group. CTX was high in 57%,21% had high osteocalcin, 53% had low Vitamin D and 25% had high PTH. There was a positive relationship between age and osteocalcin and a significant correlation (P=0.044). In this study it was found that CTX levels were significantly higher in patients than in controls,(0.7± 0.42 ng/mL vs 0.35± 0.2 ng/mL). Mean serum ferritin level was 1189± 1059 ng/mL vs 43.7± 28.39 ng/mL in control group.
Conclusion: BMD should be evaluated regularly and bone markers should be measured for early diagnosis to prevent morbidity.

W303
LABORATORY MONITORING OF ORAL ANTICOAGULANT THERAPY IN BLOOD TRANSFUSION INSTITUTE NIŠ, SERBIA

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Introduction: Oral anticoagulant therapy (OAT) is effectively used for the prevention and treatment of venous thrombosis and thromboembolic complications. It is based on the use of oral anticoagulant drugs who prevent synthesis of coagulation factors FII, FVII, FIX and FX and anticoagulant protein C and protein S. The therapeutic response to OAT is individual and requires regular laboratory control.
Aim: To show the practice of laboratory control and management of OAT in Blood Transfusion Institute Niš (Serbia) in period 2009. - 2011.
Materials and methods: The effect of OAT for outpatients is controled by determining the prothrombin time (PT) expressed by INR (International Normalized Ratio, INR = 1) from capillary blood on the unit Trombotrack Solo (Axis Shield, Norway), while for the hospital patients INR is determined from blood samples with sodium citrate as anticoagulant (1 ml of 3.8% sodium citrate and 9 mL of blood) on the device ACL Elite Pro-IL (Instrumentation Laboratory, USA).
Results: In the three year follow-up period there was a total of 103.807 outpatients INR controls and 14.746 INR determinations for hospital patients, where there is a steady increase in the number of analyzes in 2010. and especially in 2011 (34.121 outpatients and 5.337 inpatients INR controls in 2010. and 37.071 outpatinets and 5.994 inpatinets in 2011. compared to 32.615 outpatients and 3.415 inpatients INR controls in 2009). The most common diagnosed diseases in the monitored patients were deep vein thrombosis, prevention of thrombosis in the presence of artificial valves, coronary heart disease, arrhythmia, pulmonary embolism, etc. Total of 76,30% of analyzed samples were within the therapeutic range, 14,50% of the patients had INR <2,0, while 9,20% of patients had INR values >4.5 (out of which 3.2% of the patients had serious bleeding). The side effects of OAT were apparent bruising, bleeding from the nose and gums.
Conclusion: Laboratory monitoring of OAT and good cooperation between the patient, physician and clinical transfusion are the key to the proper conduct of OAT.

W304

HIGH LEVEL OF COAGULATION FACTOR VIII AND SYSTEM OF PROTEIN C DEFICIENCY IN THE SICKLE CELL ANEMIA

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Background: Sickle cell anemia is associated to hemolysis, oxidative stress and vaso-occlusions. Our objective was to seek the existence of a thrombotic process worsening the sickle cell anemia in Algerian patients.
Methods: Our study has concerned 20 controls and 40 patients affected by sickle cell anemia, of whom 11 are homozygous S/S, 10 heterozygous S/β thalassemic and 19 with sickle trait S/A, without any vaso-occlusive crises and not receiving any treatment interfering with the assessment of thrombosis. For each patient we identified the plasma level of protein C, protein S, antithrombin and the coagulation factor VIII. A Student t-test was performed in order to assess the difference between patients (S/S, S/β thalassemic, S/A) and controls A/A.
Results: Significantly higher levels of Factor VIII and lower levels of protein C and Protein S were found in S/S and S/β thalassemic compared to controls. Whereas no significant difference were noted between our patients compared to controls for Antithrombine. Factor VIII increase was allele dependant. Increases by 137%, 56% and 30% Were respectively observed In S/S, S/β thalassemic and S/A compared to controls. Contrary to S/S and S/β thalassemia group, sickle trait was associated to a high level of protein C and antithrombin.
Conclusion: These anomalies of the coagulation translate the existence of a pro thrombotic state during the sickle cell disease which would support the occurrence of vaso-occlusions . Coagulation factor VIII, which is constantly high with the sickle cell anemia, can thus constitute a privileged therapeutic target in the treatment of the thrombotic demonstrations of this disease.

W305

PATIENTS COMPLIANCE FOR A NEW EHEALTH. A VARIETY OF DELIVERY CHANNELS FOR PATIENTS IN ANTICOAGULANT ORAL TERAPHY

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Background: Oral Anticoagulant Treatment (OAT) is very effective and safe both with old drugs (warfarin) and with new drugs: dabigatran, rivaroxaban, apixaban. Adherence/ Persistence to medicines is a major determinant of their effectiveness. No method has been established to assess compliance with the drugs. Our aim was to implement and validate multichannel transmission of laboratory data at home and/or on mobility and patient continuing education.
Methods: At ASL Bari a digital communication system-Digital Health (DH) was tested which integrates management software of the OAT. It sends the reports to the patients in multi-modes (digital TV, mobile devices, web, sms). The experiment was focused on transmission of reports OAT via digital TV, iPhone, iPad,(iOS-Apple), Nokia Lumia 800 (Window Phone) e Samsung (Android-Google). Software functionality has been implemented, for all devices, to alert patients for their own therapy through invasive techniques of communication that, given the importance of the message to be delivered, are efficient and effective such as a popup window while viewing the TV channel in "standard mode". Patients can receive the report on television (channel TGNorba24Telenorba) with access by a smart card and the use of the remote control.
Results: With DH, the time between blood collection and delivery of therapeutic report was reduced from 4 to 2 hours and 48 minutes (-30%) .Management costs were reduced by 15% These results encourage us to use the same technology to verify compliance/persistence with the new drugs that are not subject to deliberate control of the laboratory.
Conclusions: This system of delivery report may have multiple benefits on the community, including the reduction of chronic patients transport. However because there is no systematic monitoring by the laboratory for new drugs it's very important for us to identify a system to control the medicines compliance. In conclusion the DH system is appreciated both for old and new drugs for ensuring OAT: ease of visualization and interpretation of transmitted data, doctor-patient interaction, reducing the "digital divide" and operating costs.

W306
THE IMPACT OF ALCOHOL ABUSE ON SELECTED LABORATORY PARAMETERS OF COAGULATION

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Introduction: Hemostasis is a complex process, which on the one hand protects against blood loss after blood vessel damage, on the other hand allows to maintain blood flow. It is known that moderate consumption is associated with a beneficial effect on the processes of coagulation and fibrinolysis, but has not yet been fully understood effect of prolonged drinking on homeostasis.
Aim: To evaluate changes in the coagulation and fibrinolysis in men diagnosed with alcohol addiction.
Material and methods: The study included 450 men aged 19 to 60 years who were diagnosed with chronic alcoholism. Patients were divided into groups depending on the duration of the disease: I group - 100 patients with addiction from 1 to 5 years, the second group - 100 patients with addiction lasting from 6 to 10 years, the third group - 100 patients with addiction lasting from 11 to 15 years, the fourth group - 100 patients with addiction lasting from 16 to 20 years and Control Group - 50 patients. The study included a one-off estimation of plasma: PT, APTT, INR, FBG, DD concentration and AT III activity.
Results: Treating the values of the Control Group as 100% we have shown statistically significant differences between groups, which were for: PT (I-104%, II-115.08%, 132.89% III-IV-146.88%), aPTT (I-102.38% 116.28% II-, III-127.43% 134.58% IV-), INR (I-107.2%, 116.2% II-, III-134 , 5%, IV-148.7%), FPG (I-113.96% 126.16% II-, III-128.63% 149.50% IV-), DD (I-102.59 %, II-219.98% 423.60% III-IV-478.13%) and AT III (I-88.65%, II-73.71%, 55.24% III-IV-47.26%)
Conclusions: During long-term alcohol addiction we have observed progressive decompensation in the coagulation and fibrinolysis, which may lead to progressive liver failure.

W307
GALECTIN-1 RECEPTORS IN RESTING HUMAN PLATELETS

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Galectins are a family of animal lectins defined by their β-galactoside-binding specificity and a consensus sequence in their carbohydrate-recognition domain. Galectin-1 (Gal-1) is expressed as a non-covalently linked homodimer present in different tissues including human platelets (HPlts). In previous works we detected Gal-1 in HPlts and observed that co-purified with actin. In order to extend our studies, we performed preliminary studies for Gal-1 receptors isolation from HPlts membranes and cytosol homogenates. Washed platelets were obtained from fresh blood blank platelet-enriched plasma. Cytosol and membran fractions were obtained by lysis by two different methods (detergent lysis or freeze thaw procedures). The presence of several protein receptors were confirmed by SDS-PAGE and Western blot onto nitrocellulose membranes. Analysis of electrotransferred proteins was done incubating the nitrocellulose membrans with and without recombinant Gal-1 1 μM, later revealed with anti- Gal-1 antibodies (1/5000) obtained in rabbits and a second peroxidase anti- rabbit Igs (1/500). Molecular weights were calculated from a regression curve of Rf vs MW of MW protein standards. Several bands corresponding to molecules of 30, 45 and 66 kDa MW were detected. Further isolation studies by affinity chromatography on a Gal-1-linked Sepharose column confirmed the presence of three bands. Preliminary studies of peptides from those protein receptors by mass spectrometry are in course in order to proceed to their chemical identification. The binding of Gal-1 to these receptors could activate HPlts, which conclude in platelet aggregation.

W308
COAGULOMETRIC AND CROMOGENIC METHODS COMPARATION FOR ANTIXA IN ENOXAPARIN ANTICOAGULATION

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Objectives: The use low molecular wigth heparin(LMWH) doens't require monitoring,except in newborns,pregnants with high risk,renal patients and obese. In these cases the jaundice in newborns interferes with the cromogenic methods leanding to use coagulometrics methods.
Material e methods: 31 patients studie, in venous blood with 3.2% citrate Na, proportion 1/9.Centrifugate to 2000G. The samples were collected 4hrs. after the enoxaparin shot. 10 patients with profilactics doses de 40 mg each 24hrs.The rest of the patiens with deep venous thrombosis (DVT) and anticoagulant doses of 1 mg/kg w.12hrs each. Stago,Rotacrom and Staclot heparin reactifs were used for cromogenic and coagulometric determination respectively, according laboratory instructions.
Results: The difference between the coagulometric and cromogenics methods is show with a media of 0.29 y 0.48 and a median of 0.19 and 0.35 respectivaly with a P <0.0001.
Conclusions:according to the results we consider thath the anticoagulantion control has be done with an accurateknoledge of the technique used in its mesurement to avoid bleending and/or failures in the treatment due to the subvaluation of the antiXa in methods coagulometrics.

W309
TWO CASES OF IDIOPATHIC ACQUIRED HEMOPHILIA A

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Background: Acquired hemophilia A is a rare (1.4 case/million each year) bleeding diathesis caused by autoantibodies against clotting factor VIII. The incidence of acquired hemophilia A increases with age. We report two cases of acquired hemophilia A in elderly patients and their clinical characteristics.
Methods: Case 1: A 70 year old female was referred to our hospital with massive subcutaneous and conjunctive hemorrhage. Case 2: a 62 year old female was referred to our hospital with intramuscular hemorrhage and dyspnea due to a sub mucosal larynx hematoma.
Results: Both these patients presented a prolonged APTT, without correction after a mixing test, low factor VIII activity. Factor VIII inhibitor was detected and quantified in Bethesda Units (BU): 12 BU/mL for case A and 8 BU/mL for case B were observed, confirming the diagnosis of acquired hemophilia A. For both these patients, due to absence of underlying disease, a diagnose oh idiopathic acquired hemophilia A was proposed. Recombinant activated Factor seven concentrates were successfully used to control acute bleeding. Long term immunosuppressive therapy with prednisone, rituximab and methotrexate was successfully performed.
Conclusions: In patients who have developed antibodies to factor VIII, a number of options are available. In patients in critical clinical conditions or with higher titers of inhibitor, activated factor VII can be used. Recombinant activated coagulation FVII (rFVIIa) by directly activating FX on the surface of activated platelets at the site of injury can circumvent the actions of inhibitory antibodies present in patients with acquired hemophilia. Our patients responded well to immunosuppressive therapy and they will remain on tapering doses of corticosteroids with monitoring of factor VIII activity and factor VIII inhibitor levels.

W310
GENEXPERT IN DETECTION OF GENETIC TRHOMBOPHILIA RISK FACTORS: AN YEAR EXPERIENCE IN AN HIGH PREVALENCE AREA

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Background: Factor V Leiden (FVL) and prothrombin G20210A (GPro) mutations were the most common inherited genetic thrombophilia risk factors. Chioggia is characterized by a high prevalence of FVL and GPro. In this study we evaluated performances of GeneXpert analyzer in our Laboratory.
Methods: Were considered 211 consecutive patients, observed between April and August 2011 in our Laboratory. All these samples were evaluated by using the GeneXpert analyzer in comparison with Roche Light Cycler assay. Analytical performance, organization impact and cost effectiveness and were evaluated.
Results: Among the considered 211 subjects, by using both assays, were identified 51 FVL heterozygous, 3 FVL homozygous, 1 GPro homozygous, 10 GPro heterozygous, 5 combined FVL and GPro heterozygous and 141 normal subjects. In six months we observed 15 invalid samples results (7.7%). We observed 100% concordance between results obtained by using GeneXpert analyzer in comparison with results obtained by using Roche Light Cycler analyzer. In our experience, in this patients series, the ratio tests/results was 1.07.
Conclusions: GeneXpert HemosIL Factor II and Factor V assay is a fully automated assay that is able, in less than 35 minutes, simultaneously detect the presence of FVL and GPro. Test format, based upon single test cartridge, was designed to minimize waste and to permit daily analytical session. In our experience this assay was affordable and characterized by a good rate between test used and results licensed. Moreover we observed an optimal concordance with the method previously adopted in our Laboratory. Further theoretical advantages to such assay include improved standardization across varying healthcare environments, more thorough sample manipulation and reduced human error.

W311
NORMAL LEVEL RANGES FOR A NEW LATEX AGGLUTINATION IMMUNOASSAY INNOVANCE® FREE PS AG IN A PORTUGUESE POPULATION OF BLOOD DONORS

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Background: Protein S (PS) deficiency increases risk of thrombosis, but accurate and correct diagnosis is difficult, due to interfering factors. Free PS (fPS) assay measures unbound PS fraction and can be used as a surrogate marker for PS activity. We evaluated normal level ranges for a new immunoassay, INNOVANCE® Free PS Ag (Siemens, Marburg, Germany) in a portuguese population of blood donors. Patients and Methods: A study cohort of 152 healthy blood donors, 88 women, mean age 38.8 (18-65) and 63 men, mean age 38.3 (18-65) was evaluated. The blood samples were taken before donation, by venipuncture into both 3.2% and 3.8% sodium citrate tubes. Plasma was separated into aliquots within 4h of collection and stored at -70 ºC until analysis. Free Protein S was measured in duplicate in each tube, by latex agglutination using INNOVANCE® Free PS Ag kit (Siemens, Marburg, Germany), a two-component kit consisting of reagent (polystyrene particles coated with monoclonal mouse anti-Free PS antibody) and buffer vials. Free PS-induced particle agglutination is measured by turbidimetry on a BCS® XP System. Calibration has been performed with Standard Human Plasma, Control Plasma N and P (Siemens, Marburg, Germany).
Results: A histogram showed a normal distribution of fPS levels in the study cohort. The effects of 3.2% and 3.8% sodium citrate concentration on the results of fPS assays were evaluated, and no statistical difference was found. Sex was significantly correlated with fPS levels, with men showing higher mean levels than women. Subgroup analysis was performed within women; the use of hormonal contraceptive methods had a statistically significant effect, with a lowering of the fPS levels. Increasing age in men showed a non-significant trend to increasing the mean fPS levels. In women, increasing age was significantly associated with increasing fPS levels, although the significance level was lower after adjustment for use of hormonal contraceptive methods.
Conclusion: Our normal ranges results for men and women are similar to previous reported. Nevertheless, as we found significantly lower levels within women under hormonal contraceptive methods, we recommended using targeted normal ranges in this group.

W312
BENEFICIAL EFFECT OF PHOSPHATIDYLETHANOLAMINE ON PREVENTING VENOUS THROMBOEMBOLISM: A RAT TAIL THROMBOSIS MODEL EXPERIMENT

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Background: Both of the activated protein C (APC) resistance in Caucasians and APC dysfunction in Asians could be the triggering mechanism for the development of thrombosis in thrombophilic carriers. We have previously reported the effects of phospholipids on blood coagulation and APC anticoagulation reactions in vitro, and found phosphatidylethanolamine (PE) acts Factor Xa-prothrombin reaction system toward decreasing thrombosis formation. In this study, we examined the effects of PE on the blood coagulation system in vivo using an improved Bekemeier's carrageenan-induced rat tail thrombosis model.
Methods: The evening before the carageenan injection (approximately 14 h prior), 500 μL of the liposome suspension solution was injected subcutaneously into the rats' tails. The following morning, rats were anesthetized using ether, and Kappa-carageenan (1 mg/mL PBS) was injected intravenously. The progression of thrombosis formation was observed at 24 and 48 hours after injection. The degree of thrombosis formation was determined by measuring the length of discoloration in the tail.
Results: Each group received an injection containing different phospholipids: PE (+), PE (-), and PBS. A significant difference in discoloration of the tail was found between rats treated with PE (+) and those that were not. In the PE specimen, the discoloration only progressed close to the middle of the tail. This suggests that pre-injection of phosphatidylethanolamine either effectively suppresses thrombosis formation, or functions to dissolve blood clots.
Conclusion: We found that the efficacy of carageenan was maintained even after PE was injected subcutaneously 12 hours prior to the carageenan injection, and we confirmed the utility of PE. This suggests that PE is not only an effective drug to treat thrombosis, but may be used to prevent it as well. Additionally, as this was confirmed through the use of a subcutaneous injection rather than an intravenous one, we can surmise that PE permeates blood vessels.

W313
"THE EFFECTS OF COMBINED ORAL CONTRACEPTIVES ON PROTEIN C PATHWAY IN A GROUP OF ALBANIAN WOMEN"

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Background: The role of hormones in development of higher risk of thromboembolic complications is well known in application of hormones for therapeutical reasons, administered in the form of oral contraceptives or as a substitution therapy. The objective of the study was to determine the effect of oral contraceptives on the Protein C pathway in the coagulation system of a group of healthy women taking oral contraceptives.
Methods: The study included 30 women between ages 24 and 45 taking femoden (ethinyloestradiol 30 μg and gestodene 75 μg) for one to two months. The subjects had no history of thromboembolic disease. Plasma was used for measuring PT, Fibrinogen, Protein C Factor V and Factor VIII, before using the pill and after stopping it. We used coagulometry (BFT II Siemens analyzer) to measure PT, Factor V, Factor VIII and Fibrinogen. While protein C is measured with ELFA (Vidas Biomerieux). We used SPSS 20 software for the statistical analyses.
Results: Comparison of the values of these parameters between two periods (before and after treatment) showed the following results: concentrations of fibrinogen, Protein C, Factor VIII were significantly increased after treatment ($P < 0.01$) while PT was reduced ($P < 0.05$). There was no significant change in the level of Factor V ($P > 0.05$).
Conclusions: We conclude that changes in haemostatic system, Protein C pathway, after oral contraceptives use, might increase the risk for thrombotic situations. Predictive testing of haemostatic parameters is recommended in women who are supposed to use oral contraceptives.

W314
NOVEL HAEMOSTATIC BIOMARKERS IN ACUTE CARDIOEMBOLIC STROKE

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Background: We studied the usefulness of haemostatic biomarkers in assessing the pathology of thrombus formation, subtype diagnosis, prognosis in the acute phase of cerebral infarction, and differences between various haemostatic biomarkers.
Methods: Our study included 69 patients (50 men and 19 women; mean age 68±12.0 years) with acute cerebral infarction who had been hospitalized within 2 days of stroke onset. Fibrin monomer complex (FMC), thrombin-antithrombin complex (TAT), D-dimer, and fibrin/fibrinogen degradation products (FDP) were assayed as haemostatic biomarkers on days 1, 2, 3 and 7 of hospitalization. FMC, D-dimer and FDP were assayed by turbidimetric immunoassay (TIA), and TAT was measured by time-resolved fluoroimmunoassay (TR-FIA). Changes over time in FMC, TAT, D-dimer and FDP were analyzed using the paired t test. P <0.05 was considered statistically significant.
Results: In the cardioembolic (CE) stroke group, FMC levels were significantly higher on day 1 (37±69 μg/mL) compared to the non-cardioembolic (non-CE) stroke group (6.6±8.6 μg/mL) (P <0.01), and D-dimer levels were also significantly higher on 1 day (5.4±8.9 μg/mL), compared to non-CE stroke group (1.5±1.5 μg/mL) (P <0.01). Both markers were decreased on days 3 and 7 of hospitalization. FDP levels were significantly higher at all times in the CE group compared to the non-CE group (P <0.05), whereas levels of TAT was not elevated. Neither the National institute of Health Stroke Scale (NIHSS) score during hospitalization nor the modified Rankin Scale (mRS) used at discharge found any significant correlations to haemostatic biomarkers, but the NIHSS score during hospitalization was significantly higher in the CE group than in the non-CE group (P <0.05).
Conclusions: Measurements of haemostatic biomarkers such as FMC and D-dimer on the early stage of cerebral infarction are useful for distinguishing between CE and non-CE stroke.

W315
AUTOMATION OF LIGHT TRANSMISSION PLATELET AGGREGOMETRY

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Background: The investigation of platelet function disorders by light transmission aggregometry has changed little over the last 50 years. The use of manually operated instrumentation makes the process time consuming and labour intensive. In the current study we have used samples from normal healthy donors to develop the potential of using a high throughput coagulation analyser (Sysmex CS-2000i) to automate platelet aggregation.
Methods: We assessed the ability of a CS-2000i with prototype software to perform platelet aggregation and examine the effect of: varying the platelet count in platelet rich plasma (PRP) using ADP (0.5–10 uM) and collagen (0.5-10 ug/mL); dose response with ADP (0.5-10 uM), Epinephrine (0.5-10 uM), Collagen (0.5-10 ug/mL), Ristocetin (0.75-1.25mg/mL), Arachidonic Acid (0.12-1.0 mM); imprecision of response to ADP (2uM and 5 uM). All platelet agonists were from Hyphen Biomed, and an AggRAM (Helena Biosciences) aggregometer was used as the reference instrument.
Results: There were no clinically significant changes in aggregation response when the PRP platelet count was 150-480 x 10^9/L, but below this there were changes in the maximum amplitude (MA) and slope (rate). For further experiments a standardised PRP count of ~250 x 10^9/L was then used. Dose response with ADP, Epinephrine, Collagen, Ristocetin and Arachidonic Acid were comparable between CS-2000i and AggRAM. Aggregation imprecision was similar on both systems (CS-2000i: MA for ADP 2 uM cv 5%, 5 uM, cv 12% slope 2 uM cv 6%, 5 uM cv 10%. AggRAM: MA for 2 uM cv 9%, ADP 5 uM cv 6%, slope 2 uM cv 7%, 5 uM cv 3%).
Conclusions: Our preliminary studies with platelets from normal healthy donors indicated that the PRP should be adjusted with autologous PPP to a count of 200-300 x 10^9/L. Aggregation with a PRP platelet count of less than 150 x 10^9/L showed poor sensitivity. Aggregation imprecision was comparable between the CS-2000i and AggRAM, with similar aggregation dose response profile from the commonly used platelet agonists. These data are encouraging but further studies are underway using clinical samples from patients with platelet disorders and subjects receiving various anti-platelet drugs.

W316

FREQUENCY OF JOINT BLEEDING IN HEMOPHILIA PATIENTS AND RELATION WITH THE INHIBITORS TITER IN PLOVDIV REGION

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Background: Hemophilia A (HA) and Hemophilia B (HB) are congenital bleeding disorders caused by a deficiency or complete absence of coagulation factor VIII (FVIII) or factor IX (FIX), respectively. As a more frequent complication almost all severe adult hemophiliacs suffer from arthropathy in one or more joints. In children, happily the use of contemporary factor replacement therapy hopefully will improve their chances of reaching adulthood without the development of arthropathies. The main obstruction of recent therapy is generation of inhibitors to the respective factors. In our study we aimed to check if there is relationship between generation, terms of hemophilia, level of factor, factor inhibitors and joint bleeding in Plovdiv region population.
Methods: A total of 53 patients diagnosed with HA - 48 and HB - 5 respectively were observed during the period 2010 to 2012. For each of these patients, all available inpatient and medical records were obtained. Measurements of FVIII and FIX respectively were done at least two times as part of the clinical trial by chronometric method with factor deficient plasma (Siemens/Dade Actin FS). The presence of factor inhibitors was evaluated by Bethesda method.
Results: In all of observed adult patients were multiple joint bleeding and complications. Five out of 27 children were with joint bleeding and registried joint complication. In children group with high level of factor inhibitor titer we found more severe hemorrhages. In all observed children the frequency and level of factor inhibitors were higher than in adult patients.
Conclusions: Despite the therapy, patients with high-level inhibitors are at increased risk of developing devastating joint disease. This study helps to clarify the presentation of hemophilia complication and laboratory evaluation approach to be useful for clinicians. More deep and prospective studies are needed to determine the efficacy of laboratory evaluation and treatment.

W317

CLINICIANS AND LABORATORISTS COLLABORATION MAY IMPROVED ANTIPHOSPHOLIPID ANTIBODIES LABORATORY DETECTION AND REPORT IN WESTERN ITALY

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Background: Antiphospholipid antibodies (aPL) (LA, aCL and aB2GpI) face problems common to all autoantibody assays including pre-analytical, analytical and post analytical issues. Several attempts have been made to standardize aPL tests including workshops, external quality assessments and consensus guidelines. Despite these efforts, a considerable degree inter-laboratory variation still exists, mainly due to the use of assays or commercial kits not conforming to guidelines for these tests. Aim of APS Piedmont and Aosta Valley Consortium was to introduce "Consensus Regional Report", according to proposed guidelines.
Methods: 1) We sent a questionnaire to all laboratories of our two regions asking about LA, aCL and aB2GpI methods, reference values and reports forms; 2) Evaluation of upper limit reference ranges (99th percentile) of LA, aCL and aB2GpI commercial assays; 3) The analytical performances of these assays, by using the normal reference ranges obtained were calculated; 4) Coagulation group and autoimmunity group (GAL) discussed with Consortium clinicians updated guidelines and organized laboratory reference values evaluation.
Results: 1) We received 17 questionnaires (only a part of laboratories of the geographical area). 2) We calculated upper limit reference ranges with most of LA assays (SCT and DRVVT IL and PTT-LA and DRVVT Stago) and aCL and aB2GpI immunoassays (EliA Phadia, CliA Zenit RA, Orgentec, Aesku and Inova ELISAs) utilized in Piedmont and Aosta Valley. 3) To investigate the analytical performances of LA, aCL and aB2GpI reference ranges calculated we evaluated 30 healthy individuals, 20 APS patients and 15 patients with infectious diseases. 4) Coagulation and immunoassay laboratory results were discussed with the Consortium clinicians. Starting from these results a meeting with the clinicians has been scheduled in order to share guidelines and produce a "Consensus Regional Report".
Conclusions: Clinicians and laboratorists collaboration gave us the possibility to improve in Western Italy laboratory detection of aPL antibodies according to proposed guidelines.

W318
APTT REFLEX ALGORITHM WITH TWO APTT REAGENTS CAN DO THE TRICK

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Background: In the investigation of a prolonged activated partial thromboplastin time (APTT) clinicians tend to order just pieces of the puzzle without getting the complete answer, or they erroneously order tests that are unrelated to the APTT, such as factor VII. A recent study suggests that investigation of a prolonged APTT with specific clotting factor assays is unnecessary if Actin FS APTT is normal. Furthermore, an algorithm for investigation of a prolonged APTT is helpful in sorting the potential clinical outcomes. Aim of our study was to investigate whether, even in our laboratory, further investigations of a prolonged APTT with our routine reagent (STA APTT, Stago) should be undertaken only if a second APTT (Actin FS, Siemens) reagent is also prolonged, unless there is a history of haemorrhage, in which case assays are indicated irrespective of the APTT.
Methods: 80 (55 females and 25 males) consecutive patients referred to our Laboratory over a 6 month period (may-october 2012) with request for intrinsic coagulation factors and LA detection were investigated with Actin FS APTT.
Results: Only 23 out of 80 samples (28.7%) yielded an abnormal result with both, Actin FS and Stago, APTT and revealed a coagulation abnormality [4 LA, 2 Vitamin K Antagonists therapy, 3 von Willebrand Disease (vWD) type I, 4 factor XII, 4 factor XI, 3 factor VIII 2 factor IX and 1 factor X deficiencies]. 29 out of 80 (36.2%) showed abnormal Stago and normal Actin FS APTT. All of them were found to be positive to LA and gave intrinsic factors within the reference range. 27 out of 80 (33.7%) were found to be normal with both APTT (3 vWD type I, 2 mild factor XII deficient, 1 LA and 19 normal coagulation screening). 2/80 (2.5%) yielded normal Stago and abnormal Actin FS APTT (1 factor X and 1 factor XII deficient).
Conclusion: 61.5% of all intrinsic factor requested in a 6 month period gave results within the reference range. Our study shows that using the algoritm, the laboratory can provide the clinician an answer without performing unnecessary tests and from a single blood draw, avoiding delays. In conclusion the use of a second APTT reagent strongly reduce the cost and time spent performing these assays.

W319
EVALUATION OF THE CHROMOGENIC TEST FOR THE DETERMINATION OF ADAMTS-13 ACTIVITY IN HUMAN PLASMA

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Background: To evaluate the chromogenic test (TECHNOZYM® ADAMTS-13 Activity, Technoclone, Austria) for the determination of ADAMTS-13 activity in human plasma. ADAMTS-13, the thirteenth member of the ADAMTS family, is the plasma metalloprotease responsible for regulating the multimeric structure of VWF. Deficiency of ADAMTS13 (congenital or acquired) is associated with thrombotic thrombocytopenic purpura (TTP), a serious disorder usually diagnosed with clinical criteria.
Methods: Patient and normal sodium citrate plasma were used. The assay was performed according to the manufacturers' package insert protocol. The reference values (2 SD limits from the mean) were determined in the group of 47 blood donors. Four patients suspected of having TTP were also included in the study. The repeatability of the method was determined on the three samples within a single run with six replicates of each. The reproducibility of the method was determined on three samples in 3 runs with six replicates of each per run. The linearity was assessed using four samples diluted with the appropriate diluent provided in the kit (undiluted;1/2;1/4;1/8). The ratio between the observed (O) and expected (E) result was calculated and expressed as O/E%. The correlation coefficient of linear regression was determined on diluted samples.
Results: The reference interval of ADAMTS-13 activity for our population of 58,0 - 105,8 % was thus calculated. The average coefficient of variation of the ADAMTS-13 activity (%) in the repeatability test was 2,43% (mean1=72,10; SD1=1,12, CV1= 1,56%; mean2=82,02; SD2=2,64, CV2=3,22%; mean3=15,93; SD3=0,40, CV3=2,52%). The average coefficient of variation of the ADAMTS-13 activity (%) in the reproducibility test was 7,34% (mean1=68,04; SD1=5,11, CV1= 7,50%; mean2 =86,06; SD2=5,20, CV2=6,04%; mean3=16,21; SD3=1,38, CV3=8,49%). The method showed acceptable linearity, the correlation coefficient of linear regression was 0,998. As was expected, the suspected TTP patients ADAMTS-13 activity measurements showed variable results (49%, below 1%, 48% and 90%).
Conclusions: The method showed very good repeatability, reproducibility and linearity. It could be clinically useful in diagnosing TTP together with other ADAMTS-13 assays usually performed for this purpose.

W320

CROMOGENIC METHODS MODIFICATION FOR DETERMINATION ANTI XA IN ICTERICS PATIENTS

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Object: The use of low molecular weight heparin (LMWH) doenst require monitoring, except in newborns (NB), pregnants with high risk, renal patients and obese.due to the subvaluation of the Anti Xa through coagulometric methods and the interference of the bilirrubin (BIL)in NB in the cromogenics methods (CrM) we have modified the last one to allows its use in the patients above mentioned.
Material and methods: samples of 40 icterics NB (total bilirrubine: 80 mg/L to 200 mg/L) were studied in venous blood (citrate Na 3.8%, proportion 1/9). Centrifuged 10' to 2000G 4 hours after extraction.Stago rotacrom Heparin were used for cromogenic determination.The calibration curve with diferents concentration of enoxaparine was done in each sample.We did a modification of the technique with a rection of diazotation for paranitroanilina (pNA) and its later copulation giving a pink colour to allow its reading at 540 nm for avoid the Bilirubin's interference with pNA.
Results: The curves done with different concentations of LMWH used in plasma samples taken for patients,have show a linear order.The absorvance of each reading was concurrent with the concentration of LMWH added in each tube is not modified by the value of BIL and corresponded to the readins obtened in anicterics plasma.
Conclusions: The modification done to the CrM allows its reading avoiding the superposition of BIL with the pNA.The adaptatin mentioned could be use in jaudice NB achieving a more exact mesurement of the heparinemia

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BLOOD CELLS DERIVED MICROPARTICLES AND VASCULAR MARKERS STUDY IN ESSENTIAL THROMBOCYTHAEMIA PATIENTS

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Background: Cellular microparticles (MP) are plasma membrane vescicles of <1.5 μm in diameter, which are released into the circulation during cellular activation by PLT (PMP), EC (EMP) or red cells (RMP). MP may affect endothelial modulators such as nitric oxide (NO), adrenomedullin (ADM) and endothelin-1 (ET-1). Previous studies demonstrated that MP are present in elevated numbers in essential thrombocytemia (ET). However, the relationship between MP with treatment and MP with endothelial modulators in ET is unknown.
Methods: 52 patients with ET diagnosis were studied: 18 on hydroxyurea (HU), 15 on anagrelide (AN), and 19 on aspirin (ASA). Platelet-poor-plasma aliquotes were stored at -80°C. Samples were analysed for MP numbers and functional markers by flow cytometry. PMP was identified using CD62P, CD36, and CD63, EMP using CD105 and RMP using CD235a. Endothelial modulator markers NO, ADM and ET-1 were measured by ELISA. Statistical analysis included Kruskal-Wallis test, Bonferroni test, correlation matrix, principal component (PCA) and partial least square regression analysis (PLSR).
Results: Total-MP were increased in all patients groups compared to controls. Bonferroni test, showed higher MP total number in the AN group compared to the HU and the ASA group. The principle components (PC) responsible for value variations were PC1 [positive loading for WCC, PMP and EMP] reflecting PLT and EC activation, and PC4 [positive loadings for PLT, PMP; negative loadings for RMP, NO]. This study confirms previous findings that MP are present in ET. We also showed that MP are affected by treatment and that MP are mainly PMP and express CD62P and CD36, which suggests that reticulated PLT participate in these processes. We also showed that HU increase NO and ADM levels, while AN reduces ET-1.
Conclusions: The finding that treatment influences PC1 (PMP and EMP) supports the idea that in ET, treatment impacts directly on MP number. The finding that NO and RMP have a negative loading within PC4, suggests that they have an antagonist-action to PMP and EMP in ET. As NO and ET-1 have antagonistic actions on endothelial cells, we suggest that HU and AN may have overlapping effects on endothelium, although using different biochemical pathways.

W322
HEPARIN INDUCED THROMBOCYTOPENIA - A LABORATORY EXPERIENCE

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Heparin induced thrombocytopenia (HIT), is an acquired usually transient pro-thrombotic disorder caused by Heparin, more commonly un fractionated than fractionated even in our experience. HIT is caused by platelet activated antibodies IgG class, that recognizes complex of Platelet Function 4 and heparin. The samples were analyzed on an ELISA technique (Asserachrom HPIA-STAGO). Functional assay was not available. The optical density (OD) was the mode of measurement and the cut off positive was >0.5 OD. This enabled the subjects to be classified as negative <0.5 OD, borderline positive 0.5 to <1.5 OD, positive >1.5 OD, strongly positive >2.0 OD to >3.0 OD. In a retrospective analysis from (December 2008 to October 2012) 954 assays were performed on samples referred for suspected HIT. These were patients mainly from the surgical cardiothoracic, intensive coronary care and critical care units. Of the 954 samples assayed positivity of the test was seen in 59 patients. The laboratory classification of <0.5 normal was 93.8%, borderline positive was 62.3%, positive 15%, strongly positive was 2%. This type of segregation according to OD was seen as the best way to interpret results for therapeutic measure. Morbidity was high in the categories of positive and strongly positive and three cases of mortality were seen. The aetiology of which was definitely thought to be HIT. A close correlation with the clinical picture and platelet monitoring helps overcome the suspicion of HIT and this simple approach of categorization has helped to a large degree in aiding clinical and therapeutic measures at our centre.

W323
TESTING PLASMA FOR LUPUS ANTICOAGULANT PRESENCE ACHIEVING ADEQUATE PERFORMANCE CONCERNING SPECIFICITY AND SENSIBILITY

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Background: Phospholipid-dependent antibodies are often associated with thrombosis and late pregnancy lost. Testing for lupus anticoagulant (LA) in patients with significant probability of having antiphospholipid syndrome (APS), or who have unexplained prolonged aPTT is imperative. Criteria for identification of positive LA in plasma samples are proposed by the Scientific and Standardization Committee on Lupus Anticoagulant/Antiphospholipid Antibodies of the International Society of Thrombosis and Hemostasis. Two different tests that represent different assay principles and contain different concentrations of phospholipids should always be associated. In LA positive cases confirmation of results must be carried out, commonly after 12 weeks, to evaluate transient or persistent antibodies.
Methods: 150 patients with suspicion of APS were evaluated for LA using diluted Russell Viper Venom Time (dRVVT) and an aPTT with Silica as an activator and low phospholipid content, HemosIL LAC and HemosIL Silica Clotting Time (SCT) respectively. All patients presented prolonged aPTT and were screened for coagulation inhibitors by using mixing aPTT test with normal pool plasma. All samples were collected and processed according to laboratory protocol.
Results: 32% of patients (n=48) presented positive result for LA. From these, 46% (n=22) were positive for both reagents used, 40% (n=19) were positive using SCT and 14% (n=7) presented positive results using dRVVT. The sensitivity and specificity obtained for dRVVT reagent was 73.1% and 66.2% respectively, whereas sensitivity was 62.1% and specificity 66.2% for SCT. The obtained areas under corresponding receiver operating characteristic curves were 0.917 for dRVVT and 0.877 for SCT.
Conclusions: The dRVVT method which activates directly factor X, associated with Silica Clotting Time, an aPTT based technique that is sensitive to antiβ2glycoprotein I, brings high benefits for LA testing, for no reagent alone is sensitive to all phospholipid-dependent antibodies, due to the high heterogeneity of LA antibodies. Therefore a positive result obtained with one of the two reagents tested is needed for indication of high probability LA presence and thus an essential tool for APS diagnosis.

W324

SIMULATION OF THE COST/EFFECTIVENESS OF PHARMACOGENETIC TESTING PRIOR TO THE INITIATION OF ANTICOAGULOTHERAPY

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Background: In industrialized countries, anticoagulation therapy is a leading cause of visits to the emergency department and of hospitalizations related to adverse drug events. Three main approaches may be actually offered: standard warfarin dosing (without genetic information), warfarin dosage under the guidance of CYP2C9 and VKORC1 genotypes and dabigatran (a new class of anticoagulants). Our study aimed to compare the cost/effectiveness of these approaches applied to anticoagulotherapy initiation. Computer simulations are a well-established method to perform certain types of comparative cost/effectiveness studies, as it allows to directly compare several approaches and to study the impact of varying some critical parameters.
Methods: A decision tree was built to simulate the cost-effectiveness of initiating anticoagulation therapy over a period of 90 days in a virtual cohort of 2000 patients without contraindications to anticoagulotherapy. The model considers three options: 1) warfarin with standard dosing 2) warfarin under CYP2C9 and VKORC1 genotype-guided dosing and 3) dabigatran 150 mg twice daily. Model inputs were derived from extensive literature research and government's data bases. The outcomes considered were the number of total major events (thromboembolic and hemorrhagic events) and the direct medical costs for the Quebec public healthcare system.
Results: Base case results showed that anticoagulant therapy initiation with dabigatran dominates the two other options. It is the most effective option (32% less major events) and the less costly. Results were robust to sensitivity analyses (univariate and probabilistic). Dabigatran remains the dominant option even at a cost of CAD $7/day. Compared to warfarin with standard dosing, warfarin with pharmacogenetic guided dosing was not cost-effective even if a ceiling ratio is set to CAD$100 000/major event averted.
Conclusion: At the current costs of CYP2C9 and VKORC1 testing, pharmacogenetic-guided warfarin initiation does not appear to be cost/effective and anticoagulant therapy initiation with dabigatran is the most cost/effective option for the initiation of anticoagulotherapy.

W325

LABORATORY MONITORING OF ANTIPLATELET THERAPY

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Introduction: Platelet aggregation, as a laboratory test for assessment of platelet function, is very efficient for optimal antiplatelet treatment and also to identify individuals who have suboptimal response to antiplatelet drugs, such as aspirin and clopidogrel. This suboptimal platelet response is defined as a platelet resistance and is associated with bad clinical outcome.
Aim: To determine the platelet function using two different monitoring methods - PFA-100 and impedance aggregometry, in patients on single or dual antiplatelet therapy.
Materials and methods: The examination included 275 patients, 158 patients were treated with aspirin and clopidogrel, 79 patients with aspirin alone and 38 with clopidogrel alone. Platelet function was measured on citrated blood with the PFA-100® (Platelet Function Analyzer, Siemens), for collagen/ADP closure times (Col-ADP) and for collagen/epinephrine closure times (Col-EPI) and using a new generation impedance aggregometer Multiplate (Multiplate Platelet Function Analyzer, Dynabyte, Germany) in blood samples with heparin for ASPI and ADP-HS (the area under the aggregation curve (AUC) was used to express the aggregation response over the measured time (AU*min).
Results: There is statistically significant increase in Multiplate test sensitivity than PFA-100 in patients treated with aspirin and clopidogrel ($\chi2=7,47$, $P<0,05$ for aspirin, $\chi2=52,70$, $P<0,001$ for clopidogrel), but testing on both devices showed statistically significant increase in sensitivity to aspirin than to clopidogrel ($P<0,001$). There is not statistically significant difference in test sensitivity in patients treated with aspirin alone ($\chi2=0,28$, $P>0,05$), but statistically great increase in Multiplate test sensitivity compared to PFA-100 in patients treated with clopidogrel alone ($\chi2=41,26$, $P<0,001$).
Conclusions: Impedance aggregometry is more sensitive to dual antiplatelet therapy and to clopidogrel alone than PFA-100, but there is not significant difference for patients treated with aspirin alone. Clinical studies are required to show which parameter of platelet function correlates best with an elevated risk for thromboembolism in patients stratified as aspirin and/or clopidogrel non-responders in a laboratory assay.

W326
PLATELET REACTIVITY IN PATIENTS WITH ACUTE GASTRODUODENAL BLEEDING

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Background: Currently, recommendations for the step-wise management of patients with gastroduodenal bleeding are based on clinical, laboratory and upper endoscopy data. However, this approach is not useful for analysis of the mechanisms of alteration in thrombogenesis and un-sustained hemostasis. The aim of this work was to assess platelet functions as a key link of hemostasis in patients with gastroduodenal bleeding.
Methods: We analyzed the platelet aggregation in 247 patients with acute gastric and duodenal ulcer bleeding according to clinical, laboratory and endoscopic data before treatment. The following agonists were used: ADP (5 mkM), epinephrine (2.5 mkM), 5-hydroxytriptophane (10 mkM), collagen (1 mkM) and thrombin (1.2 NIH). Measurement of platelet aggregation was carried out with the SFP aggregometer.
Results: Acute peptic ulcer bleeding occurred in 185 (74.9±2.8%) men, aged 54±1.4 years old and in 62 (25.1±2.8%) women, aged 70.2±1.9 years old. Under endoscopic observation the active bleeding (Forrest class 1) was found in 21 patients (8.5±1.8%). Recent bleeding was diagnosed in 204 cases (82.6±2.5%), Forrest class 3 – in 18 (7.3±1.7%) patients. There were no significant differences in coagulation system indexes ($P > 0.05$) and platelet count in patients with ulcer bleeding. Significant differences in platelet aggregation induced by ADP ($P < 0.01$), collagen ($P < 0.01$) and thrombin ($P < 0.001$) in patients with different Forrest class were detected. The lowest platelet response to collagen and thrombin ($P < 0.001$) was in patients with continuing bleeding - 12±6.7% (CI 3-18%) and 20±10.2 (CI 13-28). Platelet aggregation induced by epinephrine was associated with chronological characteristics. It was high during the first 6 hours after bleeding manifestation, but the decrease of platelet adrenoreactivity was detected in patient after 12 h of bleeding beginning. A multivariate linear regression model proved thrombin- and ADP-induced platelet aggregation to be significant prognostic markers for sustained thrombogenesis in patients with gastroduodenal ulcer bleeding.
Conclusions: Platelets can be used for assessment of mechanisms of thrombogenesis failure predicting the gastroduodenal ulcer bleeding outcome.

W327
OLD PROBLEM – NEW SOLUTION: THE WAY TO DETERM PLATELET AND ENDOTHELIAL DYSFUNCTION IN CEREBROVASCULAR OR CARDIOVASCULAR DISEASES

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Platelet and endothelium play a leading hand in the pathogenesis of cardiovascular diseases. Nonetheless the compound of different methods for determination of platelet and endothelial dysfunction is weak and a number of assays lack for standardization. The aim was to form the new laboratory approach in platelets' reactivity and endothelial dysfunction assessment based on flow cytometry.
Methods: We examined 65 healthy men and women, 164 patients with cerebrovascular/cardiovascular disease (48/118) including 32 subjects treated with clopidogrel (Clop) 75 mg/day + aspirin (ASA) 100 mg/day and 19 – ASA only. The number of GP IIb-IIIa receptors and P-selectin expression per platelet were analyzed by flow cytometry with antibodies to CD61, CD62P-PE, CD45 and CD146 before and after 10 mkM ADP induction (pat. №2442167 RF). The quantity of circulating endothelial cells (CEC) was determed as a number of nucleate cells with specified size and positive binding to anti-CD146, but negative binding to anti-CD45.
Results: We evaluated the platelet reactivity after ADP induction by ΔGPIIb-IIIa(%) and ΔP-selectin (%) which were significantly decreased in Clop+ASA treated patients compared with controls and ASA-treated groups: 7.9±1.5% vs. 13.0±2.0% and 14.9±2.5%, 48.0±6.0% vs. 75.0±5.0% and 70.3±6.7% ($P < 0.05$), respectively. The CEC level was significantly higher in selected group with acute coronary syndrome (ACS) or ischemic stroke (IS) compared with sex and age matched control – 6.3±0.9 cells/mL and 4.3±0.8 cells/mL in ACS and controls, respectively ($P=0.04$) and 7.0±1.3 cells/mL and 3.6±1.1 cells/ml in IS and controls, respectively ($P=0.05$).
Conclusion: A combination concept of determination of platelet and endothelial dysfunction by flow cytometry allowed to assess vascular wall's damage and effects of anti-platelet therapy in acute stage of cardio- or cerebrovascular diseases.

W328
THE NEW ASSAY FAECAL CALPROTECTIN IN RANDOM ACCESS: WHAT CHANGES FOR THE LABORATORY?

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Background: Calprotectin, a calcium binding protein accounting for 60%in the cytosol of neutrophil leukocytes. Close correlation between faecal calprotectin and faecal leukopcyte excretion quantified with 111 indium has been described, the faecal calprotectin has been proposed as a non-invasive marker of intestinal inflammation in inflammatory bowel diseases (IBD). Faecal calprotectin , a marker of intestinal inflammation, can be used to distinguish between functional and organic bowel disease (e.g. Crohn's disease, ulcerative colitis). In the study, we evaluated a new assay random access for the measurement faecal Calprotectin: EliA CalprotectinTM Phadia. Materials and methods: Random stool samples were collected from patients with abdominal discomfort attending the Department of Gastroenterology at the Fondazione IRCCS Policlinico San Matteo, Pavia, Italy. Calprotectin was measured in all 27 stool samples using two enzyme-linked immunosorbent assays: Phical ELISA (also called Calprest)manufactured by Eurospital, Trieste, Italy and EliA CalprotectinTM Phadia Laboratory Systems Thermo Fisher Scientific Ab Uppsala, Sweden. We also evaluated the performance of the two commercial sample extraction devices (respectively ScheBo Biotech for Eurospital and EliA Stool extraction kit) to the manual weighing method. Results The average and standard deviation of values of calprotectin that were obtained respectively equal to 220 and 231mg/Kg to the dosage Calprest Eurospital with kit device and 300 and 348 mg/Kg manual weighing method; while the mean and standard deviation of the values obtained for Calprotectin EliA were 671 and 1269 mg/Kg with kit device and 531 and 963 mg/Kg manual weighing method. Patients were classified follows respectively.We got a good agreement in the classification of patients, only one patient was negative with assay Calprest Eurospital and positive with the new assay.
Conclusions: Laboratories should be aware of the lack of the assay standardization, as demonstrated by the between-assay variability, of the high biological variability and of the criticality of the extraction. Therefore, the possibility to have a dosage random access allow us to improve the diagnostic accuracy of the test as well patient clinical menagement.

W329
SELECTED BIOCHEMICAL PARAMETERS AND CHRONIC ALCOHOLISM

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Background: Alcoholism is a permamently progressive, chronic disease causing a number of serious complications for health and life. Changes in chronic alcoholism can lead to cirrhosis of the liver with symptoms of its failure, jaundice, anemia, consciousness disorder, coma, bleeding from esophageal varices, coagulation failure, which in the worst stage ends in death.
Aim: To estimate changes in biochemical parameters in men diagnosed with alcohol addiction.
Material and methods: The study included 450 men aged 19 to 60 years who were diagnosed with chronic alcoholism. Patients were divided into groups depending on the duration of the disease: I group - 100 patients with addiction from 1 to 5 years, the second group - 100 patients with addiction lasting from 6 to 10 years, the third group - 100 patients with addiction lasting from 11 to 15 years, the fourth group - 100 patients with addiction lasting from 16 to 20 years and up Group - 50 patients Serum concentrations: CRP, BIL-T, and activity of the following liver enzymes: AST, ALT, ALP, and GGTP.
Results: Treating the values of the control group as 100% we have shown statistically significant differences between groups, which were for: AST (I-157.18%, 318.71% II, III and IV 435.49% 607.03%-), ALT (I-139.98% 274.37% II-, III-372.08% 354.18% IV-), ALP (I-96%, 93.45% II-, III-97 , 86%, 115.60% IV-), GGT (I-170.13% 302.23% II-, III-596.47% 641.94% IV-), T-BIL (I-139 , 60%, II-213.58% 291.98% III-IV-360.63%) and CRP (178.9% I-II-504.05% 1223.68% III, IV - 1800.46%).
Conclusion: During the length of lasting alcoholism we have observed deepening changes in the biochemical parameters which may lead to liver failure and to cirrhosis of this organ.

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THE POTENTIEL VALUE OF THE RELATIONSHIP BETWEEN MSH2 AND MUC2 TO IMPROVE THE EFFICIENCY OF IDENTIFICATION OF COLORECTAL CANCERS WITH MICROSATELLITE INSTABILITY

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Background: The malignant potential of colorectal adenomas increases with the number and order of genetic and epigenetic aberrations. The majority (~85%) of the sporadic carcinomas is characterized by chromosomal aberrations, referred to as a chromosomal unstable phenotype (CIN), whereas the smaller group (~15%) typically shows microsatellite instability (MSI) caused by defect DNA mismatch repair genes (MMR) like MSH2 gene. This inactivation lead to subsequent mutations in the microsatellite repeat sequences of genes linked to tumor progression such as MUC2 gene. Colorectal tumors with MSI have distinctive features. They have a slightly better prognosis than CRC without MSI. Discovery of MSI has increased awareness of the diversity of CRCs and implication for specialized management of patients.
Aim: we investigated whether there are a mechanistically relationship between MSH2 and MUC2 in colorectal carcinogenesis. Design: we performed a comparative immuno-histochemical analysis of MSH2 and MUC2 proteins in 286 primary colorectal tumors.
Results: Herein, we provide that all cases (MSH2-/MUC2-) with aberrant expression of MSH2 were every-time associated to MUC2 loss-expression (P=0.0026). Thus, we suggest a direct mechanic interaction between MSH2 and MUC2. Indeed, MUC2 shown to be one of major target gene of microsatellite instability. Moreover, we found that the MUC2 loss-expression was significantly associated to mucinous histological subtype (P=0.0001) and to poorly differentiated carcinomas (P=0.008). However, in the cases without instability, the aberrant expression of MUC2 may be explained by hyper-methylation of the CpG Island of exon1 which has been reported to be associated with MUC2 gene silencing and low expression levels. But, this biological action, if it does remains to be studied. In general, our results support the theory that initiation and progression of CRCs may involve different mechanisms explaining the distinct clinicopathologic features and behavior of tumors. There may be different genetic pathways (MSI / LOH / CIMP) by which MUC2 is involved to develop CRCs.
Summary: Our present results supported the evidence that MUC2 was a valuable diagnostic and prognostic marker to identify and predict better outcome for CRCs with MSI.

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THE DIAGNOSTIC PERFORMANCE OF THE COMBINED USE OF FECAL CALPROTECTIN AND OCCULT BLOOD TESTS FOR SCREENING OF ORGANIC BOWEL DISEASE IN PRIMARY CARE SETTING

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Introduction: Almost all the previous published studies evalvated the fecal markers in Secondary Care and not the Primary Care setting. The aim of our study was to assess the utility of the combined use of two fecal biomarker tests (calprotectin and fecal occult blood) to differentiate between organic bowel disease (OBD) and non-OBD in primary care patients with symptoms suggestive of OBD.
Patients and methods: This prospective cross sectional study was performed on 142 primary care patients complaining of persistent lower abdominal pain. Fecal Calprotectin and occult blood test (iFOBT) were performed followed by endoscopic and histological examination within five days.
Results: Histological examination revealed OBD in 36 patients (25.1%); the majority of them had neoplastic disease [7 (19.1%) carcinoma and 19 (53%) adenocarcinoma], followed by irritable bowel diseases[8 (19.15)] and diverticulitis [3(8%)] .Five patients had advanced adenomas. Sensitivity for OBD was 0.62(94%) for calprotectin and 0.55 (0.40-0.60) for iFOBT; specificities were 0.53 (0.48-0.59), 0.83 (0.78-0.87) respectively. Negative predictive values (NPVs) were 0.080 (073-085) and 0.84 (0.80-0.87) respectively. Statistical significant increase in the concentration of fecal calprotectin was found in patients with OBD compared to those with non-OBD (P <0.001). iFOBT showed higher specificity than calprotectin for OBD. When the two tests combined together sensitivity and NPVs improved to 0.78 and 0.87 respectively, while specificity lowered to 0.47. In cases of advanced adenoma (Secondary end point) the using of combined tests improved NPV up to 0.96 and sensitivity to 0.79 without a change in specificity.
Conclusions: The use of the two tests ,alone or combined were not very useful for discriminating OBD from non-OBD when all adenomas were considered OBD, except in cases of advanced adenoma(>1 cm size) where using the combined tests could rule out OBD. Discriminating power of the tests may be improve further when these tests are used in combination with signs and symptoms or being a part of scoring systems to identify patients with OBD.

W332
EVALUATION OF THE DIASORIN LIAISON AUTOMATIC STOOL ANTIGEN TEST FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION

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Background: Helicobacter pylori bacterium is well recognized as a major cause of gastrointestinal diseases, such as chronic gastritis, duodenal and gastric ulcers and is also associated with an increased risk of gastric cancer. Several methods are used to diagnose H. pylori infection including the invasive method of gastric biopsy and noninvasive methods such urea breath test, serological blood test (only for seroprevalence studies) and stool antigen test. The H. pylori stool antigen test provides a simple alternative to the urea breath test and is appropriate for diagnosis and follow-up of the infection.
Methods: The LIAISON H. pylori assay (DiaSorin Inc., USA) is a new automatic modified two-step, two-site sandwich assay, which uses monoclonal antibodies for detection of H. pylori stool antigen. In this study stool specimens from outpatients children and adults of both genders, with gastrointestinal complains, were collected from clinics in the north of Israel during the period from August to November 2012, and were examined for the existence of H. pylori stool antigen by the LIAISON assay. The results were compared to the Amplified IDEIA Hp StAR assay (Oxoid Ltd., UK), a monoclonal known ELISA test for the detection of H. pylori stool antigen. Discrepant samples were confirmed by molecular methods and clinical diagnosis.
Results: Whereas the LIAISON method revealed 43% positive stool specimens for H. pylori antigen, the Amplified IDEIA Hp StAR assay detected only 41% of the samples as positive. If the Amplified IDEIA Hp StAR assay was considered as a gold standard, the sensitivity and specificity of the LIAISON test was 97.4% and 94.5%, respectively. The PPV and NPV values of this test were 92.7% and 98.1%, respectively.
Conclusions: We thus conclude that the new automatic LIAISON test is an accurate test for the detection of H. pylori antigen in stool samples. The LIAISON platform advantages, the random access and speed of proceeding enabled us the successful implementation of H.pylori stool test on an automated analyzer.

W333
HELICOBACTER PYLORI CORRELATION WITH LIPID DISORDERS

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Background: Helicobacter pylori is a gram negative bacterium which is one of the most important cause of gastritis and peptic ulcer but also have been associated with several extra digestive diseases. The association between Helicobacter Pylori infection and serum lipid profiles is still quite unclear. The aim of this study was to investigate possible relationship between Helicobacter Pylori infection and lipid levels in Kosovo population.
Materials and methods: In this study were examined 82 persons (18 – 60 years old) with chronic gastritis (45 Male, 37 Female) diagnosed with chronic gastritis. Blood samples were collected and serum was analyzed for levels of serum IgG antibodies against H. pylori using ELISA method (HUMAN GmbH Wiesbaden, Germany). Total cholesterol, triglyceride, HDL-cholesterol concentrations were measured by routine enzymatic methods.
Results: From 82 examined people with chronic gastritis, 61 were positive in Helicobacter Pylori IgG antibodies (values higher than 10.0 U/L) and were selected as patients group, other 22 patient with chronic gastritis but negative in Helicobacter Pylori IgG antibodies were classified as control group. Mean value of Total Cholesterol was 5.4mmol/l in patient group and 4.9 mmol/l in control group. Triglycerides mean value in patient group was 2.2 mmol/L while mean value in control group was 1.54 mmol/l. HDL-Ch mean value in patient group was 1.29 mmol/l and 1.7 mmol/L in control group. Patient group mean value of LDL-Ch was 4.2 mmol/l and 3.5 mmol/L for control group.
Conclusions: Based on our study findings patients with chronic gastritis resulted by H. pylori had increased values of Total Choleterol, LDL-Chol and especially Triglycerides compared to control group, while HDL-Chol values were lower in patient group compared to control group. Helicobacter Pylori infection as one of the most frequent cause of digestive diseases can also cause a lipid metabolism disorders which can be risk factor for cardiovascular diseases.

W334

KILLER IMMUNOGLOBULIN-LIKE RECEPTOR (KIR) AND HUMAN LEUKOCYTE ANTIGEN (HLA) LOCI PREDICT OUTCOME OF HEPATITIS C VIRUS (HCV)-RELATED HEPATOCELLULAR CARCINOMA (HCC)

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Background: Natural killer (NK) cells are involved in anti-tumor immune response through the interaction of inhibitory and activating receptors with their ligands. We evaluated the impact of the Killer Immunoglobulin-like Receptors (KIRs) of NK cells and of their Human Leukocyte Antigen (HLA) ligands over the outcome of HCV-related hepatocellular carcinoma (HCC) after treatment by either surgical resection (SR) or radiofrequency thermal ablation (RTA).
Methods: Sixty-one patients with HCV-related HCC were included in this study. Typing of 10 KIR genes was performed by real-time PCR coupled with melting analysis. HLA typing was carried out by PCR-Sequence Specific Priming followed by high resolution typing by PCR-Sequence Specific Oligonucleotide Probes when definition of the HLA-B or C supertypes was ambiguous. Functional characterization of NK cells was performed on PBMC samples obtained just before treatment and available for analysis. The expression of interferon-γ and of CD107a, a marker of cytotoxic function, was evaluated in basal condition and after stimulation. Survival curves were estimated by the Kaplan-Meier method and compared by log-rank test. Cox proportional hazards regression model was used for multivariate survival analysis.
Results: Activating KIR2DS5 was associated with significantly longer time to recurrence (TTR) and overall survival (OS) ($P < 0.05$). Homozygous HLA-C1 ($P=0.01$) and HLA-Bw4I80 ($P=0.05$) were expressed by patients with longer OS. Multivariate analysis identified the type of treatment (SR vs RTA) and HLA-C1 as independently related to longer TTR (both $P < 0.05$), whereas only KIR2DS5 was an independent predictor of longer OS ($P < 0.05$). Compound KIR2DL2-C1 and KIR3DS1-Bw4T80 genotypes were associated with better TTR and worse OS, respectively ($P < 0.05$ each). A prevalent cytotoxic NK phenotype was detected in patients with both longer TTR and OS, and cytotoxic capacity was significantly higher in subjects with HLA-C1 alone or combined with KIR2DL2/KIR2DL3 ($P < 0.05$ and < 0.01, respectively).
Conclusions: These results support a central role of NK cells in the immune response against HCC, providing a strong rationale for personalized post-treatment monitoring schemes and therapeutic strategies enhancing NK cell response.

W335

GREEN TEA SUPPLEMENTATION AMELIORATES CARBON TETRACHLORIDE INDUCED HEPATIC FIBROSIS EXPRESSION IN RAT

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Background: In order to evaluated the long-term effects of green tea extract (GTE) supplementation on oxidative stress, biliary acute phase protein expression, and liver function in CCl4-induced chronic liver injury. The antioxidant activity of GTE in comparison with those of vitamin C, vitamin E, and b-carotene in vitro by using an ultrasensitive chemiluminescence analyzer.
Methods: Chronic liver injury was induced by intraperitoneally administering carbon tetrachloride (CCl4) to female Wistar rats for 8 weeks. The effects of low (4 mg/kg/day) and high (20 mg/kg/day) doses of intragastric GTE on CCl4-induced liver dysfunction and fibrosis were examined by measuring the bile and blood reactive oxygen species levels and biochemical parameters by using Western blot and two-dimensional polyacrylamide gel electrophoresis (2D-PAGE).
Results: GTE has greater scavenging activity against O2e, H2O2, and Hypochlorous acid (HOCl) in vitro than vitamin C, vitamin E, and b-carotene do. In vivo, CCl4 markedly increased bile and blood reactive oxygen species production, lipid accumulation, number of infiltrated leukocytes, fibrosis, hepatic hydroxyproline content, and plasma alanine aminotransferase and aspartate aminotransferase activities, and reduced plasma albumin levels. 2D-PAGE revealed that CCl4 increased the acute-phase expression of six biliary proteins and decreased hepatic B-cell lymphoma 2 (Bcl-2), catalase, and CuZn superoxide dismutase protein expression. GTE supplementation attenuated CCl4-enhanced oxidative stress, levels of biochemical parameters, pathology, and acute-phase protein secretion, and preserved antioxidant/antiapoptotic protein expression.
Conclusions: GTE supplementation attenuates CCl4-induced hepatic oxidative stress, fibrosis, acute phase protein excretion, and hepatic dysfunction via the antioxidant and antiapoptotic defense mechanisms.

W336

THE EFFECT OF LIVER CIRRHOSIS SEVERITY ON THE LEVEL OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT)

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Background: Carbohydrate-deficient transferrin (CDT) is recognized as a marker of chronic alcohol abuse. However, the diagnostic accuracy of CDT is influenced by liver damage. The elevated CDT results can decrease the specificity of CDT testing as a marker of alcohol abuse. More elevated CDT results are generally considered to be associated with the severity of hepatic dysfunction and in such patients may become a marker for the degree of liver injury. Therefore, the aim of this study was to assess the effect of severity of liver cirrhosis, of alcoholic and non-alcoholic origin, on the results of CDT.
Methods: Studies were carried out on 59 patients suffering from alcoholic cirrhosis and 34 patients with non-alcoholic cirrhosis. CDT, as the percentage of total transferrin (%CDT), was assayed by particle-enhanced immunononephelometry with monoclonal CDT antibodies using N-Latex CDT test on BN II System. Child-Pugh score and classes were calculated for each subject.
Results: The mean value of %CDT in alcoholic cirrhosis (total group) (mean±SD, 2.33±0.68%) was significantly higher than that in the control group (1.79±0.24%; P <0.001) and in the cirrhosis of non-alcoholic origin (1.91±0.55; P=2.999). The serum level of %CDT in non-alcoholic cirrhosis appears to be different according to the degree of liver damage evaluated by Child-Pugh score (P=0.005). Post-hoc analysis revealed that the mean %CDT level was highest in score C of non-alcoholic liver cirrhosis (2.52 ± 0.60%), and was the higher than that in score B (1.72 ± 0.37%; P=0.008) and score A (1.69 ± 0.40%; P=0.012). Meanwhile, there was no significant difference in %CDT results in alcoholic cirrhosis according to the severity of liver damage (P=0.096). However, there was significant difference in %CDT results between alcoholic and non-alcoholic cirrhosis in the score B (P=0.016). The results in alcoholic cirrhosis were higher than that in non-alcoholic ones.
Conclusions: Although the CDT results in alcoholic cirrhosis are generally higher than that in cirrhosis of non-alcoholic origin, but only the CDT results in non-alcoholic liver cirrhosis can reflect the severity of liver dysfunction during that disease.

W337

NEOPTERIN AND CALPROTECTIN : FECAL MARKERS FOR PREDICTING ENDOSCOPIC SEVERITY IN PATIENTS WITH INFLAMATORY BOWEL DISEASES.

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Background : Assessment of inflammatory bowel disease (IBD) require investigating clinical, radiological and histological criteria. Gut inflammation is associated with an acute-phase reaction and the migration of leucocytes to the gut. The fecal stream should contain specific markers of mucosal disease and novel fecal inflammatory markers have emerged to differentiate and monitor IBD disease activity. The aim of this propective work was to comparatively evaluate the diagnostic accuracy of fecal calprotectin (fCal), neopterin (fNeo) and CRP in predicting endoscopic disease severity in IBD patients.
Methods: 133 patients (78 Crohn's disease (CD), 55 ulcerative colitis (UC)) were enrolled. Before endoscopy, all patients enderwent an evaluation including clinical scores, serum CRP measurement (immunoturbidimetry Beckman®), fCal determination (ELISA Bühlmann®) and fNeo determination by an in-house ELISA technique (IBL Tecomedical®). Endoscopic activities were scored independently according to the Simple Endoscopic Score for CD (SES-CD) and to the Rachmilewitz index in patients with UC. The performances of fecal markers with endoscopic disease severity were assessed by computing correlations, sensitivities, specificities and overall accuracies at adjusted cutoffs and also test operating characteristics.
Results: FCal and fNeo differed significantly in endoscopically active CD (986 ±1827 μg/g and 336±233 pmol/g) with those in patients without mucosal lesions (159±142 μg/g and 179±222 pmol/g). UC patients with active endoscopic lesions had significantly higher concentrations of fCal (3405±2582 μg/g) and fNeo (432±265 pmol/g) compared with those with endoscopically inactive disease (199±259 μg/g and 43±32 pmol/g). Fecal markers correlated closer with endoscopic scores in UC (r=0.75 and r=0.72, respectively) than in CD (r=0.53 and r=0.47). Using cutoffs of 250 μg/g for fCal and 200 pmol/g for fNeo determined by the ROC curves, fecal markers had similar overall accuracies to predict endoscopic activity in CD patients (74 %) and higher and similar accuracies (88 and 90 %, respectively) in UC.
Conclusion: FNeo is a novel biomarker able to identify IBD patients with active mucosal lesions and represents an alternative marker as accurate as fCal in this context.

W338
THE EFFECT OF SEVERITY OF LIVER CIRRHOSIS ON THE LIPID AND LIPOPROTEIN LEVELS

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Background: The liver plays a key role in the synthesis and metabolism of lipids and lipoproteins, therefore lipids disturbances are often found in patients with chronic liver diseases. It is known that the severity of liver injury affects the level of lipids in blood serum. However, the results of these studies were not clearly the same. The reason of that may be the different etiology of liver damage. For example there is no information if alcoholic (AC) and non-alcoholic liver cirrhosis (NAC) equally affect the lipids metabolism. Therefore, the aim of this study was to evaluate the effect of severity of liver cirrhosis, of alcoholic and non-alcoholic origin, on the results of lipids and lipoproteins.
Methods: The tested group consisted of 59 patients with AC and 34 with NAC. The severity of liver cirrhosis was classified into Child-Pugh class A (29 patients), B (26 patients), and C (38 patients). Total serum cholesterol (TC), HDL, LDL and triglicerydes (TG) were measured by enzymatic methods.
Results: Serum TC, HDL and LDL levels in AC patients (mean±SD, 4.11±1.67 mmol/L; 0.72±0.48 mmol/L; 2.72±1.38 mmol/L, respectively) and NAC patients (3.68±1.04 mmol/L; 0.89±0.46 mmol/L; 2.25±0.78 mmol/L, respectively) were significantly decreased in comparison with the control group (4.98±0.65 mmol/L; 1.32±0.25 mmol/L; 3.04±0.54 mmol/L; P<0.001 for all, respectively). Mean TG level was decreased only in NAC patients (1.11±0.51 mmol/L) when compared to the controls (1.25±0.37 mmol/L; P=0.017) and was significantly higher in AC patients (1.47±0.83 mmol/L) than that in NAC patients (P=0.017). TG concentrations in class A, B, and C in AC patients appeared to be different (P=0.036). In NAC patients, HDL level was higher in class A Child-Pugh (1.06±0.43 mmol/L) than that in class C (0.56±0.25 mmol/L; P=0.029), and LDL concentrations were lower in class A (2.02±0.33 mmol/L) and B (2.02±0.79 mmol/L) than that in class C (3.04±0.74 mmol/L; P=0.034; P=0.009, respectively).
Conclusions: In the liver cirrhosis the changes in the lipids and lipoproteins levels occur and they depend on the origin and severity of liver disease.

W339
RISK FACTORS IN THE PROCESS OF CHOLESTEROL GALLSTONES FORMATION

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Background: Cholesterol gallstones has been regarded primarily as a metabolic liver disease induced by environmental or genetic factors. Biliary cholesterol is transported by vesicles and micelles, but the cholesterol microcrystals derived from physicochemically unstable vesicles. Rapid aggregation of cholesterol-phospholipid vesicles in gallbladder bile seems to be the first event in the production of cholesterol crystals, a prerequisite for cholesterol gallstone formation. The aim of this study is to establish certain risk factors in the process of cholesterol gallstones formation, particularly the incidence of female parity and LDL-cholesterol.
Methods: Bile samples were obtained from 49 patients (47 women, 2 men) underwent cholecystectomy. In the biliary samples we analyzed the biliary lipids, cholesterol saturation index, crystalization time, and vesicular and micellar cholesterol content, separated by gel filtration chromatography. Moreover the lipid profile was determined in serum of these patients.
Results: 1) It was confirmed the high prevalence of this disease in women (96%) 2) The number of births was positively correlated with the cholesterol saturation index (r =0.47, P <0.05), and with the vesicular cholesterol/phospholipid ratio (r = 0.68, P <0.05) 3) The LDL-cholesterol was positively correlated with the vesicular cholesterol/phospholipid ratio (r = 0.44, P <0.05)
Conclusions: Female sex, parity and LDL-cholesterol are risk factors prevailing in the mechanisms of cholesterol gallstones formation.

W340

SERUM LEPTIN AND BILE ACIDS IN BILIARY ACUTE PANCREATITIS

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Acute pancreatitis(AP), autodigestive disease, is an inflammatory response to pancreatic injury. The magnitude of the response and its complications are highly variable and unpredictable. While multiple etiologies are known, bile remains the most common (BAP). Bile is composed of cholesterol, bile acids (BA) and bilirubin. Most episodes of BAP are associated with gallstone impact which, in periampullary location, enabling reflux of bile into the Wirsung. It's known that central distribution of fat and obesity are risk factors related to the BAP's severity. Leptin (Lp, a protein secreted by adipocytes in proportion to the mass of adipose tissue, triglyceride content and nutritional status. Peripancreatic visceral adipose tissue secretes a high amount of this protein. The aim is to measure the serum concentration of Lp and BA in the sera of patients controls and BAP, and to find an association between these parameters.
Methods: We studied 31 patients, without significant differences in body mass index, age and male/female ratio, classified:-Controls: patients with non-pancreatic digestive diseases, normal abdominal ultrasound, no hyperlipidemia, alcoholism or hepatobiliary disorders, n=10, age (62±13) years, 4men/6women,-BAP: patients with biliary acute pancreatitis, 24-48hs.evolution, Ranson:1-4, abdominal ultrasound studies support the BAP, no hyperlipidemia or alcoholism, n=21, age (71±16) years, 8 men/13 women. Ethics Committee consent-FFyB-UBA. Lp were determined by Quantikine-Elisa-R&D, BA by enzymatic methods-Randox,in Autoanalyzer Hitachi917-Roche. Statistical Analysis:Tests Student, Mann-Whitnney and Spearman.Significance P <0.05 (SPSS-16)
Results:We observed in the BAP population in relation to controls: Decreased concentration of Lp (P=0.001);Increased concentration of BA (P=0.031) and loss of association Lp/BA:r (P=0.042)
Conclusions:In BAP patients: decrease of the leptin could be related to the amount of other factors that influence on adipose tissue, such as glucose tolerance and insulin resistance, through deregulation of the endocrine pancreas with exocrine alteration; increase in BA should be caused by an hepatobiliary alteration.The association between leptin and BA could be due to still unknown, a mechanism linking visceral fat and hepatobiliary alteration.

W341

PROGNOSTIC VALUES OF PROCALCITONIN AND CRP IN ACUTE PANCREATITIS

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Early assessment of AP severity is essential for adequate and timely treatment. Moreover, it helps in reducing the mortality rate and can help in preventing numerous complications during the course of the disease. The objective was to determine the value of procalcitonin (PCT) and C-reactive protein (CRP) as prognostic marker in early stages of AP and the evolution of the disease in relation to the course and outcome. In a prospective study were 51 patients with AP (29 with severe AP). In the first 24 hours of patient admission CRP and PCT were determined. The values of PCT were compared with values of CRP. Serum CRP concentration levels were measured using a quantitatively immunoassay method (Cardio Phase, hsCRP BN2, Siemens, Germany) with the cut-off value of 120 mg/L were accepted as an indicator for severe inflammation. PCT serum concentration levels were measured with a commercial quantitative assay (PCT sensitive, Kryptor, Brahms, Berlin, Germany) with the cut-off value of 0,25 ng/mL. Values of PCT and CRP were highly significantly elevated in patients with severe AP. There was a highly significant correlation between PCT and CRP in assessment of the severity of disease. In no survivors it was highly significantly elevated values of PCT on admission and highly significant correlation between maximal values of PCT and outcome. In predicting severity of disease, sensitivity and specificity of CRP was (cutoff 120 mg/L 75.9% and 13.6%) and PCT (cutoff 0.25 ng/mL 89.7% and 54.5%). It was found that the PCT is highly significant predictor of outcome (c^2=23.592; P <0.01). Between survivors and nonsurvivors there was a significant difference in serum values of CRP (Chi-square=6.317, P=0.012; Z=-2.434, P=0.015), and a highly significant difference in serum concentrations of PCT (Chi-square=23.592, P <0.001; Z=-4.177, P <0.001). In early prediction of AP severity, PCT has a better predictive value than CRP. PCT is a better predictor of fatal outcome in AP measured at 24 h of admission than CRP. PCT is a good marker for early assessment of AP severity, with better specificity than CRP. Increased values of PCT suggest a possible outcome. PCT analysis is simple, routinely available and a highly accurate method in early assessment of AP severity and outcome.

W342
ROUTINE ASSAY OF 7A-HYDROXY-4-CHOLESTEN-3-ONE IN SERUM AS A MARKER FOR THE BILE ACID SYNTHETIC RATE: DIAGNOSTIC APPLICATIONS FOR LIVER AND INTESTINAL DISEASES

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Background: 7α-hydroxy-4-cholesten-3-one (C4) is an intermediate in bile acid synthesis. It is previously concluded that C4 levels in serum (S-C4) can be used as a quantitative marker for the bile acid synthetic rate. The bile acid synthesis is highly regulated and shows great variations, both displaying a circadian rhythm and alterations in conjunction with disease conditions. It is strongly upregulated in patients with fecal losses of bile acids due to intestinal malabsorption, and suppressed in patients with many liver diseases. Determination of S-C4 is now established as a routine analysis in our unit, and utilized for gastro-enterological diagnostics.
Methods: Quantitative analysis of S-C4 is carried out by LC-MS/MS, using a deuterium-labelled internal standard. Approximately 0.1 ml of serum is needed, and results are expressed as the ratio between S-C4 and the serum total cholesterol level.
Results: Our reference interval was determined to 2.5-25 nmol of S-C4/mmol of total cholesterol. Of patients with chronic diarrhea, those individuals with specific malabsorption of bile acids could easily be diagnosed since they displayed high levels of S-C4, about 100-150 nmol/mmol, indicating an upregulated bile acid synthesis due to faecal losses. In contrast, patients with cholestatic conditions had very low levels of S-C4, generally having good inverse correlations between their levels of serum bile acids or serum bilirubin and S-C4. In this way, patients with bile acid metabolic diseases have been subjected to long-term follow-up, whereby cholestatic episodes were characterized by low S-C4 levels. Altered levels of S-C4 were also detected in patients with genetic defects within the bile acid synthesis. In patients with advanced liver diseases such as cirrhosis or reduced liver function, lower S-C4 levels were found, indicating compromized bile acid synthesis under these conditions.
Conclusions: Establishing the S-C4, a marker for the bile acid synthetic rate, as a routine analysis for medical diagnostics will offer better possibilities to evaluate patients with chronic diarrhea and liver diseases, especially conditions with cholestasis and liver insufficiency.

W343
A FURTHER STEP TO IMPROVE THE COST-EFFECTIVENESS OF CALPROTECTIN DETERMINATION

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Background: Fecal calprotectin (FC) is used as screening test for inflammatory bowel disease. To markedly improve its turnaround time, in 2010 we introduced a quantitative immunochromatographic POCT [Bühlmann Quantum Blue Calprotectin (QBC), previously estimated cut-off, 200μg/g stool]. Considering the QBC measurement range (30-300μg/g stool), our current laboratory's workload (a total of ~340 tests per year) results in 27% of samples with FC below the detection limit (<30), 49% with FC between 30-300, 13% with FC between 300-1500, thus implying a 1:5 dilution, and 11% with FC above the upper concentration measurable after dilution (>1500). Consequently, ¼ of our samples have to be diluted and measurements repeated one or more times. Quite recently, a QBC High Range (HR) version has become available, expanding the measurement range to 100-1800 μg/g stool and thus reducing the number of diluted samples. The aim of this study was to show result comparability between QBC and HR before introducing the latter in our laboratory. Methods: Method comparison was performed on 41 stool samples (FC concentration range, 111 to 1501 μg/g stool by HR). In addition, three extracts of stool samples with normal, borderline and abnormal FC concentrations were tested for evaluating HR imprecision. Results: Median (25th-75th percentiles) of FC concentrations were 219 μg/g (177–405) for QBC and 264μg/g (163-351) for HR, respectively. The agreement (by concordance correlation coefficient) between QBC and HR was 0.87 (CI: 0.78-0.93). The Passing–Bablok regression model (QBC in x-axis) showed a slope of 1.2 [CI: 0.97-1.4] with no significant intercept (−19 μg/g, CI: −114 to 61), and a correlation coefficient of 0.89 (CI: 0.80-0.94). HR imprecision (CV) was 18.6% at 209 μg/g, 11.5% at 271 μg/g, and 15.5% at 1500 μg/g, respectively. Conclusions: Our results showed a quite good agreement between QBC and HR, permitting the implementation of the latter in our setting without any change in the employed threshold. In our situation, this should decrease the yearly test costs of ~900 € due to a significantly lower number of samples to be diluted. Furthermore, higher FC concentrations become measurable with acceptable imprecision allowing a better characterization of the explored disease.

W344
DETERMINATION OF HELICOBACTER PYLORI ANTIGEN IN STOOL IN COMPARISON TO BREATH TEST

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Background: Helicobacter pylori (HP) is one of at least 28 known members of the Helicobacter family, which is adapted to the human stomach. HP is a spiral, microaerophilic, Gram-negative bacteria with 2-7 polar sheathed flagella, measuring 0.3 mm × 3 to 5 μm. It is adapted exclusively on life in the acidic environment of the stomach. The highest concentrations have been demonstrated in the antral region of the stomach. HP infection causes different effects, depending on the individual's immune response to bacteria, age, environmental influences virulence and strain to which an individual is infected. Therefore, its early detection and treatment is of high importance. Methods with which HP can be confirmed infection are invasive and non-invasive diagnostic methods. We chose urea breath test as the gold standard to compare results of the HP antigen determination in stool.
Methods: In order to evaluate the usefulness of a diagnostic test determining antigen in stool, we collected data on 115 patients aged 11 to 86 years. Stool samples were processed and tested by LIAISON® H. pylori SA Immunoassay (Diasorin Inc., Stillwater US). The cut-off value discriminating between the presence and the absence of H. pylori stool antigen was < 1.0 for negative and ≥1 for positive. We assessed the diagnostic value (specificity, sensitivity, positive and negative predictive values, and accuracy) of the test and compared the results with urea breath test presented as ROC curve.
Results: In our group of patients, we found that the sensitivity of the test for determining HP antigen in stool was 89,6%, and its specificity was 98,5%. The positive predictive value 97.7%, negative predictive value of 93% and 96,3% accuracy.
Conclusions: We have shown that test for the determination of HP antigen in stool give close reliability, and comparable specificity and sensitivity with urea breath test.

W345
THE EFFECT OF LIVER DISEASE ON THE CONCENTRATION OF TOTAL AND FREE SIALIC ACID

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Background: Protein's glycosylation plays an important role in the pathogenesis and progression of liver disorders. Therefore, the liver function may affect the synthesis of carbohydrates, such as sialic acid, that occupied the terminal position in the oligosaccharide chains of serum glycoproteins and glycolipids. The aim of this study was to evaluate the changes in the serum concentration of total (TSA) and free sialic acid (FSA) depending on the presence of liver diseases of different etiologies.
Methods: The tested group consisted of 278 patients (99 females and 179 males) suffering from liver disease. The patients were divided into the subgroups according to the diagnosis of liver diseases. Control group consisted of 50 healthy subjects (18 females and 32 males) recruited from hospital workers. Serum TSA concentration was measured according to an enzymatic method using the colorimetric procedure. FSA concentration was determined using the thiobarbituric method of Skoza and Mohos.
Results: In the most of liver diseases the mean TSA level was significantly lower than that in the control group, but there were no significant differences in the serum TSA concentration between liver diseases of different etiologies (P=0.143). In contrast to TSA, the mean concentration of FSA appears to be different between liver diseases (P=0.015). The concentration of FSA was significantly higher in toxic hepatitis than that in non-alcoholic cirrhosis. Serum concentration of FSA in patients with toxic hepatitis and alcoholic cirrhosis was significantly higher in comparison to the control group. There were positive correlations between TSA and FSA in alcoholic and non-alcoholic cirrhosis, chronic non-viral and viral C and B hepatitis and autoimmune hepatitis patients. The highest correlation coefficients were in autoimmune hepatitis and reached value above 0.8.
Conclusions: We conclude that the FSA concentration can be useful to differentiate between toxic hepatitis and non-alcoholic cirrhosis. In the some liver diseases, especially in alcoholic cirrhosis, the changes in TSA and FSA concentrations are parallel, what indicates on the significant disturbances in sialylation of glycoproteins in these diseases.

W346
VARIATIONS OF THE ACTIVITY OF PARAOXONASE 1 (PON1) IN LIVER DISEASES

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Introduction: The aim of this study is to evaluate the variations of PON1 activity in some liver diseases.
Patients and methods: Our study included 103 patients (49 cases of cirrhosis, 32 cases of hépatitis, 11 cases of cholestasis, 9 cases of cancerous and 2 cases of cysts) and 50 healthy individuals aged respectively 55 ± 15 years and 50 ± 15 years. PON1 activity was determined by kinetic method at 405 nm on Konelab 30® (Thermo Electron Corporation). The determination of albumin, total cholesterol, triglycerides, HDL-cholesterol and total bilirubin, and ALT, AST, alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) activities were performed on Integra 400® (Roche Diagnostics). All values were expressed as median and interquartile range.
Results: PON1 activity was significantly lower in patients compared to controls even after adjustment for confounder factors (133 IU / L [73-250] vs. 232 IU / L [110-380]). The lowest activity was found in patients with cirrhosis. This decrease was noted in patients with cytolysis, cholestasis or hepatic failure. We showed negative significant correlations between PON1 activity and ALT, AST, GGT, PAL activities and total bilirubin concentrations (r=-0.127, -0.338, -0.174, -0.205 and -0.293 respectively). Moreover, a significant positive correlation was observed with albumin (r=0.349) and HDL cholesterol (r=0.370).
Conclusion: The determination of PON1 activity during liver disease can inform about the organ dysfunction and the risk of oxidative stress associated with the decrease of this enzyme.

W347
PERMEABILITY INDEX IN CELIAC DISEASE PATIENTS

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Background: The primary function of the small intestine is the digestion of the intestinal contents, the absorption of nutrients and electrolytes and water homeostasis maintenance. However, small intestine has a very important role as a barrier between the human organism and the external environment. The mechanism of the barrier enables selective permeability for some macromolecules. Some diseases have an important influence on small intestine's integrity. The small intestine's integrity differs when comparing healthy people and people with Coeliac disease. Due to the intestinal mucosa balance and changes of tight junctions among enterocytes as a result of the inflammation, the people with Coeliac disease express typically higher values of the permeability index in the urine. The higher values are the result of the intensively absorbed lactulose through modification in tight junctions among enterocytes and a weak absorption of mannitol due to the balance of mucosa.
Methods: The permeability index is a suggestion for a new method, based on small intestine's permeability for the two molecules of different sizes: the smaller molecule mannitol and the larger molecule lactulose. Our group consisted of 10 patients and 10 controls. We measured the concentration of sugars in the five-hour urine samples after drinking the solution containing lactulose and mannitol. The permeability index is the quotient of the lactulose and mannitol concentrations. The principle of the detection of lactulose and mannitol in the urine was absorption spectroscopy.
Results: The study shows that the values of the permeability index significantly differ between the two groups of the people. We assessed the diagnostic value (specificity, sensitivity) of the test and compared the results between groups and presented as ROC curve. The specificity of the method was 100% and the sensitivity 60%. The AUC value is 0.990.
Conclusions: The study proves that the permeability index is an appropriate marker of the small intestine's permeability when it comes to people with Coeliac disease. Different permeability is the consequence of changes in Zonulin protein fraction of tight junctions and result is different permeability for macromolecules. Despite the small samples of the study it can be concluded that the permeability index is an important potential diagnostic test when it comes to people with Coeliac disease.

W348

SERUM OSTEOPROTEGERIN IN PATIENTS WITH ACUTE PANCREATITIS

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Background: Acute pancreatitis (AP) is a self-limiting disease in most patients, but its severe form develops in up to 20-30% of cases. Early diagnosis of severe form of AP has been considered a key determinant of successful therapy and patients' survival. Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily that functions as a soluble decoy receptor and can promote cell survival by binding to the TNF-related apoptosis-inducing ligand (TRAIL). The aim of this study was to evaluate the diagnostic value of OPG in patients with AP.
Methods: The study included 40 patients with AP (28 mild, 12 severe); 24 (60%) males and 16 (40%) females with mean age 47 years admitted to the surgical department. Serum OPG concentrations were measured by ELISA. Additionally, complete blood count, levels of interleukin 6 (IL-6), interleukin 18 (IL-18), polymorphonuclear elastase (PMN-elastase), soluble receptor of TNF-alpha (sTNFRII), C-reactive protein (CRP) and serum amyloid A (SAA) were determined and Glasgow score was calculated. In data analysis Mann-Whitney and Spearman tests were used as appropriate; $P < 0.05$ was considered statistically significant.
Results: Serum OPG concentrations was significantly higher in severe than in mild AP on 3rd, 5th and 7th day after admission to hospital(median: 8.51 vs 4.49 pmol/L; 7.94 vs 4.12 and 5.56 vs 4.0 respectively; $P < 0.05$). Statistically significant correlation between OPG levels and concentrations of SAA (R=0.45), sTNFRII (R=0.57) and RDW-Red Cell Volume Distribution Width (R=0.49) were found on day 3 ($P < 0.05$). After 48 hours, OPG showed positive correlation with Glasgow score expanded of IL-6 (R=0.53); IL-18 (R=0.49); CRP (R=0.57); SAA (R=0.60) and PMN-elastase (R=0.64); $P < 0.05$.
Conclusions: Single determination of OPG levels does not offer better diagnostic accuracy over novel markers used in the current AP diagnostics including interleukin 6 and procalcitonin. Measurement of OPG together with the calculation of Glasgow score could be a new and useful diagnostic tool for the early assessment of AP severity.

W349

EVALUATION OF A NEW COLLECTION TUBE FOR AT ROOM TEMPERATURE CONSERVATION OF HUMAN HEMOGLOBIN IN FECAL SAMPLES IN COLORECTAL CANCER SCREENING PROGRAMS

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Background: Colorectal cancer screening programs have been established in many European countries. Good quality of the screening services are an essential prerequisite for the success of these screening programs to reduce the mortality and/or incidence of the disease. The control and the reduction of hemoglobin degradation are fundamental at higher ambient temperatures.
Methods: The FOB Gold Tube Screen (REF 11561H) is a device for the collection and conservation of fecal samples. The buffer contained in this device is able to reduce the hemoglobin degradation at room temperature. In the protocol were used single fecal samples and pooled fecal samples. The fecal samples were collected with 3 different buffers: FOB Gold Tube Screen, FOB Gold Tube NG (REF 11561N), Eiken Buffer (REF V-PZ25). The buffers were evaluated on their capability to preserve hemoglobin by degradation for at least 10 days at 2-8 °C, 8 days at +25 °C, 3 days at +30 °C. For the determination of hemoglobin concentration in the samples was used the FOB Gold NG reagent (REF 11560N).
Results: After 10 days at 2-8 °C the recoveries (% bias) vs Time 0 concentration on single fecal samples were: FOB Gold Tube Screen 94.0%, FOB Gold Tube NG 76.0%, Eiken Buffer 81.5%, while on pooled fecal samples were: FOB Gold Tube Screen 94.4%, FOB Gold Tube NG 76.6%, Eiken Buffer 80.4%. After 8 days at +25°C the recoveries (% bias) vs Time 0 concentration on single fecal samples were: FOB Gold Tube Screen 68.5%, FOB Gold Tube NG 44.1%, Eiken Buffer 57.7%, while on pooled fecal samples were: FOB Gold Tube Screen 58.7%, FOB Gold Tube NG 47.9%, Eiken Buffer 50.3%. After 3 days at +30°C the recoveries (% bias) vs Time 0 concentration on single fecal samples were: FOB Gold Tube Screen 68.6%, FOB Gold Tube NG 27.6%, Eiken Buffer 49.7%, while on pooled fecal samples were: FOB Gold Tube Screen 85.6%, FOB Gold Tube NG 41.6%, Eiken Buffer 56.4%.
Conclusion: The use of FOB Gold Tube Screen (REF 11561H) has allowed to reduce the hemoglobin degradation in the fecal sample, in comparison with results obtained with other buffers. These results confirm the ability of FOB Gold Tube Screen to reduce the hemoglobin degradation at high temperatures, with clear advantages in the execution of screening programs.

W350

THE ENHANCED LIVER FIBROSIS (ELF) SCORE: NORMAL VALUES, INFLUENCE FACTORS AND PROPOSED CUT-OFF VALUES

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Background: Progressive fibrosis is a major cause of morbidity and mortality in chronic liver disease. To replace liver biopsy for disease staging, multiple serum markers are under evaluation with multiparametric panels yielding most promising results. The ELF score is an ECM marker set consisting of tissue inhibitor of metalloproteinases 1 (TIMP-1), amino-terminal propeptide of type III procollagen (PIIINP) and hyaluronic acid (HA) showing good correlations with fibrosis stages in chronic liver disease.
Methods: The Enhanced Liver Fibrosis (ELF) score was measured in 400 healthy controls and 79 chronic hepatitis C patients using an ADVIA Centaur automated system. The ELF score was calculated using the published algorithm combining TIMP-1, PIIINP and HA values. Patients' fibrosis stage was defined histologically. ROC analyses were performed to study marker validity. Reference values und influence factors for the ELF score were validated.
Results: ELF score reference values ranged from 6.7 to 9.8 and were significantly higher for men vs. women (7.0-9.9 vs. 6.6-9.3, resp.). Afternoon values were slightly higher than morning values (6.7-9.9 vs. 6.6-9.5, resp.). Age was a notable influence factor. We identified three cut off values: 7.7 for a high sensitivity exclusion of fibrosis, 9.8 for high specificity identifcation of fibrosis (sensitivity 69%, specificity 98% for moderate fibrosis), and 11.3 to discriminate cirrhosis (sensitivity 83%, specificity 97%). ELF score validity was superior to the results of the single tests.
Conclusion: The ELF score can predict moderate fibrosis and cirrhosis. However, influence factors such as gender and age need to be taken into account.

W351

EVALUATION OF SENTINEL PANCREATIC AMYLASE ASSAY ON BECKMAN COULTER AU5800 CLINICAL CHEMISTRY ANALYZER

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Background: Sentinel Pancreatic Amylase [PAMY] assay was evaluated on Beckman Coulter AU5800. Study goals: verify analytical performances and establish assay agreement with desirable analytical specification based on biological variability as provided by Ricos et al.
Methods: PAMY reagent, manufactured by SENTINEL CH, is a ready to use format. α-amylases are hydrolytic enzymes originated from various organs from which the corresponding name is derived, which hydrolyze starch into maltose. Pancreatic α-amylase is produced form pancreas and released into the intestinal tract. PAMY assays are suitable for diagnosing and monitoring acute pancreatitis, as well as for identifying acute attacks during chronic pancreatitis. The enzymatic colorimetric assay for PAMY determination develops in two steps: 1) Incubation: human salivary α-amylase is inhibited by two different monoclonal antibodies with no effect on the pancreatic α-amylase; 2) hydrolysis of the EPS substrate (Ethylidene Protected Substrate) p-nitrophenylmaltoheptaoside 4,6-ethylidene-blocked (ethylidene-G7PNP) by pancreatic α-amylase, forming: G2PNP, 2 G3PNP and G4PNP, which are hydrolyzed by α-glucosidase into p-nitrophenol (PNP) and glucose. PAMY activities is proportional of the absorbance increase due to PNP formation. Beckman Coulter AU5800 is a fully automated, high-throughput clinical chemistry system, which performs colorimetric, enzymatic, as well as turbidimetric assays. Study protocols were based on CLSI guidelines. Acceptance criteria were defined to meet desirable analytical specifications as per biological variation (TE <17.7%) at clinical decision level (51 U/L). Total Imprecision (EP05A): CV ≤6%; Linearity (EP06A): bias +/-5%; Method comparison (EP09A): compare the results of serum samples on Beckman Coulter AU5800 and AU480, and on Abbott Architect c8000 analyzers.
Results: Total Imprecision: CV%=1.9% at 39.9 U/L: 1.8% at 109.8 U/L. Linearity: from 0.4 up to 2800 U/L. Comparison: A) AU5800 vs AU480: n 100, slope 1.01, intercept -0.34, r 1.00, P <0.001; B) AU5800 vs c8000: n 100, slope 1.05, intercept -4.98, r 0.99, P <0.001.
Conclusions: performance of Sentinel CH. PAMY assay on Beckman Coulter AU5800 met Acceptance criteria based on Biological Variation database specifications.

W352
EFFICIENT SEMI-AUTOMATED EXTRACTION OF HUMAN DNA FROM STOOL SAMPLES

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Background: Colorectal cancer is common neoplastic disease with a high mortality rate, mostly because the tumor growth is secretively progressing, why diagnosis often is late. Malignant cells are shed into the stool, which could provide a potential tool for earlier detection of the disease. The first step of this noninvasive approach requires extraction of human DNA from stool samples. Our aim was to investigate whether intestinal DNA can be extracted in a rapid and efficient way from stool samples collected from patients with colorectal cancer in order to probe for cancer specific genetic aberrations.
Methods: DNA was isolated from human stool samples using a fully automated system for processing of fecal specimens (SoniC, S2G Scandinavia AB), where after DNA was extracted using the QiaCube extraction system. The robotic system for pre-analytical processing of stool material performs weighting of the samples, addition of appropriate buffer volumes, homogenization by ultrasound and centrifugation of fecal samples. The final homogenate consists of 2-4 mL of a buffer suspension, of which 1,4 mL was used for DNA extraction according to instructions from QIAGENE.
Results: DNA from each individual was extracted from 200-400 mg of stool material resulting in a total yield of 50 – 350 ng of DNA /mikroliter, and the 260/280 ratio was estimated to 1,8-2,0. The presence of human genomic DNA was monitored by melt curve analysis of Faktor V Leiden Mutation using LightCycler (Roche) and TaqMan probe based analysis of LCT-13910C>T using 7500 Fast Real Time PCR System (Life Technologies). In total 10 samples were analyzed, and human DNA was identified in all cases.
Conclusion: Our results show that efficient extraction and amplification of human DNA can be performed from stool samples by the use of automatic robot systems. The system for processing of fecal samples has a capacity of processing up to 160 samples per 8 hours, and require very few manual moments. The technology is intended to be used for studies of genetic markers in patients being investigated for occurrence of colorectal neoplasia, by i.e. screening the obtained DNA for the presence of specific mutations in the K-Ras gene.

W353
A NEW METHOD FOR IMMUNO-TURBIDIMETRIC MEASUREMENT OF CALPROTECTIN IN FECES, PLASMA AND OTHER BODY-FLUIDS

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Background: The interest in calprotectin has been increasing during last few years due to its potential as a non-invasive, cheap and sensitive marker for inflammation, particularly for intestinal inflammation. Currently, calprotectin is measured with several commercially available ELISA and EliA methods, which are time-consuming and used only in clinical laboratories. Moreover, determination of calprotectin in feces requires often manual and long pre-analytical processing of the fecal samples, which may lead to very long turn-a-round time for the calprotectin results. We have validated a new immune-turbidimetric assay for determination of calprotectin in combination with fully automatic system for pre-analytical processing of fecal samples in order to improve efficiency and generate shorter turn-a-round time for the fecal Calprotectin results.
Methods: A new latex particle-enhanced immune-turbidimetric assay (Gentian, Moss, Norway) for determination of Calprotectin was validated. Fecal Calprotectin was assayed on a Cobas c111 system (Roche AG). Pre-analytical processing of fecal samples was performed with a fully automated robotic system (SoniC, S2G Scandinavia AB) and turn-around time for reporting of results was well within a working-day.
Results: Linearity was proven throughout the measuring range from 1 to 50 mg/L for plasma samples and 50 to 2500 mg/kg for fecal samples. Within-run CVs for fecal Calprotectin ranged from 2,2 - 9,6 %, for concentration range 50 – 700 mg/kg. Good agreement was achieved in the comparisons between the Gentian-Calprotectin assay and the commercially available ELISAs (Calpro AS and BÜHLMANN Laboratories AG: slope range 1,08 – 1,38, R2 = 0,89-0,92).
Conclusions: The immune-turbidimetric Calprotectin assay was shown to be precise and accurate with proven linearity over the measuring range. Good comparability was obtained with other commercially available ELISAs. The automatization of both pre-analytical processing of fecal samples and measurement of Calprotectin concentration resulted in improved efficiency and significantly shorter turn-around-time for reporting the fecal Calprotectin results.

W354
I-FABP AND INTESTINAL BOWEL DISEASES

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Background. Fatty acid-binding proteins (FABPs) are a class of cytoplasmic proteins that bind long chain fatty acids. FABPs are small intracellular proteins (~13-14 kDa) with a high degree of tissue specificity. There are at least nine distinct types of FABP. Due to its small size, FABP leaks rapidly out of ischemically damaged necrotic cells leading to a rise in serum levels.
Methods. The human I-FABP ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle. During the incubation is used Streptavidin-peroxidase conjugate which bind to the biotinylated tracer antibody; it react with the substrate, tetramethylbenzidine. A color reaction is occurring where the intensification is in a direct ratio to I-FABPs levels in tested serum.
Results. For a period of 3 months we studied 20 patients with symptoms for acute abdomen. 11 women, 9 men; the age was between 25 and 55 years. The results were compared to a control group of 20 patients. In control group minimum 0.09 ng/ml, maximum 1.10 ng/mL, avarage 0.50 ng/mL for males; correspondingly to 0.15 ng/mL to 1.21 ng/mL to 0.72 ng/mL for females. The presence of serum I-FABP in patients with intestinal bowel diseases was minimum 4.00 ng/mL, maximum 11.58 ng/mL, average 7.08 ng/mL in males; correspondingly to 5.15 ng/mL to 10.87 ng/mL to 7.50 ng/mL in females. The reference ranges are up to 2.0 ng/mL.
Conclusions. Serum I-FABPs levels are the newest and earliest marker to determine intestinal bowel diseases.

W355
EVALUATION OF SERUM CASPASE-3 AND AGGRECAN LEVELS IN PATIENTS WITH CHRONIC HEPATITIS C VIRUS

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Chronic hepatitis C virus(HCV) liver disease is a major health problem worldwide. Early diagnosis is mandatory to hinder progression into cirrhosis and hepatocellular carcinoma (HCC).Apoptosis orchestrated by a group of proteases where caspase-3 is the chief mediator has an important role in early HCV-induced liver cell damage.Aggrecan synthesized by hepatic stellate cells is chiefly indulged in fibrosis. Its serum level mirrors its hepatic level .Our aim was to evaluate serum levels of caspase-3 and aggrecan as non invasive markers in patients with chronic HCVinfection for early detection of HCV-induced liver cell apoptosis and fibrosis respectively.
The study was conducted in Theodore Bilharz Research Institute.It included 69 male subjects; 54patients with clinical &/or laboratory evidence of HCV-induced chronic HCV liver affection {+veHCV IgG Ab(ELISA) &/or positive HCV-RNA(PCR)} divided into 2 groups(Gp):I :with normal ALT (n=27)and II: with elevated ALT (n-27).15 healthy subjects as referenceGp (III).All cases underwent clinical examination and abdominal ultrasonography.12 ml venous blood withdrawn and collected on: -citrated tubes for prothrombin time (PT),& -plain tubes for routine(hepatic&renal function tests) and specific laboratory investigations. Estimation of:serum α feto protein level(αFP)ELISA, serumcaspase-3 levels(colorimetry) and serum aggrecan (ELISA).
Results:In GpI significant rise in mean caspase compared to reference value.Significant +ve correlation between caspase level and ALT: r^2=0.269525 P <0.05. In Gp II significant elevation in aggrecan (106.1±97.6 ng/mL) compared to bothGpIII (11.57 ±9.88 ng/ml) &GpI (12.38 ± 8.63 ng/mL) mean values (P <0.001, <0.001 respectively).Significant positive correlation between serum aggrecan and AST/ALT ratio r2=0.28421; P <0.05. Serum caspase-3 can be used as an indicator of liver cell injury in chronic HCV patients with normal ALT and to monitor persistently normal ALTpatients. Early therapeutic or anti-caspases may halt progression of the disease.Aggrecan can be a non-invasive marker of liver fibrosis in chronic HCV patients.Longitudinal studies are needed to study the role of apoptotic markers in perpetuation of chronic HCV hepatic affection and as potentiators of HCC.

W356

INHIBITORY EFECT OF PURIFIED ACETAMINOPHEN-GLUTATHIONE CONJUGATE IN HUMAN GLUTATHIONE REDUCTASE

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Background: Acetaminophen (APAP) is a frequently used analgetic and antipyretic drug. After overdose (> 140 mg / kg), it may cause a number of pathophysiological processes that can even lead to acute liver failure. The entire mechanism of acetaminophen toxicity is still unknown. APAP is metabolized in hepatocytes throught various pathways. The most important pathway acting in overdose is oxidation of APAP by cytochrome P450 to a substance, which is detoxified by reaction with glutathione. This product, APAP-SG, has been recognized as non-toxic generally. However, the mail goal of our study was to estimate the possible toxic effect of purified APAP-SG on glutathione reductase (GR), a key enzyme of glutathione metabolism. This enzyme catalyzes reduction of glutathione disulfide to the reduced form.
Methods: Glutathione reductase (human, recombinant) activity was estimated in presence of 0.1 – 4 mM APAP-SG. GR activity was determined using spectrophotometric method in presence of glutathione disulfide (0.5 - 4 mM) and NADPH (0.8 mM). This method is based on measurement of absorbance decline (λ=340 nm) due to oxidation of NADPH. We determined decline of absorbance in 365 nm based on spectral properties of APAP-SG. APAP-SG conjugate was purified using preparative liquid chromatography and structure of the compound was confirmed using mass spectrometry. The purity of prepared APAP-SG was >98 %.
Results: We proved that APAP-SG is able to decrease GR activity dose dependently. Activity of GR was inhibited by 2%, 11% and 12%, in presence of 1 mM, 2 mM and 4 mM APAP-SG, respectively (in 4 mM GSSG). In addition, we found that the rate of enzyme inhibition depends on GSSG concentration. Glutathione reductase activity was inhibited by 18%, 24%, 25% and 27%, in presence of 0.1 mM, 1 mM, 2 mM and 4 mM APAP-SG, respectively (in 0.5 mM GSSG). Here presented concentrations of APAP-SG are similar with concentrations occuring in the cell.
Conclusion: We found that glutathione reductase, the essential enzyme of the antioxidant system, was dose-dependently inhibited through a product of acetaminophen metabolism, i.e. through the conjugate of APAP and glutathione. Our results likely show a new important mechanism of acetaminophen toxicity.

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ERADICATION OF HELICOBATER PYLORI – WHICH SCHEME GIVES US THE BEST OUTCOME?

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Background: Infection with Helicobacter pylori (HP) is the most common infection in humans, as it infects more than half of humanity. It infects 40-50% of the population in developed countries and 80-90% of the population in developing countries. HP infection causes different effects, depending on the individual's immune response to bacteria, age, environmental influences virulence and strain to which an individual is infected. Therefore, its early detection and treatment is of high importance. In Slovenia, we treat patients with gastric and duodenal ulcer, chronic gastritis, intestinal metaplasia and/or atrophy, patients with gastroesophageal reflux disease before chronic treatment with proton pump inhibitors (PPIs) and long-term treatment with non-steroidal anti-inflammatory drugs or salicylates. In addition, we treat also patients with mucosa-associated lymphoid tissue (MALT) lymphoma, gastric cancer after surgery and in first degree relatives of patients with gastric cancer.
Methods: In our study we treated 132 patients, 100 women and 32 men, age range was from 4 to 80 years, mean age 40 years. We present data on 201 regimens. We used the following antimicrobial agents in different combinations: amoxicillin, metronidazole, clarithromycin, amoxicillin, azithromycin, ciprofloxacin, oxytetracycline, levofloxacin, all with PPIs.
Results: The most successful treatment regimen was combination of amoxicillin, clarithromycin and PPIs, which was successful in 24 (41) patients or in 58.5% [95% confidence interval, CI: 43.5% - 73.6%]. In the group treated with the scheme clarithromycin + metronidazole + PPIs, treatment was succesfull in 38 (80) patients or in 47.5% [95% confidence interval, CI: 36.6% - 58.4%]. 59 patients received treatment with amoxicillin, metronidazole, and PPIs, treatment was succesfull in 24 patients or in 40% [95% confidence interval, CI: 27.5% - 52.5%]. 21 pateients were treated with the use of other scemes. Treatment was succesfull 7 cases or in 33.3%.
Conclusions: Our results show that different eradication schemes have different outcome. As in the literature, the best results were obtained with the use of the combination of amoxicillin, clarithromycin and PPIs. In the second line is the combination of clarithromycin + metronidazole + PPIs.

W358

PANKRIN, HOW MUCH WE GAIN IN THE DIAGNOSIS OF ACUTE PANCREATITIS

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Background: Acute pancreatitis (AP) is an inflammatory disease of the pancreas, causing sudden and severe abdominal pain associated with nausea and vomiting. Pancreatic damage occurs when the digestive enzymes are activated before they are secreted into the duodenum and begin attacking the pancreas. There are many possible causes of AP, but 60 to 75% of all cases in adults are caused by gallstones or alcohol abuse. Other causes include medications, infections, trauma, metabolic disorders and surgery. Most cases of AP are mild and self limiting, needing only brief hospitalisation. However, 20% of patients develop a severe disease with local and extrapancreatic complications characterised by early development of hypovolaemia, and multiple organ dysfunction. Diagnosis is usually based upon a medical history, physical examination, and chemistry test results. A new serum assay for diagnosis of AP is Pankrin, which measures a mixture of pancreatic proteins using polyclonal antibodies towards elastase and different epitopes of other pancreatic enzymes.
Methods: The aim of this study was to evaluate serum pankrin levels in 24 patients with AP and 26 healthy volunteers. Serum pankrin concentrations were determined with a solid phase enzyme immunoassay based on the double sandwich technique. The receiver operating characteristics (ROC) and the area under the curve (AUC) were calculated in order to evaluate the diagnostic value of the pankrin. Non-parametric test was used for evaluation and a p-value of 0.05 was considered statistically significant.
Results: In healthy volunteers, the median value of pankrin was 61.2 U/mL (range 30.3-108.5 U/mL) and in patients with acute pancreatitis was 236.9 U/ml (range 92.6-718.1 U/mL, P <0.0001). The AUC of pankrin calculated from the ROC curve was 0.97. When the cut off value of pankrin was set at 77.8 U/mL, its clinical sensitivity was 95.8% and its clinical specificity was 88.5%.
Conclusion: This study shows that Pankrin assay could be of help to improve the diagnosis of AP. Pancreatic enzymes detected with the assay persist in high concentrations for a much longer period of time after onset of AP than does conventional chemistry tests, and consequently, the disease should not be overlooked even in patients who come late to the hospital.

W359

ALPHAFETOPROTEIN LEVELS IN PATIENTS WITH HEPATOCELLULAR CARCINOMA AFTER TREATMENT WITH SORAFENIB

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Background: Hepatocellular carcinoma (HCC) is the most common primary liver cancer. In 60-90% of HCC it arises in cirrhotic liver. Among the factors associated with liver cancer included in cirrhosis of the liver are chronic infection with hepatitis B virus (HBV) or hepatitis C (HCV), alcohol abuse, metabolic disorders, infection with aflatoxin, resulting in contamination of cereals by the fungus Aspergillus flavus. Sorafenib (Nexavar) is the only approved oral anti-angiogenic target drug, which has proved to be effective in the systemic treatment of advanced primary liver cancer. Of particular importance is the monitoring of AFP, where the AFP concentrations above 1200 mg/L confirm HCC. AFP is a non-specific biomarker, elevated in 50-70% of patients with HCC. Its value is also higher in some patients with germ tumors (testicular and ovarian cancer) and in active hepatitis B and C.
Methods: Our group of patients with confirmed diagnosis, consisted of 18 patients, all were treated with Nexavar. We wanted to determine the importance of AFP concentrations, and see how these levels change during treatment and what is happening to the size of the tumor. AFP concentrations were measured in sera, with a sandwich immuno-chemiluminescence method using the Liaison analyzer.
Results: It was assumed that the high concentration of AFP indicate a larger and more numerous tumors and metastases. We found, however, that a high concentration of AFP does not imply the presence of a large tumor and metastases and, conversely, a low level of AFP does not mean that the tumor is small. Further, the concentration of AFP in the range of reference values does not mean that a tumor is not present. Our results show that they are in line with expectations the size of the tumor in 16.7% of patients did not change in 44.4% of the size decreased for 56.5%, and in 22.2% of those it was contrary to expectations, tumor size increased for 31.2%. During the treatment with Nexavar at a dose of 800 mg daily, the concentration of AFP dropped in 77.8%, while in 22.2% increased. We have found that in 11.1% of patients who discontinued treatment, AFP concentration increased, but as the treatment is continued, AFP level dropped.
Conclusions: Monitoring of serum tumor marker AFP in patients with HCC is very important. In patients before diagnosis of HCC is confirmed, its significance relates to the fact that the increased concentration of AFP arouse the suspicion of HCC, while in patients on treatment changing in AFP concentrations is important indicator of the success of the therapy.

W360

APPLICATION OF CALPROTECTIN, NEOPTERIN AND PENTRAXIN 3 IN DIAGNOSTICS OF ULCERATIVE COLITIS AND ASSESSMENT OF DISEASE ACTIVITY

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Background: The diagnosis of ulcerative colitis (UC) is based on the histopathology, endoscopy or X-ray, what makes it costly invasive and time-consuming.The aim of this study was to evaluate inflammatory markers in the UC diagnosis and assessment of disease activity.
Methods: 26 patients with UC (group I) and 44 controls(group II) were enrolled in the study.The UC patients were divided into active disease (group IA) and inactive disease (group IB) according to the Truelove-Witts index(assessing amount of defecation per day and systemic disorders).Serum CRP was measured using immunonephelometry(reagents from Siemens Healthcare Diagnostics) using the Nephelometer II Analyzer (Siemens Healthcare Diagnostics). Plasma calprotectin was measured using ELISA (Hycult Biotech, the Netherlands), plasma neopterin was measured using ELISA (IBL International, Germany) and plasma pentraxin 3 was measured using ELISA (R&D Systems, USA). All ELISAs were performed on the Biotek Elx 800 spectrophotometer(Biotek Instruments, USA).The result were not normally distributed and are presented as ranges, medians and quartiles.
Results: Serum CRP was significantly higher in group I than in group II (11.61 [6.08-27.20] vs. 1.18 [0.08-4.32] mg/L, P <0.0001). Similarly, plasma calprotectin was significantly higher in group I as compared with group II (82 [55-220] vs.42.5 [10-231] ng/mL, P=0.0106).Pentraxin 3 and neopterin levels did not differ significant between group I and II.Serum CRP and plasma pentraxin 3 were significantly higher in group IA than in group IB: 17.3 (11.6 – 42.6) vs.4,13 (2.20-7.16) mg/L, P=0.0003 and 1,10 (0.75-1.68) vs.0,43 (0.32-0.61) pg/mL, P=0.0005, respectively.No significant differences in calprotectin and neopterin levels between group IA and IB were found.The correlation between pentraxin 3 and CRP levels in group I was statistically significant (P <0.04, r=0.61). CRP(r=0.70; P <0.0001) and pentraxin 3 (r=0.68; P <0.0001) significantly correlated also with the severity of the UC.
Conclusion: It was found that CRP and calprotectin levels in patients suffering from UC were higher than in control group.Plasma calprotectin can be considered a useful biomarker in the diagnosis of UC.Both inflammatory markers, pentraxin 3 and CRP may be useful in treatment monitoring and detection of exacerbations.

W361

ACUTE–PHASE PROTEINS AS INDICATORS OF BACTERIAL INFECTION IN PATIENTS WITH CIRRHOSIS

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Background: Infections in the cirrhotic patients can have serious implications that vary from functional and quality of life decrements to an accelerating progression of the disease and increased risk of death. Early and accurate diagnosis of the infection is crucial in patients with liver impairment. One of the earliest signs of infection is the acute-phase response.
Methods. Serum markers of infection as C-reactive protein (CRP), Beta 2 microglobulin (B2-μg), Ferritin (Fer) and Haptoglobin (Hpt) were measured using immunoturbidemetric and immunophlorescence techniques.
Results: The present study was carried on 40 cirrhotic patients divided into 2 equal groups:Group I: 20 cirrhotics complicated by infections and Group II: 20 cirrhotics non complicated by infections. CRP(mg/L) ranged between 10.80-101.0 and 2.10-33.70 with the mean of 58.79±28.23 and 11.75±9.46 for infected and non infected groups respectively. Infected group have values statistically higher than non infected group, (P=0.001). Fer (ng/mL) ranged between 51.60-1018.0 and 42.0-682.0 with the mean of 647.42±338.71 and 242.43±179.04 for infected and non infected groups respectively. Infected group have values statistically higher than non infected group, (P=0.001). B2-μg (mg/L) ranged between 1.90-10.50 and 1.20-9.90 with the mean of 5.54±2.60 and 3.18±1.98 for infected and non infected groups respectively. Infected group have values statistically higher than non infected group. (P=0.001). Hpt (mg/dL) ranged between 0.02-282.0 and 0.20-123.0 with the mean of 67.15±87.84 and 45.47±41.46 for infected and non infected groups respectively. There were no statistical significant differences between the studied groups, (P=0.968).
Conclusions: These results showed that CRP, Fer and B2-μg were significantly increased when cirrhotic patients are affected by bacterial infections, irrespective of the underlying cause of cirrhosis. Also showed that CRP seems to be the best test to identify bacterial infection among cirrhotics, because of the statistically significant difference of its levels between the two groups, and its levels mostly not affected by the severity of liver cirrhosis.

W362

COMPARISON OF IMMUNOLOGICAL CHANGES INDUCED BY THERAPIES IN IMMUNE-MEDIATED GASTROINTESTINAL DISEASES BY FLOW CYTOMETRIC PHENOTYPE ANALYSIS OF PERIPHERAL BLOOD LYMPHOCYTES

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Background: The research was aimed at studying the immunological alterations and their association with different therapies in immune-mediated gastrointestinal diseases. Coeliac disease (CD) is an autoimmune disorder of the small intestine, which occurs in genetically predisposed people, showing a reaction to gliadin (gluten) found in wheat and has been linked with a number of conditions. Inflammatory bowel disease (IBD) refers to ulcerative colitis and Crohn's disease, sometimes also present with extra-intestinal manifestations (such as liver problems, arthritis, skin manifestations and eye problems). The treatment of CD is a gluten-free diet. A variety of medications are available in IBD, several of them induce immunological alterations.
Methods: Anti-gliadin, anti-tissue transglutaminase and anti-endomysial antibodies were measured in CD, presence of anti-saccharomyces cerevisiae and anti-neutrophil cytoplasmic antibodies (MPO, PR3) was tested in IBD to specify disease groups. Proportions and absolute numbers of main lymphocyte subgroups: cytotoxic, helper and regulatory T cells; B1 and B2 B cells; NK and NKT cells were determined, activation and memory markers were also tested with flow cytometry. Disease related differences were analyzed first, followed by the comparison of groups created according to the given therapy: none, 5-asa compounds, immunosuppressive drugs, biological therapy.
Results: Phenotypic analysis of peripheral blood lymphocytes showed several significant differences among the examined diseases. The majority of them could be related mainly to the use of immunosuppressive drugs, which provoked further dissimilarities. The combination of the immunosuppressant with biological therapy moderated the alterations. No significant differences were found in lymphocytes phenotypes of patients receiving 5-asa compounds, biological therapy or no medication at all.
Conclusions: Detailed phenotypic analysis of peripheral blood lymphocytes according to the clinical picture, considering the different therapies applied could facilitate better understanding the immunological differences among these diseases, aid in precise diagnosis and help to evaluate usefulness of available medications.

W363

IL-6 AND TNF-A LEVELS IN CHRONIC HEPATITIS B PATIENTS

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Objectives: The aim of this work was to measure serum IL-6 and TNF-a levels in patients with chronic HBV infection and evaluate their correlation with AST, ALT and viral load.
Material and methods: We examined sera of 50 patients, 27 men and 23 women, aged 28-78 years, (mean 51,8 years). All patients were HBsAg(+)/anti-HBc(+)/anti-HBe(+) and HBVDNA(+) by real-time PCR. They were negative for other forms of viral hepatitis and HIV. AST and ALT levels were measured on the Siemens ADVIA 1800 automated analyzer. Measurement of viral load was performed by COBAS Taqman Amplicor (Roche). IL-6 and TNF-a levels were measured with Human Cytokine/Chemokine Panel I (Cat. No. MPXHCYTO-60K) Milipore Co, USA.
Results: AST levels ranged from 11,6 to 242,4 U/L (mean 31,0) and ALT levels from 19,9 to 319,1 U/L (man 36,8). IL-6 levels ranged from 1,433 to 16,123 pg/mL (mean 2,287). TNF-a levels ranged from 1,522 to 46,944 pg/mL (mean 7,112). Viral load ranged from 14,8 to 3650000,0 U/mL. High positive correlation was observed between IL-6 levels and AST (r=0,900), ALT (r=0,918) and viral load (r=0,911). Significant positive correlation was also observed between TNF-a levels and viral load (r=0,408).
Discussion: Our data show that serum IL-6 levels are correlated with serum AST and ALT levels, as well as with viral load, while TNF-a is correlated only with viral load. Both IL-6 and TNF-a play an important role in liver injury induced by HBV. Changes in their serum patterns are related with viral persistence, host immune response and liver damage.

W364

HIGH SEROPREVALENCE OF HUMAN HERPESVIRUS TYPE 8 IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background: Immunologic abnormalities and high seroprevalence of human herpesvirus type 8 (HHV-8) have been found in cirrhotics without human immunodeficiency virus (HIV) infection. Almost all hepatocellular carcinoma (HCC) patients are cirrhotic and have immunoabnormalities. However, the association between HCC and HHV-8 is unclear.
Methods: Blood samples from 97 HCC patients and 97 age- and sex-matched healthy controls were collected and analyzed for lymphocyte and monocyte counts, HHV-8 antibody and DNA, HBsAg, anti-HCV, and anti-HIV.
Results: The mean lymphocyte and monocyte counts were extremely less and greater in HCC patients than in healthy controls, respectively (P < 0.0001, both). The seropositive rate of HHV-8 antibodies in HCC patients was significantly greater than in healthy controls, particularly in male patients, those with Child–Pugh class B cirrhosis, HBV infection, or monocytosis, and those without lymphopenia (P=0.0002, <0.0001, =0.0003, <0.0001, =0.0003, and <0.0001, respectively). Seropositive male patients were significantly younger than seropositive female patients (P=0.0186). Antibody titers for HHV-8 in patients also significantly exceeded those in healthy controls (P <0.0001). The mean lymphocyte count was significantly less in seronegative than in seropositive patients (P=0.0216). All participants were negative for anti-HIV; only one female HCC patient positive for HHV-8 antibody was positive for HHV-8 DNA (340,800 copies/mL).
Conclusions: High seroprevalence of HHV-8 in HCC patients was discovered, comparable to that in cirrhotics in our previous study, and associated with sex, age, and lymphocyte counts, and seemed to be independent of tumor stages.

W365

EFFECT OF PIOGLITAZONE, QUERCETIN AND HYDROXY CITRIC ACID ON VASCULAR ENDOTHELIAL GROWTH FACTOR MESSENGER RNA (VEGF MRNA) IN EXPERIMENTALLY INDUCED NON ALCOHOLIC STEATOHEPATITIS (NASH)

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Background: Non-Alcoholic Fatty Liver Disease (NAFLD) consists of a spectrum of liver diseases ranging from simple fatty infiltration to progressive fibrosis. Non-Alcoholic Steatohepatitis (NASH) is a severe form of NAFLD, which progresses to the end stage liver disease. The prevalence of NAFLD in Indian population is estimated around 7 – 13%. Vascular endothelial growth factor (VEGF) attributes to various ischemic and inflammatory diseases, in particular it plays an important role in the development of liver fibrosis and hepato carcinogenesis in NASH. In this study, the comparative effect of pioglitazone, quercetin and hydroxy citric acid on VEGFmRNA in experimentally induced NASH has been studied.
Methods: The experimental protocol consists of 5 groups viz. Control (n = 6); NASH Induced (n=6); NASH + Pioglitazone (n=6); NASH + Quercetin (n=6); NASH + Hydroxy Citric Acid (n=6). Quantitative real-time polymerase chain reaction (RT-PCR) analysis of vascular endothelial growth factor (VEGF) messenger RNA (VEGF mRNA) was analyzed in all the groups.
Results: High expression of VEGF mRNA in hepatic cells was observed in experimentally induced NASH group when compared to the expression of VEGF mRNA in control group. Very mild increase in the expression of VEGF mRNA was observed in experimental NASH treated with quercetin. Where as mild increase in the expression of VEGF mRNA was observed in experimental NASH treated with pioglitazone and in experimental NASH treated with hydroxy citric acid. The drug quercetin showed an effective inhibition of VEGF mRNA expression and perhaps only smaller inhibition of VEGF mRNA level seen in hydroxy citric acid and pioglitazone treated rats.
Conclusion: By virtue of our findings, it could be concluded that the drug quercetin showed an effective inhibition of VEGF mRNA expression and perhaps only smaller inhibition of VEGF mRNA level seen in hydroxy citric acid and pioglitazone treated rats. This study showed the therapeutic value of quercetin, pioglitazone and hydroxy citric acid.

W366

BIOLOGIC VARIATION OF COPPER, CERULOPLASMIN AND COPPER/CERULOPLASMIN RATIO IN SERUM

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Background: Diagnostic algorithms for Wilson's disease recommend the determination of serum ceruloplasmin (Cp), while measurements of total serum copper (Cu) and/or Cp-unbound ("free") Cu are of value in monitoring pharmacotherapy. Despite their clinical role, aspects related to biologic variation (BV) of Cp, total and "free" Cu have not received enough attention, even because the direct measurement of "free" Cu is still difficult and not routinely available. To overcome this issue, the use of Cu:Cp ratio has been proposed. Here we performed an assessment of BV components of total Cu, Cp and Cu:Cp by an accurately designed protocol.
Methods: We collected five blood specimens from each of 19 healthy volunteers (10 men, 9 pre-menopausal women) every 2 weeks for 2 months. Serum specimens were stored at –80 °C and analyzed in a single run in duplicate using a colorimetric (Cu) and an immunoturbidimetric (Cp) assay on Roche Cobas c501 platform. Cu:Cp was calculated as Cu (μmol/L) x 0,132/Cp (g/L). Cochran's test and Reed's criterion were employed for outlier identification and Shapiro-Wilk test was used to check data distribution. Data were analyzed by ANOVA.
Results: Cu:Cp was slightly higher (P=0.02) in women than in men, whereas no sex-dependent difference was found for Cu and Cp concentrations. Intra- and inter-individual CVs were 5.8% and 14.5% for Cu, 6.2% and 14.4% for Cp, and 2.4% and 3.8% for Cu:Cp. Only Cu:Cp showed an index of individuality >1. Therefore, if classical reference intervals have little use in the interpretation of serum Cu and Cp results, they are useful for interpretation of Cu:Cp values. An average reference change value of 19% for both Cu and Cp can be assumed as a figure to guide clinical decision making. Desirable analytical goals for imprecision (as CV), bias and total error were ≤2.9%, ±3.9% and ±8.7% for Cu, and ≤3.1%, ±3.9% and ±9.1% for Cp, respectively.
Conclusions: The smaller variability of Cu:Cp, when compared with those of Cu and Cp, is fitting with the reason of its clinical use directed to minimize variations in each of the two analytes. However, Cu:Cp has the potential weakness to behave differently depending on the Cp assay, as the measurement of this protein is not standardized.

W367

VALIDATION OF COOPSCORE AS A SCORE-BASED BLOOD TEST FOR LIVER FIBROSIS IN HIV-HBV CO-INFECTED PATIENTS

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Background: Non invasive methods for the assessment of liver fibrosis are increasingly used as an alternative to liver biopsy. Recently, a score-based blood test: Coopscore, including alpha-2-macroglobulin, apolipoprotein-A1, AST, collagen IV and osteoprotegerin, has been developed on a chronic hepatitis C (CHC) cohort. Coopscore showed higher diagnostic performances than Fibrometer®, Fibrotest®, Hepascore® and FibroscanTM (Bosselut N. Clin Chim Acta. 2013). Here, we assess the Coopscore on an independent cohort of HIV/HBV co-infected patients in order to validate this performance.
Methods: Ninety-seven HIV/HBV co-infected patients were enrolled from a previously described cohort (Bottero J. J Hepatol. 2009). METAVIR histological fibrosis stage was used as reference. Blood samples had been collected close to liver biopsy (less than 6 months), and were analyzed retrospectively for osteoprotegerin and collagen IV measurement. Fibrotest®, Fibrometer® and Hepascore® were computed as previously described; Obuchowski index and ROC curve analysis were used to compare Coopscore with these tests.
Results: The Obuchowski index was higher for Coopscore than for other scores reflecting a better ability to discriminate between fibrosis stages (0.840 vs 0.808, 0.791, 0.807 respectively for Fibrometer®, Hepascore® and Fibrotest®). The AUROC value was greater for Coopscore than for other tests, especially for significant fibrosis (0.836 vs 0.790, 0.727, 0.778 respectively for Fibrotest®, Fibrometer® and Hepascore®), though the differences did not reach significance.
Conclusions: This study assesses the diagnosis accuracy of Coopscore in HIV-HBV co-infected patients and suggests that Coopscore could lead to a better diagnosis of significant fibrosis than other blood tests. However, results failed to reach significance possibly due to the small sample size and should be confirmed on a largest cohort.

W368
VISCERAL ADIPOSITY INDEX IN NONALCOHOLIC FATTY LIVER DISEASE: ASSOCIATION WITH HEPATIC AND SYSTEMIC INFLAMMATION

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Background: Nonalcoholic fatty liver disease (NAFLD) represents the most common hepatic disorder in western countries. It is now well accepted that NAFLD is the hepatic manifestation of the metabolic syndrome (MetS) and is associated with insulin resistance, visceral obesity and dyslipidaemia. Visceral Adiposity Index (VAI), a novel marker of visceral fat dysfunction, showed a strong association with insulin resistance and visceral adipose tissue. We aimed to investigate the relationship of VAI with hepatic and systemic inflammation in subjects with NAFLD.
Methods: The consecutive 215 patients with histologically proven NAFLD were enrolled the study. Plasma levels of adiponectin, tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured by ELISA method. High sensitive C reactive protein (hs-CRP) were measured by immunoturbidimetric method. Insulin resistance (IR) was assessed by homeostasis model assessment-estimated insulin resistance (HOMA-IR) index.
Results: High GGT (P=0.04), high total cholesterol (P <0.001), high triglyceride (P <0.001), low HDL (P <0.001) and presence of MetS (P=0.04) were associated with higher VAI, although only higher GGT (P=0.02), and TC (P <0.001) were independent factors on multiple linear regression analysis. On the other hand, no significant association was found between VAI, hepatocellular ballooning, lobular inflammation and fibrosis. In the 101 patients assessed for adipocytokines, no significant association was found between VAI and adiponectin, TNFa, IL-6 and hsCRP levels.
Conclusions: The results of this study demonstrate that VAI is not associated with the severity of hepatic and systemic inflammation in NAFLD.

W369
ASPARTATE TRANSAMINASE'S REFERENCE RANGE REVISITED?

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Background: 12,000 people died from liver disease in the UK in 2009. Liver disease is the only major cause of death in the UK still increasing on a yearly basis. This increase is fuelled mostly by obesity and excessive alcohol consumption, which costs the NHS around £6 billion each year. If diagnosed early, liver disease can be treated at a far lower cost and with greater success. Currently liver function tests (LFTs) including AST as indicators of liver disease have poor predictive value. Modulating the reference range for AST should help identify liver disease at an early stage. We now wish to reassess the current reference range for AST by minimising the effect of alcohol consumption using serum carbohydrate deficient transferrin (CDT). The measurement will at least partially exclude the effect of alcohol abuse on AST levels. The clinical utility of AST in the diagnosis of fibrotic liver disease may be increased.
Methods: AST and ALT were measured using routine lab methods on the Abbott Architect Ci8200. CDT was measured using the Sebia method on capillary electrophoresis. The results were analysed using "Minitab" software. Non Gaussian data was compared using the Mann Whitney test-P<0.05 was considered statistically significant.
Results: AST activity in patients with normal CDT levels <1.7% are lower than those in patients with high CDT levels >1.7% P <0.000. The upper limit of AST is 30 IU/L at CDT levels <1.7%. The manufacturer's upper limit is 35 U/L.
Conclusions: Alcohol abuse as assessed by raised CDT levels is consistent with higher AST activity which is still within the accepted reference range. This suggests that the current reference range for AST is too wide to be useful in diagnosing liver disease at mild or early stages. This observation is in keeping with the observation that 15% of patients with hepatic fibrosis have AST activity within the conventional normal range. Alcohol is not the only factor affecting AST levels; other factors such as drugs, pollutants, viruses and diabetes are important. After further work, the reference range for AST should be reassigned to include the above co-founders.

W370

EVALUATION OF HEPATIC FIBROSIS RISK WITH SOME NONINVASIVE SERUM MARKERS IN PATIENTS WITH CHRONIC HEPATITIS B

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Background. It is important to determine early stage fibrosis on behalf of preventing the formation of liver cirrhosis. Although liver biopsy is the gold standard for diagnosis of liver fibrosis, many noninvasive markers are being investigated. We therefore evaluated the diagnostic value of serum transforming growth factor-beta1 (TGF-β1), tissue inhibitor of metalloproteinase (TIMP-1), fibroblast growth factor-21 (FGF-21), fetuin-A levels and noninvasive fibrosis models, including aspartate aminotransferase to platelet ratio index (APRI), FIB-4 and Forns index, to predict the extent of liver fibrosis and to compare their diagnostic potential in patients with biopsy-confirmed fibrosis due to chronic hepatitis B (CHB).
Methods. Seventy three patients with biopsy-confirmed different stages of fibrosis were included in this study. Patients were divided into two groups according to METAVIR stage when fibrosis stage ≥ F2. Serum levels of TGF-β1, TIMP-1, FGF-21 and fetuin-A were measured by using enzyme-linked immunosorbent assay (ELISA). APRI index, FIB-4 and Forns index were calculated from published formulas. The area under receiver operating characteristics curve (AUROC) was determined. Sensitivity, specificity, positive predictive value and negative predictive value were determined according to the optimal cut off points.
Results. No significant difference was reported in serum concentrations of TGF-β1, TIMP-1, FGF-21 and fetuin-A between two groups (P >0.05). APRI, FIB-4 and Forns index were significantly higher in patients with significant fibrosis (P <0.05). The AUROC of TGF-β1, TIMP-1, FGF-21, fetuin-A were 0.445, 0.483, 0.595 and 0.436 respectively, APRI, FIB-4 and Forns index were 0.662, 0.687 and 0.680 respectively. For predicting fibrosis of F2 or more, Forns index cut-off point of 4.05 had a highest sensitivity (75.6%) and FIB-4 index cut-off point of 1.085 had a highest specificity (62.5%) among noninvasive fibrosis models.
Conclusions. These data suggest that serum TGF-β1, TIMP-1, FGF-21 and fetuin-A were not predictive for the extent of liver fibrosis in CHB. APRI, FIB-4 and Forns index have a better diagnostic value in patients with significant fibrosis than those with no/minimal fibrosis.

W371

THE RELATIONSHIP BETWEEN OXIDATIVE STRESS AND ISCHEMIA IN INFLAMMATORY BOWEL DISEASES

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Background: Ulcerative colitis (UC) and Crohn's disease (CD) are two distinct forms of inflammatory bowel diseases (IBD). Intestinal ischemia and oxidative damage have been known to play a part in the pathogenesis of IBD. The purpose of the present study was to evaluate the relationship between serum ischemia modified albumin levels and serum natural antioxidants including total bilirubin, uric acid and GGT levels.
Methods: In 36 ulcerative colitis, 30 Crohn's disease and 19 control subjects serum levels of IMA were determined manually using a spectrophotometric Co(II)-albumin binding assay method. Total bilirubin, uric acid and GGT levels were measured by routine methods.
Results: Median(min-max) GGT levels were 21(7-221)U/L, 16(5-111) U/L and 14(2-41) U/L in UC,CD and control subjects, respectively. GGT levels of ulcerative colitis patients were significantly higher than normal control subjects ($p<0.05$). There was no statistically significant difference in concentrations of other parameters between UC ,CD and control groups.
Conclusions: We suggest that serum GGT levels in patients with UC might have been increased to protect the effect against oxidative stress. There was no significant difference between CD and control subject in regard to GGT levels ($p>0.05$). However, GGT levels in patients with CD were higher than control subject levels. This data may explain why IMA levels did not increase in patients with IBD. However, further investigations are required with larger sample size in this line.

W372

AFP-L3 - SCREENING MARKER FOR A HEPATOCELLULAR CARCINOMA IN PATIENTS WITH ALCHOCOLIC CIRRHOSIS

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Background: Hepatocellular carcinoma (HCC) is the most common complication of liver cirrhosis. Screening of patients with cirrhosis is necessary to confirm the diagnosis of HCC in an early phase of the limited and small tumor. The recommended monitoring strategy in patients with cirrhosis is still based on ultrasound examination and determination of α-fetoprotein (AFP) in the serum. The limitations of ultrasound diagnostics are the inability to distinguish HCC from hemangioma and cirrhotic nodules, limited sensitivity to detect small tumors as well as the impact of human factor. On the other hand, AFP tumor marker has a low specificity. Considering that, it is necessary to find and evaluate new serum and molecular markers for monitoring high risk population as well as for early diagnosis of HCC. The aim of this study was to evaluate whether the determination of AFP-L3 isoforme can improve early non-invasive diagnosis of HCC.
Materials and Methods: Sera samples from 32 patients with HCC with a background of alcoholic liver disease (ALD) as well as 28 sera samples from age adjusted alcohol related cirrhosis control group, were assessed by specific ELISA assay for AFP-L3 (Cusabio Biotech CO., LTD. Wuhan, P.R.China). AFP concentrations were determined using Cobas e411 (Hitachi High Technologies Corporation, Tokyo, Japan) analyzer. The diagnostic accuracy of each biomarker was evaluated using receiver operating characteristic (ROC) curve analysis reporting the area under the curve (AUC) and its 95% confidence interval (CI).
Results: Our results showed that AFP concentration of 14.62 ng/mL distinguishes patients with HCC and patients with liver cirrhosis with diagnostic sensitivity of 68,75% and 100% diagnostic specificity. AFP-L3 concentration of 4.26 ng/mL distinguishes tested groups with diagnostic sensitivity of 75% and 100% diagnostic specificity. AUC (95% CI) for AFP was 0.888 (0.781-0.955), and for ALP-L3 0.929 (0.832-0.979).
Conclusion: Our study indicates that AFP-L3 is an improvement in HCC screening of high risk ALD patients in relation to AFP due to better diagnostic sensitivity and higher AUC.

W373

ELEVATED CARDIAC MARKERS ARE ASSOCIATED WITH HIGHER MORTALITY IN PATIENTS AFTER TRANSJUGULAR INTRAHEPATIC PORTOSYSTEMIC SHUNT INSERTION

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Background: It has been known for more than four decades that liver cirrhosis is associated with cardiovascular abnormalities. Transjugular intrahepatic portosystemic shunts (TIPS) have become widely accepted tool in the treatment of patients with symptomatic portal hypertension. Aim our study was to assess the value of cardiac markers before and after TIPS insertion for the prediction of 1-year mortality in cirrhotic patients.
Methods: Biochemical markers were measured before and 24 hours after TIPS. Cardiac troponin T (hs-cTnT) was tested by high-sensitivity immunoassay (Roche Diagnostics). Concentrations of other cardiac markers were determined by the Evidence Investigator protein biochip system (Randox Laboratories, Crumlin, UK). The cardiac array included the following markers: creatine kinase MB isoenzyme (CKMB), myoglobin (MYO), glycogenphosphorylase BB isoenzyme (GPBB), and heart type of fatty acid binding protein (FABP). The study population consisted of 55 consecutive patients (38 men and 17 women, age 55.6 ± 8.9 y) with liver cirrhosis treated with elective TIPS.
Results: Twenty-four hours after the TIPS procedure, we found a significant elevation in serum GPBB in comparison with pre-procedural values ($P < 0.001$). There was an association between concentrations of cardiac markers (pre-procedural hs-cTnT, FABP and post-procedural of MYO, hs-cTnT, FABP) and overall survival.
Conclusions: Measurement of cardiac markers, mainly hs-cTnT and FABP, may be useful for mortality prediction in cirrhotic patients after TIPS.

W374

EFFECT OF GARCINIA MANGOSTANA LINN PERICARP EXTRACT ON CYCLOOXYGENASE – 2 (COX – 2) ENZYME EXPRESSION IN DIETHYL NITROSAMINE (DEN) INDUCED HEPATOCELLULAR CARCINOMA IN EXPERIMENTAL RATS

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Background: Diethyl Nitrosamine (DEN), an hepatocarcinogen is known to cause perturbations in nuclear enzymes involved in DNA repair and replication and induces hepatocellular carcinoma in experimental animal models. Garcinia Mangostana Linn pericarp extract has abundant source of xanthones which neutralizes free radicals. Cyclooxygenase is the rate limiting enzyme involved in the conversion of arachidonic acid to prostaglandin, the precursor of important inflammatory mediators. In this study, the effect of Garcinia Mangostana Linn pericarp extract on COX – 2 enzyme expression in DEN induced hepatocellular carcinoma in experimental rats was studied.
Methods: The experimental protocol consists of 4 groups viz. Group I: Control rats (n=6); Group II: DEN Induced hepatocellular carcinoma (n=6); Group III: DEN alone was administered for 4 weeks and pericarp extract of Garcinia mangostana Linn treated (n=6); Group IV: Pericarp extract of Garcinia mangostana Linn alone treated (n=6). Immunohistochemistry analysis of COX – 2 enzyme expression was analyzed in all the above groups.
Results: High expression of COX – 2 was observed in experimentally induced hepatocellular carcinoma group (Group II) when compared to the expression of COX -2 in control group (Group I). Less expression of COX -2 was observed in DEN and Garcinia mangostana Linn pericarp extract treated group (Group III). No expression was observed in Garcinia mangostana Linn pericarp extract alone treated group (Group IV).
Conclusion: COX – 2 expressions may contribute to liver damage and tumor genesis in animal models. It may contribute to inflammation mediated tumor development. Mangostins can inhibit COX -2 expression activity which supports the present findings.

W375

EFFECTS OF AMINO ACIDS ON REVERSAL OF AMINOOXYACETATE-INDUCED METABOLIC DISTURBANCE

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Background: Malate-aspartate (MA) shuttle, locating on the inner membrane of mitochondria, is essential for maintaining the cellular bioenergetic states via the redox and transaminase reactions. Aspartate aminotransferase (AST) is required for the balances of the amino acids participating in the shuttle. In cytosolic compartment, NAD+ is formed to carry reducing equivalents into mitochondria. Therefore, the impairment of the MA shuttle would cause metabolic disturbance and dysfunction of mitochondria, leading to cell damage. Thus, we examine the effects of Aminooxyacetate (AOA), an aminotransferase inhibitor, on HepG2 cells; and investigate whether the treatment of amino acids would reverse the changes.
Methods: The activities of AST were analyzed to assess the inhibitive effects of AOA. Concentrations of metabolites including aspartate, glutamate, malate, α-ketoglutarate, citrulline, NAD+ and NADH were measured by a liquid chromatography tandem mass spectrometry (LC-MS/MS). The integrity of mitochondrial membrane potential (MMP) was evaluated by flow cytometry with Rhodamine dye.
Results: AOA inhibited AST activity in a dose-dependent manner (0.25-5.0 mM, $P < 0.0005$ for trend analysis). In addition, 1 mM AOA treatment decreased intracellular aspartate (mean value 13.62 vs. 7.54 μmol/g, $P < 0.0005$) and malate (21.99 vs. 5.37 μmol/g, $P < 0.0005$) and increased glutamate (102.13 vs. 147.19 μmol/g, $P < 0.005$). The ratio of aspartate to glutamate and malate to α-ketoglutarate in cells were decreased (0.14 vs. 0.05 and 3.22 vs. 0.53, respectively, $P < 0.0005$). Furthermore, 1 mM AOA treatment increased the intracellular ratio of NADH to NAD+ (0.83 vs. 0.76, $P < 0.005$) and caused the disruption of MMP ($P < 0.0005$). Supplement of specific amino acids to AOA-administrated cells changed the concentrations of metabolites. The supplement of pyruvate reversed the elevation of NADH to NAD+ ratio. And glycine treatment protected cells from the disruption of MMP.
Conclusions: The treatment of pyruvate and glycine may protect cells against the AOA-induced cell damage.

W376
TO MEASURE OXYGEN AT THE LEVEL REQUIRED BY THE GOLD DIRECTIVE

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Changing point of care instrumentation can be challenging. A change from calculated to measured saturation caused trouble in decision-making because calculations are not entirely reliable in combination with a drift in the measurement of partial pressure over time.
Background: Pulmonary disease often leads to decreased ability to maintain saturation at a required level for acquired physiological function. For chronic obstructive pulmonary disease a guideline called the GOLD Initiative (Global Initiative for Chronic Obstructive Lung Disease) makes recommendations about when oxygen therapy should be initiated. The recommendations includes parameters such as pO2 and sO2 in levels that are difficult to measure (sO2 <88%, pO2 <7.3). We experienced these problems when changing bloodgas instrumentation (Radiometer ABL77 to Radiometer ABL 80) at the pulmonary ward at one of our hospitals - a switch from calculated to measured saturation.
Methods: The connection between saturation and partial pressure of oxygen was studied. Measured values of saturation from unidentified patients with pulmonary disease was compared to calculations of saturation from partial pressure using i) the inbuilt calculation in the ABL77 instrument, ii) the Siggaard-Anderssen algorithm and iii) an algorithm published by Severinghouse.
Results: Data plotted as saturation vs partial pressure of oxygen (oxygen dissociation curve) showed deviations in the area below 90% of saturation in the calculated results. The data showed an overestimation of saturation when calculated and a small variation in measured partial pressure of oxygen resulted in deviation of calculated saturation.
Conclusion: We concluded that measured saturation gives a more accurate value of saturation in the required area since the algorithms does not include all important variables such as 2,3-DPG in combination with an observed drift of pO2 measurement stability over time.

W377
ISO 22870 ACCREDITATION OF BLOOD GAS POINT-OF-CARE TESTING (POCT)IN A FRENCH MEDICAL LABORATORY: A 2-YEAR EXPERIENCE

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Background: In January 2010, a new legislation was implemented in France: among various requirements, the new law makes accreditation according to ISO 15189 and 22870 (POCT activities) mandatory for medical laboratories. Cofrac is the independent organism in charge to authenticate the competences of the medical laboratories in France.
Methods: The public medical laboratory of the Saint-Antoine Hospital in Paris has 3 blood gas analyzers in 3 different locations. We present our 2-year experience of POCT accreditation. To achieve this goal, we focused on specific requirements of the ISO 22870 standard, compared to the ISO 15189 requirements (quality policy, process control, creation of a point-of-care coordination group…), as well as additional specific requirements of the French legislation (external quality control program, a posteriori biological validation of results).
Results: Late 2010, a 1.5-day initial audit was carried out by 2 Cofrac evaluators. The audit of point-of care activities revealed few gaps and appropriate answers (under brackets) were sent to Cofrac: 1) the name of the quality responsible for POCT was not specified (now added in the quality manual) ; 2) the way the Cofrac logo will be used on laboratory reports was not specified (a procedure is written) ; 3) habilitations were not sufficiently formalized (new procedures are written) – 4) there was no external quality control for CO-oxymetry (a new program began in January 2011) – 5) some documents should be integrated to the documentary system (these documents are now codified and integrated). POCT accreditation was finally obtained in 2011. One year after this initial audit, Cofrac evaluators came back for the first follow-up audit: our accreditation was renewed after correction of 3 minor gaps. Moreover, accreditation was extended to a new blood gas location previously accredited according to ISO 17025
Conclusions: This was the first experience of an ISO 22870 accreditation of blood gas in France. Extension of accreditation to other POCT activities (cardiac markers, glycated haemoglobin...) is scheduled before 2016. France is the first country to take such drastic measures to give medical laboratories recognition of their practices.

W378

COMPARISON BETWEEN ABL837® RADIOMETER AND DIMENSION VISTA1500® SIEMENS FOR CREATININE MEASUREMENTS

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Background: Routine creatinine testing is mandatory before radiological examinations (CT scans/MRI). To decrease the turn around time, a point of care test for creatinine will be implemented on the blood gas analyser ABL837® in the emergency department (ED) of Bichat hospital. We thus compared the enzymatic creatinine assay of ABL837® to the routine methods used in the central laboratory on Dimension Vista1500®.
Methods: 141 heparinised whole blood samples, collected from patients with different degree of renal failure were first measured on the ABL® and following centrifugation plasma samples were assessed on Vista® using both the enzymatic (E) and the colorimetric Jaffe (J) methods. Comparison between methods was performed using Passing-Bablok regression and Bland-Altman analysis.
Results: All methods showed a good precision (between-assay CV <5%). Creatinine results from ABL® and Vista® were statistically different (ranges [26-804], [20-792] and [33-848] µM for ABL®, Vista-E and Vista-J respectively; P <0.0001). but highly correlated (ABL= 1.048Vista-E + 1.984; r^2= 0.996 and ABL= 0.960Vista-J - 2.091, r^2= 0.996). A mean positive bias of 5.5 +5.1% with ABL837® was found compared to Vista-E. This positive bias was 7.2 + 7.1% when creatinine <90 µM, cut-off for renal failure (n=77) and 5.4 + 5.2 % when >90 µM. As expected, a mean negative bias of -4.9 9+ 5.9% was observed as compared to Vista-J. The bias between enzymatic methods was similar in hemolysed (plasma haemoglobin range [60-200] µM; n=54) and non hemolysed samples (<60 µmol/L) suggesting the lack of interference of hemolysis. As opposed when compared to Vista-J, bias was different in hemolysed (-2.12 + 13.03) and in non-hemolysed samples (-8.03 + 7.09). No influence of hematocrit was found. Based on creatinine measurements in the central lab by Vista-J, there was no misclassification of patients with renal failure.
Conclusions: Although creatinine results from ABL837® showed a positive bias compared to the routine Vista-E method, the specificity of this enzymatic method allowed a confident use especially when hemolysis is present on whole blood. Further, the good practicability is suitable for the use in the ED. The immediate availability of the result might improve patient care.

W379

A MICROFLUIDIC UNIT FOR FAST DETECTION OF BACTERIAL DNA AS MENINGITIDIS DIAGNOSTIC AMENDMENT

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Shifting of demand for analysis from central laboratories to the point-of-need-politics, enhances the need for simplified but functional disposable test units. A miniaturized diagnostic set-up was realized by microfluidic structures in disposable plastic modules. Fluidic transport needed for dilution, supply with reactive components and sample flow is directed by valves and pumps automatically. After isolation of genomic nucleic acid is performed, the microfluidic structure guides the sample to 4 heating zones. The amplification mix for polymerase chain reaction (PCR) is pushed through the microfluidic channel, thus passing the denaturing zone, the annealing zone, and the amplification zone repeatingly. Capturing molecules are printed as arrays in the last part of the PCR chip channel. This part is warmed to room temperature. Fluorescence labelled amplicons created during PCR are bound when they pass the array. To enhance binding probability, capturing oligonucleotides are printed in a three dimensional gel matrix. Results get visible as a line of fluorescent dots. Assay sensitivity was monitored in clinical samples spiked with meningitis inducing bacteria (Neisseria meningitidis, Streptococcus ssp. (group B)). The conventional method of silica bead binding for DNA uptake and recovery was adapted to the laminar flow conditions present in the small channel volumes. The flow through procedure was evaluated by the comparison of DNA extraction efficiency, DNA quality, handling time and material costs. In a cavity with up to 120 µL volume, 1x10exp9 beads with 1 µm diameter were sufficient for the quantitative uptake of 2x10exp2 to 4x10exp4 bacteria flushed in in 100µL blood or liquor. Increasing the number of beads was not necessary for optimal PCR success. In opposite, high numbers in beads resulted in the inhibition of PCR if high numbers of bacteria were present as well. Positive test range was 1x10exp2 to 1x10exp9 bacteria in 100 µL sample. Detecting meningitis inducing bacteria as automated sample processing of liquor or blood culture is supposed to give useful additional information with clinical value.

W380

COMPARATIVE ASSESSMENT OF THE PERFORMANCES OF THE STATSTRIP POINT-OF-CARE TESTING DEVICE FOR THE MEASUREMENT OF KETONE BODIES

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Background. Ketone bodies are produced by fatty acid metabolism in hepatic mitochondria during a range of physiological and pathological conditions. Their monitoring can be useful in critically ill patients and a POCT device could be an efficient solution provided a good quality of the obtained results. Aim of this evaluation is to assess the precision, linearity, method comparison, interference studies and reliability of a new handheld POC glucose/ketone device: the StatStrip bedside meter (Nova and Menarini) specifically designed for use in the hospital settings.
Methods. Whole blood lithium heparin specimens were collected and spiked, when necessary, with beta-hydroxybutyrate 0.5 mol/L. Precision, linearity, method comparison and interferences studies were performed according to CLSI EP5, EP17, EP9 and EP7 respectively. The effect of hematocrit was evaluated. All the experiments were performed with two different lots of strips and in comparison with both a competitor POCT system (Abbott Optium Free Style) and a manual spectrophotometric measurement based on an enzymatic reagent (Randox Ranbut).
Results. The following results were obtained for StatStrip and Optium Free Style (in brackets). Overall CV <10.2%; linearity: both systems appear linear up to the maximum concentration tested (6.5 mmol/L); method comparison: StatStrip=1.009 manual method + 0.16 mmol/L, r^2=0.9815; Optium Free Style =1.365 manual method -0.28 mmol/L, r^2=0.9794; interferences: an interference of less than -10% (-8%) was found for acetaminophen 0.66 mmol/L, an interference of about -10% (+10%) was found for ascorbic acid 0.29 mmol/L, an interference of less than +10% (+8%) was found for acetoacetate 10 mmol/L. Hematocrit variation from 22% to 66% caused an apparent maximum change of +0.3 (-0.9) mmol/L of ketone bodies each 10% hematocrit increase at a concentration level of about 4.5 mmol/L.
Conclusions. The StatStrip analyser showed a good reproducibility at all the concentration levels, excellent comparability with the manual method, high linearity and robustness versus interferents in a wide range of hematocrit variation. A lower bias versus the reference method and a superior immunity to hematocrit variation was observed in comparison with the Optium Free Style.

W381

EVALUATION OF AN ADVANCED BLOOD GAS ANALYSIS SYSTEM IN A CENTRALIZED LABORATORY

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Background: The aim of this work is to demonstrate that analytical performances of results obtained from using an advanced blood gas analysis system can be qualitatively aligned with lab reference systems, while also ensuring a considerable decrease in major pre-analytical errors, which are most commonly to be found at POCT sites.
Methods: The following analyzers were used for this evaluation: Radiometer Medical ABL 825 FLEX evaluation analyzer (spectroscopy for ctHb and potentiometry for electrolytes); Horiba ABX Pentra DX 120 reference analyzer (absorbance for ctHb); Beckman Coulter AU 480 reference analyzer (ISE – Ion Selective Electrode for electrolytes). For ctHb correlation, analyses were carried out by running 43 successive patient samples, obtained from various departments and measured randomly on both analyzers. Evaluation of within-run repeatability for glucose and electrolytes (K+, Na+, Cl-) was performed by replicate analyses of a sera pool repeated 21 times.
Results: The result obtained from hemoglobin correlation test yielded an R2 index=0.9298, as compared to the reference analyzer. Results from within-run repeatability test for glucose and electrolytes yielded following results: ABL825FLEX showed CV% =1.022 K+, 1.043 Na+, 0.896 Cl-, 1.480 Glucose; AU480 showed CV% =1.168 K+, 0.265 Na+, 0.265 Cl-, 1.129 Glucose.
Conclusions: The results obtained confirm that the ABL825 FLEX system shows a good correlation with the reference analyzer, when evaluating hemoglobin, as a result of both combined safePICO sampling devices and built-in FLEXQ module. Analytical performances for evaluation of glucose and electrolytes obtained from point of care testing are in line with the analytical quality of performances obtained from laboratory instruments

W382
EVALUATION OF TRIAGE NT-PROBNP TEST AS POCT

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Background: The development of new technologies are contributing to the significant increase of techniques that can be performed as Point of Care Testing (POCT). Is unquestionable the need of clinical laboratory professionals in all aspects of the POCT development, including the evaluation and selection of methods. Elevated NT-proBNP levels are a sensitive and specific biomarker for congestive heart failure (CHF) and allowed physicians to differentiate between heart failure (HF) and lung disorders with similar symptoms. The test is also to be used as an aid for the risk stratification of patients with HF and acute coronary syndromes. The aim of this study is the evaluation of Triage®NT-proBNP Test, Biosite (fluorescence inmmunoassay, quantitative determination in whole blood and plasma) as POCT, by comparison with the method currently used in our laboratory, electrochemiluminescence assay, modular analytics E170 (Roche diagnostics).
Methods: We included 60 patients suspected of having CHF. The samples were analyzed in modular analytics E170 and Triage platform using plasma and whole blood samples respectively. Two groups were established: Group 1 (n=60): including all the samples; group 2 (n=46): including pro-BNP values between 0-6000 ng/mL, values that are clinically significant.Data were analyzed using Passing-Bablok regression and Bland-Altman plot, Method Validator 1.19
Results: Bland-Altman plots showed statistically significant differences in group 1. Passing-Bablok equation was: pro-BNP (Triage, group 1)= 0,874proBNP [modular analytics E170] -25,2; r=0,955; slope=0,874(95% CI 0,811 to 0,944); intercept=-25,2 (95% CI -58,2 to 43,4). And pro-BNP(Triage, group 2) =0,931pro-BNP [modular analytics E170] -43,7; r= 0,968; slope=0,931 (95% CI 0,863 to 1,025); intercept= -43,7 (95% CI -75,8 to 8,4).The statistic analysis of the results from both methods showed a good linearity and an excellent correlation in group 2.
Conclusion: The Triage®NT-proBNP Test can be used in clinical practice to assist clinicians to assessing patients with breath symptoms with suspected of CHF. It's a reliable, easy to use, fast and economic procedure and results can be integrated into the patient's history, so it could be excellent for the classification of risk patients and for implantation as POCT.

W383
ENRICHING IN VITRO DIAGNOSTICS POINT-OF-CARE TESTING (IVD-POCT) BY DEVELOPING APPROPRIATE SOFTWARE ON A CHARGE-COUPLED DEVICE (CCD) BASED DIGITAL MICROSCOPE

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Background: Bedside Microscopy is limited first, by the lack of acquaintance in Microscopy of the Clinicians in charge and second, by equipment restrictions, such as lack of portability, high costs due to the use of expensive optics and regular maintenance. The purpose of this paper is to present the development status of a system that expands in vitro Diagnostics Point-of-Care testing (IVD-PoCT) capabilities, as well as, enables bedside in vivo loci and in vitro samples' microscopy, as well as, morphological data acquisition and processing.
Methods: The system was developed by employing custom-made software and adjustments, on a commercially available portable, lightweight and extremely inexpensive Bresser USB-digital Microscope (Meade Instruments Europe GmbH & Co. KG), adapted thus, to operate also, as a reliable visual spectrophotometer. We have developed image processing and pattern recognition software tools, allowing for the minimization of the necessary prerequisite laboratory skills of the potential user. The Modulation Transfer Function (MTF) for each application is being thoroughly compared, after proper standard color-calibrations, with the corresponding performance of a custom developed reference microscope-spectrophotometer (UV-VIS-NIR) that combines an optical microscope and a highly sensitive spectrophotometer, as an external additional component, measuring the intensity of light vs. wavelength.
Results: As example, a bedside Hemoglobin (HGB) test is being developed, by comparing HGB-values of samples evaluated in a reference SEAC-HeCo Analyzer and in our system. 50 μL finger-tip blood diluted and lysed (4-16 % v/v) are introduced into a Plexiglas microscopy-slide, custom-engraved annularly (R: 3 mm, depth: 1.8 mm~50 μL) and the reflectance is evaluated. The system's accuracy is (15 read-outs of reference-blood Equinox 8 High) is quite acceptable < 2-5 % (V.C. %), depending on dilution (Analyzer: <1 %.).
Conclusions: The developed system offers an inexpensive approach towards merging microscopy and spectroscopy techniques with modern microfluidics and ensuring connectivity to digital medical records, expanding thus, bedside IVD-PoCT support of the treating clinical personnel.

W384
EVALUATION OF THE ADAPTABILITY OF THE ABL 90 IN THE MANAGEMENT OF THE POCT PROCESS

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Background: The blood gas analysis (BGA) in Point-of-care testing (POCT) technology has been increasingly used for the management of patients in Critical Care (CC), and this is appropriate, but it is important that their management is included in the "Quality System" of the Laboratory (LAB). Therefore, the choice of a "good tool", simple to use and which does not need maintenance, is essential. In 2011 we have shown an average of 20 NC for month (90% of these caused by operator of CC) on instrument's management for which we evaluated the possibility of changing tool. For this reason, in September 2012, a qualitative comparison was performed between the traditional current analyzer in use Radiometer ABL835 FLEX(A835F) to new, for cartridge-technology, ABL90 FLEX (A90F).
Methods: Instrument correlation: 1334 samples on A90F vs A835F (244 venous and 1090 arterial samples) to define Passing and Bablok regression and correlation coefficient (r). Operating modes: Evaluation of TAT (Turn Around Time) through the laboratory information system, analysis of clinical risk management with FMECA (Failure Mode Effects and Critically Analysis) simplified methodology; evaluation of organizational impact assessment according to methodology inspired by TQM (Total Quality Management).
RESULTS: Instruments correlation:
pH: Y = -0.0698 +1.0117 X, r =0.9686 P <0.0001, pCO2: Y = 2.2382 +0.9549 X, r =0.9964 P <0.0001, pO2: Y =-3,0950 + 1,0287 X, r =0,9962 P<0,0001, K+: Y =0.0000 +1.0000 X, r =0.9957 P <0.0001, Na+: Y =2.0000 +1.0000 X, r =0.9825 P <0.0001, tHb: Y =0.3000 +1.0000 X, r =0.9933 P <0.0001
Lactate (82 samples): Y =0.0000 +1.0000 X, r =0.9974 P <0.0001, TAT: 5' saved for BGA testing (3'on CC vs 8' on LAB). Time for LAB supervision: 12' saved/day (30' with A90F vs 42' with A835F). NC: 2 with A90F Vs 20 with A835F.
Conclusions: The A90F can be analytically correlated to the A835F, it is very fast, very simple to use, and almost maintenance-free. As shown in our evaluation, for the CC the A90F is safer because detects a probability of error of less than 90%. Moreover, with the A90F decreases both the Tourn Around Time Therapeutic and the time of supervision for the process by the LAB.

W385
NANOPARTICLE-BASED IMMUNOASSAY DESENSITIZATION

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Background. Highly luminescent polystyrene nanoparticles enable the development of immunoassays for targets requiring high sensitivity. On the other hand, D-dimer - an analyte used in critical care testing - requires wide dynamic range and a cut-off value of 500 ng/mL (FEU). The challenge for a D-dimer sandwich assay is to shift the range upwards to accommodate for this.
Methods. Desensitization was performed with an immunoassay exploiting D-dimer specific antibodies (Mab and F(ab')2-fragmented). The assay was performed in streptavidin microtitration wells coated with a biotinylated capture antibody. The second antibody was attached to internally dyed europium(III)-chelate polystyrene nanoparticles. Variable amounts of free capture or detection antibody were added to particle solution in a one-step assay with preincubation of the nanoparticles and the sample. Signal from the assay was measured with a plate fluorometer.
Results. Addition of free F(ab')2-fragment had little effect on the dynamic range. When Mab was employed as the capture, 1 μg/ml free Mab shifted the linear range from 10-1000 ng/mL (LOB; 0.130 ng/mL) to 50-10000 ng/mL (LOB; 11.2 ng/mL). In reversion of capture and detection antibodies, the addition of 15 μg/ml free Mab extended the linear range from 10-100 ng/mL (LOB; 0.0630) ng/mL) to 10-10000 ng/mL (LOB; 12.4 ng/mL).
Conclusions. Addition of free capture or detection antibody can have remarkable effects on the dynamic range of an immunoassay. With the correct antibody combination a 100-fold extended dynamic range was obtained. With this approach nanoparticles can be employed in immunoassays requiring wide dynamic range rather than high sensitivity.

W386

ACCURACY EVALUATION OF TWO BLOOD GLUCOSE MONITORING SYSTEMS ACCORDING TO NEW PREDICTION-ERROR CRITERIA

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Background: Blood glucose (BG) monitoring systems allow diabetes patients to control and adjust their therapy. Although numerous performance standards have been proposed, consensus guidelines lack. The International Organization for Standardization (ISO) 15197:2003 requires that 95% of results fall within ±20% at glucose concentrations ≥4.2 mmol/L (≥75 mg/dL) and within ±15 mg/dL at glucose concentrations <4.2 mmol/L (<75 mg/dL). The 2011 AACC/NACB guidelines have proposed to limit total allowable error for 95% of samples to ±15% at glucose concentrations ≥5.6 mmol/L (≥100 mg/dL) and within ±15 mg/dL at glucose concentrations <5.6 mmol/L (<100 mg/dL).
Methods: We evaluated two BG monitoring systems (MENARINI StatStrip Xpress and BAYER Breeze®2) for system accuracy according to ISO 15197, AACC/NACB guidelines and to Clarke error grids. BG measurements of 148 patients were performed on both systems and then compared with quantified results using hexokinase reaction on ROCHE Cobas 8000.
Results: Passing-Bablok linear regression and Bland-Altman plots showed that both BG monitoring systems fulfilled ISO 15197 requirements (the percentage of results showing the minimum acceptable accuracy was 100.0% for either StatStrip Xpress or Breeze®2). Nevertheless, only Breeze®2 meets the AACC/NACB guidelines, while StatStrip Xpress does not (95.9% vs. 86.5% of results showed the minimum acceptable accuracy, respectively). According to Clarke error grids, both StatStrip Xpress and Breeze®2 had 100.0% of their results in error zones A and B, considered as “clinically uncritical”.
Conclusions: Both BG monitoring systems fulfilled the minimal accuracy requirements of the ISO standard and didn’t present clinically critical errors, but only Breeze®2 met the more stringent AACC/NACB guidelines. Because inaccurate results lead to the risk of false therapeutic decisions and subsequent possible severe health injury, regular and standardized evaluation of BG meters and test strips should be performed by manufacturers in order to ensure adherence to more recent guidelines.

W387

POINT OF CARE INR REDUCES DOOR-TO-NEEDLE TIME FOR INTRAVENOUS TPA IN ACUTE ISCHEMIC STROKE

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Background: Stroke is characterized by the sudden loss of blood circulation to an area of the brain, resulting in a corresponding loss of neurologic function. For every hour lost in stroke treatment, the brain loses neurons- the average patient loses 1,9 millions brain cells per minute. Thrombolytic therapy for the administration of rt-PA (recombinant tissue-type plasminogen activator) administered between 3 and 4,5 hours after the onset of symptoms was found to be efficacious in improving neurologic outcomes. According to Acute Stroke Evaluation and treatment: 60 Minute or Less Protocol – time for INR test must be ≤15 min. The aim of this study was to assess the turnaround time (TAT) and analytical performance of POC INR monitor, the CoaguChek XS Pro (Roche Diagnostics, Germany) by comparison to a laboratory method STA Compact (Diagnostica Stago, France).
Methods: The study was performed over a three weeks period using samples obtained from the Emergency Department of the Anesthesiology and Intensive Care Clinic of Tartu University Hospital. Method correlation was performed by analyzing 20 capillary and venous blood specimens on the CoaguChek compared to laboratory. Mean INR value was 1,71 (range = 0,90-5,14). INR turnaround time was estimated from blood drawing to result communication.
Results: The CoaguChek correlated well with the laboratory standard method (R2-0,935) and had not significant difference (p-0,697). Linear regression analysis demonstrated a slope of 0,79 an intercept of 0,33. Maximum difference was 27% (1,24) at the value 3,9 and mean difference was -0,03. TAT: median and 90th percentile of laboratory INR was 62 min and 118 min respectively. The CoaguChek mean time to result was 2 min.
Conclusion: We conclude that the CoaguChek is adequate for monitoring INR for stroke patients. Data indicates that the CoaguChek reduce the INR time to result from 1 hour to 2 minutes, improve the over workflow while preserving the patient`s quality of life.

W388
VALIDATION OF A POINT-OF-CARE INR COAGULOMETER IN INTENSIVE CARE PATIENTS NOT ON ORAL ANTICOAGULANT THERAPY

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Background. Point-of-Care (POC) INR determination is widely used for monitoring oral anticoagulant therapy (OAT) in general practice and by patient self testing. Short assay turn-around time is desirable especially in intensive care units (ICU), including rapid INR results. As most ICU patients are not on OAT, validation of POC INR in this setting is required prior to clinical use. Our purpose was to validate the CoaguChek XS Plus (CCXS+, Roche Diagnostics) in ICU patients not on OAT.
Methods. Blood samples were drawn from a Vamp System (Edwards Lifesciences) connected to arterial lines in 76 ICU patients. Blood was immediately applied onto two CCXS+, and a citrate tube was collected for routine INR determination on Sysmex CS-2100i. A single lotno. CCXS+ test strips was used. Analytical coefficient of variation (CVa) was calculated from normalized differences in the duplicates. Method comparison was based on CCXS+/Routine ratios. Validation involved quality specifications for POC INR/Routine ratios in general practice set forth by the Danish Society of Clinical Biochemistry and the Organization of General Practitioners in Denmark.
Results. CCXS+ INR's averaged 1.5 (range 0.9-4.5). CVa was 1.9%. The CCXS+/Routine ratio averaged -1.3% (SD 13.3%, range -29.4% to 34.1%). 72/76 (95%) of ratios met the preset quality specification of ±26%. Low molecular weight heparin prophylaxis in 55 patients did not influence the CCXS+ results.
Conclusions. CCXS+ is useful in ICU patients not on OAT, and performs in such patients with the same analytical quality as is known from a vast number of patients on OAT monitored in general practice or by self testing.

W389
THE FINAL FRONTIER - POINT OF CARE CULTURE!

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Background: We identify current deficiencies in performance evaluation for which proven solutions can improve bedside decision-making; prioritize useful initiatives (e.g., noninvasive screening and monitoring) for resiliency in low-resource countries and island nations; and introduce the concept of "point of care culture." These initiatives point to a future where high performance, minimally invasive, and culturally aware point of care (POC) becomes a primary modality empowering individual healthcare.
Methods: Methods comprised non-parametric performance evaluation using locally-smoothed median absolute difference and maximum absolute difference (LS MAD-MaxAD) curves; a new health resources demographic scoring system that helps position POC where it is needed most; review of Cambodian field resources; needs assessment surveys; small-world network (SWN) strategies; geographical information systems; cultural factors analysis by the Peace Corps; and analysis of POC policy and guidelines in the ASEAN.
Results: Need for POC testing in low-resource settings is striking. Often, we found POC testing was used in lieu of diagnostic testing in hospital laboratories, since conventional services were too distant, prohibitively costly, or simply unavailable. MAD-MaxAD enables method fidelity. Rapid noninvasive testing facilitates integrated screening, diagnosis, monitoring, and treatment in care paths for diabetes. POC speeds the critical path of the acute myocardial infarction patient. Remarkable progress with policy and guidelines in Malaysia demonstrates the impact of POC national directives.
Conclusions: Advances in policy and guidelines and the new IFCC Task Force on POC Testing are vital for addressing future cultural challenges in nations adapting to increasing populations of both young and old persons, despite significant scarcity of resources. Global harmonization of POC performance will accelerate progress in these countries by improving the quality, usefulness, and impact of decision-making. Culturally sensitive POC strategies implemented in SWNs worldwide will enhance standards of care, including crisis standards of care for public health pandemics, complex emergencies, and natural disasters. Nurturing POC culture builds resiliency.

W390

COMPARISON OF C-REACTIVE PROTEIN EQA RESULTS OBTAINED FROM ROUTINE LABORATORIES AND POCT PROFESSIONAL USERS

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Background: Number of POCT systems used by practical doctors grows up exponentially. We will show the results of EQA rounds focused on the CRP measurement where identical samples were sent to the routine laboratories and to the professional POCT users.
Methods: We use 2 samples in each EQA round. All results are evaluated together (are not divided into groups). Assigned values (AV) are calculated as robust means (according to ISO 13528) and maximal deviation of the result from AV (Dmax) is 24 %. More than 1300 participants are engaged in EQA CRP POCT programme. Although users of four POCT systems participate in this programme, only two of them are represented significantly (QuikRead approximately 70 % and NycoCard 15 %). We organise 6 EQA rounds for POCT users per year. The concentration of CRP in samples ranges from 10 to 95mg/l. About 400 laboratories participate in the EQA CRP programme for clinical laboratories. We organise 3 EQA rounds for laboratories per year. In some EQA rounds we use identical samples for POCT users and clinical laboratories.
Results: Average success of the POCT users in all 6 rounds of 2012 is 85 %. Total reproducibility (CV) is 12 % in the average. Average success of the routine laboratories in all 3 rounds of 2012 is 98 % and average CV is 7 %.
Conclusions: We have no doubt about commutability of the samples as we observe very good agreement of robust means of all results and of individual groups (instruments in laboratories and individual POCT systems). But total reproducibility (CV) of POCT results is twice worse than CV of laboratories and it is significantly dependent on the concentration of CRP in the sample which is a phenomenon never observed in the routine laboratories. As the result of higher CV of POCT results we observe lower success of this group. We are not using in EQA POCT programme the samples with CRP concentration lower than 10 mg/L because under this limit the CV rises exponentially.

W391

EVALUATION OF THE EPOC BLOOD GAS ANALYZER COMPARED TO THE SIEMENS RAPIDLAB 1265, SIEMENS DIMENSION VISTA AND SYSMEX XE-2100 ANALYZERS

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Background: Intensive care and neonatology units, operating rooms and emergency departments need rapid measurements of blood gases, electrolytes and metabolites. These analyses can be performed in the central laboratory or in the clinic with traditional or compact cassette type blood gas analyzers. The expanding selection of point-of-care analyzers has continued to raise the interest in implementing these systems in acute care setting. Enterprise point-of-care or EPOC (Epocal Inc, Ottawa, Ontario, Canada) system is a blood gas analyzer for testing whole blood samples at the bedside. The EPOC system consists of a test card containing the sensors, a wireless card reader, and a data assistant running software for data analysis.
Methods: We collected 75 heparinized samples from intensive care unit and analyzed them using EPOC, Siemens Rapidlab 1265, Siemens Dimension Vista and Sysmex XE-2100 analyzers. The samples were first analyzed with Rapidlab and EPOC, followed by Hb analysis with Sysmex XE-2100. Finally, the samples were centrifuged and the Na and K values were analyzed by Dimension Vista.
Results: The between-day imprecision of EPOC was determined with two different levels (high and low). The CV% for pH, pCO2, pO2, Na, K, glucose and lactate were <6.0 except for low level lactate (CV% 8.0). The CV% for Hb was < 1.5%. Within-run imprecision for pH, pCO2, pO2, Na, K, glucose, and Hb were <3.0. For lactate the CV% was 5.0. The correlation (R2) of EPOC with Siemens Rapidlab was >0.93, with the exception of Na and Ca (>0.83). Na and K correlation (R2) between EPOC and Dimension Vista was 0.73 and 0.89, respectively. Correlation (R2) of Hb between EPOC and Sysmex XE-2100 analyzer was >0.94.
Conclusions: Blood gas parameters of the EPOC analyzer correlated well with Rapidlab. The correlation of electrolyte levels was not as good. However, the different measuring principles between EPOC (direct ISE) and Dimension Vista (indirect ISE) might partially affect the results. The storage of test cards in room temperature should provide operational advantage and wireless information transfer can provide real-time connection with laboratory information system. Altogether, the analytical and technical properties of the EPOC analyzer look promising.

W392
UNIQUE BUILT-IN QC IN A POCT 5-PART DIFF SYSTEM

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Background: A new point-of-care hematology system has recently been introduced by HemoCue. Whole blood is sampled directly into a microcuvette from the finger or from a venous sample. The measurement starts automatically when inserting the microcuvette into the analyzer. The novel system uses stat-of-the-art imaging technology to count the white blood cells and perform a 5-part differentiation. The analyzer will flag all samples containing pathological white blood cells (blasts and immature granulocytes). The system is factory calibrated and needs no further calibration by the user. An advanced built-in QC-system will automatically at start-up and between each measurement control the electronics, software and the optics. The WBC DIFF system also has a unique check during the measurement. Using the imaging technology, the system will check for correct filling and correct position of the microcuvette, reagent functionality, dirt, blurred cells, non-homogenous sample, small coagula etc. An error code will be displayed if any of those QC checks fails.
Method: The built-in QC system was validated in different user error cases identified by FMEA risk analysis. EDTA venous blood samples were used to test the different cases. The identified cases were blood outside on the surface of the cuvette instead of in the cavity of the cuvette, air bubbles in the cavity of the cuvette, moisture on the optics of the analyzer, finger prints, grease, blood smears, scratch, powder or reagent placed on the measuring eye on the outer surface of the cuvette, measuring with an empty cuvette or with no cuvette, measuring with a partly filled cuvette and measuring with a cuvette filled with blood in two steps.
Results: Totally 567 blood samples were analysed on the different cases identified in the risk analysis. Either correct results or error codes from the built-in QC system were displayed in all samples. No incorrect results were obtained.
Conclusions: The novel POCT HemoCue® WBC DIFF technology is based on state-of-the art imaging technology. The unique and advanced built-in QC system will secure that results from the system are not influenced by user or instrument errors. A white blood cell count including a 5-part diff at the point of care will increase the availability of already well established and frequently used lab parameters. Rapid and easy access will be a valuable tool for physicians in making direct and more well based decisions in several clinical situations.

W393
EVALUATION OF A NEW HEMATOLOGY AND BLOOD CHEMISTRY ANALYSER SUITABLE AS POINT-OF-CARE TESTING (POCT) IN NEONATAL CARE UNIT: THE MICROSEMI CRP ANALYSER

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Background. POCT can have a profound impact on newborns and premature babies outcomes. Precision of the Microsemi CRP (HORIBA) methods (C-Reactive Protein, CRP; hemoglobin, Hb; white and red blood cells, WBC, RBC; platelets, PLT) and agreement with laboratory methods (Dimension Vista and ADVIA 2120) were assessed.
Methods. The precision was determined by testing 3 commercial control sample on 20 consecutive days. For the comparison studies, blood samples were taken from 70 newborns (group A) and 20 premature babies (group B), admitted to the neonatal care unit (NCU). Samples were collected in capillaries (POCT) and in paired tubes with silicone gel barrier and EDTA (laboratory test). POC tests were performed in NCU.
Results. The CVs% for POC methods range from 0.27 to 3.6. Methods comparison between capillary blood samples matched with serum and EDTA samples, yielded intercept, slope, 95% CIs for the regression analysis (Passing and Bablok regression) and absolute bias (Bland and Altman analysis) as follows: Group A. CRP Horiba=0.06 (-0.42 to 0.40); CRP Vista=0.92 (0.88 to 1.0); bias = -0.2 (-2.4 to 1.9). WBC Horiba=-0.21(-0.9 to 0.3); WBC Vista=1.01 (0.9 to 1.0); bias=0.15 (-0.11 to 0.43). RBC Horiba=-0.5 (-0.93 to -0.09); RBC Advia=1.0 (1.0 to 1.2); bias= 0.09 (-0.01 to 0.21). PLT Horiba=-2.44 (9.0 to 12.8); PLT Advia=0.97 (0.9 to 1.0); bias=-5.57 (-11.2 to 0.1). Hb Horiba = 0.0001 (-0.4 to 0.1); Hb Advia=1.0 (1.0 to 1.04); bias=0.1 (-0.03 to 0.16). Group B. CRP Horiba=-0.19 (-0.66 to 0.3); CRP Vista=1.0 (0.99 to 1.1); bias=2.13 mg/l (-0.23 to 4.5). WBC Horiba=-0.12 (-0.75 to 0.4); WBC Advia=0.99 (0.93 to 1.0); bias=-0.05 (-0.36 to 0.25). RBC Horiba=-0.32 (-1.45 to 0.06); RBC Advia=1.04 (0.93 to 1.3); bias=-0.21 (-0.37 to -0.04). PLT Horiba=-4.0 (-14.2 to 6.7); PLT Advia=1.0 (0.93 to 1.0); bias=-5.1 (-13.2 to 2.9). Hb Horiba=-0.70 (-2.7 to 0.07); Hb Advia=1.0 (0.98 to 1.2); bias=-0.07 (-0.17 to 0.03).
Conclusions: The Horiba methods provide reproducible results. Comparison between methods showed for RBC only, a sistematic non-significant difference (group A), and a negative significant bias (group B). Precision and comparison data as well as the limited volumes of blood requested, could justify the use of the analyzer as POCT in the NCUs.

W394
CORRELATION BETWEEN SFRI AND PROLYTE ISE ANALYZER

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Background: Clinical decision making is confirmed by laboratory test results. When dealing with sick patients, the speed and accuracy of tests to detect metabolic derangements is very important. It is, therefore, required that laboratory results be timely accurate, reliable and fit for the purpose. We evaluated agreement between two ion selective electrode analyzers used for the quantitation of plasma sodium and potassium in our laboratory.
Materials and methods: 42 samples originally meant for electrolytes were employed for the study. After blood receipt the samples were spun at 4,000r.p.m immediately. Plasma sodium and potassium were analyzed simultaneously with Prolyte and SFRI ISE analyzers. The agreement between the two analyzers was assessed using Bland-Altman Method.
Results: Na+ Coeffient of variation PROLYTE2%, %, SFRI,1.2 %, Regression Analysis; Correlation Coefficient r^2=0.93, slope=1.08, intercept,-13.24, maximum difference, 4mmol/L, minimum difference, -4 mmol/L, Bland-Altman analysis mean difference between PROLYTE and SFRI ion selective method is 1.4 ± 3.42 with 95% confidence interval of -2.02 to 4.82; 2. K+ , Coeffient of variation 0.8%, PROLYTE, % SFRI,%, Regression Analysis; Correlation Coefficient r^2=0.98, slope=1.1, intercept ,-0.2,maximum difference =0.2, minimum difference,-0.5, Bland-Altman analysis mean difference between PROLYTE and SFRI ion selective method is 0.21±0.26 with 95% confidence interval of -0.05±0.47.
Conclusion: Good degree of agreement was observed on comparing the two ISE analyzers for the measurement of sodium and potassium. The two analyzers can be used without fear of inaccuracy and imprecision.

W395
EVALUATION OF THE CLINICAL UTILITY OF THE HEMOCUE WBC DIFF POINT OF CARE DEVICE- A COMPARISON TO THE SYSMEX XE2100 FULLY AUTOMATED ANALYSER AND THE REFERENCE MANUAL DIFFERENTIAL

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Background: The clinical utility of the white cell differential is high and there are demands for quicker results to circumvent bottlenecks. Its accuracy is also important for effective monitoring and clinical diagnosis. The Hemocue WBC Diff is a portable point- of-care device requiring 10 ul of blood and produces a differential in less than 5 min. We evaluate the manufacturer's claims of accuracy and assess its comparability to the routinely used XE2100 automated analyser and the manual differential.
Methods: Samples were obtained from 500 blood specimens (48.6% females and 56.4% men), (age range: 16–79 years). Corresponding blood films were prepared and examined by two morphologists performing 200- white cell differentials. Total WBC comparability. The Hemocue WBC Diff results were compared with the manual differential and those analysed on the XE 2100. Reproducibility Reproducibility of the Hemocue WBC Diff was checked by performing triplicate analysis on 50 samples. The flagging capability was assessed by processing samples with pathological leucocytes confirmed by blood film morphology. Specificity and sensitivity indices were calculated for each of the major conditions. P-values <0.05 were considered significant.
Results: The correlation coefficient for the total white count = 0.996 (range 0.1 × 109/L - 24.1 × 109/L) with 3.5% varying significantly by greater than 10% (range: 10.1%-14.2%) The manual differential and the Hemocue Diff were insignificantly different for neutrophil (P=0.854) eosinophil (P=0.593) and lymphocyte (P=0.706) sub-types. Monocytes were marginally over- estimated by the XE-2100 (P=0.055) and under-estimated by the Hemocue WBC Diff (P=0.059). Flagging: There were no significant differences between the hemocue WBC Diff and the XE2100 for specificity (P=0.067) and the sensitivity (P=0.099)
Conclusion: The assessment of the utility and reliability of the HemoCue WBC Diff has shown good precision, accuracy and comparability to the more complex Sysmex XE2100 analyser and the manual differential method. Its flagging was effective and sensitive, sufficiently alerting the operator to the presence of abnormal white cells. This easy- to- use device is fast and could effectively optimize patient care.

W396

ESTABLISHING REFERENCE VALUES FOR UMBILICAL CORD VENOUS BLOOD GASES, ELECTROLYTES AND METABOLITES IN OUR HEALTH CARE AREA

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Background: Establishing reference values for analytical methods is a must for laboratory medicine despite of it not all centers do it. Reference values should be established according to the population of medical care attendance. Umbilical cord blood gas analysis is performed routinely following all deliveries in our center. The aim of this study was to evaluate cord blood venous reference ranges for pH, pO2, pCO2, lactate, glucose, Ca++, K+, Na+ and hemoglobin in newborns, delivered by Spontaneous Vaginal Delivery (SVD) included instrumental delivery (forceps and vacuum) and by Cesarean Section (CS) performed in our Hospital. The aim of our study is to check results with former literature.
Methods: Subjects were selected for the study if APGAR test score was greater than 7 and medical record showed no inborn/newborn complications. Exclusion criteria for metabolic acidosis in fetal blood: pH <7.20, EB ≤-7.2 and Lac >3.7 mmol/L was taken into account only if they met the three criteria in the sample. 461 samples of umbilical venous blood were collected. The samples were taken from the umbilical cord (double clamp) in sodium heparin syringes. Cord blood samples were analyzed for standard blood gas, electrolytes, metabolites and pH, using the analytical device GEM4000 as POCT at the Delivery Department (DD). After evaluation the group consisted of 295 newborns with SVD and 87 newborns delivered by CS. We tabulate all cord blood gases data realized in the DD over the period of 06-02-2012 to 20-04-2012 for its evaluation and subsequent assessment of ranges of reference in our hospital. Statistics were performed in SPSS 20 software (IBM)
Results: We obtained reference values expressed as range (lower and upper reference value expressed as 2.5 and 97.5 percentiles) for vein cord blood in newborns for non-parametric variables (KS): pH=7.04-7.38; pCO2=34.20-81 mmHg; pO2=6-33 mmHg; Na+=128-138 mmol/L; K+ 3.80-6.04 mmol/L; lac= 1.40-7.87 mmol/L. We also obtained reference values for parametric variables (KS) expressed as mean ±SD: Ca++=1.46±0.86; glu=80.42±18.71 mg/dL and tHb=16.58±1.61.
Conclusion: Result in our Hospital differ from those found in the literature. This remarks the necessity for a strict laboratory control of devices situated outside the core laboratory.

W397

EVALUATION OF BD PRESET™ AND BD A-LINE™ CRITICAL CARE SYRINGES WITH OTHER CURRENTLY MARKETED DEVICES FOR IONIZED CALCIUM, POTASSIUM, SODIUM AND PH

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Background: Blood gas analysis is essential for patient care and provides physicians with detailed information regarding cardiopulmonary and metabolic homeostasis. BD Preset™ and BD A-line™ syringes contain calcium balanced lithium heparin spray dried on the sides of the syringe and can be used to perform critical care tests. The syringes were evaluated at two time intervals (15 and 60 min). These are typical of the times at which stat samples are tested and allow for delays which may be encountered in a hospital setting.
Methods The clinical performance of BD Preset and BD A-line critical care syringes was compared with currently marketed syringes (Sarstedt Blood Gas Monovette® and Radiometer Pico™ 50) for the testing of ionized calcium (iCa), potassium (K), sodium (Na) and pH. Venous specimens were collected from 30 adult subjects into each syringe. Testing was performed at 15 and 60 minutes post blood collection and analyzed on the Siemens RAPIDPoint®. Mean between-syringe biases with 90% simultaneous intervals (for 95% two one-sided test) were calculated for the syringe comparisons per time interval. Clinical criteria for the bias—the maximum allowable difference in test results—were defined for each comparison and analyte. Equivalence was established if the mean bias and 95% limits were within the clinical criteria.
Results: Clinical equivalence was demonstrated for all comparisons except those listed below. For these comparisons, the mean biases were within the clinical criteria, but the 95% limits exceeded the criteria. Following data review, the results were considered clinically acceptable.
Comparison/analyte [clinical criteria, mean bias (95% confidence limits)]: BD Preset and BD A-line vs Sarstedt at 60 minutes/Na [3.0 mmol/L, 1.55 (0.03, 3.06); 1.60 (0.08, 3.11)], respectively. BD Preset and BD A-line vs Radiometer at 60 minutes/pH [0.03 units, -0.0104 (-0.0337, 0.0129); -0.0130 (-0.0363, 0.0104)], respectively BD Preset vs Radiometer at 15 and 60 minutes/iCa [0.05 mmol/L, -0.021 (-0.051, 0.009); -0.023 (-0.053, 0.007)], respectively.
Conclusions: Clinical equivalence or clinical acceptability was demonstrated for all analytes for each syringe comparison at both time intervals.

W398
CORRELATION OF I-STAT WITH CONVENTIONAL METHODS: MEASUREMENT OF ELECTROLYTES AND UREA

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Background: Rapid electrolyte and urea measurement is of great importance in intensive care and emergency patients. Electrolyte measurements often influence therapeutic decisions. The study was designed to investigate the agreement between point of care whole blood electrolytes and urea and that of conventional methods.
Materials and methods: 42 blood samples for which I-STAT was requested for at the Chemical Pathology Department, University College Hospital, Ibadan were employed for the study. The samples were assayed immediately for i-stat analysis. After the i-stat test the same sample is spun without delay and plasma is separated out from the cells. The sample were analyzed along with quality control samples for electrolytes and urea the same day using conventional methods. Results: 1. Na+ Coeffient of variation, ISE,1.35%, Regression Analysis; Correlation Coefficient r^2=0.97, slope=1.02, intercept, -1.86, Bland-Altman analysis mean difference between i-stat and ion selective method is 0.29± 1.50 with 95% confidence interval of -2.71 to 3.30; 2. K+ , Coeffient of variation, ISE,1.1%, Regression Analysis; Correlation Coefficient r2= 0.99, slope=0.97, intercept , 0.15, Bland-Altman analysis mean difference between i-stat and ion selective method is 0.026 ± 0.09 with 95% confidence interval of -0.15 to 0.21; 3. HCO3- Coeffient of variation, back titration method, 3.8 %, Regression Analysis; Correlation Coefficient r^2= 0.67, slope=1.52, intercept ,-7.92, Bland-Altman analysis mean difference between i-stat and back titration method is 3.07 ± 4.10 with 95% confidence interval of -5.13 to 11.27 ; 4. Urea, Coeffient of variation, DAM method, 5.6 % Regression Analysis: Correlation Coefficient r^2= 0.99, slope=1.21, intercept ,-13.81, Bland-Altman analysis, mean difference between i-stat and DAM is 1.98 ± 23.93 with 95% confidence interval of -45.87 to 49.83.
Conclusion: I-STAT as a POCT performs favourably well as traditional methods in the analysis of electrolytes and urea in our laboratory. Considering its good turnaround time it should be used in emergency cases without fear of inaccuracy or imprecision.

W399
EVALUATION OF ENDOTOXEMIA LEVEL WITH SHORT-TERM TESTS

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Background: massive inflow of endotoxin to the systemic circulation can be the reason of organ failure formation, DIC and most of other clinical development of sepsis. To date, there is a limited number of tests that allow defining the activity and the number of endotoxin in blood during 30-60 min. To estimate informational content of various laboratory short-term tests of endotoxemia level in patients postoperatively on the heart and vessels.
Methods: 55 patients of ICU were examined. Inclusion criteria to the group of the study were hyperthermia, leucocytosis, the level of procalcitonin was more than 0.5 ng/ml. The level of endotoxemia was defined according to the data of LAL-test (Limulus Amebocite Lisate), the level of endotoxin activity - EAA (Endotoxin Activity Assay), with methods of activated species – MAS-Endotox.spp (SCCVS Bakoulev). The estimation of endotoxemia level was performed before and after sorption to the patients who had procedures of LPS sorption during complex intensive treatment. According to the values of endotoxin-tests, the patients were divided into three groups, depending on the level of endotoxemia.
Results. The values of MAS-test were 7.5-30 pg/mL (7.5-125 pg/ml). 84% of patients had exceeded physiological standard. LAL-test was positive in all examined patients and it was 0.72 (0.36-1.44) EU/mL, EAA was 0.54 (0.36;0.68). 28-day survival of examined patients, depending on the level of endotoxemia, studied by various methods: at the level of EAA=0-0.4 – 67%, at the level of 0.4-0.6 – 62%, 0.6-1.0 – 50%; survival at the level of MAS was 7.5-30 pg/mL – 67%, 30-125 pg/mL-62%, 125-500 pg/mL – 56%; survival of patients was 82% where LAL-test was less than 0.72 EU/mL, survival was 50% at 0.72 EU/mL, survival was 60% where the level of endotoxin was more than 0.72 EU/ml. After LPS sorption, significant decrease of endotoxemia level was noted with EAA (EAAbefore=0.73 (0.63; 0.79), EAAafter =0.53 (0.47;0.58), P=0.001) and MAS (MASbefore=30-125 (30-500), MASafter=7.5-30 (7.5;125), P=0.013).
Conclusions. The methods, based on antigen-antibody interaction (EAA and MAS-Endotox.spp) are more informative for instant diagnosis of endotoxemia. Short-term tests allow defining the indications for appropriate therapy and monitoring of its efficacy.

W400
POINT-OF-CARE TESTING (POCT) IN DECENTRALISED SCREENING FOR DYSLIPIDAEMIA: INDEPENDENT EVALUATION PROTOCOL OF THE POCT DEVICE

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Background: PoCT decentralised laboratory testing is performed at sites of immediate patient care and its results are used for clinical decision-making. Quality control is required to ensure that PoCT laboratory testing is high quality and cost effective, in order to contribute to optimal patient care. Few studies have assessed the clinical agreement between lipid PoCT results compared to laboratory results. The aim of this study is to evaluate the accuracy and precision of PoCT devises for lipid screening compared with laboratory lipid test results in healthy subjects and patients with dyslipidaemia.
Methods: 2 CardioChek PA Analysers (CCA) (PTS, Indianapolis, USA), which employ light reflectance to measure enzymatic chemical reactions using PTS PANELS Lipid Panel test strips to measure total cholesterol, HDL cholesterol and triglycerides in whole blood, were evaluated on 20 consecutive days by designed quality control kit (ChekMate) and PTS Panel Quality Control materials. Fasting venous samples from 50 subjects were analysed on both CCA whose results were compared with the routine clinical laboratory assay of plasma lipids (COBAS 6000, Roche Diagnostics, Milano, Italy). Fasting finger-stick samples of 25 subjects were analysed on one CCA and compared with laboratory venous results.
Results:There was no statistically significant difference between portable measurements of total cholesterol, HDL cholesterol, and triglycerides vs. clinical laboratory results using paired Student t test. Capillary values of total cholesterol, HDL cholesterol and triglycerides well correlated with laboratory results on venous blood (r from 0.96 to 1.0, P <0.001). Within-run variation coefficient was 1.8 and 0.8% (total cholesterol 146±3 and 275±2 mg/dL, respectively), 8.3 and 3.8% (HDL cholesterol 29±2 and 78±3 mg/dL), 2.3 and 1.1% (triglycerides 153±3 and 126±1 mg/dL).
Conclusions:Preliminary results suggest that CCA provides sufficiently high-quality results. At it completion, in order to validate lipid measurements with the PoCT analyser, the quality evaluation protocol intends to recruit 200 subjects (venous blood) and 80 subjects (capillary blood) in addition to determining repeatability (within-run precision) of portable measurements at multiple plasma lipid levels.

W401
OPTIMIZING THE PARAMETERS OF A LATERAL FLOW ASSAY WITH FPSA AS ANALYTE

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Background: Lateral flow (LF) immunoassays are ideal for point-of-care testing due to their simplicity and ease of use. There is room for improvement however, especially in quantitative and sensitive LF assays. Significant improvements in these aspects of the assay are possible by substituting the normal visual labels with fluorescent labels, but the LF assay format also has inherent limitations that can be charted through systematic testing of different parameters of the assay. The goal of this work was to investigate these parameters and develop a highly sensitive yet simple and rapid LF assay for free prostate specific antigen (fPSA).
Methods: We developed a highly sensitive yet simple and rapid LF assay for PSA using long-lifetime fluorescent labels and a streptavidin binder surface, and tested this assay in different configurations with fPSA spiked serum as analyte. The assay sensitivity, linear range, simplicity and speed were optimized.
Results: At the most sensitive format, the detection limit of the assay was improved to 0.002 ng/mL from 0.44 ng/mL when compared with traditional colloidal gold labels. The linear range reached up to 25 ng/mL of PSA in undiluted samples. The assay could be simplified by eliminating the supplementary wash step. Without the wash step the detection limit was 0.012 ng/mL. The kinetics of the assay can be investigated by scanning the LF strip continuously with a fluorometer, starting immediately after adding the sample. The sensitivity optimized fPSA assay reached the maximum signal to background ratio 20 min after the start of the assay. In order to shorten the assay time to 15 min, the nitrocellulose pore size could be increased, but this pushed the detection limit up to 0.003 ng/ml. The assay strip can be measured before maximum signal is reached, but this lowers the sensitivity and makes quantitative calibration difficult.
Conclusions: We have developed a highly sensitive LF assay for fPSA. However, in developing a simple LF assay, the sensitivity of the assay must be balanced with the simplicity and speed of the assay procedure. Using instrument-read luminescent labels, increasing the assay time and adding a wash step improve the sensitivity of the LF assay at the cost of simplicity.

W402

COMPARATIVE EVALUATION OF TWO POINT OF CARE BLOOD GAS ANALYZERS IN INTENSIVE CARE UNIT

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Background: Point of Care Blood Gas Analyzers (POC BGA) in Intensive Care Unit (ICU) provide valuable advantages, mainly allowing rapid and appropriate clinical decisions with reduction of complications and decreased morbidity and mortality. The aim of our work was to evaluate the analytical performance of 2 POC BGA (direct potentiometry): NOVA Phox Ultra (GEPA) and ABL 837 FLEX Radiometer (De Mori), measuring pH, pCO2, pO2, tHb, Na, K, Cl, Ca, Glucose, Lactate and Creatinine.
Methods: The evaluation was performed by testing 100 whole blood (WB) samples from critically ill patients; each sample was tested with the two instruments artenately to minimize the variability of exposure to room air, within 1 to 2 minutes of each other. Further we compared all obtained clinical results of ABL and Phox with the reference methods (Roche Cobas 8000). The obtained results were analyzed statistically with Passing Bablock linear regression and Bland-Altman plot.
Results: Reproducibility was good: CV <2% for electrolytes, glucose, pH, CV <5.6% for lactate, blood gas and creatinine. Linearity concentrations spanning the clinically relevant ranges were verified for all analytes. For all parameters, a good correlation (0.96< R2< 0.99) was observed for the 2 analyzers and the total bias of the mean differences (±2SD) were very low for all tests, confirming the good agreement of the 2 systems, except for creatinine with R2=0.93. In fact the WB creatinine results of ABL and Phox compared to plasma creatinine results showed for the former a R2=0.97 and for the latter a R2=0.91. This statistical difference between Phox and Roche was more evident for the creatinine values ≤0.7 mg/dL and ≥1.3 mg/dL. Further the Bland-Altman plot shows these differences are tightly dependent on values.
Conclusion: The checked POC BGA produce clinically acceptable and interchangeable results. For the ease of use, small sample requirement and low response time are perfectly suitable for ICU, even if the different performance of critical values of creatinine, especially with the opportunity of evaluating eGFR, could suggest the adoption of the former compared to the latter.

W403

ANALYTICAL EVALUATION OF THE STATSTRIP XPRESS AND THE STATSTRIP HOSPITAL METERS AS A GLUCOSE MONITORING SYSTEM IN A SMBG AND POCT

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Background: Glucose meters are widely used in self –monitoring of blood glucose (SMBG). On the other hand, they are used for point-of-care (POC) glucose testing. In this study an evaluation of the analytical performance of the StatStrip Xpress and the StatStrip Hospital glucose meters (Nova Biomedical, Waltham, MA, USA) utilizing glucose oxidase method and amperometric measurement, designed for SMBG and POCT, respectively, was carried out.
Methods: The assessment of precision and between-lot variability was based on glucose measurements in EDTA venous blood samples. The glucose concentrations measured in 200 fresh EDTA venous blood samples using the evaluated glucose meters and the laboratory glucose oxidase method on the Maxmat PLII analyzer were compared. The hematocrit (HCT) effect was assessed using EDTA blood samples with HCT modified by adding or removing defined aliquots of plasma. Glucose concentration was measured using each meter in 21 series of blood samples, with HCT ranging from 20 % to 60 % in 10 % increments.
Results: The within-run imprecision coefficient of variation (CV) for the StatStrip Xpress and the StatStrip Hospital amounted to 2.74% and 3,31%, respectively. The relative lot-dependent differences assessed for three strips lots were equal to 2.9% and 5.1%. The inter-method difference calculated for the whole range of glucose concentrations amounted to 2.93% and 2.63%, respectively. The Passing-Bablok agreement test showed slightly better results concordance for the StatStrip Hospital. Error grid analysis yielded all measurement points reflecting paired results in zone A and B with 95% and 96.5% of results in zone A, respectively. Measured glucose concentration change per 1% increase in HCT amounted to -0.009 mmol/L (-0.16 mg/dL) and -0.025 mmol/L (0.45 mg/dL), respectively.
Conclusions: For both evaluated glucose meters, the StatStrip Xpress and the StatStrip Hospital, similar imprecision and concordance with the laboratory method was demonstrated. The HCT effect found despite the compensation system utilized by these appliances can be considered negligible. Both evaluated glucose meters meet the analytical requirements for their use as a coherent system for blood glucose monitoring both in SMBG and POCT setting.

W404
IMPLEMENTATION OF A POCT FOR FETAL PH MEASURE

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Background: Intrapartum fetal pH measure is an important parameter which can help deciding gynecologists whether to finish the labour with a caesarea or with a vaginal delivery. The main problem we found in fetal pH intrapartum measure is the amount of capillary blood needed for it. In our Core Lab we work with two GEM3000 IL blood gas analyzers where the minimum amount of blood needed is 150 uL. This is small amount for blood gas or umbilical post partum pH but too much for fetal intrapartum pH.
Methods: We decided to evaluate the EPOCAL (Alere Healthcare S.L.U.) POCT blood gas analyzer where the sample needed is only 90 uL. The use of EPOCAL is very simple even for a non expertise staff. Pre calibrated individually sealed cards stored under room temperature are allocated straightforward in the analyzer. The EPOCAL will check the lot and after 2 min it is ready for use. 1 min after introducing the sample the results will ready. The card will be blocked 8 min after, and a new card would be needed.To assure the analytical quality 50 whole blood samples were checked simultaneously in both GEM3000 and EPOCAL, this way the pre-analytical status was the same for both analyzers. We also performed the tests with our external quality program samples with both analyzers.
Results: Three samples of our external quality control scheme were no taken in account for calculations because ph was too low for Gem3000 the mean difference between Gem3000 and Epocal was 0.023 ranging from 0 to 0.04 pH units and we found no statistical significant differences (P <0.001) between both analyzers. Correlation analysis showed an R=0.996. No other parameters were evaluated.
Conclusions: After a consensus meeting with the obstetricians and midwives we decided that the EPOCAL was a good solution for pH measure even though the main problem is still the handling of the sample and the analyzer during the labor because of the unpredictability of the moment when the physician/midwife will need it. A Plan of online education will be introduced as to assure the quality of the whole process.
Our next goal is to validate the EPOCAL for capillary blood gas and capillary ionic calcium measure in neonatal patients.

W405
A NEW WHOLE BLOOD CONTROL SAMPLE AND ITS UTILIZATION FOR THE SMALL NETWORK OF QUALITY CONTROL ASSESSMENT SCHEME OF POINT OF CARE GLUCOSE TESTING

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Background: This study was to invent and use new whole blood samples treated with several blood additive agents as control samples in the Small Network of Quality Assessment Scheme (SNQAS) for point of care (POC) glucose testing.
Methods: Expired donation whole blood was prepared for low, medium, and high glucose concentrations. All samples at each concentration were treated with four combinations of blood additive agents and normal saline as a negative control. All blood samples were incubated in a refrigerator for 8 hours. Plasma glucose was determined by using glucose oxidase, and hemolysis was investigated by visual inspection at 0 to 72 hours after incubation. The statistical ANOVA was used to compare means of glucose. Three levels of optimal blood samples treated with additive agents was used as QC samples in the SNQAS for point of care (POC) glucose testing at District Health Promoting Hospitals surrounding Naresuan University for three times.
Results: Plasma glucose was stable at least 16 h at low and at least 48 h at medium and high glucose concentrations in blood samples treated with 10 mmol/L and 20 mmol/L of glyceraldehydes respectively. Hematocrit of all samples was ranged from 42-46%. Hemolysis was not found within 72 hours inspections. Triglyceride, bilirubin, and lactate dehydrogenate of all sample were within acceptable limits of glucose meter manufacturer. Overall means of glucose testing of all participants were very close and standard deviation indexes of glucose testing in the IQAS were ranged from 0 to 2.5 (n=15 sites). There were 10% of mean of POC glucose testing sites that were significantly difference from mean of group (P <0.001).
Conclusions: Whole blood samples were prepared by using expired donation blood treated with glyceraldehydes and could be used as QC samples for 16-48 hours in the IQAS. This is the first introduce a new whole blood sample and pilot model of the Small Network for Quality Assurance of POC glucose testing at health care and primary care settings in Thailand by using new whole blood samples.

W406

PRELIMINARY STUDY OF AN IMPLEMENTATION OF THE QUALITY CONTROL ASSESSMENT PROGRAM OF POINT OF CARE GLUCOSE TESTING AT PRIMARY CARE UNITS BY USING WHOLE BLOOD CONTROL SAMPLE

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Background: Point of care (POC) glucose testing was used the most at primary care unit (PCU) in Thailand. However, the policy of quality control (QC) for point of care testing (POCT) in Thailand has not been unclear. This study was first to introduce the Quality Assessment Program (QAP) for POC glucose testing at PCU by using whole blood samples as control samples.
Methods: Expired donation whole blood was prepared for low, medium, and high glucose concentrations and treated with 10 mmol/L of glyceraldehydes. All samples were aliquot into 0.5 mL and kept in refrigerator until used. All QC whole blood samples were sent out to our health system including one Allied Health Sciences Faculty, three District Health Promoting Hospitals, and thirty health volunteers in the QAP for POC glucose testing. QC samples were tested for blood glucose within sixteen hours after preparations. All participants performed QC once per month from August to October. The statistical standard deviation index (SDI) of blood glucose was calculated. Mean of glucose of each participant was compared to mean of group by using t-test. Data of POC glucose, glucose meter, and QC were survey at beginning. Satisfaction of QAP was evaluated at the end of the program.
Results: All participants used the same brand of glucose meter (n=29) in the QAP and blood glucose was performed approximately 120 tests per day. Assessed time to test blood glucose was ranged from 3-4 h and there was no significantly different among participant ($P > 0.05$). SDI of blood glucose testing was ranged from 0 to 2.5. There were three meters (10.3%) those SDIs were exceeded 2.0 at the first month, but decreased to less than 2.0 at the second and third months. All participants were satisfied of QAP and satisfaction score was 5.0.
Conclusions: This is the first preliminary study of the QAP for POC glucose testing at primary care units in Thailand by using whole blood samples. This study provides an evidence for continuous improvement of quality of blood glucose testing at PCUs.

W407

POINT OF CARE TESTING - INDISPENSABLE BUT NOT WITHOUT THE PITFALLS!

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Background: Point of Care Tests (POCT) such as urine dipsticks and glucometers are used routinely in clinical practice and are indispensable for instant screening and monitoring. Galactosaemia, an autosomally inherited disorder due to deficiency in the enzymes involved in galactose conversion to glucose, results in galactosuria, hypoglycaemia, neurodevelopmental delay, failure to thrive, prolonged jaundice and hepatomegaly.
Methods and Results: We present a 7.5 months old baby who at the age of 4 weeks had staphylococcal septicaemia, prolonged neonatal jaundice, hepatomegaly, central hypotonia and hyperglycaemia up to 12.7 mmol/L on glucometer (Accucheck) readings which was treated with insulin. There was a family history of diabetes. He was diagnosed with transient hyperglycaemia due to intraventricular haemorrhage and was discharged. Home POCT glucose readings were around 7 mmol/L with one peak of 18 mmol/L. At 4 months he was hospitalised with lower respiratory tract infection and was noted to have hepatosplenomegaly, central hypotonia and peripheral hypertonia with brisk reflexes, and failure to thrive. His 3rd admission (at 7.5 months) was with jaundice, failure to thrive, global hypertonia and intermittent hyperglycaemia on glucometer readings (13.9 mmol/L, 14.6 mmol/L) and glycosuria on urine dipsticks. Laboratory glucose measurements did not show hyperglycaemia. Clinitest was positive for urine reducing substances (4+) and Thin Layer Chromotography showed increased Galactose (3+). Galactose-1-phosphate uridyltransferase was almost undetectable in red blood cells confirming the diagnosis of galactosaemia.
Conclusions: Accucheck and other POCT devices use a nonspecific Glucose Dehydrogenase method that can produce false positive results in the presence of monosaccharides such as galactose. Similarly, urine dipsticks lack specificity for glucose. Most laboratory analysers use Glucose Hexokinase method which is specific for glucose and is based on the reference method for measuring glucose. Therefore formal glucose measurements should always be obtained to confirm the abnormal results obtained on POCT and prior to commencing any treatment, especially in the paediatric patients where the delayed diagnosis may result in life-long consequences.

W408
HOW SENSITIVE ARE URINE TESTSTRIPS TO ASCORBIC ACID?

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Introduction: A change of automated urine teststrip analyser in a hospital laboratory is almost impossible since the scale of ratings that are used in the individual systems, has never been normalized. Secondly the influence of ascorbic acid (AA) mainly on hemoglobin, glucose and nitrate readings is unclear. Peer reviewed studies are scarce, company disclosures not always reliable. AA is ingested by patients in variable amounts. L. Pauling suggests an intake of up to 5 g AA per day. In Europe as opposed to the USA, intake is somewhat more moderate. However, up to 30% of patients drink one or more servings of fruit juice per day which leads to concentrations of AA in the urine that may interfere with haemoglobin and glucose measurements (50 - 250 mg AA/L).
Methods: Three commonly used testrip readers Urisys 2400 (Combur 9), iChem Velocity (iChem 10 SG) and Clinitec Advantus (Clinitec 9) were used to measure dipsticks. Urine was spiked with various concentrations of glucose, hemoglobin (Hb), protein and nitrate and exposed to several concentrations of AA (0, 50, 100, 200, 500 mg/L). The measurements were performed twice in duplicate.
Results: At Hb concentrations <5 the impact of AA is concentration dependent in all systems. In Urisys and Clinitec AA has no effect at higher Hb concentrations (>2+). iChem Velocity is susceptible at all Hb concentrations tested. Hb at > 5 mg/L is not impaired by AA in Urisys and Clinitec, but is strongly so in iChem. Glucose >5 g/L is hardly impaired by the presence of AA. Clinitec yields lower readings at very high concentrations of AA. The iChem system and to a lesser extent Urisys, are quite sensitive at concentrations around 2 g glucose/L, but not at higher concentrations. Protein-readings did not differ more than half a “step” in any system examined.
Conclusion: The Urisys and Clinitec dipsticks perform excellent and very similar in the presence of AA in patient urines, with Clinitec being somewhat more susceptible. The i-Chem system does not have an oxidizing layer, that can eliminate AA, Urisys uses jodate, Clinitec has an oxidant as well, but not jodate. A change of urinanalysis system should be well arranged, the instruction of physicians must include knowledge about scales and impairment by AA.

W409
A BLENDED-LEARNING COURSE FOR THE GENERAL PRACTITIONER IN SWITZERLAND (FAPL)

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Introduction: For the general practitioner (gp) aiming to run a POCT-laboratory, it is almost impossible to know all the legislatory and quality items that he is subject to. Reliability of generated results can thus be compromised with harmful consequences to the patient. Therefore the Swiss health authority (Bundesamt für Gesundheit, BAG) has comissioned the society of general practitioners (Kollegium für Hausarztmedizin KHM) in 2004 to create a mandatory course for gp’s. Since 2012 the course is conducted in blended-learning, with an e-learning and a hands-on part.
E-learning: The course is based on an open source learning management system ILIAS, as used in many European universities. E-learning modules are uploaded in pdf , which is readily accessible for most systems. Topics are: regulatory framework to run a POCT laboratory, pre-analytics (taking blood samples, handling), specimens (serum vs. plasma), analytics on POCT instruments (problem solving), postanalytics (interpretation, CV, reference ranges, sensitivity, specificity, PPV, NPV) internal, external quality controls, disinfection and waste disposal/sterilisation. Care has been taken to use as many visual aids as graphs, drawings, pictures as possible to ease learning. Layout has been created with a specialist in learning psychology. A forum and quizzes complete the tool.
Practical course: After e-learning, attendants perform an online-test (multiple-choice), which must be passed. The evaluation of tests is done automatically according to the administrators settings.Practical courses include: clinical chemistry, internal and external quality controls (Levey-Jennings-plots, Westgard-rules), urine diagnostics (sticks and microscopy of sediments), hematology (scatter-plots, differentiation, malaria, coagulation) and microbiology (rapid tests, uricult, interpretation of results, hygiene).
Conclusions: The course has been conducted five times with about 80 physicians each. Continuous improvement of contents and tests is being made, which results in less necessary interventions by the instructor each time. An online-forum is used more frequently each time which results in interesting discussions and optimal supervision of learning.

W410

EXPERIENCE IN THE ESTIMATION OF QUALITY HOSPITAL GLUCOSE METERS

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Background: Glucose meters (GM) are devices for measurement (Mt) of blood glucose (G). The quality (Q) of Mt is very important for clinical decision-making and depends on the analytical performance of a GM and the skill of its user. The usual users of the GM in hospital are the nurses. To ensure the Q of G Mt, training of nurses and participation in the external quality control (EQC) scheme has been carried out at our hospital. The aim of our investigation was to assess the Q of G Mt by GM and the ways for Q improvement.
Methods: Management of Q control process was carried out by the central hospital laboratory. The central laboratory received control samples of unknown to GM users G concentrations by mail from EQC organization 3 times a year. These control samples were sent from the laboratory to each user for analyzing. The results of all Mt were collected by the central laboratory, but only some were reported to the EQC because of limited funds. The Q assessment was performed following EQC requirements according to which the controlled meters were compared with the target value and the analytical performance was recognized as “acceptable” if deviation from target value was up to 10%.
Results: 9 control samples of different levels were measured using hospital GM, overall 713 times during the 3-year observation period. The analytical performance of almost all GM (exept 2) was acceptable at G concentration of 6.6; 8.1; 12.4; 15.3 and 17.8 mmol/L. The coefficient of variation (CV) at these levels was between 3.1% – 5.3%. However, at concentration of 2.9; 3.2 and 5.7 mmol/L the CV was between 7.0% - 9.8% and 50 results were unacceptable because of overestimations of the target.
Conclusions: The data from the EQC scheme demonstrated that the Q of the Mt by GM was worse at hypo- and normoglycaemic than at hyperglycaemic level. After examination of the potential sources of errors it was confirmed that GM were working properly while insufficient skills of the users were suggested as the only possible reason. It is known that even slight changes in testing technique may lead to imprecision in the result, which is especially significant at low levels. Despite the overall good performance of GM it was decided that the skills of the GM users need to be improved.

BS01

A PARTICULAR CASE OF λ CHAIN MULTIPLE MYELOMA

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Background: The measurement of serum Free Light Chains (s-FLC) is recommended for diagnosis, prognosis and monitoring of monoclonal gammopathies and specific assays on automated nephelometers and turbidimeters are available. FLC produced by patients with plasma cell disorders are highly heterogeneous and therefore it is necessary to have antibodies that can recognize the diverse FLC. In addition many factors need to be considered when deciding upon the most appropriate method because the identification of the monoclonal component can be unexpected and require further testing for confirmation.
Methods and results: Woman, 47 years old, with bone pains, admitted to the Orthopaedic Day Surgery, Istituto Nazionale Tumori, Naples, Italy. Serum electrophoresis and capillary electrophoresis performed on a Sebia System, did not show abnormalities and the nephelometric assays for immunoglobulins and total light chain were within the reference ranges. A relevant difference in the results was obtained using s-FLC assays, performed by two commercial kits: the N latex FLC on the BNP ProSpec (Siemens Healhtcare Diagnostics) and the Freelite TM , Binding Site on the Cobas C 6000 (Roche). The s-FLC λ concentration was highly abnormal with the Freelite TM assay, whereas in the N latex FLC assay the levels were within the reference ranges. An agarose high resolution electrophoresis, home made, in serum and urine samples, showed the presence of monoclonal component in the gamma zone. We performed serum and urine immunofixation electrophoresis that showed a λ monoclonal component confirmed by a follow immunofixation using also IgD, IgE and FLC λ antibodies. Immunoblotting and SDS Page confirmed the presence of FLC and showed that the monoclonal component was consisted of proteins with molecular mass of 22000 and 44000 Da. The woman was admitted to the Hematology-Oncology Unit and one month after initiation of therapy serum and urine immunofixation showed the disappearance of the monoclonal component and the s-FLC assay (Freelite TM) returned to the reference ranges.
Conclusions: Bone biopsy confirmed the diagnosis of λ Chain Multiple Myeloma as detected by Freelite assay. The difference in results obtained with the two assays can depend on the specificities and affinities of the antibodies.

BS02

A SIMPLE WAY TO DIAGNOSE MYELOMA WITH FREE LIGHT CHAINS (FLC) FREELITE® ASSAY

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Background: Plasma cell disorders are characterized by uncontrollable production of the same immunoglobulin, complete or not, from a proliferating plasma cells clone. The risk factors for the disorders are: being middle aged, being male and exposed to chemical compounds or radiation. Plasma cell dyscrasias are classified into Myeloma, MGUS, Macroglobulinemia and Heavy Chain Disease. We want to show the uselfuness and importance of Freelite® Assay for Myeloma diagnosis.
Methods and results: S.G., a 65-year-old man, was hospitalized in San Giuseppe Moscati Hospital to undergo an operation to his inguinal hernia. He had only a feeble pain at the abdomen, increasing with deambulation. Laboratory tests, performed before the surgery, didn't report important alterations. Haematological parameters had little variations compared to average values and reported a slight pancytopenia (RBC=3,7x106/μL, Plt=129x103/μL, WBC =3,8x103/μL) and a slight macrocytic and hyperchromic anemia (Hct=38,6%, MCV=104fL, MCH=36,1 pg). Calcemia was normal (9,1 mg/dL), serum protein electrophoresis showed a little hypogammaglobulinemia and a little thickening-band before gamma globulins. We measured FLC in S.G serum using Freelite® Assay and unexpectedly it reported high levels of kappa free-chains (177 mg/dL) and a κ/λ ratio of 17,4. This data were then confirmed by other methods. Serum immunosubtraction identified a signal breakdown of thickening-band from kappa anti-serum in pre-gamma position. Then, we measured k and λ chains in urine by nephelometry and performed urine electrophoresis. Nephelometry identified a massive excretion of kappa chains (κ/λ urinary ratio = 140) , urinary electrophoresis identified a strong band of immunoglobulins in pre-gamma position and an intermediate excretion of albumin. For the diagnosis of Myeloma we waited for the results of NMR, X-ray and bone marrow biopsy. X-ray reported radiolucency areas only in the cranial theca and NMR reported diploic hyperintense nuclei in L1 on the right side and in L2 on the right peduncle.
Conclusions: Bioptic test of bone marrow, taken from iliac crest, allowed the identification of a conspicuous plasmocytosis (30%) and confirmed Myeloma diagnosis, which was previously and correctly formulated through FLC laboratory data.

BS03

DFLC DATA PROCESSING TO MONITOR THE RESPONSE TO THERAPY IN PATIENTS WITH AMYLOIDOSIS AL

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Background: The aggregation and abnormal deposition of misfolded proteins in the tissues is the pathological basis of systemic AL Amyloidosis (AAL). Recently, clinical studies have indicate that the serum free light-chains (FLCs) measurement is a well-established assay for identifying and monitoring the response to therapy. Furthermore, although there is a significant correlation between the extent of reduction of amyloidogenic FLCs and improvement in survival, few clinical tools in use allow to monitor globally the evolution of the disease according to the variation of the difference between involved and uninvolved FLCs (dFLCs). We propose a statistical dFLCs analysis to monitor the disease course from the beginning of therapy. Considering dFLCs values, it is easy to extrapolate a regression line like a generic straight line y = mx + d.
Methods: We performed the FLCs analysis on 7±1 samples of 10 AAL patients collected in a time period of at least 3±1 years from the therapy start. First, we have calculated the values of dFLCs and then those of the percentage variations of each dFLCs (pv-dFLCs), and analysed the correlation with dFLCs value obtained at the beginning of therapy. Finally, we plotted pv-dFLCs in a graph, and calculated the regression line using the appropriate statistical formulas.
Results: We observed that the positive or negative slope (m) of the regression line correlates with the patient's response to therapy over the time. Notably, this analysis is useful to monitor therapeutic response, but also allows to appreciate, in the follow-up, a turnaround prognosis of a disease even in the absence of other blood parameters alterations. This simple statistical analysis can help in choosing the best treatment approach even in cases with equivocal findings (e. g. when the values of the dFLCs and the κ/λ ratio are normal but the other values are slightly altered indicating a possible early progression).
Conclusions: In this case, the slope symbol of the regression line may be used to asses, in real time, a first change leading to pathological evolution. This is possible because the pv-dFLCs is a highly sensitive parameter, able to identify variations more deeply and accurately than the dFLCs value.
However, further studies are needed to confirm the goodness of the method.

BS04

FREELITE™, HEVYLITE™ AND SERUM PROTEIN ELECTROPHORESIS (SPE) ASSAYS FOR MONITORING OF A PATIENT WITH IGGλ MULTIPLE MYELOMA

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Background: Serum Free Light Chains (sFLC) and "Heavy/Light Chains" (sHLC) are found in patients with B-cell dyscrasias, including multiple myeloma (MM). HLC is a new immunoassay based on the recognition of epitopes spanning the junction of the immunoglobulin's heavy and light chains, which separately identifies the different light chain types of each immunoglobulin class: IgGκ, IgGλ, IgAκ, IgAλ, IgMκ, IgMλ. Serum Ig'K/Ig'λ ratio (sHLCr) and serum K free/λfree ratio (sFLCr) are useful in MM monitoring because they account for both involved and uninvolved Ig classes. In this IgGλ case, SPE, which is directly related to the tumor mass, clearly allowed monoclonal protein monitoring. We evaluated and compared the role of sFLC and sHLC vs SPE. Patient and Methods: A 60 years old female MM patient was studied. At diagnosis she presented with 35,2 g/L IgG, positive SPE and Immunofixation Electrophoresis (IFE); Bence-Jones proteins were absent. The patient reached a partial remission after therapy and autologous transplantation; the follow-up period we considered ranged from the remission phase until monoclonal protein consistently increased, before relapse occurred, and lasted 19 months. sFLC [FREELITE™, The Binding Site, Birmingham, UK] and sHLC [HEVYLITE™,The Binding Site] were evaluated on a Binding site SPAPLUS™ Analyzer. SPE was performed by Hydrasis [Sebia, France]. A total of 56 determinations were performed.
Results: During the whole follow-up period, SPE was positive in all the samples; the monoclonal spike ranged from 3.2 to 7.4 g/L. sHLCr was always below the normal values; sFLCr was in the normal range until month +3, became below the normal range from month +5 until month +12, then became again normal till month +15, and was normal again until the end of the follow-up (+19).
Conclusion: In this MM patient, sHLCr had the same trend as SPE, while sFLCr showed different kinetic behaviour. In this particular case sHLCr demonstrated to be more suitable than sFLCr in disease monitoring.

BS05

MEASUREMENT OF CIRCULATING FREE LIGHT CHAIN AND CARDIAC BIOMARKERS IDENTIFIES PATIENTS WITH AL AMYLOIDOSIS AT VERY HIGH RISK OF EARLY DEATH

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Background:The ability to accurately identify patients with AL amyloidosis at high risk of early death is crucial in the design of treatment strategy. A staging system based on amino-terminal pro-natriuretic peptide type-B (NT-proBNP) and troponins (cTn) is currently used for prognostic stratification. Recently, the measurement of FLC has been incorporated in this staging system. We prospectively assessed the prognostic role of measurement of FLC and cardiac biomarkers in 531 consecutive newly diagnosed patients enrolled between 2004 and 2010.
Methods and results: Serum FLC concentration was measured by the Binding Site assay on a BN ProSpec nephelometer. Median age was 65 years. 393 (74%) patients had cardiac involvement by standard diagnostic criteria. Mayo cardiac stage was I (NT-proBNP <332 ng/L and cTnI <0.1 ng/mL) in 18% of patients, II (NT-proBNP ≥332 ng/L or cTnI ≥0.1 ng/mL) in 43% and III (NT-proBNP ≥332 ng/L and cTnI ≥0.1 ng/mL) in 39%. In the overall population studied, Mayo stage (Hazard Ratio [HR] 3.4, P <0.001) and difference between involved and uninvolved FLCs (dFLC) >500 mg/L (HR 1.8, P <0.001) were independent prognostic determinants. In stage III subjects, NT-proBNP >10,000 ng/L was an additional independent prognostic factor. Median survival was 2 months if both NT-proBNP and dFLC were above the cutoff (84% of patients dying before completing 2 cycles of treatment). Stage III subjects with only 1 marker above the cutoff had a median survival of 6 months. Median survival was 12 months if both FLC and NT-proBNP were below the cutoff. Stage III patients are not an homogeneous group, but comprise subjects who enjoy relatively long survival, as well as patients who have little chance of surviving long enough to benefit from available treatments.
Conclusions: These subjects can be identified by adding FLC measurement to cardiac biomarkers and should be considered for innovative treatment approaches.

BS06

MONITORING OF THE FREE LIGHT CHAINS CONCENTRATION DURING A COMBINED THERAPEUTICAL APPROACH TO MULTIPLE MYELOMA (CHEMOTHERAPY AND EXTRACORPOREAL REMOVAL)

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Background: Acute Renal Failure (ARF) due to deposition of immunoglobulin free light chains (FLC) in the renal tubule (cast nephropathy, CaN) often complicates Multiple Myeloma (MM). If CaN does not recover, chronic renal insufficiency and chronic dialysis are the consequence. The extracorporeal removal of FLC, combined with chemotherapy, presents as a rational therapeutical approach. Different techniques for an extracorporeal removal are possible (plasma-exchange, high cut-off dializers, adsorption) with different efficiency and cost. To define the type and timing of the removal however free lambda and kappa immunoglobulin light chains should be identified and quantitatively measured separately. A nephelometric and turbidimetric serum FLC assay (Freelite) has been recently developed, to be run on most major immunology and clinical chemistry analyzers.
Method and results: We described a case report of a male patient, 65 years, with kappa MM and CaN with severe ARF (plama creatinine 7.5 mg/dL). His initial level of the kappa chains was very high (14000 mg/dL); we decide to treat him with the high cutoff dialysis, in which the removal of FLC and the uremic solutes is simultaneous and very efficient. At the same time a first chemotherapy cycle was started. After 4 treatments the kappa FLC went down to 4000 mg/L, and the renal function was improved (creatinine 3 mg/dL); however, over 12 days there was a new progressive increase of the k LC up to 8000 mg/L. A second chemotherapy cycle was introduced, with the addition of melphalan. Simultaneously, a second cycle of extracorporeal removal was performed with an adsorptive technique, with a reduction of the k LC to levels <500 mg/L, that persisted over the following weeks.
Conclusion: The quantitative measurement of the FLC has a strong and powerful value in supporting the therapeutical decision making. Since the artificial removal of LC needs complex and expensive treatments, the most appropriate technique and the timing of its application have to be defined on the basis of the circulating levels and the response to chemotherapy. Monitoring the FLC level throughout the treatment serves as a guide for the whole therapy, including the extracorporeal removal.

BS07

NEGATIVE PREDICTIVE VALUE OF SFLC IN DOUBTFUL HISTOLOGICAL PATTERN

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Methods: In January 2011 a 48-year-old Chinese woman was admitted due to back pain. Laboratory examination showed mild anemia (Hb=119 g/L) and a mild increase of acute phase proteins; immunofixation electrophoresis was negative; κ and λ serum Free Light Chains (sFLCs) were 2.80 and 7.91 mg/L, respectively; creatininemia was 29 umol/L; Quantiferon test was negative. Magnetic Resonance (MR) of the spine showed diffuse multiple substitutive lesions. A Positron emission tomography-computed tomography scan was consistent with multiple glucose-avid lesions at the whole spine, sternum and pelvis level. A bone marrow biopsy showed 10% clonal plasma cells. The cytogenetic investigation was normal. The body of the 2nd lumbar vertebra was biopsied and showed clonal plasma cells, consistent with plasma cell neoplasia.
Results: A non-secretory multiple myeloma was diagnosed. The patient underwent chemotherapy according to Lenalidomide-Dexamethasone regimen, but a subsequent MR showed progression of the disease. Serum and urine immunofixation was negative; sFLC concentrations ranged from undetectable to 10.3 mg/L, κ/λ ratio ranged from 0.35 to 1.17 and did not change over the time. The patient underwent unsuccessful radiation therapy of the spine (T7-T11 and L3-L4). Because of refractory disease, a D8-D9 laminectomy with multiple biopsies was performed: the histologic diagnosis was still consistent with plasma cell neoplasia. The patient underwent sequential unsuccessful chemotherapies. The histologic specimens were reviewed elsewhere and the diagnosis was consistent with probable metastasis of primary unknown cancer. The patient underwent a surgical iliac bone biopsy. Mycobacterium tuberculosis was shown both on cultural and molecular grounds. Interestingly, the Quantiferon test became positive. Eventually a diagnosis of Pott's disease was made. Abnormal sFLC ratio identifies a high proportion (68% to 100%) of non-secretory multiple myeloma patients. In our patient despite anemia, bone lesions and several histologic diagnosis of clonal plasma cell disease, sFLC concentrations were always <11 mg/L, and did not change upon different chemotherapy regimens.
Conclusions: This finding suggests that sFLC must be taken into account as negative predictive marker in patients with doubtful histological patterns.

BS08

RATING FLC IN MONOCLONAL GAMMAPATHIES AND IN PATIENTS WITH MULTIPLE MYELOMA: A EXEMPLARY CLINICAL CASE

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Background: The assay of serum free light chains (Serum Free Light Chains, SFLC) together with the relationship between SFLC Kappa (SFLCK) and Lambda (SFLCL) types is used in the diagnosis, monitoring and prognosis of monoclonal gammopathies and during multiple myeloma therapy. The SFLC proves to be an early marker of remission or relapse during treatment. This is possible thanks to the rapid turnover of the SFLC and the good sensitivity of the method.
Materials and methods: The protocol in use in our centre provides quantitative and qualitative assessment of serum Monoclonal Component (CM) detectable by electrophoresis and the quantization of SFLCK, SFLCL and their ratio (SFLCR) to assess the SFLCs involved (iFLC) or not (uFLC) in the pathology and their difference (dFLC). These parameters were correlated with monthly disease progression and response to treatment according to the current guidelines. Serum protein electrophoresis (ESP) and immunofixation electrophoresis (IFE) were carried out on agarose gel (Sebia). SFLC was performed on the same samples with the kit Freelite - The Binding Site on Integra 400 - Roche. We followed up 60 patients, both with monoclonal gammopathy and treated for multiple myeloma, for a six months period. ESP and SFLC measurement were performed monthly and results were compared and correlated with IFE every three months.
Results: In all cases, CM and iFLC confirmed a parallel trend. A specific case of multiple myeloma, previously treated, shows evident and early increase of iFLC and CM, identifying disease relapse following usual chemotherapy cycles. During a subsequent therapy, dFLC values changed as expected, according to treatment response. IFE showed to be a less sensitive method, becoming positive for free light chains at a later stage compared to SFLC test (band not detectable in the previous examination).
Conclusions: In our experience, the dosage of SFLC has now a well established diagnostic role; it is for us an important criterion for the diagnosis and progression of monoclonal gammopathy and a key tool in the assessment of response to treatment in multiple myeloma. We propose the completion of an electronic chart for each patient in order to better follow the evolution of the disease and the effects of therapy.

BS09

SLOW RENAL FUNCTION RECOVERY AFTER ACUTE KIDNEY INJURY IN A CASE OF MYELOMA KIDNEY: ROLE OF FREE LIGHT CHAIN MONITORING

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Background: Up to 10% of patients with myeloma develops acute kidney injury (AKI) which requires dialysis support. Unless fast reduction of toxic free light chain (FLC) is achieved by means of aggressive chemotherapy (CT) coupled to high cut-off membrane hemodialysis (HCO-HD), most of them become dialysis dependent.
Methods: A 66 year woman, on follow-up for IgG λ smouldering myeloma (40% λ monotypic plasma cells), was admitted at our hospital for AKI. Laboratory findings were as follows: serum creatinine (sCr) 9.8 mg/dL; IgG λ monoclonal component (MC) 1.05 g/dL , FLCλ 16160 mg/L, κ/λ 0.0014 and Bence Jones λ 156 mg/24 h. Renal biopsy confirmed "cast nephropathy" and the patient started dialysis with HCO-HD (Theralite 2100 Gambro; 6 hours /3 times a week) in addition to Bortezomib and Dexamethasone according to a biweekly schedule (4 cycles) plus Thalidomide treatment. FLC concentrations were measured by nephelometry (Freelight Binding Site; Beckman Immage 800) in blood samples collected at 5,180 and 360 min.
Results: Dialysis independence(sCr 5.2 mg/dL) was achieved after 10 dialysis sessions and a very good partial hematological response after 2 cycles of CT with a remarkable low value of FLCλ (33.9 mg/L). After two subsequent cycles of CT, sCr was reduced to 2.5 mg/dL, CM to 0.14 g/dL, FLCλ to 12.7 mg/L and the absence of clonal cells in bone marrow was documented. One month later the patient underwent high dose CT in view of autologous stem cell transplantation.After 6 months the hematological response was stable (FLCλ 18.3 mg/L) and renal function was further improved (sCr 1,9 mg/dL).
Conclusions: In the short-term HCO-HD was more effective on FLC levels (99% reduction), than on sCr (47% reduction), but the present case shows that, even after CT interruption, renal function may further improve. The observed slow renal recovery may be caused by the slower clearance of pathological FLC from kidney, due to the potential unique biochemical characteristics of these high variable molecules. FLC monitoring seems to be an useful tool to guide extent of HCO-HD, tailoring treatment of single myeloma kidney patient.

BS10

THE RELEVANCE OF SERUM FREE LIGHT CHAIN MONITORING IN IGD KAPPA MULTIPLE MYELOMA MENAGEMENT

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Background: The IgD Multiple Myeloma (MM) is a rare disease almost exclusively Ig Lambda Chains restricted. In this report we describe a case of IgD Kappa MM.
Methods: A 51 y/o woman with anaemia and bone lesions was admitted in our Institute. A Bence Jones (BJ) positive Kappa light chain in the urine and a serum free light chain ratio of 709.5 with Kappa free 5889 mg/L and Lambda free 8.3 mg/L values, was documented. The plasmacell (PC) flow cytometry characterization identified an infiltration (23% of bone marrow population) of CD38, CD138, CD28, CD56, CD117 positive, CD45 CD19 negative tumour plasmacells, with Kappa free light chains (FLC) restriction. Patient was treated with 2 cycles of Vincristine, Adriblastine, Dexamethasone (VAD), 2 cycles of Endoxan and 2 cycles of Bortezomib, Myocet, Dexamethasone (BMD). Response to treatment was monitored with Kappa / Lambda free light chains ratio, bone marrow (BM) trephine and plasmacell flow cytometry. A high serum level of Kappa FLC, with an abnormal FK/FL ratio was documented, supported by a tumour PC immunophenotype, confirming no treatment response. An autologous stem cell transplantation (autoSCT) was performed.
Results: The FLC monitoring identified a progressive K/L ratio reduction as well as an increase of CD45 CD19 positive, CD56 CD117 negative normal BM PC, leading to a near Complete Remission (nCR).
Conclusions: IgD Kappa MM are seldom reported in the literature. In this case report, we describe the relevance of a non invasive approach, the FLC Kappa and Lambda ratio, in disease monitoring and in the identification of lack of treatment response. FLC appears to be an important and crucial tool for classification of responders and non responders and could deeply contribute to a better management of MM patients.

BS11

THERAPY MONITORING WITH "FREELITE" ASSAY IN A PHARMACORESISTANT MICROMOLECULAR MYELOMA

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Methods: A 78-years-old man was admitted to hospital with strangury, fever and dyspnea in Nephrology ward. Abdomen CT scan and echography were all unremarkable. Urine test showed proteinuria (7.9 g/L in 24 h), but normal renal function. Serum and urine immunofixation showed a faint monoclonal λ band. Serum Freelite assay was performed and showed an abnormal κ/λ ratio =0.0008 (κ 1.52 mg/L, λ1890 mg/L) indicating a monoclonal component of λ free light chain (FLC). This abnormal values highlighted the suspect of myeloma and nephrologists prescribed a osteo-medullary biopsy that revealed aggregates of plasmacytosis, accounting for 20% of the marrow cellularity; the cellular aggregates stained positively for λ FLC and negatively for κ FLC. Periumbilical fat was negative at Congo red staining indicating no amyloidogenic λ FLC.
Results: The patient diagnosis was II A λ micromolecular myeloma and treatement with bortexomib, melphalan and prednisone was started. During the following days κ/λ ratio was still altered indicating therapy failure. A week later he experienced anury and Lab tests showed acute renal failure with blood creatinine increasing (13 mg/dL) without obstruction of excretory apparatus and increase of λ FLC in serum (κ 0.42 mg/L, λ 4540 mg/L). According to nephrologists and hematologists advices the patient began with bicarbonate 1/6 M and mannitol against the cast nephropathy and parenteral desametasone 20 mg/die. The therapy did not show any effect: λ values were still high with anuria. Freelite assay helped to define the best therapy: clinicians decided to suspend pharmacological therapy and to dialyze the patient with PMMA filters (6 h treatment, changing filter every 2 h) to remove FLC from the serum. Every 2 h Freelite assay was performed to follow-up proteins removal. After 6 h FLC concentration decreased (κ 0.5 mg/L, λ 534 mg/L), diuresis was restored and renal function was improved (creatinine 1.7 mg/dL). The myeloma treatment was established with steroids because of lenalidomide treatment patient incompliance and, after kidney recovery, the dialysis was suspended.
Conclusions: Freelite assay helped laboratorists to highlight the suspect of myeloma and helped nephrologists and hematologists to define the best therapy and patient follow-up process.

BS12

USEFULNESS OF SERUM FREE LIGHT CHAIN ASSAY FOR DIAGNOSIS AND THERAPEUTIC MONITORING OF PLASMA CELL DISORDERS: A CASE REPORT

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Background: According to the International Myeloma Working Group guidelines, serum Free Light Chain (FLC) assay can be used for evaluation and management of plasma cell disorders.
Methods: We report the case of a 40 year-old woman who arrived at our department with the following clinical history: progressively increased serum creatinine value (from 1.0 to 2.8 mg/dL) and non-selective glomerular proteinuria (1-2 g / 24 h) starting from 32 week of pregnancy; transitory reduction of creatinine value after treatment with Metilprednisolone (40 mg/die) for pre-sacral pain (no lesions evidenced by X-Ray); delivery at 35 week and rising of creatinine value (3 mg/dL) and proteinuria (2.5 g / 24 h) after metilprednisolone reduction; electrophoresis and immunofixation displaying k FLC with no evidence of osteomedullary alteration.
Results: In order to exclude a plasma cell disorder, patient was hospitalized and biochemical evaluation evidenced: serum creatinine (1.24 mg/dL), hemoglobin (12 g/dL), non-selective glomerular proteinuria (3 g/dL), elevated serum k FLC level (1176 mg/L, measured by turbidimetric method, Freelite™, The Binding Site Ltd, Birmingham, UK; reference interval: 3.3-19.4 mg/L) with consequent abnormal FLC κ/λ ratio (79.5; reference interval 0.3-1.2). Serum and urinary immunofixation confirmed the presence of κ FLC. Renal biopsy revealed a cast-nephropathy condition due to κ FLC deposition. Further investigations were conducted: total body PET was negative; backbone NMR highlighted a wide replacement of pre-sacral region with pathological tissue characterized by plasma cell infiltration (CD138+, CD56+, CD20-) with low proliferative index (ki67 <5%). Multiple myeloma was excluded by bone biopsy. The definitive diagnosis was of solitary plasmacytoma of sacral region, secreting κ FLC, with consequent cast nephropathy. After peripheral stem cell collection, chemotherapy (Velcade, Thalidomide and Desametasone, VTD) together with focused radiotherapy of sacral region were carried out. During VTD therapy, FLC ratio gradually decreased and now it is almost normalized, representing the only marker of positive response.
Conclusions: In this case report, the evaluation of serum FLC level and its ratio has allowed to diagnose and to monitor the therapy efficacy.

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