Blood biomarkers associated with autism spectrum disorder may provide early diagnosis

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BACKGROUND
Autism Spectrum Disorders (ASD) are a collection of neuropsychiatric disorders. ASD affects 1 in 87 in Qatar and is expected to increase. The high variability and heterogeneity of the symptoms makes diagnosis of ASD difficult and uncertain, particularly at the early stages of development. If detected early, clinical support can be given to promote optimal development and well-being of children with ASD and even achieve complete remission. The current method of diagnosing ASD is by observations and interviews made by experts in child development to assess child behavior, communication and cognition: the ADOS test. There is often delay in referral for expert diagnosis; delay is typically >18 months in Qatar and >4 years in EU and USA. The diagnostic accuracy is 60 – 70%.

RESULTS
Changes in protein glycation and oxidation in children with autism spectrum disorder, compared to children with normal development Children with ASD had plasma protein with increased glycation adducts - N-carboxymethyl-lysine (CML) and Nω-carboxymethylargine (CMA), and increased oxidation damage marker, dityrosine (DT), with respect to children with normal development.

Aim: To Develop a blood test for autism to provide early and swift diagnosis by measure the levels of damaged amino acids in the blood of children with ASD and children with normal development and use artificial intelligence machine learning techniques to develop diagnostic algorithms for diagnosis of ASD.

METHODS
Subject recruitment: Sixty-nine children were recruited for this study: 38 had a diagnosis of ASD (29 males and 9 females) and 31 were classified as Typically Developing (TD) children (23 males and 8 females) – Figure 1. Subject age was: ASD group, 7.6 years ± 2.0 years, range 5 – 12 years and TD group, 8.6 ± 2.0 years, range 5 – 12 years (P=0.05). All ASD subjects received a diagnosis of ASD by two child development experts at the Child Neurolgy and Psychiatry Unit of the Bellaria Hospital of Bologna (IRCCS Institute of Neurological Sciences), according to the Diagnostic and Statistical Manual of Mental Disorders V (DSM 5 criteria, Autism Diagnostic Observation Schedule (ADOS), Childhood Autism Rating Scale (CARS) and characteristics of onset pattern of ASD defined according to Ozonoff et al. All subjects were recruited at the Child Neurolgy and Psychiatry Unit of the Bellaria Hospital of Bologna, Bologna, Italy.

Analysis: Plasma proteins undergoing continuous spontaneous modifications by sugars (glycation), oxidation and other processes. The adducts formed may be quantified in a small volume of blood by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry (LC-MS/MS). A blood plasma sample (50 µl) from cases and control is processed through 4 cycles 10-fold dilution and concentration by ultrafiltration over a 10 kDa cut-off filter to wash the protein. The protein is delipidated and treated with 5 µl of 0.5 M iodoacetic acid, and then hydrolysed exhaustively to amino acids by successive addition of pepsin, Pronase E, Prolidase and leucine aminopeptidase over 4 days. The digest is then mixed with stable isotopic standards and analysed for ca. 20 chemically-defined protein glycation and oxidation adducts by LC-MS/MS.

Machine learning development of diagnostic algorithms: support vector machines (SVM) Use of hyperplanes and maximum margin in SVM algorithm development:
(a) Two-dimensional scatter plot for a two-class-labelled dataset of outcome, o & +. The data can be separated by an infinite number of hyperplanes - three are shown (11, 12, 13). (b) Hyperplane maximizes the margin (m) between the two classes of outcome, o and +. These are specified by the ‘support vectors’ – maximum differences from data at the margins.

DISCUSSION & CONCLUSION
A blood test has been developed for early detection of autism. It could be widely implemented with diagnostic outcome provided within one week. Improved access to diagnosis of autism would resolve current long periods of uncertainty for suspected cases and their carers and, where diagnosed and confirmed, would allow for an appropriate care plan to be implemented and produce remission if implemented at the early stages of ASD. The validation of the test in independent cohorts of cases and controls is in progress in collaboration with QBRI and Sidra Medicine. Data driven combination of these biomarkers gave diagnostic algorithms of high sensitivity, specificity and accuracy for ASD than achieved by current in-service clinical assessments.

REFERENCES