Mediterranean Journal of Hematology and Infectious Diseases

Original Article

Detection of Antinuclear Antibodies Targeting Intracellular Signal Transduction, Metabolism, Apoptotic Processes and Cell Death in Critical COVID-19 Patients

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Competing interests: The authors declare no conflict of Interest.

Abstract. ***Background and Objectives*: The heterogeneity of the coronavirus disease of 2019 (COVID-19) lies within its diverse symptoms and severity, ranging from mild to lethal. Acute respiratory distress syndrome (ARDS) is a leading cause of mortality in COVID-19 patients, characterized by a hyper cytokine storm. Autoimmunity is proposed to occur as a result of COVID-19, given the high similarity of the immune responses observed in COVID-19 and autoimmune diseases. Here, we investigate the level of autoimmune antibodies in COVID-19 patients with different severities. Results: Initial screening for antinuclear antibodies (ANA) IgG using ELISA revealed that 1.58% (2/126) and 4% (5/126) of intensive care unit (ICU) COVID-19 cases expressed strong and moderate ANA levels, respectively. An additional sample was positive with immunofluorescence assays (IFA) screening. However, all the non-ICU cases (n=273) were ANA negative using both assays. Samples positive for ANA were further confirmed with large-scale autoantibody screening by phage immunoprecipitation-sequencing (PhIP-Seq). The majority of the ANA-positive samples showed "speckled" ANA pattern by microscopy and revealed autoantibody specificities that targeted proteins involved in intracellular signal transduction, metabolism, apoptotic processes, and cell death by PhIP-Seq; further denoting reactivity to nuclear and cytoplasmic antigens. Conclusion: Our results further support the notion of routine screening for autoimmune responses in COVID-19 patients, which might help improve disease prognosis and patient management. Further, results provide compelling evidence that ANA-positive individuals should be excluded from being donors for convalescent plasma therapy in the context of COVID-19.**

Keywords: Autoimmunity; ANA; ICU; COVID-19; Coronavirus.

Citation: Nasarallah G.K., Fakhroo A.D., Khan T., Cyprian F.S., Al Ali F., Ata M.M.A., Taleb S., Zedan H.T., Al-Sadeq D.W., Amanullah F.H., Hssain A.A., Eid A.H., Abu-Raddad L.J., Al-Khal A., Al Thani A.A., Marr N., Yassine H.M. Detection of antinuclear antibodies targeting intracellular signal transduction, metabolism, apoptotic processes and cell death in critical COVID-19 patients. Mediterr J Hematol Infect Dis 2022,14(1): e2022076, DOI: http://dx.doi.org/10.4084/MJHID.2022.076

Published: November 1, 2022 **Received: August 3, 2022** **Accepted: October 13, 2022**

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Introduction. The severity of COVID-19 is diverse, with a wide range of symptoms characterized from mild to lethal. A robust immune response is usually initiated upon viral infections, involving both the innate and adaptive immune systems to irradicate the virus.[1] Typically, viruses evade these immune responses through one of the following mechanisms; (1) Molecular Mimicry, (2) bystander activation, and (3) epitope spreading.[1,2] In COVID-19 patients, several immunological impacts have been observed, including hyper-immune response, abnormal cytokine/chemokine production, T cells hyperactivation, increased monocytes, macrophages, and neutrophils count.[1,3] The cytokine storm (i.e., abnormal cytokine secretion) is associated with fatality in COVID-19 patients, who usually experience a hyper-pro-inflammatory immune response leading to ARDS.[1,3] These COVID-19 immune responses are quite similar to those observed in autoinflammatory and autoimmune conditions.[1,4] It is known that infectious diseases trigger autoimmunity, specifically through molecular mimicry, and several viruses have been associated with autoimmune diseases.[1,5,6] For example, enteric viruses have been associated with type 1 diabetes, and herpesviruses infections have led to the development of several autoimmune disorders, including multiple sclerosis, systemic lupus erythematosus, and rheumatoid arthritis.[1,6,7,8] In addition, mice infected with murine coronavirus developed immune-mediated encephalomyelitis.[9] Further, rhinovirus and coronavirus were shown to be the highest frequently detected pathogens in patients with psoriasis flares following respiratory tract infections.[10]

Severe COVID-19 patients have been suspected of developing autoimmunity. Several reports have suggested a link between SARS-CoV-2 infection and Kawasaki-like disease, acute inflammation of the blood vessels affecting children.[5,11] Moreover, patients with severe COVID-19 pneumonia reported neutralizing IgG autoantibodies against type I IFNs.[12] In addition, other autoantibodies, such as anti-platelet autoantibodies (APA), were reported in COVID-19 patients, leading to immune thrombocytopenia.[13] Accordingly, critically ill COVID-19 patients may experience elevated levels of other autoimmune antibodies, including antinuclear antibodies (ANA).[14,15] High levels of ANA have been previously associated with several autoimmune disorders, such as lupus erythematosus (SLE) and rheumatoid arthritis (RA).[16] This study investigates whether COVID-19 severe outcome could be associated with autoimmunity. We measured ANA levels in blood sera samples collected from COVID-19 patients with different clinical severities (i.e., ICU "Severe" vs. Non-ICU "Mild or Asymptomatic"). We report a higher frequency of ANA in severe COVID-19 cases, suggesting a possible contribution of COVID-19 to autoimmunity and exacerbated disease outcome.

Method.

Sample Collection and Ethical Compliance. This study was approved by the IRB committees of Hamad Medical Corporation (MRC-01-20-145) and Qatar University (QU-IRB 1289-EA/20). Informed consent was obtained from all patients per the approved protocol, and informed consent was obtained from next of kin in case of death. The samples' numbers in **table 1** are only identifiable to the researchers and are not related to the national nor the medical IDs of the patients. Sera samples were collected from COVID-19 patients at different clinical stages and classified into two groups; (1) mild/asymptomatic (non-ICU, n=273), (2) Severe/Critical (ICU, n=126). Out of the 126 ICU patients, only 80 had sera extracted at different time points, assuming that ICU admission was on Day 1. For those with repetitive samples, at least two-time points were tested, and positive results were reported. All methods were performed following the relevant guidelines and regulations.

ANA IgG ELISA assay. All samples [mild/asymptomatic (non-ICU, n=273) & Severe/Critical (ICU, n=126)] were initially screened by ELISA. Sera samples were diluted 1:101 in sample diluent (DILSPE), and ELISA was performed using an ANA Screening IgG kit (DIA.PRO, Italy) according to the manufacturer's standards. The cutoff value was calculated as negative control (OD450nm) + 0.250. Samples with cut of value (S/Co) <0.8, S/Co (0.8-1.1), S/Co>1.1 were considered negative "normal", equivocal "moderate" and positive "abnormal" respectively. Note that samples that tested positive and equivocal were repeated in triplicates with mean and standard deviation calculated.

ANA HEp-2 IFA Assay. All ICU patients' sera (n=126) and an equivalent number of randomly selected non-ICU sera (n=121) were subjected to Indirect Fluorescent Antibody (IFA). A technique was performed using the AccuDiag™ ANA HEp-2 IFA kit (Diagnostic Automation/Cortez Diagnostics, US) according to the manufacturer's standards. A positive reaction is indicated by the presence of any pattern of nuclear apple-green staining observed at a 1:40 dilution based on a 1+ to 4+ scale of staining intensity (+1 weak → +4 strong). Samples were regarded as positive if they tested positive with either ELISA or IFA test. These samples were then

Table 1. Demographic Data of COVID-19 Patients with Moderate-to-Abnormal ANA Level.

Sample #	Gender	Age	Nationality	Comorbidities	Clinical Outcome	ANA detection/ Status
C020	M	Early 60's	Bangladeshi	-	***Deceased***	**IFA**
C024	M	Early 50's	Indian	Hypertension	Alive	***ELISA/ Abnormal***
C047	M	Early 40's	Filipino	-	Alive	ELISA/ Moderate
C083	M	Early 60's	Filipino	DM2, gouty arthritis	Alive	***ELISA/Abnormal***
F003	M	Mid 70's	Indian	Hypertension	***Deceased***	ELISA/ Moderate
F004	M	Mid 50's	Qatari	DM, Hypertension, Ischemic heart disease/First degree heart block,	Alive	ELISA/ Moderate
F026	M	Late 40's	Filipino	Hypertension, ESRD, Dyslipidemia, hyperuricemia	***Deceased***	ELISA/ Moderate
F057	M	Late 50's	Sri Lankan	DM, Hypertension, CAD, Dyslipidemia	***Deceased***	ELISA/ Moderate

DM: Diabetes Mellitus, ESRD: End-Stage Renal Disease, CAD: Coronary Artery Disease.

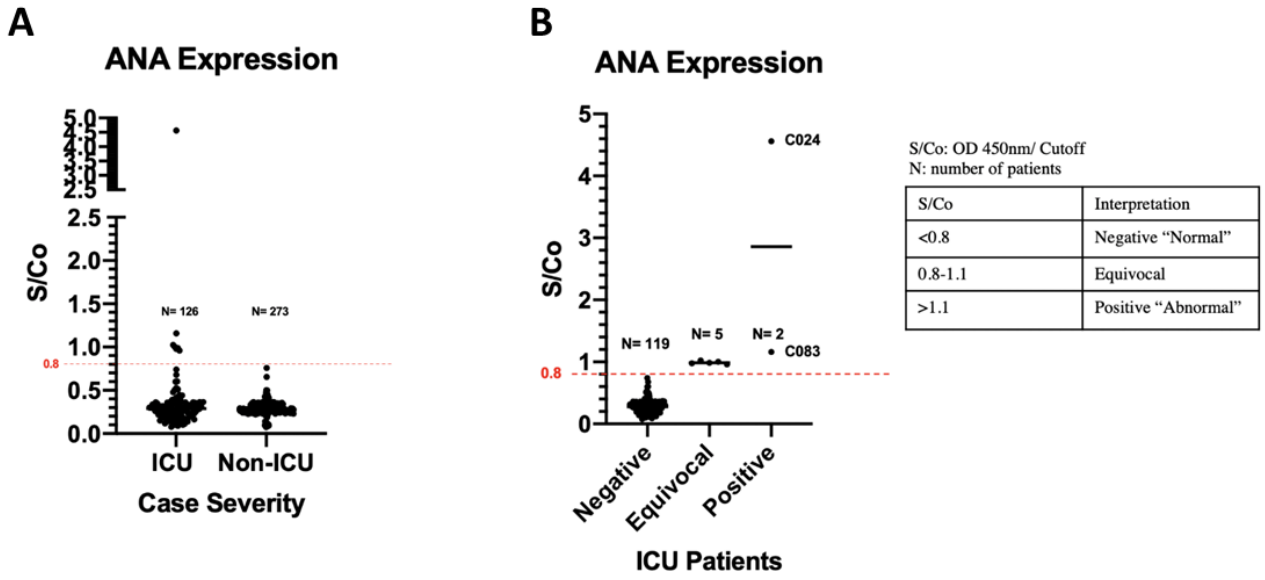
subjected to PhIP-Seq analysis.

Phage Immunoprecipitation-Sequencing (PhIP-Seq) and Peptide Enrichment Analysis. PhIP-Seq and peptide enrichment analysis were performed as previously described[17] using the T7 Human ORF 90mer library, a phage display library expressing 90-aa protein fragments tiling through the human proteome with a 45-aa overlap.[18] The T7 Human ORF 90mer Library was obtained from S. Elledge (Brigham and Women's Hospital and Harvard University Medical School, Boston, MA, USA). In brief, we imputed $-\log_{10}(P)$ values as described previously[17] by fitting a zero-inflated generalized Poisson model to the distribution of reading counts obtained from the tested samples following immunoprecipitation; then we regressed the parameters for each peptide sequence based on the read counts obtained from an input library sample (*i.e.*, prior to immunoprecipitation). These $-\log_{10}(P)$ values were considered peptide enrichment scores and reflected a quantitative measure for the presence of autoantibody specificity in a given sample. A peptide enrichment score of ≥ 2.3 was considered statistically significant. We also removed peptides from the downstream analysis enriched in mock-IP samples, which served as negative controls. We only considered peptides significantly enriched in at least two test samples. We then computed log odds ratios (LOD) for all retained peptides (n = 328) to identify autoantibody specificities that were differentially enriched between ICU patients and asymptomatic COVID-19 cases. Peptides with a $|\text{LOD}| \geq \ln(1.5)$ were considered differentially enriched (n = 79).

Gene Set Enrichment Analysis. Of the 79 differentially enriched peptides, 62 were derived from coding sequences with a defined gene annotation (Entrez) and were considered for gene enrichment analysis. We used the Molecular Signatures Database (MSigDB) for this analysis as previously described.[19] Out of the 62 queried genes, 42 were found to be significantly enriched (*P*-value < 10^{-5} and FDR q-value < 0.05) in one of the 20 gene sets.

Detection of Shared Linear B Cell Epitopes. To test for shared linear B cell epitopes between the identified autoantigens and SARS-CoV-2 antigens, we built a pairwise distance matrix that captured the maximum size of linear sequence identity of amino acids between the 79 differentially enriched human 90 aa peptides and 17 reference sequence of SARS-CoV2 proteins in UniProtKB (https://covid-19.uniprot.org; used for analysis not to deposit the data) as previously described.[20] A linear sequence identity of 7 amino acids or more was considered a shared linear B cell epitope.

Results. ANA antibodies were screened in non-ICU (n=273) and ICU (n=126) COVID-19 patients using ANA IgG ELISA. All of non-ICU patients (n=273) tested negative; (S/Co<0.8) with an average of 0.286 (+/- 0.073). On the other hand, 7/126 ICU patients (~5.6%) reported a S/Co value of >0.8, showing moderate to high ANA levels (**Figure 1a**). Four percent of the ICU patients (5/126) tested equivocal (S/Co: 0.8-1.1, moderate ANA level), whereas 1.58% (2/126) tested positive (S/Co>1.1, abnormal ANA level) (**Figure 1b**). The positive samples, C024 and C083, had a S/Co value of 4.561 and 1.159, respectively (**Figure 1b**). To confirm the presence of ANA, IFA was performed using HEp-2



S/Co	Interpretation
<0.8	Negative "Normal"
0.8-1.1	Equivocal
>1.1	Positive "Abnormal"

Figure 1. ANA ELISA levels of the COVID-19 Patients. **(A)** Sera samples of ICU (n=126) and non-ICU (n=273) COVID-19 patients; and **(B)** sera samples of ICU COVID-19 patients (n=126). Samples were tested at 1:101 dilution.

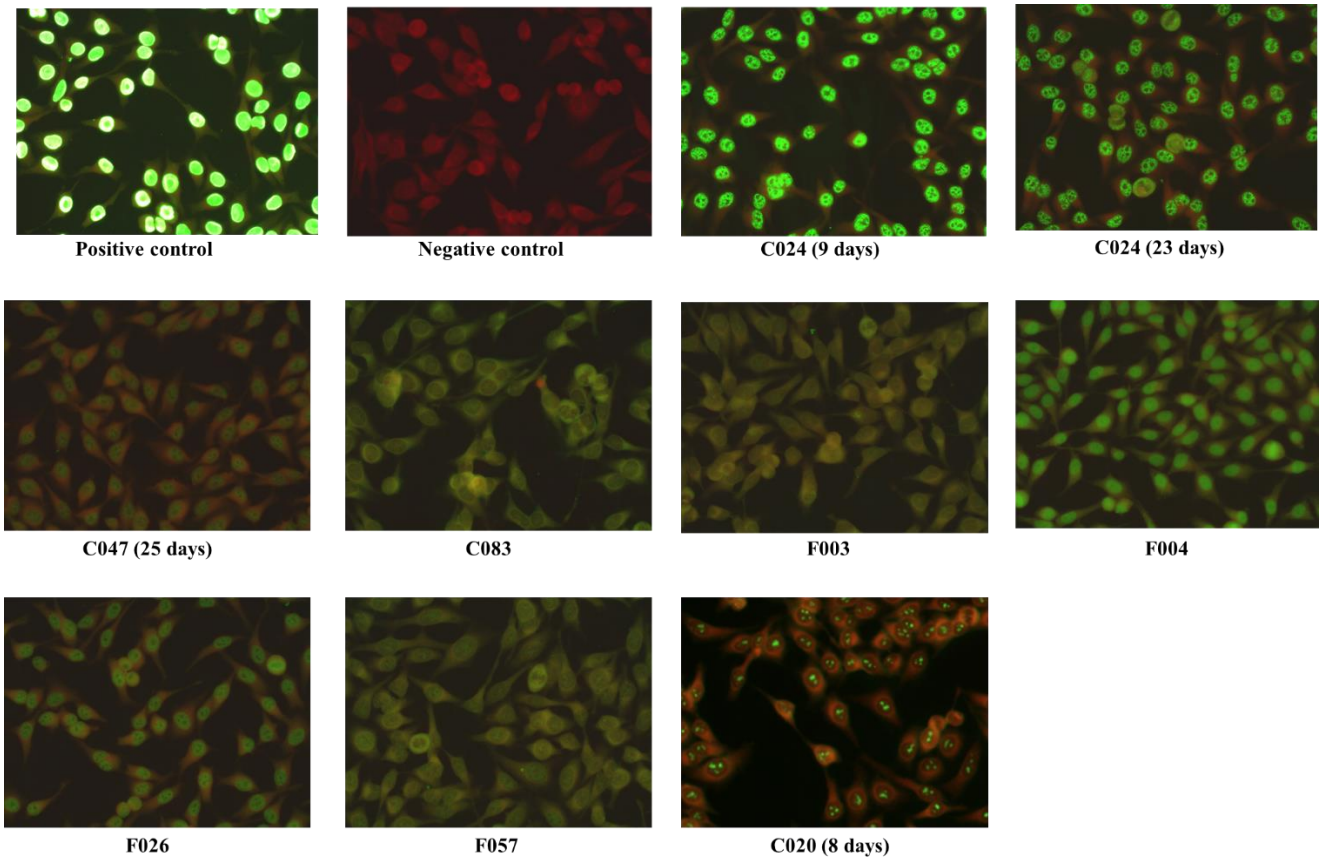


Figure 2. Immunostaining of Hep2 Cells with ANA from COVID-19 Sera Samples. Sera samples of ICU patients (n=126) and an equivalent number of randomly selected non-ICU patients sera (n=121) were subjected to Indirect Fluorescent Antibody (IFA)**.** HEp2 cells were used as a substrate to detect ANA antibodies in human serum. Samples were tested at 1:40 dilution. Note that # of days corresponds to the time of sample collection, assuming ICU admission is day 1.

cells on all ICU and an equivalent number of non-ICU samples (**Figure 2**). An additional sample (C020) tested strongly positive for IFA. The majority of the samples (C024, F026, F004, and C047) showed a "speckled"

ANA pattern (**Figure 2**). In addition to nuclear ANA level, cytoplasmic ANA level was observed in samples F003, F004, C020, F057, and C083 (**Figure 2**). Samples F057 and C083 showed a "punctate nuclear envelope" ANA pattern (**Figure 2**). All patients who tested equivocal/positive for ANA are males within the age range of 41-75 years (Average= 55 years) (**Table 1**). In relation, 71.4% of the ANA-positive patients (5/7) are co-diagnosed with hypertension, and the mortality rate was 42.9% (3/7), specifically the samples F003, F026, and F057 that showed moderate ANA Level (**Table 1**). Diabetes was also common among ANA-positive patients, accounting for 42.8% of patients (3/7), including patient C083, which exhibited abnormal ANA levels. Finally, we performed a large-scale autoantibody screen of the ANA-positive (C024, C083) and five equivocal sera samples using phage-immunoprecipitation sequencing.[9,12] Randomly selected ANA-negative samples obtained from ICU patients with COVID-19 (n = 7) and from asymptomatic COVID-19 cases (n = 15) were assayed for comparison. Principal component analysis of the peptide enrichment scores confirmed that most ANA-positive and equivocal samples of ICU cases clustered separately from the asymptomatic COVID-19 cases, except for the day 9 sample of C024 and the sample collected from C83 on day 17 after ICU admission (**Figure 3A**). We identified 79 autoantibody specificities that were differentially enriched in ICU patients in comparison to the asymptomatic COVID-19 cases (**Supplementary Table 1**), allowing a clear separation of critical versus asymptomatic COVID-19 cases by hierarchical clustering (except for one asymptomatic case) (**Figure 3B**). Interestingly, our unbiased screen revealed autoantibody specificities against several known autoantigens present in AAgAtlas 1.0, a human autoantigen database,[21] including nuclear proteins such as the DEAD-Box Helicase 42 (DDX42) and Mtr4 Exosome RNA Helicase (MTREX), as well as proteins involved in immune defenses and cellular signaling, such as Immunoglobulin Superfamily DCC Subclass Member 1 (DCC) and Ras Suppressor Protein 1 (RSU1). To functionally characterize the self-antigens that were being targeted in critically ill COVID-19 patients, we performed a gene set enrichment analysis using the Molecular Signatures Database (MSigDB)[11] and

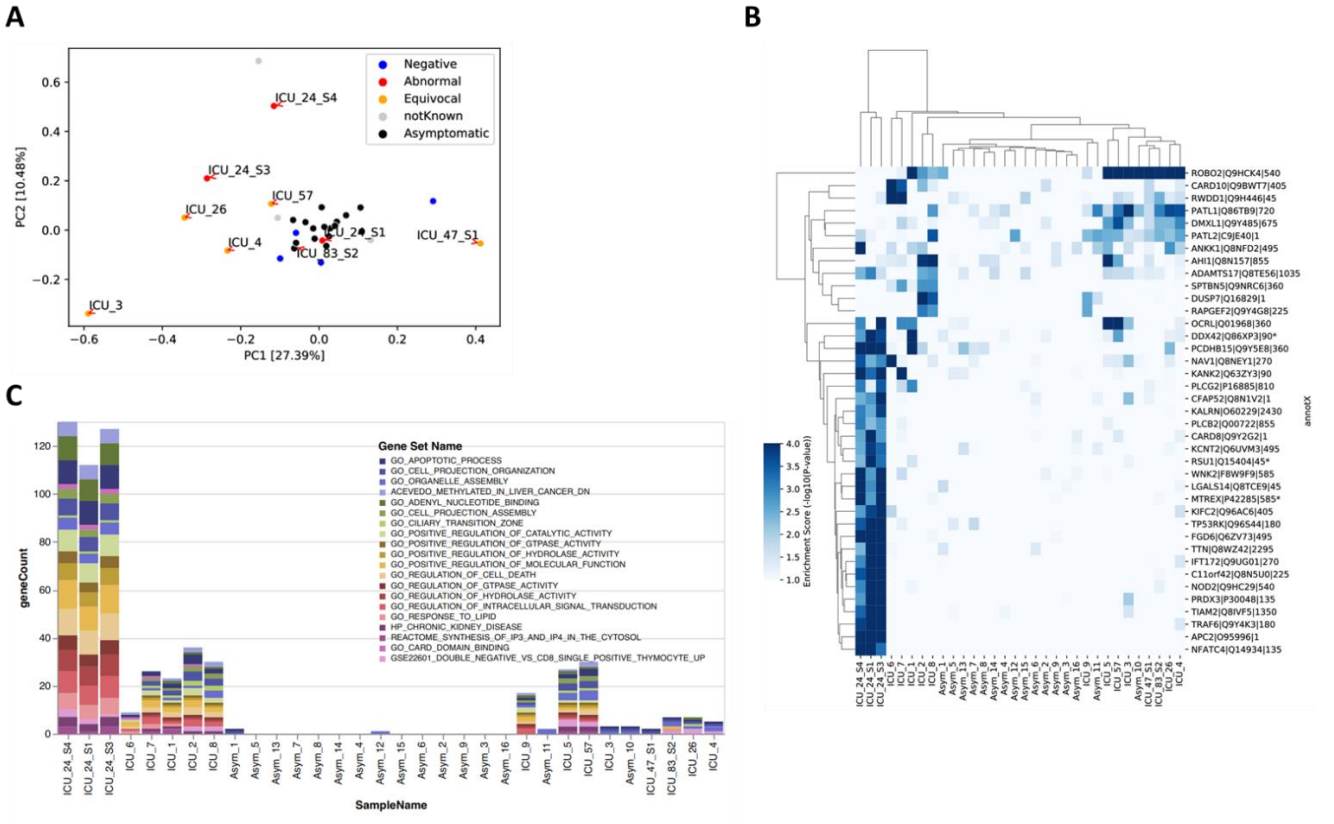


Figure 3. Autoantibody profile of selected cases assessed by PhIP-Seq. (**A**). Principal component analysis of the peptide enrichment scores reflecting autoantibody-autoantigen interactions in ICU patients and asymptomatic COVID-19 cases. The color core indicates the ANA status. (**B**). Heatmap plot showing the binding profile of the 79 differentially enriched (DE) peptides in ICU cases versus asymptomatic cases, with hierarchical clustering. Each row indicates a human peptide (90mer, start position is indicated relative to the UniProtKB entry), and each column represents a sample. The color gradient for each cell of the heatmap plot represents the peptide enrichment score ($-\log_{10}(P)$ value) for a given antigenic peptide and sample. A $-\log_{10}(P) \geq 2.3$ was considered significantly enriched; * Represents known autoantigens with an entry in the human autoantigen database (AAgAtlas 1.0). (**C**). Stacked bar plot showing the results of a gene set enrichment analysis of the peptides shown in (**B**). The color code indicates the gene sets from the Molecular Signatures Database (MSigDB) for which at least one DE peptide was enriched (P-value < 10^{-5} and FDR q-value < 0.05). Samples are sorted as shown in (**B**) according to hierarchical clustering.

including all of the 79 putative autoantigens for which we had found autoantibodies to be differentially enriched among the tested ICU cases when compared to asymptomatic COVID-19 cases. This analysis confirmed that autoantibodies in ICU patients primary targeted intracellular proteins involved in intracellular signal transduction, metabolism, apoptotic processes, and cell death. Autoimmune responses were primarily observed in samples with moderate and high ANA levels, particularly in the samples of patient C024 with the highest ANA measurements, which appeared to increase over time (**Figure 3C** and **Supplementary Table 2**).

Discussion. COVID-19 disease progression may pass through up to four different phases. The first phase is characterized by an initial viral infection phase that is usually mild or asymptomatic in approximately 80% of patients. The host-virus interactions then delineate the progression of the disease. Some patients progress to a second phase, characterized by a hyper-immune response (i.e., cytokine storm). A state of hypercoagulability occurs in the third phase.[1] In combination, they may lead to organ damage in the fourth phase, which is usually mediated by the host's innate immune system.[1,5,14] In this present study, we assessed the generation of antinuclear autoimmune antibodies (ANA) in critically ill COVID-19 patients to better understand the disease prognosis and pave the way for the possible use of immunomodulatory drugs for the treatment of these patients. It is worth noting that several immunomodulatory drugs have been proven to be effective in relieving COVID-19 symptoms, such as tocilizumab.[1]

Interestingly, ANAs were exclusively observed in ICU COVID-19 patients (8/126, 6.34%), which suggests a potential correlation between COVID-19 severity and ANA production. In other words, SARS-CoV-2 infection may have triggered the production of ANA autoantibodies leading to possible cases of autoimmunity in severely ill COVID-19 patients. However, the mechanism is yet to be studied. Only one patient (C24) had a high level of ANA as tested by ELISA. We tested this patient's samples at different time points, and all were highly positive (**Figure 2**). It was inapplicable to follow up with this patient to see the ANA titer levels after he was discharged from the hospital.

Immunological dysregulation, including the production of autoimmune antibodies, has been previously described in COVID patients,[12,22,23] In one study, Pascolini et al. reported the presence of anti-platelet autoantibodies (APA) in three COVID-19 patients suffering from immune-mediated thrombocytopenia. Such antibodies were not detected in our samples when using the PhIP-Seq assay. In general, viral infections trigger autoimmunity through one of the following mechanisms, (1) molecular mimicry, (2) bystander activation, and (3) epitope spreading.[2,5,6] In molecular mimicry, viruses display antigens structurally similar to self-antigens activating a cross-reactive immune response against both self and non-self-antigens. During bystander activation, a non-specific hyper anti-viral immune response characterized by a pro-inflammatory environment causes the release of self-antigens from damaged tissues, which are then presented by antigen-presenting cells (APC), triggering autoreactive T cells and autoimmunity. One example is HIV, which mimics the human T-cell receptor (TCR) to a great extent where autoantibodies are produced.[24] In support of this mechanism, our large-scale autoantibody screen of selected patients by PhIP-Seq revealed several known and novel autoantigens among ICU cases, particularly those with moderate and high ANA responses. An in-depth analysis of these autoantibody specificities confirmed that these autoimmune responses were primarily directed against intracellular proteins and, therefore, likely as a consequence of extensive tissue damage during disease progression. Of note, the patient with the highest ANA serum levels and most robust autoantibody responses as assessed by PhIP-Seq (a 53-year-old male with Indian nationality) had a clinical history of hypertension but was otherwise previously healthy.

Similarly, epitope spreading is characterized by the release of more self-antigens activating autoreactive T cells that eventually spread to other autoreactive T cells (i.e., the diversification of epitope specificity). A recent study identified cross-reactive epitopes between SARS-CoV-2 and human molecular chaperones.[25] Bioinformatics analysis showed that a family of heat shock proteins (Hsp70) shared antigenic epitopes with SARS-CoV-2, capable of inducing autoimmunity against endothelial cells through the process of molecular mimicry.[25] Thus, a similar mechanism may apply to ANA production, where cross-reactive epitopes between SARS-CoV-2 and nuclear antigens may exist. However, none of the autoantigens we identified in this study shared linear B cell epitopes with any of the SARS-CoV-2 protein reference sequences (**Supplementary Figure 1**).

In terms of comorbidities, hypertension was common among patients who showed ANA levels, specifically in deceased patients (F003, F026, and F057). Thus, this may suggest a potential link between hypertension and ANA level. However, a more extensive cohort study is needed to validate this hypothesis. Autoimmune diseases such as lupus and RA have increased risk for hypertension and cardiovascular disease.[26] Therefore, we speculate that hypertension may act as a risk factor promoting a pro-inflammatory environment, possibly leading to autoimmunity through bystander activation. Although these samples showed moderate ANA levels, it is inconclusive because extra time points of the

samples are needed to check ANA level change over time. The second most common comorbidity was diabetes, specifically patient C083, who experienced both type 2 diabetes and abnormal ANA level. Type 2 diabetes is suspected to be an autoimmune condition given the presence of circulating autoantibodies against β cells;[27] thus, this may contribute to abnormal ANA levels (i.e., risk factor).

Furthermore, ANA level was confirmed using IFA, where HEp-2 cells were immunostained with ANA expressed in sera samples. ANA level comes in different patterns depending on the antigens to which ANA binds. According to the results, most positive/equivocal samples showed speckled patterns suggesting potential antigens such as n-RNP, Sm, and SSB/La.[28] Interestingly, only one patient had a detectable ANA level with a speckled pattern. Further, one sample (C020) showed a nucleolar pattern. Previous studies have shown a correlation between ANA nucleolar pattern and systemic sclerosis.[29] According to the study, a nucleolar pattern of ANA was associated with pulmonary fibrosis (i.e., lung scarring) ($P<0.01$), suggesting a critical organ involvement with a decreased chance of survival in systemic sclerosis patients.

Regarding COVID-19, a similar association was observed between the presence of the ANA reactivity with nucleolar pattern and severe COVID-19 disease.[14,15] Further, in a small study by Chang et al.,[30] autoantibodies were detected in moderate and critical cases of COVID-19. The study involved 47 PCR-confirmed COVID-19 hospitalized patients. The total ANA positive rate was 21.3%, which is higher than our study but lower than other studies, as reported in their discussion. Interestingly, similar to our findings, ANA titers were mostly weak (Median 1:40), showing 50% nucleolar and 30% speckled staining. While 9.1% (1/11) of their patients with autoantibodies and 8.3% (3/36) of patients without autoantibodies died, almost 50% of ANA-positive patients in our study died. It is worth noting that ANA positivity in our study was confirmed with large-scale autoantibody screening by phage immunoprecipitation-sequencing (PhIP-Seq), which revealed autoantibody specificities that predominantly targeted proteins involved in intracellular signal transduction, metabolism, apoptotic processes, and cell death by PhIP-Seq.

Unlike other studies, we did not detect neutralizing anti-cytokine (IFN) antibodies in the PhIP-Seq assay, as previously reported.[12,31] Their study[12] identified individuals with high titers of neutralizing autoantibodies against type I IFN-α2 and IFN-ω in about 10% of patients with severe COVID-19 pneumonia. We selected only ANA-positive samples for further screening with PhIP-Seq, which could be the reason for the negative outcome. Further, with our PhIP-Seq screen, we have a limited ability to detect autoantibodies that target conformational epitopes (due to the smaller size of peptides, 90 aa in length, that are being used for phage display) which may have limited the sensitivity.

Screening randomly selected pre-pandemic samples (n=2655) from Qatar Blood Bank donors (unpublished results) revealed ten samples with abnormal ANA levels (~0.38%). Among these positive ANA samples, 5 (50%) were positive for documented viral infections (**Supplementary Table 3**). Four samples (A, B, C, and D) tested positive for B19 Virus IgG, two samples (A and E) for WNV (West Nile Virus) IgG, and one sample (A) for Dengue virus. B19 virus has been associated with several autoimmune diseases, such as rheumatoid arthritis, systemic lupus, antiphospholipid syndrome, systemic sclerosis, and vasculitides.[32] It has also been shown to induce cross-reactive autoantibodies utilizing molecular mimicry between parvovirus VP1 protein and host proteins (e.g., human cytokeratin and transcription factor GATA 1).[32] Likewise, WNV infection has been reported to promote autoimmune conditions, including myasthenia gravis (MG), through the process of molecular mimicry.[33] In addition, several studies have shown cross-reactivity between antibodies directed against dengue virus nonstructural protein 1 (NS1) and human platelets/endothelial cells damaging them.[34] As observed, molecular mimicry and autoimmunity are common among these viruses, suggesting a similar mechanism taking place during SARS-CoV-2 infection.

Conclusions. This study sheds light on a potential relationship between COVID-19 and autoimmunity, particularly ANA production. Nevertheless, some limitations are associated with this study. First, the sample size is relatively small, and a larger-scale study may be needed. Second, some of the sera samples were only taken at one-time points, which makes it harder to explore ANA change over time. Third, people are admitted to ICU mostly seven days after infection, so the time points of the ICU samples are calculated from their admission to the ICU rather than the beginning of the infection. Thus, the acute vs. convalescent phase inconsistency of the ICU and non-ICU samples may affect the accuracy of the results. Still, this study provides a closer insight into the immunological progression of the disease and its prognosis. Therefore, we propose including the screening for autoimmune antibodies as a routine test for COVID-19 patients.

Acknowledgment. The authors would like to thank all the nurses and staff who facilitated the sample collection.

Statement of Ethics. This study was approved by IRB committees of Hamad Medical Corporation (MRC-01-20-145), Sidra Medicine (IRB Protocol #: 1511001953), and Qatar University (QU-IRB 1289-EA/20). In addition, we have received written informed consent from the

participants.

Funding Sources. This study was supported by funds from QNRF, grant # NPRP11S-1212-170092.

Authors' contribution. HMY and GKN designed the study. ADF, TK, FA, MMA, HTZ, DWA, and FHA ran the experiments. FSC, ST, and AHH collected samples. AHE, LAR, AA, AAA, and NM helped in logistics and supervision. All authors read and approved the manuscript.

Data Availability Statement. All data are provided in this manuscript either in the main text or in the supplemental files. Inquiries about additional information can be requested directly from the corresponding author.

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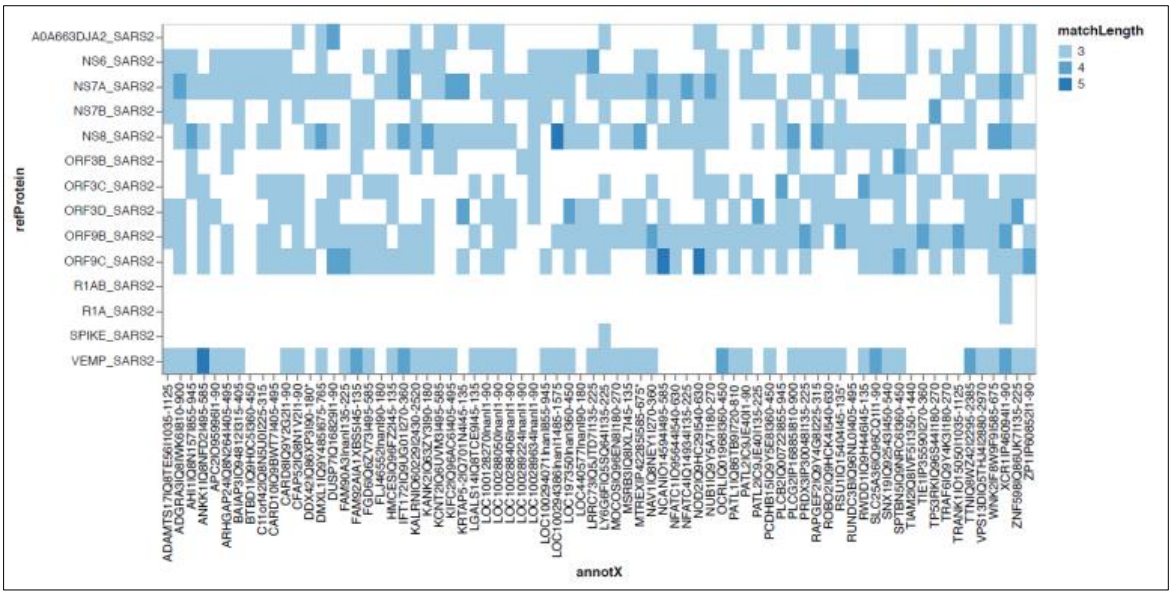
Supplemenary Files



Supplementary Figure 1

Supplementary Table 1

#	Gender	Age	Nationality	Severity	Living Status (Alive, Dr Deceased)	HbA1C %	Comorbidities: Diabetes Mellitus	Hypertension	Cardiovascular disease	Cerebrovascular disease	GERD/Peptic ulcer disease (PUD)	Renal Disease/Transplant	Liver Disease/Transplant	Asthma	Dyslipidemia	Previous infectious diseases (TB, HBV, HCV, Malaria etc)	Other Comorbidities	Day(s) of sample collection (relative to ICU admission)	ANA test results	Medications
ICU_1	M	70	Non Qatari	Critical	D	NA	1	0	Ischemic HD	0	0	0	0	0	0	0	0		negative	amoxicillin-clavulanate azithromycin Cefuroxime enoxaparin hydroxychloroquin levocetirizine Acetylcysteine Beta-Sitosterol cefepime Chlorhexidine Gluconate Esomeprazole Insulin Aspart tigecycline dexmedetomidine EPINEPHrine heparin norepinephrine propofol remifentanil rocuronium vasopressin
ICU_2	M	40	Non Qatari	Severe	A	6,9	1	0	0	0	0	0	0	0	0	0	0		negative	Hydroxychloroquine Azithromycin Lopinavir / RitonavirMethylprednisolone 40mg BID Beta-Sitosterol enoxaparin Esomeprazole Insulin Aspart Insulin Glargine Metoprolol Tartrate Multivitamin and Minerals Bisacodyl Polyethylene Glycol Senna extract Sodium Phosphate Noradrenaline
ICU_7	M	54	Non Qatari	Severe	A	6,3	1	1	CAD/CABG	0	0	0	0	0	0	0	0		negative	hydroxychlorquine Lopinavir / Ritonavir aspirin beta-sitosterol, ferrous sulfate mupirocin paracetamol, perindopril, povidone iodine QUEtiapine SITagliptin/metFORMIN Atorvastatin BisoprololEnoxaparin Insulin AspartInsulin Glargine/Lidocaine Nystatin/piperacillin-tazobactam Glycerin Haloperidol Lactate
ICU_8	M	56	Non Qatari	Critical	D	7,3	1	1	0	Stroke	0	0	0	0	0	0	Cancer of tounge/Cataract		negative	ColistinMTigecyclineAmpicillin/sulbactamHydrocortisone SeptrinMMeropenem hydroxychlorquine levothyroxine, pantoprazole Tazocin/kanamycin favipiravir Clopidogrel Dexamethasone Esomeprazole Eye Lubricant Metoprolol Tartrate Midodrine Multivitamin Nystatin Polyethylene Glycol Senna extract Terbinafine dexmedetomidine insulin regular norepinephrine Morphine Paracetamol
ICU_24					ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	9;23;30	positive	ND
ICU_83					ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ICU_47					ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	equivocal	ND
ICU_3					ND	ND	ND	ND	ND	ND	0	0	0	0	0	0	ND	ND	equivocal	ND
ICU_4					ND	ND	ND	ND	ND	ND	0	0	0	0	0	0	ND	ND	equivocal	ND
ICU_26					ND	ND	ND	ND	ND	ND	0	ESRD	0	0	1	0	hyperuricemia	ND	equivocal	ND
ICU_57					ND	ND	ND	ND	ND	ND	0	0	0	0	1	0	0	ND	equivocal	ND
ICU_5					ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ICU_6					ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
ICU_9					ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym4				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym5				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym9				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym11				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym13				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym17				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym18				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym103				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym107				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym580				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym645				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym754				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym1371				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym1791				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Asym18				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
AsymAll3				Asymptomatic	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Supplementary Table 2

GeneSymbol|Uniprot|start-end	prevalence ICU cases	prevalence asymptomatic cases	log odds ratio	MSigDB entry	aa sequence
ADAMTS17|Q8TE56|1035-1125	25,16	0,31	4,68		TITSPRLAALTYKCTRDQWTVVCRVIREKNLCQDMRWVQRCCQTCRDFYANKMRQFPPNS
AHI1|Q8N157|855-945	25,16	0,31	4,68		TFLFAGSEDGIVYVWNPETGEQVAMYSDLPFKSPIRDISYHPFENMVAFCAFGQNEPELYIYDFHVAQQEAEMFKRYNGTFPLPGIHQS
ANKK1|Q8NFD2|495-585	18,94	0,31	4,32		TPLHVAAYFGHVSLVKLLTSQGAELDAQQRNLRTPLHLAVERGKVRAIQHLLKSGAVPDALDQSGYGPLHTAAARGKEYLICKMLLRYGAS
APC2|O95996|1-90	18,94	0,31	4,32		MASSVAPYEQLVRQVEALKAENSHLRQELRDNSSHLSKLETETSGMKEVLKHLQGKLEQEARVLVSSGQTEVLEQLKKALQMDITSLYNLK
ARHGAP24|Q8N264|405-495	18,94	0,31	4,32		SVHKLDVSRSPPLMVKKNPAFNKGSGNTNGSFSSSNAEGLEKTQTTPNGSLQARRSSSLKVSGTKMGTHSVQNGTVRMGELNSDTLGNP
BAIAP3|O94812|315-405	18,94	0,31	4,32		KGMGRYFKQVKSARANGTAGPTEDHTDDFLGCLNPVRE VPVAGVDRWFKLEPRSSASRVQGHCHLVLKLITQRDTAMSQRGRSGFLS
BTBD1|Q9H0C5|360-450	18,94	0,31	4,32		IVGFGLYGSIHGFTDFQVNIQIEYEKKQTLGQQNDTGFSCDGTANTFRVMFKEPIELPNVCYTACATLKGPDSHYGTKGLKKVVHETPA
C11orf42|Q8N5U0|225-315	18,94	0,31	4,32		VRPNLSIMPPLAPTSAPADTTEAADVPPPVPAPPTPPPQIEGPEDKPTRFSYKGRNPFWRGPQILSENWLFSPRSPPPGACQGGGPRDPDGH
LOC100128270|nan|1-90	18,94	0,31	4,32		MVTPAGCLGGRNQGPREIPGTAFPRSSRAGQTGQAVSGAQVSSWRERQPCSGSRGPLHILGTDGNVDTTGKLGLVPTPPRIQKETEQGAL
HMCES|Q96FZ2|45-135	18,94	0,31	4,32		PQSNSPVLLSRLHFEKDAQSSERIAAPMRWGLVPSWFKESDPSKLQFNTTNCRSDTVMEKRSFKVPLGKGRRCVVLADGFYEWQRCQGTN
LRRC73|Q5JTD7|135-225	56,21	0,31	6,02		LLPPDGAKSGLKEELTLSANPGITPKGWSRLAIAVAHSSQVRVLNLDYFNPLGDHVAGMLAVAVASSRTLEVLDLEGTGLTNQSACTLLDMV
CARD10|Q96WT7|405-495	18,94	0,31	4,32		QLQVSQSLIEKDCYRKQVRGLEAERDELLTTLTSLEGTKALLEVQLQRAQGGTCLKACASSHSLCSNLSSTWSLSEFPSPLGGPEATGEA
LOC100288406|nan|1-90	18,94	0,31	4,32		MPVPGSWQHSGLHTLLGFTFVAGSLQTRPMTNTVSSGQSKGRAQRQAVVQLPVRKRPTSGETGQTKGSEKCDLGVVEALGKGKGRSELPH
DDX42|Q86XP3|90-180*	31,37	0,31	4,99	autoantigen	IPAENSPTRQQFHSKPVDSDSDCDDPLEAFMAEVEDQAARDMKRLEEKDKERKNVKGIRDDIEEEEDDQEAYFRYMAENPTAGVVCQEEEEDN
DMXL1|Q9Y485|675-765	31,37	0,31	4,99		LNEEECSLTQQNKSTVDVAFQDPSAVYSELILWRVDPVGPLSFSGGVSELARINSLHVSAFSNVAWLPTLIPSYCLGAYCNSPSACFVAS
DUSP7|Q16829|1-90	18,94	0,31	4,32		MKNQLRGPPARAHMSTSGAAAAGGTRAGSEPGAGSGSGAGTGAGAATGAGAMPCKSAEWLQEELEARGGASLLLLDCRPHELFESSHIET
FAM90A3|nan|135-225	31,37	6,52	1,88		STEPSDYLRVASGPMPVHTTSKRPRVDPVLADGSATEMSDRGSVLASLSPLRKASLSSSSSLGPKERQTGAAADPQPAVRHQGREPLLV
FAM92A|A1XBS5|45-135	50,00	12,73	1,92		DKADLLVNEINAYAATETPHLKLGLMNFADEFAKLQQYRQAEVERLEAKVVEPLKTYGTIVKMKRDKLEATLTARNREAKIQLTQLERTRQ
FGD6|Q6ZV73|495-585	18,94	0,31	4,32		PKKPQRHSLPATGVLKKAASEELLEKSSFPSSEEKSSEKSLERNHLQHLCAQNRGVSSSFDMPKRASEKPVWKLPHPILPFSGNPEFLKS
FLJ46552|nan|90-180	18,94	0,31	4,32		DFANLEDNFCFHSTSRFLKICPSPLKRASHPSGLFVAFDEPRHSLRDQ
ADGRA3|Q8IWK6|810-900	37,58	12,73	1,42		VFVGGITQTRNASICQAVGILHYSTLATVLWVGVTARNEYKQVTKKAKRCQDPDEPPPPPRPMLRFYLIGGGIPIVCGITAAANIKNY
IFT172|Q9UG01|270-360	18,94	0,31	4,32		RRSIWEEAKPKEITNLYTITALAWKRDGSRLCVGTLCGGVEQFDCCLRRSIYKNKFELTYVGPSQVIVKNLSSGTRVVLKSHFGYEVEEV
KALRN|O60229|2430-2520	18,94	0,31	4,32		ILGNKVSVKETNSSEESECDDLDPNTSMEILNPNFIQEVAPEFLVPLVDVTCLLGDTVILQCKVCGRPKPTITWKGPDQNILDTDNSSAT
KANK2|Q63ZY3|90-180	25,16	0,31	4,68		ASGDSRHSAYSYCGRGFYPQVGALETRGGFNPRVRETRLLDARRRLEDQAATPTGLGSLTPSAAGSTASLVGVGLPPPTPRSSGLSTPVPP
KCNT2|Q6UVM3|495-585	18,94	0,31	4,32		ESTFFAEYEGKSFTYASFHAHKKFGVCLIGVRREDNKNILLNPGPRYIMNSTDKCFYINITKEENSAFKNQDQQRKSNVSRSFYHGPSRL
KIFC2|Q96AC6|405-495	18,94	0,31	4,32		LKGNRVLCRLRPGTSSSLSVSVEPGPGGTVTTCYRGRHRRFRLDWVFPPDASQEEVFRELEPAVLSCLRGYSVCIFTYGQTGTGKTYSME
KRTAP5-2|Q701N4|45-135	25,16	0,31	4,68		SSSCGGCGSRCYVPVCCCKPVCSWVPACSCTSCGSCGGSKGGCGSCGGSKGGCGSCGGSKGGCGSCGCSQSSCCKPCCCSSGCGSSCCQS
LGALS14|Q8TCE9|45-135	18,94	0,31	4,32		GSCVITGTPILTFVKDPQLEVNFYTGMDEDSDIAFQFRLHFGHPAIMNSCVFGIWRFEEKCYYLPFEDGKPFELCIVRHKEYKVMVNG
LOC100288050|nan|1-90	18,94	0,31	4,32		MCIFLKLYFSPISHILTSLISSSWGRRGLTSDAAASLCTGSPGAVTAHLLQLGHLARAWRGKHRGVFNTQLDVKKPRRQVHTVVYGKV
LOC100289224|nan|1-90	18,94	0,31	4,32		MPTLPRPSQTPKPEPPGKRLPTGHNTQAETRGEADQKKGRRARNPASGAQGTCVPRRTHTQTDRDRKRGRRKERERGREGERERERERLRR
LOC100289634|nan|1-90	18,94	0,31	4,32		MPSSPKPRLQPWPGRGHSQNGILSPAALEEGAGMELSQFCVETTFGEREAGFRPRSWGGGPHLPVLHRRMAPPSPCTSALLDSLKRLPEK
LOC100294071|nan|855-945	18,94	0,31	4,32		IHMEVRPYKCEECGKTFKWFPDLTNHKRHTGEKPYKCEECGKTFTCSSSLIKHKRSHTGDRPTSAKNVAKPLGGSQTLLNISKHKRHT
LOC100294386|nan|1485-1575	18,94	0,31	4,32		PERHARSEWSPLPRMVWAEQPAVCPVFPPAPLCAGLCVRQSIRECALGSLVRLARGPNLPPVFVLGAISPWGPCLPLIPLPGIAPPLAEPGH
LOC197350|nan|360-450	18,94	0,31	4,32		GYVAYRDDKGSDFIQTLVEVLRANPGRDLLELLTEVNRRVCEQEVLGPDCDELRKACLEIRSSLRRRLCLQA
LOC440577|nan|90-180	18,94	0,31	4,32		TPPVVLRFKCGSGPVHSGQHLVAVEEDAESEDEEEEDVKLLSISGKRSAPGGGSKVPQKEVKLAVDEDDDDDDDDDDDFDDEEAEEKV
LY6G6F|Q5SQ64|135-225	18,94	0,31	4,32		ADGSPCNVLLCSVVPSRRMDSVTWQEGKGPVRGRVQSFWGSEAALLLVCPGEGLSEPRSRRPRIIRCLMTHNKGVSFSLAASIDASPALC
MOCOS|Q96EN8|180-270	18,94	0,31	4,32		EERSASASNPDCQLPHLFCYPAQSNFSGVRYPLSWIEEVKSGRLHPVSTPGKWFVLLDAASYVSTSPLDLSAHQADFVPISFYKIFGFPT
MSRB3|Q8IXL7|45-135	18,94	0,31	4,32		SQQELRKRLTPLQYHVTQEIKGTESAFEGEYTHHKDPGIYKCVVCGTPLFKSETKFDSGSGWPSFHDVINSEAITFTDDFSYGMHRVETSC
NAV1|Q8NEY1|270-360	25,16	0,31	4,68		NLEETMSSLRGSQVTHSSLEMTCYDSDDANPRSVSSLSNRSSPLSWRYGQDSSPRLQAGDAPSVGGSCRSEGTPAWYMHGERAHYSHTMPM
NCAN|O14594|495-585	18,94	0,31	4,32		PEPGLQGGMEASAQPPTSEAAVNQMEPPLAMAVTEMLGSGQSRSPWADLTNEVDMPGAGSAGGKSSPEPWLWPPTMVPPSISGHSRAPVL
NFATC1|O95644|540-630	12,73	50,00	-1,92		GRKNTRVRLVFRVHVPQPSGRTLSLQVASNPIECSQRSAQELPLVEKQSTDSYPVVGGKKMVLSGHNFLQDSKVIFVEKAPDGHHVWEMEE
NFATC4|Q14934|135-225	18,94	0,31	4,32		SPPPRPAPSPGTWESQPARSVRLGGPGGGAGGAGGGRVLECPSRRTSISPTPEPPAALEDNPDAWGDGSPRDYPPPEGFGGYREAGGQG
NOD2|Q9HC29|540-630	18,94	0,31	4,32		RGRLPTLLHLGRLALWGLGMCCYVFSAQQLQAACVSPDDSLGFLVRAKGVVPGSTAPLEFLHITFQCFFAAFYLALSADVPPALLRHLF
NUB1|Q9Y5A7|180-270	18,94	0,31	4,32		LKEEKQIQRTKRGLEILAKRAAETVVDPEMTPYLDIANQTGRSIRIPPSERKALNLAMGHYHEKGRAFLERKEYGIAILPCLLDADKYFCECC
OCRL|Q01968|360-450	37,58	6,52	2,16		RFVFHNTTFCIVNSHLAAHVEDFERRNQDYKDICARMSFVVPNQTLPQLNMKHEVVIWLGDLNYRLCMPDANEVKSLINKKDLQRLLKF
PATL1|Q86TB9|720-810	37,58	6,52	2,16		ATRELLRIPQAALAKPISIPTNLVSLFSRYVDRQKLNLLETKLQLVQGIR
PATL2|C9JE40|1-90	37,58	12,73	1,42		MNCLEGPGKTCGPLASEEELVSACQLEKEEENEGEEEEEEDEEDLDPDLDPDLEEEENDLGDPAVLGAVHNTQRALLSSPGVKAPGMLG
PATL2|C9JE40|135-225	31,37	6,52	1,88		LFCSLLTSWPPRFSHLTQLHPRHQRLQQQQHSQTPSPPAKKPWSQQPDPYANLMTRKEKDWVIKVQMVQLQSAKPRLDDYYYQEYYQKL
PCDHB15|Q9Y5E8|360-450	31,37	6,52	1,88		PETEVALFRIRDRDSGENGKMCSIQDDVPFKLKPSVENFYRLVTEGALDRETRAEYNITITTDLGTPRLETECQSITVLVSDVNDNAPA
PLCB2|Q00722|855-945	18,94	0,31	4,32		NGALAPTSNGSPAARAGARIEAMKEAAEPRITASLEELRELKGVVKLQRRHEKELRELERRGARRWEELLQRGAACLAELGPPGVGGVGAC
PLCG2|P16885|810-900	18,94	0,31	4,32		YGTRIQQVFPSNVVEDSTADFEELEKQREDNPLGSLCRGALDLNTYNVVKAPQGKNQKSFVFILEPKQQGDDPVEFATDRVEELFEWF
PRDX3|P30048|135-225	18,94	0,31	4,32		SHFSHLAWINTPRKNGGLGHMNEALLSDLTKQISRDYGVLLEGSGLALRGLFIIDPNGVIKHLSVNDLPVGRSVEETLRLVKAFQYVETH
RAPGEF2|Q9Y4G8|225-315	18,94	0,31	4,32		IAQQQDYCRILNQVEKNMQKVEEEGEVVMVKEHRELDRTGTRKGHVVKGTSERLTMHLVEEHSVVDPTFIEDFLLTYRTFLSSPMEVGKK
ROBO2|Q9HCK4|540-630	56,21	0,31	6,02		PSKPQVTDVTKNSVTLSWQPGTPGTLPASAYIEAFSQSVSNSWQTVANHVKTTLYTVRGLRPNTIYLFMVRAINPQGLSDPSPMSDPVR
RSU1|Q15404|45-135*	18,94	0,31	4,32	autoantigen	VLSHNKLTMVPPNIAELKNLEVLNFFNNQIEELPTQISSLQKLKHLNLGMNRLINTLPRGFGSLPALEVLDLTYNNLSENSLPGNFFYLTT
RUNDC3B|Q96NL0|405-495	18,94	0,31	4,32		DPGQSQEGGDKQDTLNVMSEGKEDTPSLLGLCGSLTSWASYKSLTSLKSNDYLASPTTEMTSPGLTPS
RWDD1|Q9H446|45-135	18,94	0,31	4,32		ETVQTTLKFTYSEKYPDEAPLYEFSQENLEDNDVSDILKLLAICQAEENLGMVVMIFTLVTAVQEKLNEIVDQKKTRREEEKKKQKEKEAEE
MTREX|P42285|585-675*	18,94	0,31	4,32	autoantigen	EKSFYQFQHYRAIPGVVEKVKNSEEQYNKIVIPNEESVVIYYKIRQQLAKLGKEIEEYHHKPKYCLPFLQPGRLVKVKNEGDDFGWGVVV
SLC25A36|Q96CQ1|1-90	18,94	0,31	4,32		MSQRDTLVHLFAGGCGGTVGAILTCPLEVVKTRLQSSSVTLYISEVCQLNTMAGASVNRVVSPGPLHCLKVLEKEGPRSLFRGLGPNLVG
SNX19|Q92543|450-540	18,94	0,31	4,32		TADKEIEQSDVTASVTALLEGPEKTCPSRPSCLEKDLTNDVSSLDPTLPPVLSSSPPGPLSSATFSFEPLSSPDGPVIIQNLRITGTIT
SPTBN5|Q9NRC6|360-450	18,94	0,31	4,32		LSQCWAGLEWAEAARSQALQQRLLQLQRLETLARRFQHKAALRESFLKDAEQVLDQARAPPASLATVEAAVQRLGMLEAGILPQEGRFQA
TIAM2|Q8IVF5|1350-1440	18,94	0,31	4,32		TVFVFKRAVILVYKENCKLKKKLPSNSRPAHNSTDLDPFKFRWLIPSALQVRLGNPAGTENNSIWELIHTKSEIEGRPETIFQLCCSDS
TIE1|P35590|270-360	18,94	0,31	4,32		QCPGSGCRGLTFCLPDPYGCSCGSGWRGSQCQEACAPGHFGADCRLQCQCQNGGTCDRFSGCVCPSGWHGVHCEKSDRIPQILNMASEL
TP53RK|Q96S44|180-270	18,94	0,31	4,32		LIDFGLSFISALPEDKGVDLYVLEKAFLSTHPNTETVFEAFLKSYSTSSKKARPVLKKLDEVRLRGRKRSMVG
TRAF6|Q9Y4K3|180-270	18,94	0,31	4,32		DCPRRQVSCDNCAASMAFEDKEIHDQNCPLANVICEYCNTILREQMPNHYDLDCPTAPIPCTFSTFGCHEKMQRNHLARHLQENTQSHM
TRANK1|O15050|1035-1125	18,94	0,31	4,32		TGKTTCCLYRLWKKTHVYWEKAEQAGSPLLAKQVWLKRRLEVEPGKESPGGEEEEEEEEEDSIEVETVESIDEQEYEAACAGGAGVEP
TTN|Q8WZ42|2295-2385	18,94	0,31	4,32		EGKWYHNDVELKSNGKYTITSRRGRQNLTVKDVTKEDQGEYSFVIDGKKTTCKLKMKPRPIAILQGLSDQKVCEGDVQLEVKVSLESVE
CARD8|Q9Y2G2|1-90	18,94	0,31	4,32		RIASGTRLSIPITSNTLIYYHPHPEDIKFHLYLVPSDALLTKAIDDEEDRFHGVRLQTSPPMEPLNFGSSYIVSNSANLKVMPEWISSL
VPS13D|Q5THJ4|2880-2970	25,16	0,31	4,68		PKRRQPFVPFALRNHTGCTLWFATLTTTPTRAALSHSGSPGVVPEGNGTFLDDTHNVSEWREVLTGEEIPFEFEAAGKLRHRHTHDLRIH
CFAP52|Q8N1V2|1-90	18,94	0,31	4,32		MDNKISPEAQVAELELDAVIGFNDILWDYKNRELLARLSLHKGEIEALAFSPNDLYLVSLGGPDDGSVVVWSIAKRDAICGSPAAGLNV
WNK2|F8WIF9|585-675	18,94	0,31	4,32		PPEPEEPEADQHLLFPTLPTSATSLASDSTFDSGQGSTVYSDSQSSQQSVMLGSLADAAPSPAQCVCSPPVSEGPVLPQSLPSLGAYQQP
XCR1|P46094|1-90	18,94	0,31	4,32		MESSGNPESTTFFYYDLQSQPCENQAWVFATLATTVLYCLVFLLSLVGNSLVLWVLVKYESLESLTNIFILNLCLSDLVFACLLPVWISP
ZNF598|Q86UK7|135-225	18,94	0,31	4,32		QHMRRQHELFCCRLCLQHLCJFTYERKWYSRKDLARHRMQGDPDDTSHRGHPLCKFCDERVLDNDELLKHLRRDHYFCHFCDSDGAQDYY
ZP1|P60852|1-90	62,42	0,31	6,28		MAGGSATTWGYPVALLLLVATLGLGRWLQFDPGLPGLRHSYDCGIKGMQLLVTPRPGQTLRFKVVDEFGNRFDVNNCSICYHWVTSRPQE

Supplementary Table 3. Screening of positive ANA prepandemıc samples (i.e., prior Covid-19) against different viruses.

Sample	Gender	ANA IgG	Dengue	Chikungunia	HEV IgM	HEV IgG	B19V IgM	B19V IgG	WNV IgM	WNV IgG
A	M	positive	positive	Negative	ND	ND	Negative	Positive	Negative	Positive
B	M	positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Negative
C	M	positive	ND	ND	Negative	Negative	Negative	Positive	ND	ND
D	M	positive	ND	ND	ND	ND	Negative	Positive	ND	ND
E	M	positive	ND	ND	ND	ND	ND	ND	Negative	Positive

ND: Not determined.