





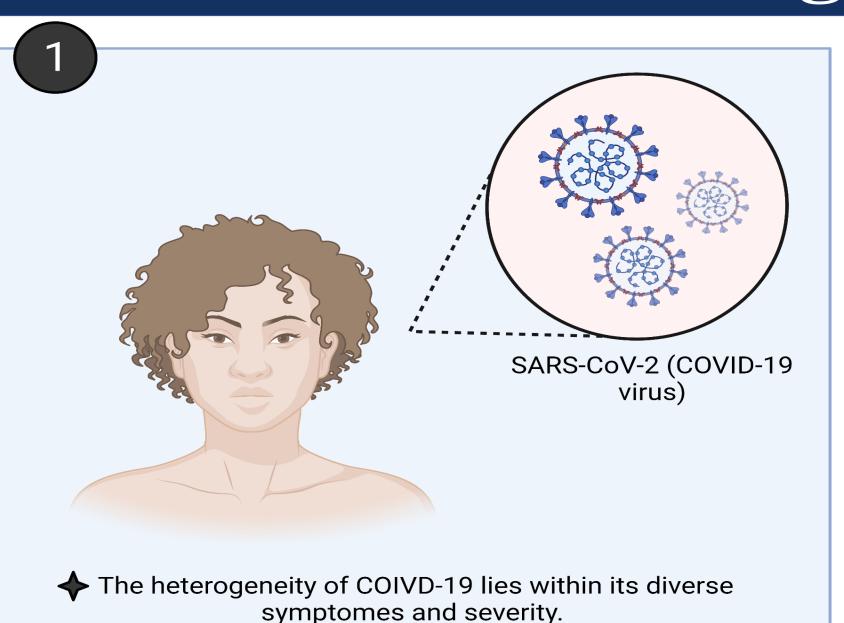
Faculty and Postdoc Health and Biomedical Sciences

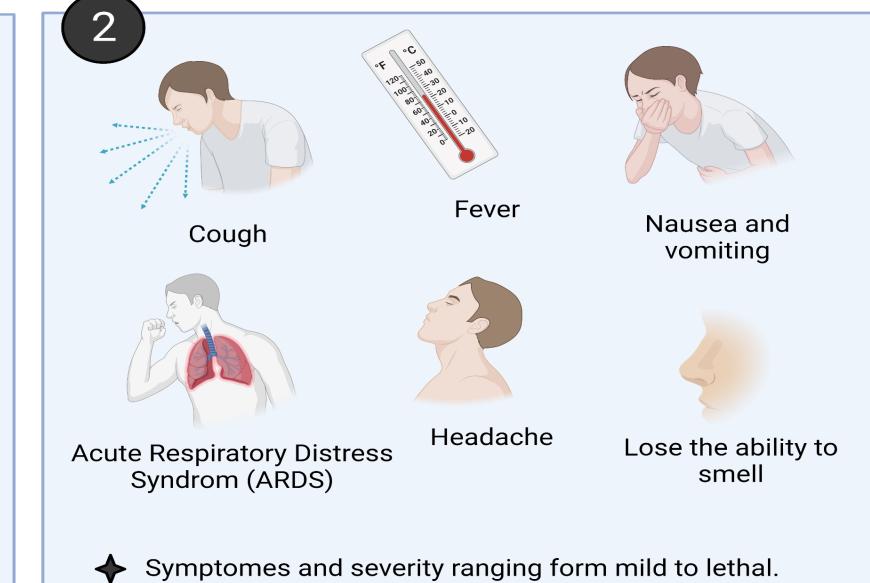
Detection of Antinuclear Antibodies Targeting Intercellular Signal Transduction, Metabolism, Apoptotic Processes and Cell Death in Critical COVID-19 Patients

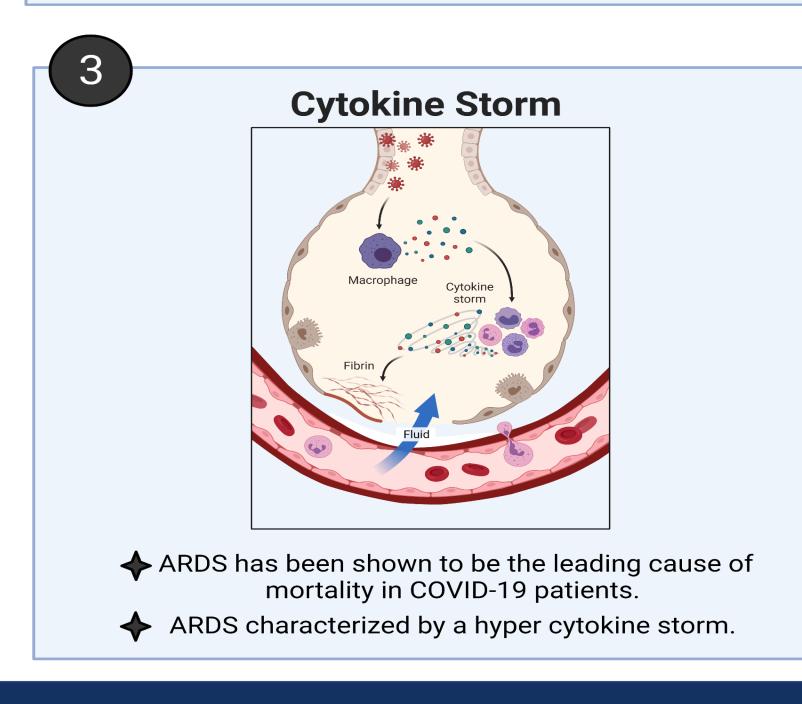
Aisha D. Fakhroo¹, Fatma Ali², Gheyath K. Nasrallah², Taushif Kahan³, Farhan S. Cyprian², Ali A. Hssain⁴, Ali A. Eid², Laith J. Abu-Raddad⁵, Abdullatif Al-Khal⁴, Asmma A. Al Thani², Nico Marr⁴, Hadi M. Yassine²*

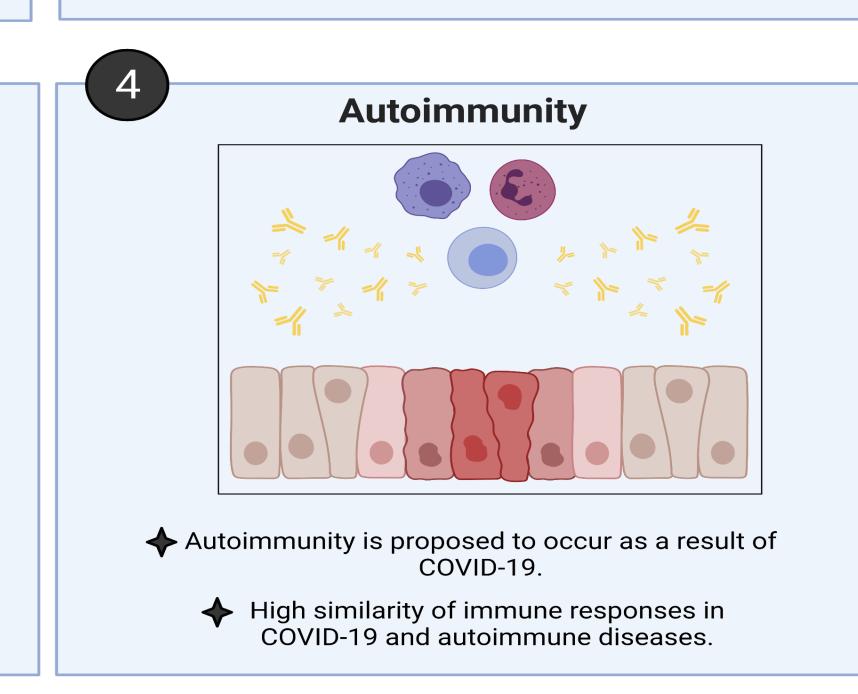
¹Barzan Holdings, ²Qatar University Biomedical Research Center, Doha, Qatar, ³Sidra Medicine, ⁴Hamad Bin Khalifa University, ⁵Weill Cornell Medicine- Qatar, Cornell University

Background







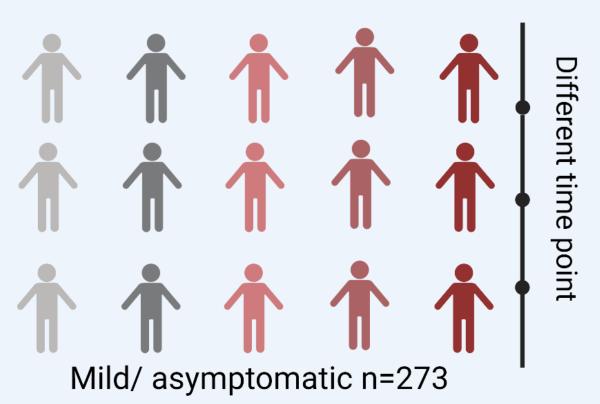


Methods

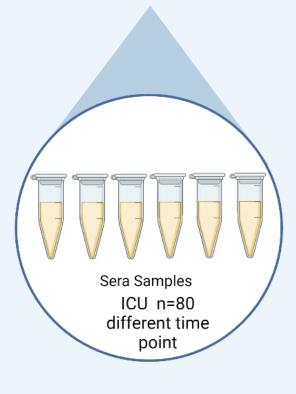




Informed consents were obtained.



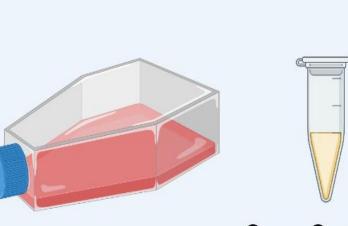
Sever/ critical (ICU) n=126



Sera samples were colleted form COVID-19 patients at different clinical stage classified to 1) mild/ asymptomatic 2) sever/ critical (ICU)



ANA HEp-2 IFA Assay



HEp-2 cells







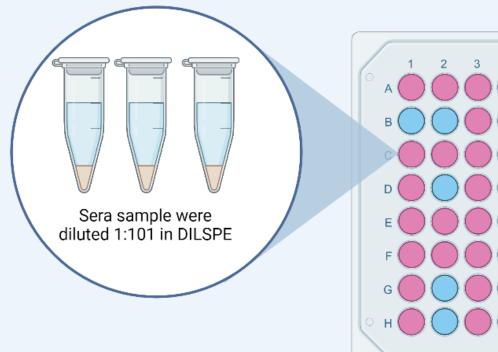
ANA HEp-2 IFA Kit

A Positive reaction is indicated by the presence of any pattern of nuclear apple- green staining.

A postive reaction was observe at diultion 1:40

> Scale of staining intensity +1 weak to +4 strong

ANA IgG ELISA



Cuttoff (Co)= Negative controt (OD 450nm)+ 0.250

Negative/ normal Samples= Sample/Co < 0.8

Moderate/ equivocal Samples= Sample/Co (0.8-1.1)

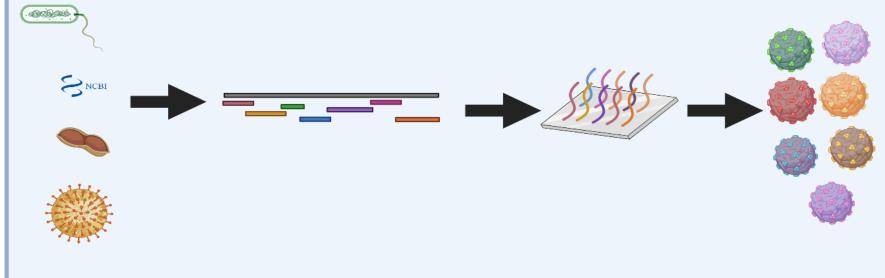
Positive/ abnormal Samples= Sample/Co >1.1

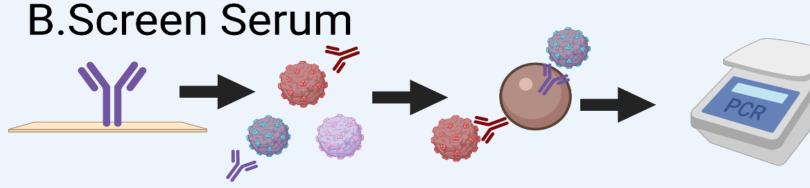
Normal/ equivocal and postive sample were repeated in triplicated with mean and SD calculated

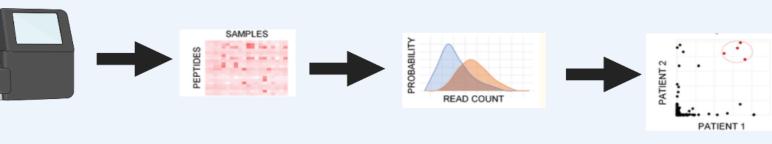
PhIP-Seq and Piptide enrichment analysis

A.Create library

C.Analyze Data







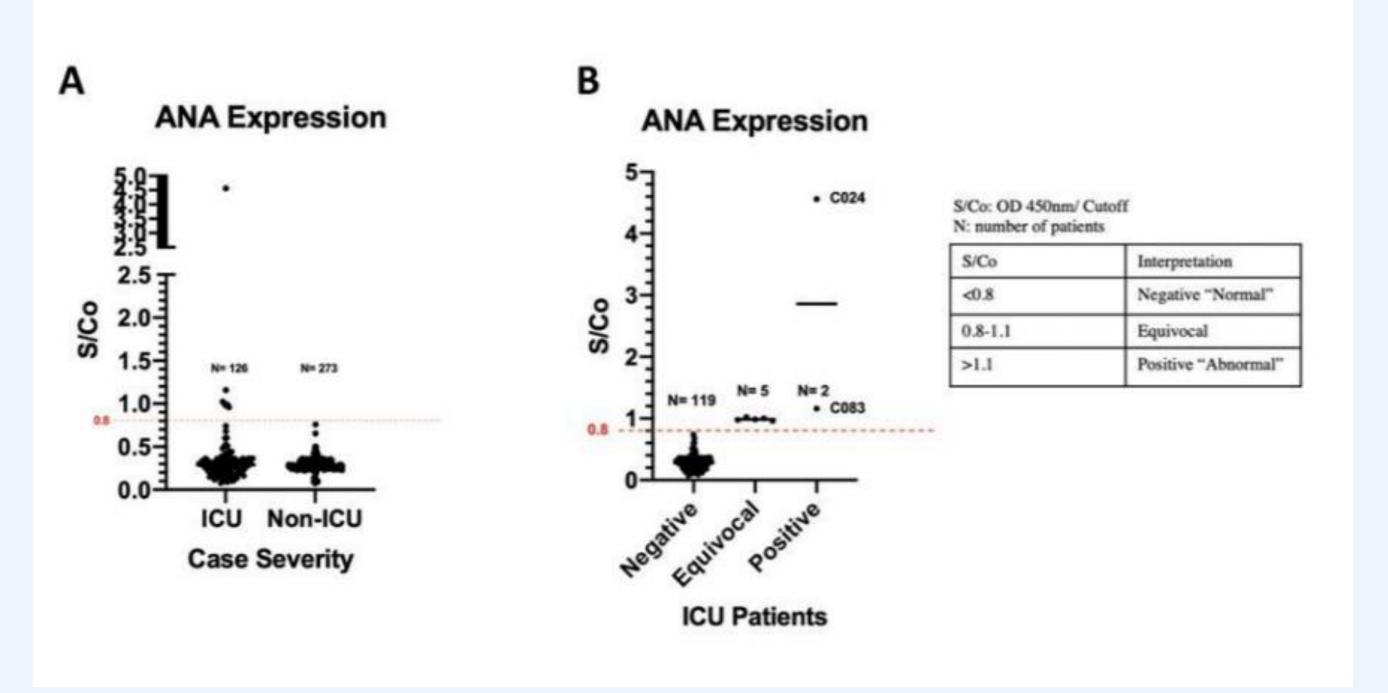


Used for this analysis Molecular Signature Database (MSigDB). 42 out 62 queried genes, were found significantly enriched p-value< 10 -5 and FDR q-value q-value < 0.05 in 1/20 genes. **Detection of shared linear B cell epitopes**

Built pairwise distance matrix. A linear sequence identity of 7 amino acids or more was considered a shared linear B cell epitope.

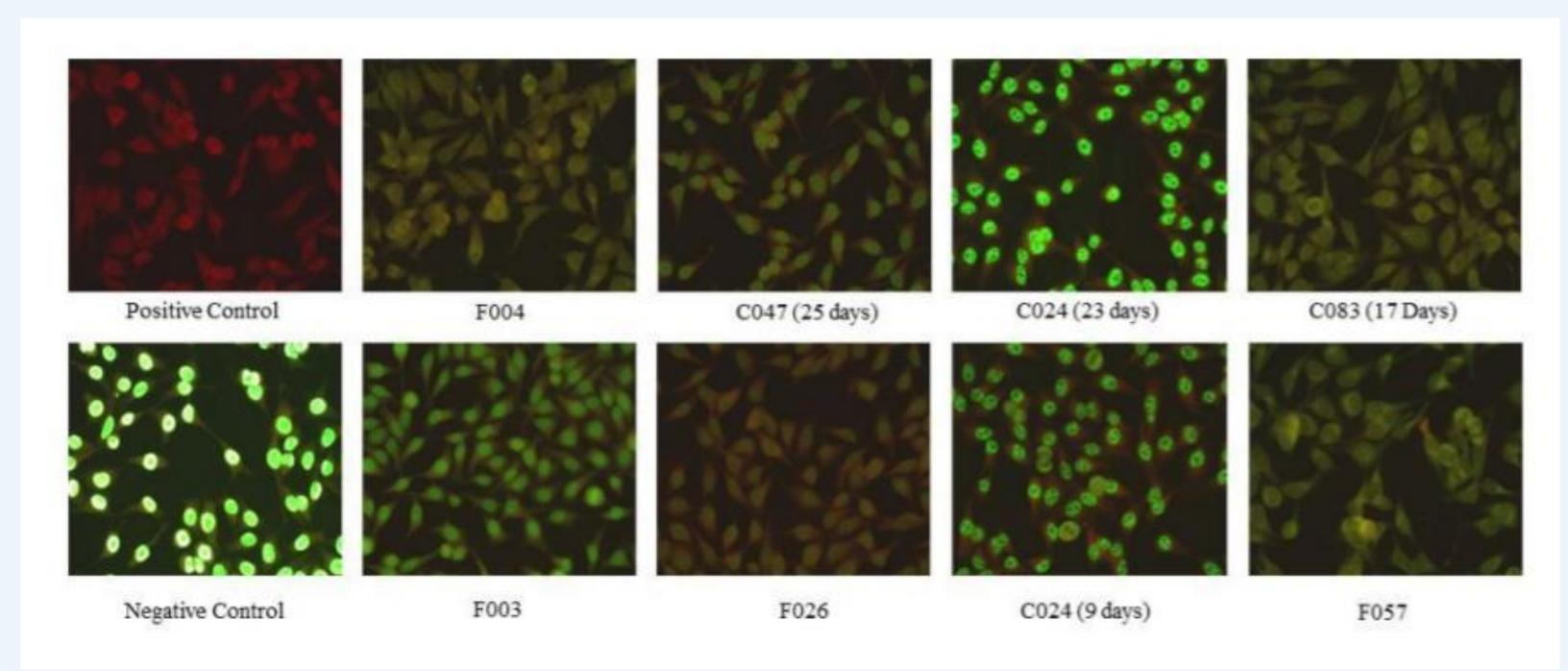
Results

ANA IgG ELISA



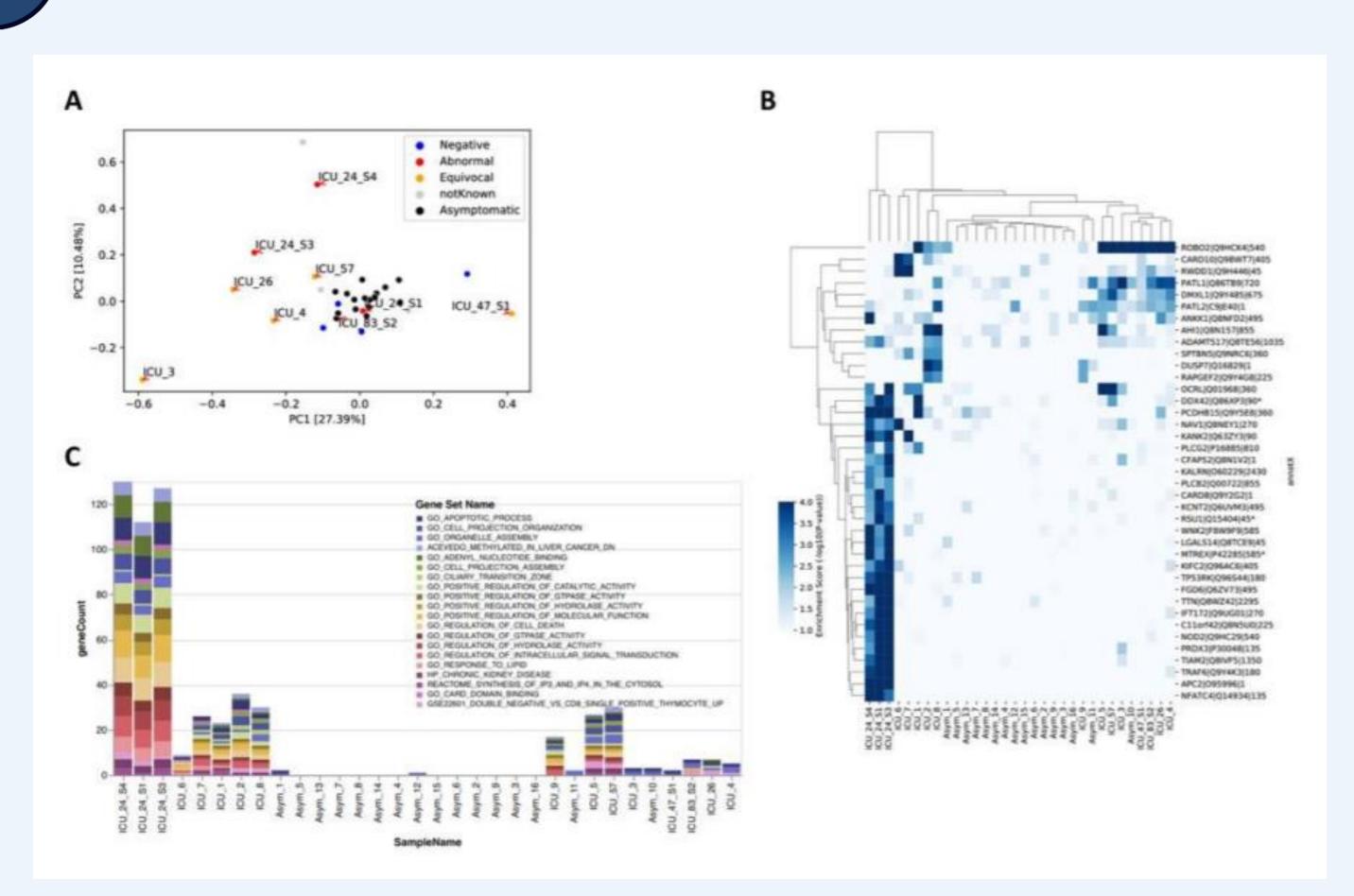
Figuer.1 ANA IgG ELISA results shows A) All non- ICU samples were negative (n=273) B) 1.6% (2/126) of ICU samples were strong ANA levels and 4% (5/125) of ICU samples were moderate ANA levels.

ANA Hep-2 IFA Assay



Figuer.2 ANAccHEp-2 IFA results confirmed the ANA level in positive and equivocal samples.

PhIP-Seq and Peptide Enrichment Analysis



Figuer.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 asympromatic COVID-19 cases. B) Heatmap plot showing the binding profile of the 79 differentially enriched (DE) peptides in ICU cases versus asymptomatic cases. C) Stacked bar plot showing the results of a gene set enrichment analysis of the peptides shown in (B).

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.



ANA-positive individuals should be excluded from being donors for convalescent plasma therapy in the context of Covid-19

Acknowledgment

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