Autoimmune effect of antibodies against the SARS-Cov-2 nucleoprotein

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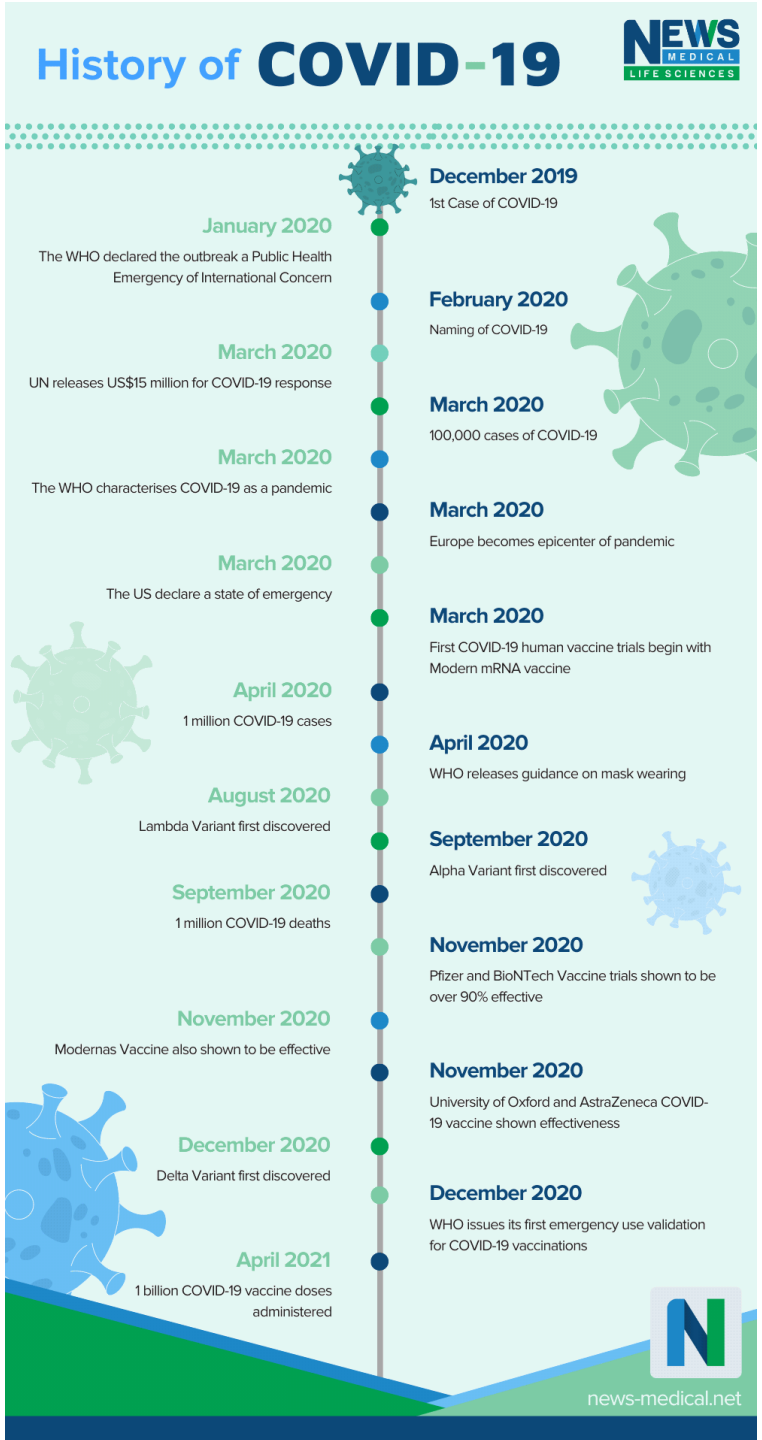
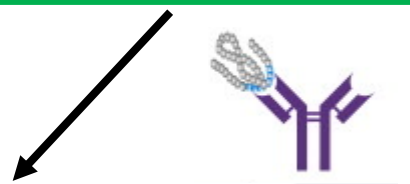


Our study design

Serum profiling
149 patients diagnosed with COVID-19;
4 points of material collection: admission to the hospital , after 48 hours, (after 7 days), discharge
Control group - 48 people (sera collected before 2019)

The patients were divided into 3 groups according to the computed tomography data:
CT = 0-1.5 - light severity patients
CT = 2-2.5 - medium severity patients
CT = 3-4 - high severity patients

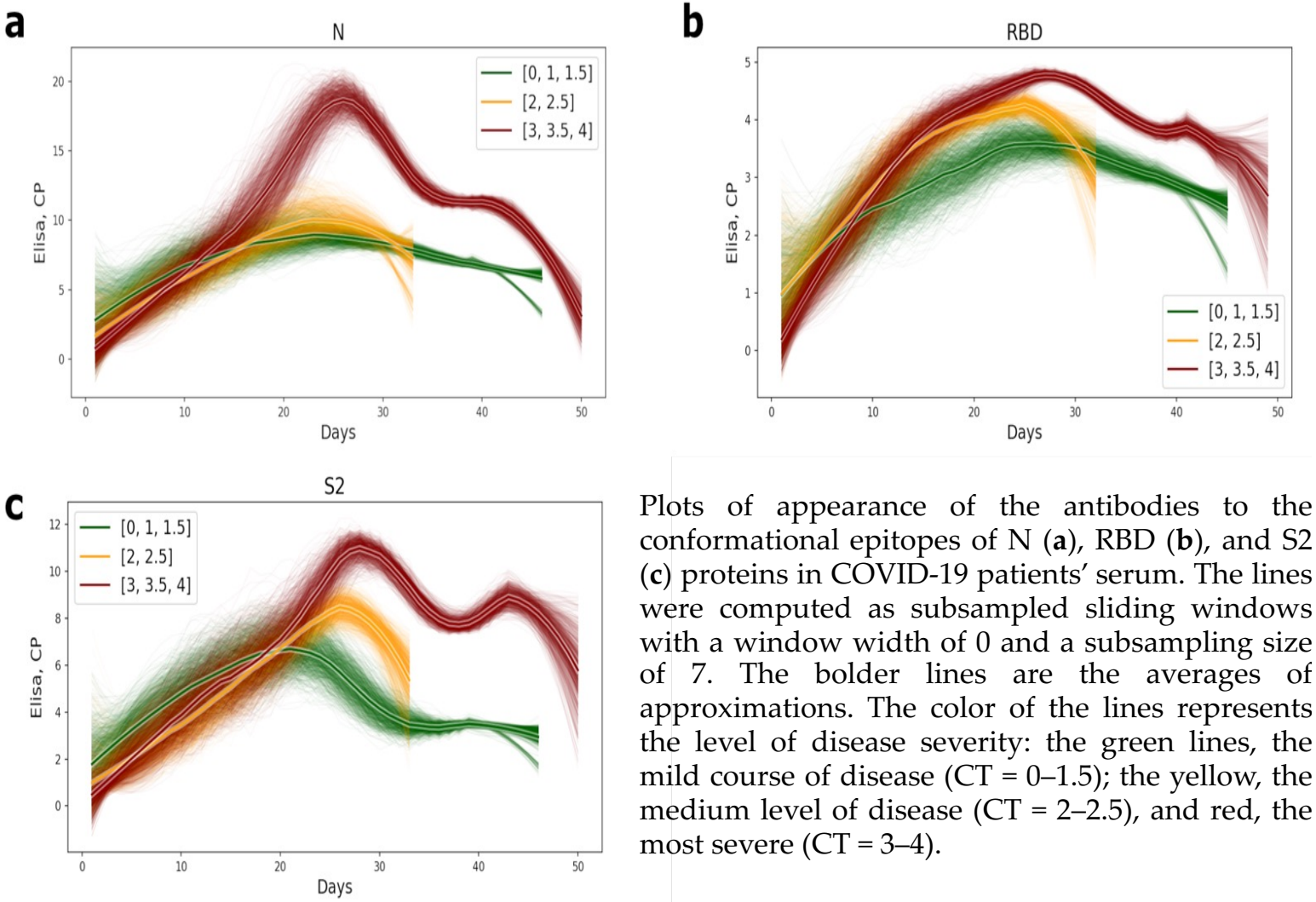
Analysis of IgG response to antigens (recombinant Nucleoprotein (NP), Spike (RBD, S2), nsp2, nsp5, nsp7, nsp9, nsp10, nsp15)



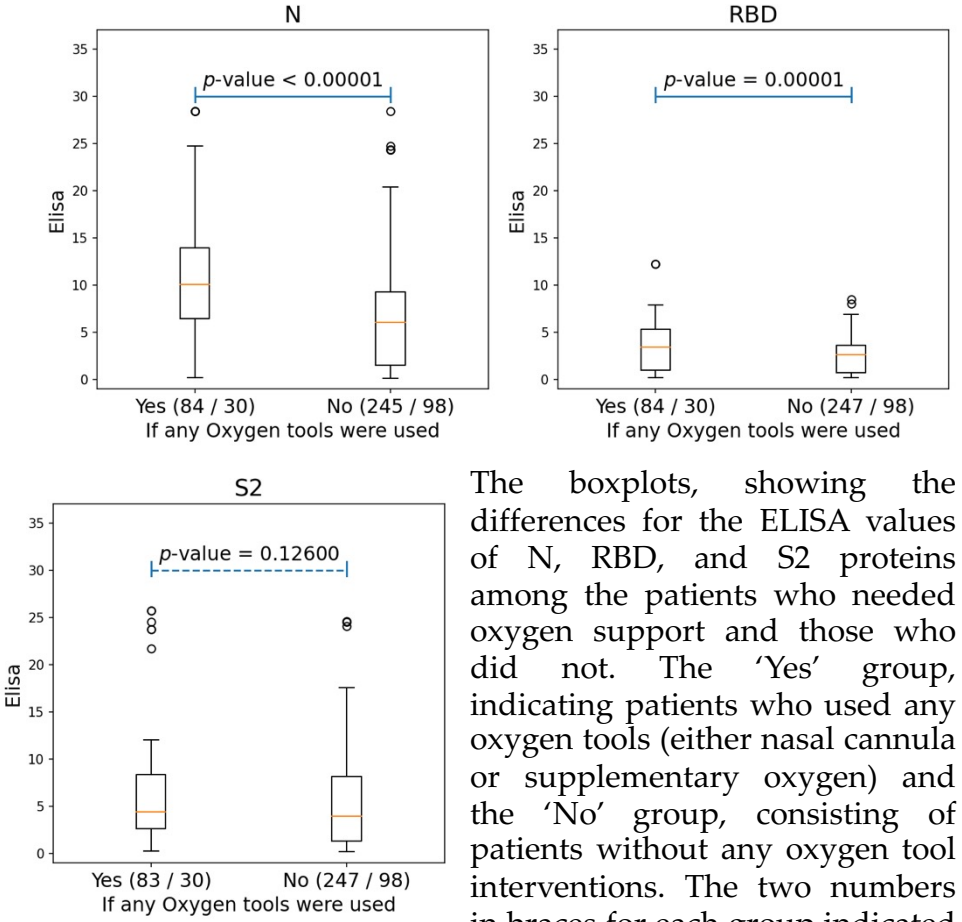
Conformational epitopes (ELISA, chip assay)

Linear epitopes (Western blot)

A high titer of IgG antibodies to the conformational epitopes of nucleocapsid protein correlates with the severity of the disease and oxygen demand.

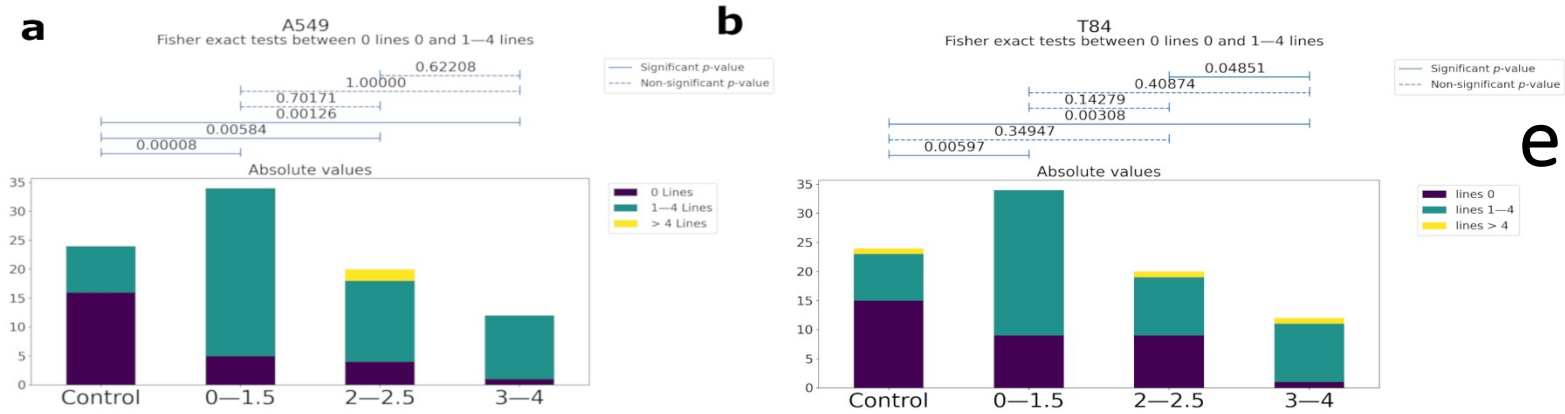


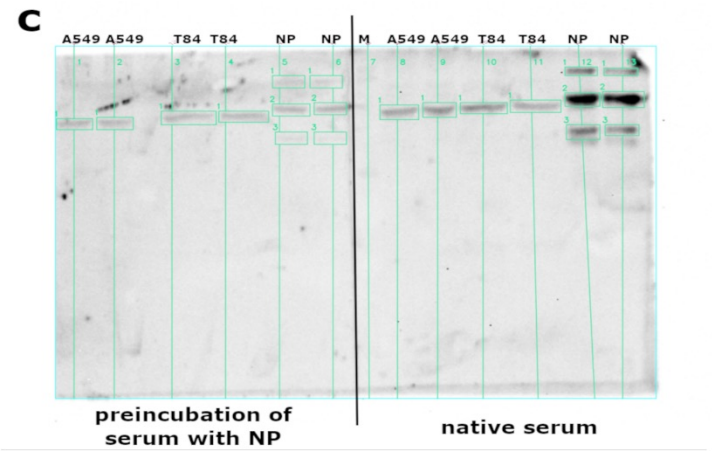
Plots of appearance of the antibodies to the conformational epitopes of N (**a**), RBD (**b**), and S2 (**c**) proteins in COVID-19 patients' serum. The lines were computed as subsampled sliding windows with a window width of 0 and a subsampling size of 7. The bolder lines are the averages of approximations. The color of the lines represents the level of disease severity: the green lines, the mild course of disease (CT = 0–1.5); the yellow, the medium level of disease (CT = 2–2.5), and red, the most severe (CT = 3–4).

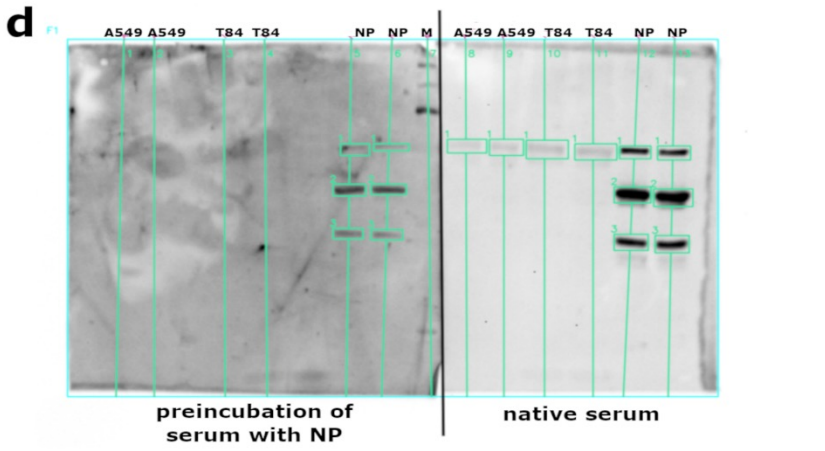


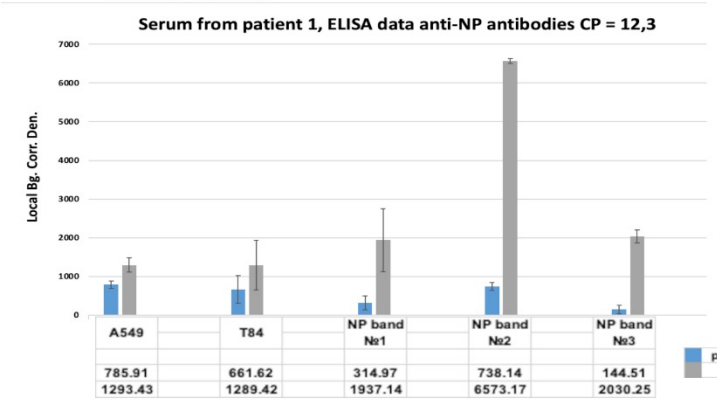
The boxplots, showing the differences for the ELISA values of N, RBD, and S2 proteins among the patients who needed oxygen support and those who did not. The 'Yes' group, indicating patients who used any oxygen tools (either nasal cannula or supplementary oxygen) and the 'No' group, consisting of patients without any oxygen tool interventions. The two numbers in braces for each group indicated the number of Elisa samples and patients correspondingly. The *p*-values were produced by performing ANOVA tests for each of the two groups.

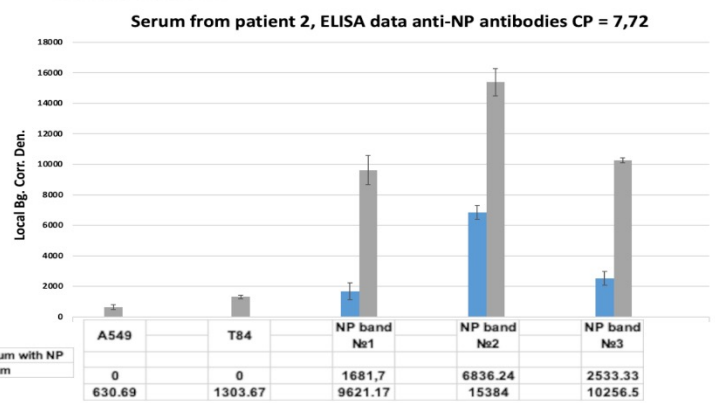
IgG antibodies to linear epitopes of the nucleoprotein can cross-reactively bind to human proteins









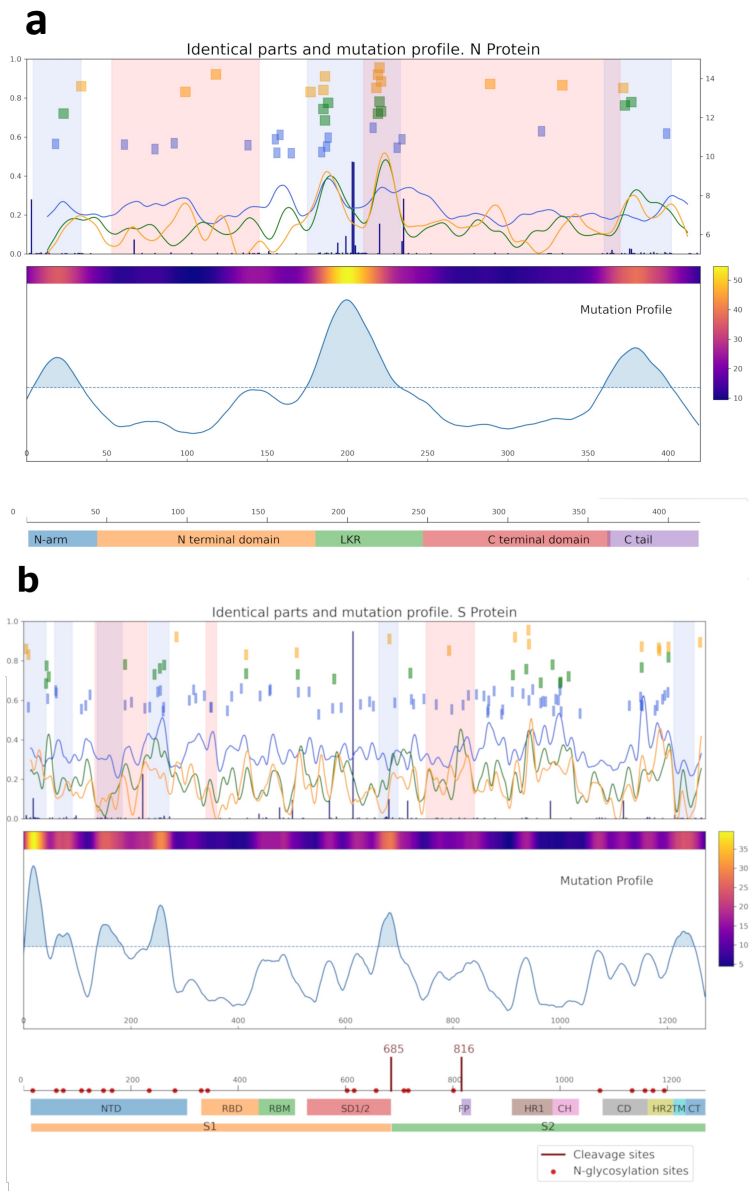


A549 - lung adenocarcinoma

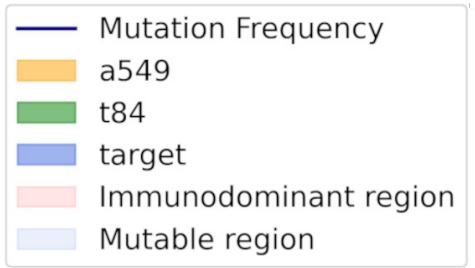
T84 - colorectal carcinoma

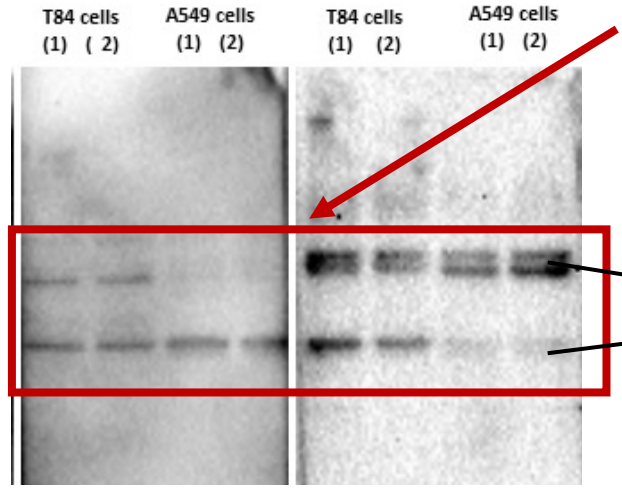
Analysis of the cross-reactive binding of IgG antibodies from the sera of patients with COVID-19 with linear epitopes of human proteins. Serum from healthy donors obtained before COVID-19 time was used as a control. (**a**,**b**) Correlations between the number of bands recognized by antibodies and groups of patients of different severity (according to CT data) for A549 cells and T84, respectively. (**c**,**d**) Analysis of the cross-reactivity of IgG from the serum before and after the separation of anti-NP specific antibodies from two different patients' serum, respectively, with eukaryotic proteins by western blot and quantitative values of bands chemiluminescence calculated using the built-in software iBright Imaging Systems (ThermoFisher). The table shows the average signal intensity values. Local Bg. Corr. Den.—Local Background Correction Density—The Local Background Corrected Volume divided by the Area.

Human proteins recognized by IgG from the COVID-19 patient sera were identified



Analysis of common regions between N (a), S (b) Sars-Cov-2 and human proteins. Proteins from A549 cell line are shown in yellow; –proteins from T84 cell line – in green; our target protein, detected by mass-spectrometry as potentially capable of being recognized by IgG antibodies. For cell lines, the intersection area was selected at 6 amino acids, for target proteins – at 4. Mutational and immunodominant profiles of proteins were taken from published data.





Recognition of human proteins by antibodies from patients' serum with COVID-19

Mass-spectrometry protein identification

Accession number	Description
T84 cell line	
K1C18_HUMAN	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2
ACTG_HUMAN	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1
K2C8_HUMAN	Keratin, type II cytoskeletal 8 OS=Homo sapiens GN=KRT8 PE=1 SV=7
VIME_HUMAN	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4
EF1A1_HUMAN	Elongation factor 1-alpha 1 OS=Homo sapiens GN=EEF1A1 PE=1 SV=1
A549 cell line	
VIME_HUMAN	Vimentin OS=Homo sapiens GN=VIM PE=1 SV=4
TUBA1B_HUMAN	Tubulin alpha-1B chain OS=Homo sapiens GN=c PE=1 SV=1
K1C18_HUMAN	Keratin, type I cytoskeletal 18 OS=Homo sapiens GN=KRT18 PE=1 SV=2
ACTG_HUMAN	Actin, cytoplasmic 2 OS=Homo sapiens GN=ACTG1 PE=1 SV=1
ENOA_HUMAN	Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2

Identified the main HLA alleles presenting viral peptides that are similar in amino acid sequence of human proteins

We used the downloaded standalone version of NetMHCpan 4.1 and NetMHCIIpan 4.0 [Reynisson, B. et al. Nucleic Acids Res. 2020] to predict protein fragments likely binding the most common alleles of HLA class I and the pan-specific binding of peptides to HLA class II alleles of all known sequences, respectively.

Peptide sequence	Sars-Cov-2 Protein	Human protein	HLA class I allele
SSPDDQIGYY	N_protein_77-87	Alpha-enolase (ENOA_HUMAN)	**HLA-A01:01**
NSSPDDQIGYY	N_protein_77-87		**HLA-A01:01**
NTNSSPDDQIGYY	N_protein_77-87		**HLA-A01:01**
SSPDDQIGY	N_protein_77-87		**HLA-A01:01**
NSSPDDQIGY	N_protein_77-87		**HLA-A01:01**
SPDDQIGYY	N_protein_77-87		**HLA-A01:01**
SVASQSIIAY	surface_glycopr	Keratin, type II cytoskeletal 8 (K2C8_HUMAN)	**HLA-A26:01**
VASQSIIAY	surface_glycopr		**HLA-B15:01**
SVASQSIIAY	surface_glycopr		**HLA-B15:01**
LQLPQGTTL	N_protein_158-1	Elongation factor 1-alpha 1 (EF1A1_HUMAN)	**HLA-B39:01**
LQLPQGTTL	N_protein_158-1		**HLA-B15:01**

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Thank you for your attention!

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