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## ANTIBODY IMMUNE RESPONSES TO SARS-COV-2 PEPTIDES IN COVID-19 CONVALESCENT PATIENTS

**Abstract:** *identifying immunogenic targets of SARS-CoV-2 is essential to develop novel treatments. The authors of the article studied humoral immune responses to spike (S) and nucleocapsid (N) SARS-CoV-2 proteins in serum from convalescent COVID-19 patients from Tatarstan, Russia. Multiple SARS-CoV-2 peptides were identified as reacting with convalescent COVID-19 serum. In addition, age and gender associated differences in the reactivity to S and N protein peptides were identified. Changing pattern of immunogenic peptide reactivity in COVID-19 serum based on age and gender was demonstrated. These data highlight how humoral immune responses to some of these peptides could contribute to SARS-CoV-2 pathogenesis.*

**Keywords:** *SARS-CoV-2, COVID-19, spike protein, peptides, antibody humoral immune response.*

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## **ИММУННЫЕ РЕАКЦИИ АНТИТЕЛ К ПЕПТИДАМ SARS-COV-2**

### **У ВЫЗДОРАВЛИВАЮЩИХ ПАЦИЕНТОВ С COVID-19**

*Аннотация:* выявление иммуногенных мишеней коронавируса SARS-CoV-2 имеет решающее значение для совершенствования стратегий диагностики и борьбы с заболеванием. Авторы статьи проанализировали гуморальный (ИФА) иммунный ответ на спайковый (S) и нуклеокапсидный (N) белки SARS-CoV-2 в сыворотке крови выздоравливающих пациентов с COVID-19 из Татарстана, Россия. В сыворотке крови выздоравливающих от COVID-19 были идентифицированы множественные пептиды SARS-CoV-2. Кроме того, были выявлены возрастные и гендерные различия в реактивности к S- и N-белковым пептидам. Таким образом, в исследовании была продемонстрирована изменяющаяся картина реактивности иммуногенных пептидов в сыворотке COVID-19 в зависимости

от возраста и пола. Полученные данные показывают, как гуморальные иммунные реакции на некоторые из коронавирусных пептидов могут способствовать патогенезу COVID-19.

**Ключевые слова:** COVID-19, SARS-CoV-2, спайковый белок, пептиды, антитела, гуморальный иммунный ответ.

Это исследование было проведено за счет гранта, выделенного Казанскому федеральному университету для выполнения государственного задания в области науки (проект №0671-2020-0058). Эта работа является частью Программы Стратегического академического лидерства Казанского федерального университета.

#### *Introduction.*

In 2019, an outbreak of a «pneumonia of unknown etiology» in Wuhan province, China was linked to a novel strain of coronavirus [1]. This virus, later named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a novel member of the beta-coronavirus family [2]. COVID-19 can be asymptomatic in a large subset of patients; however, some patients could have a broad spectrum of clinical presentation such severe pneumonia, acute respiratory distress syndrome and death [3].

Studies of SARS-CoV-2 infection have reported that the recovery from COVID-19 depends on the activation of antibody responses, where mild form shown to have higher IgG antibody titers [4]. Sun *et al* have confirmed the activation of the humoral immune responses by identifying spike (S) glycoprotein and nucleocapsid (N) proteins as the major immunogens [5]. In another report by Röltgen *et al*, S-specific IgG antibody levels were found higher in patients not admitted to the intensive care unit (ICU) whilst in ICU patients, N-specific IgG levels were higher [6]. These data provide strong support for the role of antibodies in pathogenesis of SARS-CoV-2.

In this study of patients in Tatarstan, Russia, we demonstrated that SARS-CoV-2 S and N epitopes can be identified using COVID-19 convalescent sera. Defining these epitopes could help diagnostics development. We also analyzed the SARS-CoV-2 S

and N protein reactivity depending on the age and sex of patients. These findings could have implications for the development of novel therapeutics against COVID-19.

#### *Materials and Methods.*

*Human subjects:* Convalescent serum samples were collected from 138 COVID-19 patients and 39 age-matched controls. Also, acute COVID-19 serum samples from 14 patients were collected. The diagnosis of SARS-CoV-2 infection was based on clinical symptoms and qPCR data. Convalescent serum samples were collected following standard operating procedures in the hospital for the diagnosis of COVID-19 infection and stored at  $-80^{\circ}\text{C}$ .

*Ethics Statement:* The ethics committee of the Kazan Federal University approved this study, and signed informed consent was obtained from each patient and control subjects according to the guidelines adopted under this protocol (Protocol 4/09 of the meeting of the ethics committee of the KSMA dated September 26, 2019).

*COVID-19 peptides:* Multiple S and N protein peptides for SARS-CoV-2 were synthesized by GenScript (Jiangsu, China). SARS-CoV-2 S and N protein peptide aa sequences (purity  $>95\%$ ).

*Peptide reactivity with serum antibodies:* Each peptide ( $1\ \mu\text{g}/100\ \mu\text{L}$ ) was adsorbed on a 384-well plate at  $4^{\circ}\text{C}$  for 18 h before plates were washed and incubated with serum samples (1:100;  $50\ \mu\text{L}$ ) at  $4^{\circ}\text{C}$  for 18 h. Wells were incubated with anti-human-IgG-HRP conjugated antibodies (1:10,000 in PBS-T, Amerixan Qualex Technologies, USA) for 30 min at  $37^{\circ}\text{C}$  followed by incubated with 3,3',5,5' Tetramethylbenzidine (Chema Medica, Moscow, Russia). Data were captured using a microplate reader Tecan 200 (Tecan, Switzerland) at  $\text{OD}_{450}$  with reference  $\text{OD}_{650}$ .

*Statistical analysis:* Statistical analysis was performed in the R environment [9]. Statistically significant differences between comparison groups were accepted as  $p < 0.05$ , assessed by the Kruskal-Wallis test with Benjamini-Hochberg adjustment for multiple comparisons.

#### *Results.*

*Clinical presentation of COVID-19:* A total of 152 samples (50 male and 102 female) were collected from acute and convalescent COVID-19 patients with an average age of  $38.0 \pm 11.9$  years old (Table 1).

Table 1

## Clinical characteristics of acute and convalescent COVID-19 patients

Clinical characteristics	Values
Age (years)	$38 \pm 0.05$
Sex (M/F)	48/89
Lung damage (>40%) (n)	4
Lung damage (20–40%) (n)	23
Lung damage (<20%) (n)	110
Severe COVID19 (n)	4
Moderate COVID19 (n)	43
Mild COVID19 (n)	105
Fever ( $^{\circ}\text{C}$ )	$37.92 \pm 0.66$
Fever (days)	$6.31 \pm 4.04$
Artificial ventilation (yes/no)	2/135

\*n = number of cases

*Peptides used in this study:* The position of SARS-Cov-2 peptides is summarized in Figure 1. It should be noted that many peptides are located in regions containing immunogenic epitopes [10] suggesting that they could have reactivity with COVID-19 serum.

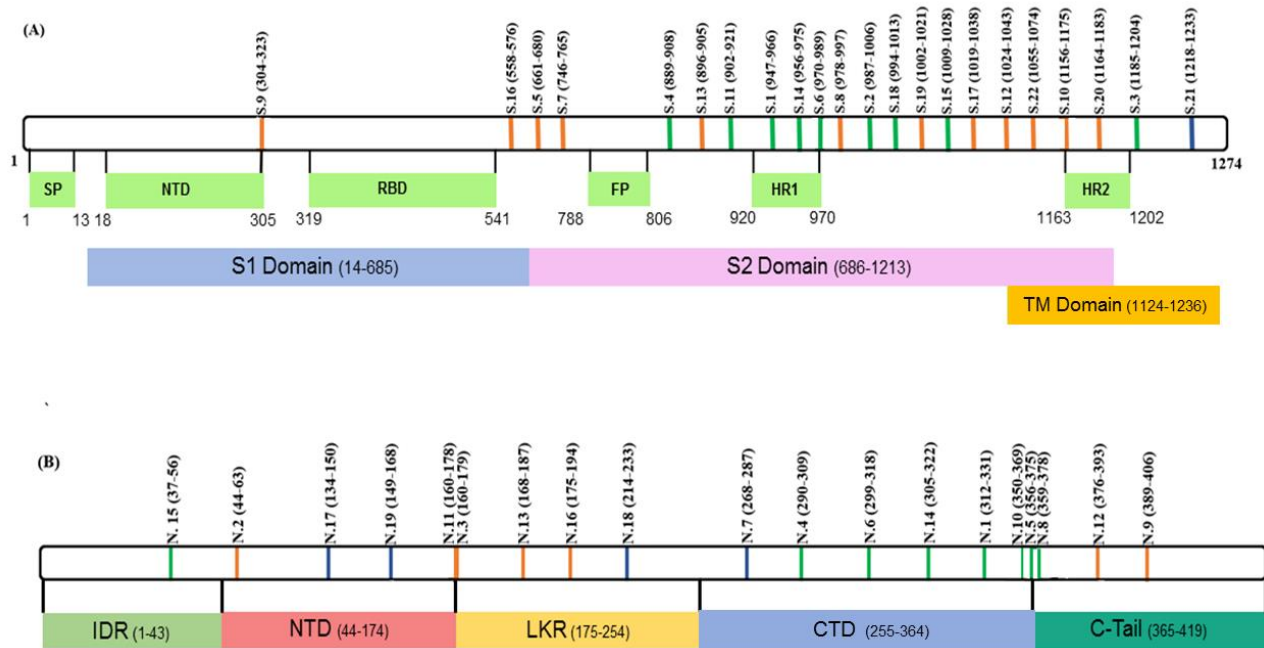


Fig. 1. Schematic presentation of SARS-CoV-2 (A) S and (B) N peptide locations

\*Green – peptides containing both, B and T cell, epitopes

Blue – peptides containing only T cell epitopes

Orange – peptides containing only B cell epitopes

*Analysis of antibody response to COVID-19 peptides:* The reactivity of all 152 COVID-19 convalescent serum samples was tested using peptides. Several S peptides (S1, S7 and S18) and the N6 peptide had significantly higher reactivity with convalescent serum as compared to COVID-19 negative controls (Table 2). Therefore, we suggest that S1, S7, S18 and N6 COVID-19 peptides could have therapeutic potential.

Table 2

Peptides with increased reactivity in convalescent COVID-19 samples compared to controls collected in 2022

<i>Covid19 Peptide</i>	<i>Control Mean ± SD</i>	<i>Covid19 Mean ± SD</i>	<i>P.adj</i>
<i>S1</i>	0.984 ± 0.336	1.17 ± 0.35	0.005
<i>S7</i>	0.39 ± 0.206	0.59 ± 0.24	2.629E-05
<i>S18</i>	0.455 ± 0.19	0.62 ± 0.33	0.009
<i>N6</i>	0.257 ± 0.153	0.44 ± 0.20	1.204E-06

*Differences in SARS-CoV-2 peptide reactivity based on sex:* Next, we sought to determine whether sex of patients contributes to serum reactivity to SARS-CoV-2 peptides (Table 3). We found that the serum reactivity with peptides S1, S7, S18 and N6 was increased in male COVID-19 patients when compared with male controls. In female, serum reactivity was only higher for S7 and N6 when compared to female controls (Table 3).

Table 3

COVID-19 serum reactivity with SARS-CoV-2 peptides depending on sex of patient

<i>Covid19 Peptide</i>	<i>Control female Mean ± SD</i>	<i>Covid19 female Mean ± SD</i>	<i>P.adj</i>	<i>Control male Mean ± SD</i>	<i>Covid19 male Mean ± SD</i>	<i>P.adj</i>
<i>S1</i>	1.08 ± 0.322	1.18 ± 0.363	0.328	0.872 ± 0.325	1.15 ± 0.336	0.02
<i>S7</i>	0.424 ± 0.161	0.591 ± 0.242	0.01	0.351 ± 0.247	0.598 ± 0.236	0.001
<i>S18</i>	0.452 ± 0.155	0.594 ± 0.312	0.118	0.458 ± 0.229	0.7 ± 0.374	0.044
<i>N6e</i>	0.316 ± 0.153	0.476 ± 0.476	0.005	0.187 ± 0.125	0.35 ± 0.177	0.015

*Reactivity to SARS-CoV-2 S and N protein peptide depending on ages:* COVID-19 patients were divided into younger (<45 years old) and older (≥45) groups. Reactivity to SARS-CoV-2 peptides was tested. We have found that younger patients serum reactivity to S1 was higher as compared to older COVID-19 patients (Table 4). Additionally, the reactivity to S7 and N6 was higher in both COVID-19 age groups as compared to controls. However, only young patients showed higher reactivity to peptides S1 and S18 as compared to the same age control (Table 4). It appears that younger COVID-19 patients have higher reactivity with more peptides (S1, S7, S18 and N6) as compared to older patients (S7 and N6). These data suggest that younger individuals have COVID-19 reacting epitopes on S and N proteins is a contributing factor to age-related disease pathogenesis.

Table 4

Age groups dependent difference to SARS-CoV-2 peptides reactivity  
in COVID-19 serum

<i>Covid19 Peptide</i>	<i>Control &lt;45 Mean ± SD</i>	<i>Covid19 &lt;45 Mean ± SD</i>	<i>P.adj</i>	<i>Control ≥45</i>	<i>Covid19 ≥45 Mean ± SD</i>	<i>P.adj</i>



				<i>Mean ± SD</i>		
<i>S1</i>	1.042 ± 0.327	1.215 ± 0.353	0.035	0.838 ± 0.328	1.045 ± 0.327	0.171
<i>S7</i>	0.437 ± 0.206	0.617 ± 0.253	0.002	0.272 ± 0.158	0.502 ± 0.157	0.014
<i>S18</i>	0.422 ± 0.164	0.63 ± 0.342	0.009	0.537 ± 0.233	0.625 ± 0.325	0.705
<i>N6e</i>	0.293 ± 0.153	0.45 ± 0.216	0.001	0.164 ± 0.116	0.432 ± 0.173	0.0005

### *Discussion.*

Our data confirm the role of anti-SARS-CoV-2 S and N protein antibodies in the pathogenesis of COVID-19. High levels of IgM and IgG were normally found in severe as compared to mild COVID-19 patients [12]. Despite this information, the immunogenic epitopes with protective capacity remain largely unknown. Here, we identified multiple peptides on S and N proteins reacting with COVID-19 serum. These data indicate that antibodies to this S protein region could have a protective role in SARS-CoV-2 infection.

We also investigated immune mechanisms in male and female COVID-19 patients by demonstrating differences in the reactivity with S and N SARS-CoV-2 peptides. Interestingly, reactivity to only *S7* and *N6* was found in female, whilst four peptides (*S1*, *S7*, *S18* and *N6*) were identified as reacting with male COVID-19 serum. We also observed that younger patients had more antibody reactivity to S and N peptides.

In conclusion, our data confirm activation of anti-SARS-CoV-2 antibodies. We also identified higher reactivity with several S and N peptides in younger patients compared older COVID-19, suggesting a contribution of reactivity with these epitopes to age-related pathogenesis. Taken together these findings identify several peptides from SARS-CoV-2 S and N proteins that are immunogenic and may be indicative of disease outcomes.

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