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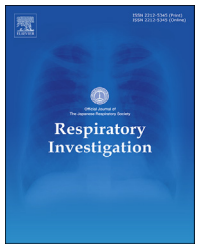

Original article

SFTPB (rs11130866) and NR3C1 (rs41423247) gene variants as potential clinical biomarkers for personalized treatment strategy selection in patients with severe COVID-19 pneumonia

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ABSTRACT

Background: Exploring the pathogenetic mechanisms behind severe lung damage in COVID-19 is crucial. In this study, we decided to focus on two molecular markers that affect surfactant metabolism and lung development: the surfactant protein B (*SFTPB*) and the glucocorticoid receptor (*NR3C1*) genes. The aim of our study was to determine the effect of *SFTPB* (rs11130866) and *NR3C1* (rs41423247) gene variants on the course of the disease in patients with COVID-19, and the treatment measures they required.

Methods: The study group included 58 patients with a diagnosis of severe "viral COVID-19 pneumonia." Determination of *SFTPB* and *NR3C1* gene variants was performed using the PCR-RFLP method.

Results: Our results indicate that the presence of the *SFTPB* gene CC genotype increases the risk of developing acute respiratory distress syndrome in patients with COVID-19 ($\chi^2 = 4.03$, $p = 0.045$, OR = 3.90 [1.19–12.78]). However, patients with the *SFTPB* gene TT genotype required respiratory support for a shorter period of time. Patients with the *NR3C1* gene CC genotype underwent a longer glucocorticoid therapy. Moreover, for patients with the CC genotype, a longer stay in the intensive care unit was detected before lethal outcome.

Abbreviations: ALV, artificial lung ventilation; ARDS, acute respiratory distress syndrome; COVID-19, coronavirus disease 2019; GR, glucocorticoid receptor; SIRS, systemic inflammatory response syndrome.

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Conclusions: The obtained results confirm the influence of the *SFTPB* (rs11130866) and *NR3C1* (rs41423247) gene variants on the therapy, course, and severity of the disease in patients with COVID-19. Of course, these results require further study, analysis, and larger, complex, systematic research.

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by the SARS-CoV-2 virus, quickly grew into a global pandemic with serious socio-economic consequences. To date, more than 550 million cases have been reported [1]. Most infected patients have mild to moderate, flu-like symptoms. However, some of the patients develop severe lung damage. This clinically severe manifestation is accompanied by hyperproduction of multiple cytokines ("cytokine storm"), loss of parenchyma, immune infiltration, and fluid filling of the alveoli, leading to the development of acute respiratory distress syndrome (ARDS) and death [2]. Numerous histopathological studies based on post-mortem biopsies of the lungs of such patients indicate the presence of diffuse alveolar damage with interstitial edema [3,4]. Therefore, the current studies examining the pathogenetic mechanisms of severe lung damage in COVID-19 are especially relevant. The predictors and triggers of its genetic determinism will help develop effective etiological and pathogenetic therapies for the severe forms of the disease, with a personalized approach to prevent severe somatic complications of organs and systems.

A team of researchers investigating the biological processes/pathways which are disrupted by SARS-CoV-2 infection identified four biological pathways that could significantly affect the condition of patients with COVID-19: response to hypoxia, lung development, respiratory processes, and surfactant metabolism [5]. In this study, we decided to focus on two molecular markers that affect surfactant metabolism and lung development: the surfactant protein B (*SFTPB*) and the glucocorticoid receptor (*NR3C1*) genes.

SFTPB is a gene that encodes the pulmonary-associated surfactant protein B (SP-B), essential for lung function. In particular, it promotes the formation and maintenance of a phospholipid-rich film at the alveolar air–liquid interface [6]. To date, more than 5500 variants of the *SFTPB* gene have been identified [7]. The study of the common single nucleotide substitution c.392C > T (or C1580T, rs11130866) in exon 4 of the *SFTPB* gene is of the most interest. This leads to the replacement of threonine (Thr) with isoleucine (Ile) at the 131th position and causes an altered modification of the N-linked glycosylation site of the protein. Researchers believe that the presence of this variant of the gene affects the processing, secretion, and folding of SP-B in certain diseases [8].

The *NR3C1* gene encodes the glucocorticoid receptor (GR), which might function both as a transcription factor and as a regulator of other transcription factors. This receptor is involved in inflammatory responses, cellular proliferation, and differentiation in target tissues. Bridges et al. revealed the cellular and molecular mechanisms by which GR signaling regulates fetal lung maturation [9]. To date, more than 55,000 variants of the *NR3C1* gene have been identified [7]. Among them, the variant g.41503C > G (also C646G or BclI, rs41423247) is one of the most common and extensively studied. This variant is located in intron 2 of the *NR3C1* gene resulting in the substation of nucleotide C with G at position 1184 + 646. This variant significantly affects the process of alternative *NR3C1* gene splicing and within that mechanism increases the sensitivity to glucocorticoids [10]. The aim of our study was to determine the effect of *SFTPB* (rs11130866) and *NR3C1* (rs41423247) gene variants on the course of the disease in patients with COVID-19 along with the treatment measures they required.

2. Patients and methods

2.1. Clinical characteristics of the patients

The study included 58 patients (26 women and 32 men) who were treated in the intensive care unit of the Poltava Regional Clinical Infectious Diseases Hospital within December 2020–June 2021. Patients were not vaccinated against SARS-CoV-2. Inclusion criteria were: age ≥18 years, confirmed severe COVID-19 pneumonia (using computed tomography of the lungs or radiological examination), need for respiratory support. Conversely the exclusion criteria were pregnancy in women or the patient refusing to give informed consent. This study was approved by the Ethics Committee of the Ukrainian Medical Stomatological Academy (protocol No. 188 of November 25, 2020) and all the patients provided informed consent.

In the department, on the first day of hospitalization (marked as 1d in the text) and during daily treatment (marked as 2d, 3d, etc. in the text) all the patients underwent clinical and laboratory examinations. Instrumental screening methods were used for all patients to verify the diagnosis of viral pneumonia, namely CT scan of the lungs and X-ray examination. The average age of the patients was 63.9 ± 14.3 years (65.4 ± 13.5 years for women, 62.7 ± 15.0 years for men) and the average BMI 29.7 ± 6.6 kg/m^2 (32.6 ± 7.2 kg/m^2 for women, 27.4 ± 5.0 kg/m^2 for men). Clinical parameters of all the patients included in the study group during hospitalization are shown in Table 1.

Fifty patients (86%) had a history of comorbidities, such as cardiovascular disease, cancer, tuberculosis, and type II diabetes. Forty-four patients (76%) had already received oxygen therapy (using an oxygen mask) on admission to the hospital. In nineteen patients (33%), hospitalized owing to the severity

Table 1 – Clinical parameters of patients on the first day of hospitalization.

Indicator	Patients (n = 58)
Temperature 1d, °C	37.6 ± 0.8
Respiratory rate 1d, per minute	23.2 ± 2.7
SpO_2/FiO_2 1d, %	167.7 ± 79.1
SOFA scale score 1d[a], in points	2 [2–3]
Glasgow coma scale 1d[a], in points	8 [7–8]

[a] Values are presented as the median [25th–75th percentile].

of the condition and respiratory failure, respiratory support was initiated in the form of artificial lung ventilation (ALV). Twenty-two patients (38%) died from complications caused by COVID-19, while other patients (36 [62%]) were subsequently transferred from the intensive care unit to the somatic unit depending on the complications associated with underlying disease and comorbidities.

Before hospitalization, the study participants had taken mucolytics, non-steroidal anti-inflammatory drugs (paracetamol), and symptomatic therapy according to existing chronic diseases, namely antihypertensives for hypertension and hypoglycemic drugs for type II diabetes. After hospitalization, patients received therapy according to the algorithm for providing inpatient care for COVID-19 in Ukraine: optimal supportive therapy in an intensive care unit/ward; oxygen support; oral or intravenous systemic corticosteroids (dexamethasone or equivalent doses of hydrocortisone, methylprednisolone); low molecular weight heparins based on blood coagulation parameters; if the bacterial flora was involved—antibacterial or antifungal agents according to local epidemiology; sedative and narcotic drugs—dexmedetomidine, midazolam, morphine hydrochloride; antiarrhythmics—beta-blockers, sympathomimetics; other symptomatic therapies.

2.2. *Genotyping*

Genomic DNA for molecular genetics research was isolated from peripheral blood using the commercial Quick-DNA Miniprep Plus Kit (Zymo Research, Irvine, CA, USA). Determination of *SFTPB* (rs11130866) and *NR3C1* (rs41423247) gene variants was performed using the PCR-RFLP method, in accordance with previously described protocols [11,12]. The same sequences of primers (Metabion, Bayern, Germany) and restriction enzymes (Thermo Scientific, Waltham, MA, USA) were used in this work. DreamTaq Green PCR Master Mix (2X) (Thermo Scientific) was used for the PCR reaction. Digested products were separated using agarose gel electrophoresis and visualized on a UV transilluminator.

2.3. *Statistical analysis*

Statistical data processing was performed using the SPSS v. 27 software and Microsoft Excel ProPlus 2016. The mean value ± standard deviation was calculated for analysis of the basic clinical characteristics. To compare the frequency distribution of genotypes among the groups we used descriptive statistics and calculation of Pearson's χ^2 criteria. The strength of the association between genotype and the risk of developing the disease, its clinical manifestations, and the need for medical interventions was assessed through the odds ratio (OR) at a 95% confidence interval. The distribution of the studied parameters was checked for normality using the Kolmogorov–Smirnov test. In case of a normal distribution, the probability of differences in the quantitative results was determined using one-way analysis of variance. If the studied parameter did not meet the criteria for a normal distribution, the comparison of this trait in carriers of different genotypes was performed using the Kruskal–Wallis test followed by post-hoc analysis with the Bonferroni correction. For all analyses, statistical significance was set at $p < 0.05$.

3. Results

ARDS is the main complication leading to the high mortality rate in patients with COVID-19. Therefore, we separately analyzed the characteristics of patients who had this complication and, accordingly, those who did not (Table 2). No significant differences were found in this subgroup of patients; however, patients with ARDS tended to be older and have a higher BMI.

The molecular genetic analysis revealed that the rates of the *SFTPB* variant rs11130866 genotypes were 36.2% CC, 43.1% CT, and 20.7% TT. For the *NR3C1* variant rs41423247, the following rates were determined: 34.5% CC, 48.3% CG, and 17.2% GG.

Table 2 – Patient characteristics based on ARDS presence.

Indicator		Patients	
		No ARDS (n = 41)	With ARDS (n = 17)
Sex	Male	23 (56.1%)	9 (52.9%)
	Female	18 (43.9%)	8 (47.1%)
Age, years		62.1 ± 15.4	68.3 ± 10.1
BMI, kg/m^2		28.7 ± 6.0	32.2 ± 7.3
COVID-19 vaccination		No	No
Comorbidities	Cardiovascular	21 (51.2%)	8 (47.1%)
	Oncology	5 (12.2%)	2 (11.8%)
	Tuberculosis	2 (4.9%)	1 (5.9%)
	Type II diabetes	7 (17.5%)	4 (23.5%)

ARDS—acute respiratory distress syndrome.

Table 3 – Analysis of genotype distribution frequency of the studied gene variants based on clinical severity.

Parameter (Complication)		Genetic variant, n (%)						Results of statistical analyses
		SFTPB rs11130866			*NR3C1* rs41423247			
		CC	CT	TT	CC	CG	GG	
ARDS	Yes	10 (58.8%)	4 (23.5%)	3 (17.6%)	6 (34.%)	8 (47.1%)	3 (17.6%)	For rs11130866: CC vs. CT + TT $\chi^2 = 4.03$, $p = 0.045$, OR = 3.90 (1.19–12.78)
	No	11 (26.8%)	21 (51.2%)	9 (22.0%)	14 (34.1%)	20 (48.8%)	7 (17.1%)	
SIRS	Yes	8 (36.4%)	9 (40.9%)	5 (22.47%)	5 (22.7%)	13 (59.1%)	4 (18.2%)	$p > 0.05$
	No	13 (36.1%)	16 (44.4%)	7 (19.4%)	15 (41.7%)	15 (41.7%)	6 (16.7%)	
ALV	Yes	7 (36.8%)	8 (42.1%)	4 (21.1%)	6 (31.6%)	10 (52.6%)	3 (15.8%)	$p > 0.05$
	No	14 (35.9%)	17 (43.6%)	8 (20.5%)	14 (35.9%)	18 (46.2%)	7 (17.9%)	
Death	Yes	8 (36.4%)	10 (45.5%)	4 (18.2%)	9 (40.9%)	10 (45.5%)	3 (13.6%)	$p > 0.05$
	No	13 (36.1%)	15 (41.7%)	8 (22.2%)	11 (30.6%)	18 (50.0%)	7 (19.4%)	

ARDS—acute respiratory distress syndrome; SIRS—systemic inflammatory response syndrome; ALV—artificial lung ventilation.

We analyzed the effect of the investigated gene variants on the risk of complications in patients with COVID-19 pneumonia in the described study group (Table 3). Our results indicate that the presence of the CC genotype in the *SFTPB* variant increases the risk of developing ARDS in patients with COVID-19 by almost four times. Conversely, the *NR3C1* variant was not associated with the development of severe complications in the patients we examined.

Next, the effect of the gene variants on clinical parameters in patients with COVID-19 was analyzed. The following were examined: temperature (in degrees Celsius: 1d, 2d, 6d); respiratory rate (per minute: 1d, 2d, 6d); SpO_2/FiO_2 (in percentage: 1d, 2d, 6d); SOFA scale score (in points: 1d, 2d, 6d); Glasgow coma scale score (in points: 1d, 2d, 6d); dose of glucocorticoids (dexamethasone or another glucocorticoid in equivalent dose, mg/day); duration of glucocorticoid therapy (days); duration of ALV (days); duration of oxygen therapy using an oxygen mask (days); total duration of oxygen support (days) (Table 4).

Therefore, for the rs11130866 variant of *SFTPB*, the relationship between genotype and total duration of oxygen support in patients was determined as: CT vs. TT, 9.0 ± 6.0 vs. 4.1 ± 6.2 days, respectively ($p = 0.022$). Hence, patients with the *SFTPB* TT genotype required respiratory support for a shorter period.

Several significant associations have been identified for the rs41423247 variant of *NR3C1*. Patients with the *NR3C1* CC genotype underwent a longer glucocorticoid therapy: CC vs. CG, 13.8 ± 5.0 vs. 9.0 ± 5.0 days, respectively ($p = 0.033$); or CC vs. CG + GG, 13.8 ± 5.0 vs. 9.3 ± 4.8 days, respectively ($p = 0.018$). Also, for patients with the CC genotype, a longer stay in the intensive care unit was detected before lethal outcome: CC vs. CG, 24.4 ± 9.6 vs. 11.9 ± 10.8 days, respectively ($p = 0.037$); or CC vs. CG + GG, 24.4 ± 9.6 vs. 12.9 ± 10.0 days, respectively ($p = 0.014$).

4. Discussion

An interdisciplinary and multimodal approach is needed to meet the challenges posed by the COVID-19 pandemic. It is already well known that in various manifestations of infectious diseases, in addition to factors such as the general state of health, age, sex, and virulence of the pathogen, the individual genetic constellation plays a key role [13,14].

In this study, we focused on the molecular markers *SFTPB* (rs11130866) and *NR3C1* (rs41423247). The *SFTPB* gene encodes SP-B, which is essential for lung function. Previous studies have indicated that the *SFTPB* variant rs11130866 is associated with numerous pulmonary diseases, such as idiopathic pulmonary fibrosis lung diseases, hypersensitivity pneumonitis, chronic obstructive pulmonary disease, and acute respiratory distress syndrome [15–18]. The *NR3C1* gene encodes GR, which has a broad spectrum of biological action. The rs41423247 variant of this gene is associated with a risk of high-altitude pulmonary edema, bronchial asthma, and influences the progression of lung function in cystic fibrosis [19–21]. Therefore, we hypothesized that the *SFTPB* (rs11130866) and *NR3C1* (rs41423247) gene variants might be associated with treatment features or adverse disease course in patients with COVID-19.

Table 4 – Effect of the studied gene variants on clinical parameters in patients with COVID-19.

Clinical parameters	Genetic variant						Results of statistical analyses
	SFTPB rs11130866			*NR3C1* rs41423247			
	CC	CT	TT	CC	CG	GG	
Temperature 1d, °C	37.6 ± 0.8	37.6 ± 0.8	37.8 ± 0.8	37.8 ± 0.9	37.5 ± 0.7	37.6 ± 0.8	$p > 0.05$
Temperature 2d, °C	37.3 ± 0.6	37.3 ± 0.6	37.2 ± 0.6	37.3 ± 0.5	37.2 ± 0.6	37.4 ± 0.7	$p > 0.05$
Temperature 6d, °C	36.8 ± 0.4	36.8 ± 0.5	37.0 ± 0.7	37.0 ± 0.5	36.8 ± 0.4	36.7 ± 0.5	$p > 0.05$
Respiratory rate 1d, per minute	23.8 ± 3.7	22.8 ± 1.8	23.0 ± 2.0	23.6 ± 3.5	23.3 ± 2.2	22.2 ± 2.2	$p > 0.05$
Respiratory rate 3d, per minute	23.4 ± 4.0	23.1 ± 2.0	22.3 ± 2.5	23.8 ± 3.9	22.9 ± 2.3	21.8 ± 1.5	$p > 0.05$
Respiratory rate 6d, per minute	23.5 ± 3.1	23.2 ± 2.8	22.8 ± 2.2	23.6 ± 2.7	22.8 ± 3.0	23.8 ± 2.3	$p > 0.05$
SpO_2/FiO_2 1d[a], %	183.2 ± 102.0	150.4 ± 57.2	173.07 ± 68.7	174.7 ± 81.2	159.2 ± 82.1	178.9 ± 71.3	$p > 0.05$
SpO_2/FiO_2 2d[a], %	217.2 ± 130.8	195.3 ± 106.5	210.1 ± 131.7	196.5 ± 105.0	201.1 ± 116.0	238.0 ± 155.6	$p > 0.05$
SpO_2/FiO_2 6d[a], %	235.4 ± 141.7	196.8 ± 123.5	225.3 ± 143.1	218.2 ± 119.5	219.1 ± 143.4	192.2 ± 139.8	$p > 0.05$
SOFA scale score 1d[a], in points	3 [2–3]	2 [2–2.5]	2 [2–3]	2 [2-2]	2 [2–3]	2.5 [2–3]	$p > 0.05$
SOFA scale score 2d[a], in points	3 [2–3]	2 [2–3]	2 [2–3]	2 [2–3]	2 [2–3]	2.5 [2–3]	$p > 0.05$
SOFA scale score 6d[a], in points	3 [2–3]	2 [2–3]	2 [2–2.5]	2 [2–3]	2 [2–3]	3 [2–3]	$p > 0.05$
Glasgow coma scale score 1d[a], in points	8 [7–8]	8 [7–9]	8 [7–9.5]	8 [7–8.3]	8 [6–9]	8 [7–8]	$p > 0.05$
Glasgow coma scale score 2d[a], in points	8 [6–8.3]	8 [7–9]	9 [6.5–10]	8 [7–8]	8 [5.5–10]	8 [6–9]	$p > 0.05$
Glasgow coma scale score 6d[a], in points	7.5 [5.3–8]	8 [7–9]	8.5 [5.8–13.5]	8 [7–8]	8 [4.3–9]	8 [4.5–8]	$p > 0.05$
Dose of glucocorticoids, mg/day	6.3 ± 1.9	6.2 ± 1.5	6.9 ± 1.0	6.3 ± 1.8	6.0 ± 0.6	8.0 ± 2.8	$p > 0.05$
Duration of glucocorticoid therapy, days	11.5 ± 6.7	11.0 ± 4.6	10.0 ± 5.7	13.8 ± 5.0	9.0 ± 5.0	11.5 ± 4.2	$p = 0.038$
Duration of ALV, days	7.4 ± 6.0	5.0 ± 4.9	3.3 ± 2.6	5.8 ± 5.9	5.1 ± 4.8	6.3 ± 5.9	$p > 0.05$
Duration of oxygen therapy using oxygen mask, days	6.1 ± 2.9	6.4 ± 4.0	5.2 ± 6.4	6.9 ± 4.1	5.4 ± 4.2	6.0 ± 4.0	$p > 0.05$
Total duration of oxygen support, days	5.3 ± 7.1	9.0 ± 6.0	4.1 ± 6.2	10.6 ± 8.4	5.4 ± 4.2	4.2 ± 5.1	$p = 0.013$

[a] Values are presented as the median [25th–75th percentile]; ALV—artificial lung ventilation 1d, 2d, 6d—day.

We found that for the *SFTPB* variant, there is a relationship between genotype and risk of ARDS (increased risk with the CC genotype) and total duration of respiratory support (shorter with the TT genotype) in patients with COVID-19. In modern scientific literature, there are not studies on *SFTPB* variants in patients with COVID-19. Meanwhile, To et al. showed that the CC genotype is an independent risk factor for severe influenza A (H1N1) infection [22]. Another study by Lin et al. also points out that the C allele may be viewed as a susceptibility factor for ARDS [18]. Moreover, interesting results were obtained by Ge et al.—they studied the functional susceptibility of transgenic mice to bacterial pneumonia and showed *in vivo* that the presence of the C allele is associated with a higher susceptibility to bacterial pneumonia than that of the T allele [23]. Therefore, our results are consistent with those of previous studies and indicate that the presence of the CC genotype is an aggravating factor in the risk of lung injury in patients with COVID-19. Moreover, SP-B concentration in the blood of premature infants depends on *SFTPB* variants, and in infants with the TT genotype the average content of surfactant protein B is significantly higher than in those with the CC genotype [24]. In addition, the results of exogenous pulmonary surfactant use in COVID-19 PCR-positive ARDS patients are very encouraging [25]. Although a recent meta-analysis suggests that exogenous surfactant administration in adults with respiratory distress syndrome does not reduce mortality and improve oxygenation rates, it is probably necessary to continue research based on genetic polymorphism [26]. Analyzing these data, we can assume that the use of exogenous pulmonary surfactant in patients with complicated COVID-19 is promising, and it is likely that patients with the *SFTPB* CC genotype will need higher doses of this drug.

The results of our study indicate that patients with the *NR3C1* CC genotype underwent a longer glucocorticoid therapy. At the same time, the length of hospitalization of these patients in the intensive care unit, among those who died from complications caused by COVID-19, was 24.4 ± 9.6 vs. 11.9 ± 10.8 days before lethal outcome, hence they lived longer. In literature, there are currently no studies on *NR3C1* gene variants that can cause pulmonary complications in

patients with COVID-19 or other viral diseases. A group of researchers showed an association of the "G" allele of the rs41423247 variant with increased risk of high-altitude pulmonary edema, the development of which may be associated with hypoxia-induced inflammatory cytokines at high altitude [19]. The GG genotype is also associated with more severe lung damage in patients with cystic fibrosis [21]. Regarding treatment, most scientists studying the effect of *NR3C1* gene variants on the effectiveness of glucocorticoid therapy in various diseases (i.e., pemphigus vulgaris, chronic obstructive pulmonary disease) did not find any significant association [27,28]. In contrast, a glucocorticoid susceptibility study in healthy volunteers revealed that the mean dose of cortisol-inhibiting dexamethasone was higher in homozygous G-allele carriers compared to heterozygous CG and homozygous CC carriers [29]. In severe inflammatory bowel disease, the GG genotype is associated with an increased response to glucocorticoid treatment [30]. These data may indicate that the *NR3C1* variant rs41423247 may have different effects, depending on the disease, tissue, and ethnicity. Because the rs41423247 variant is intronic, its effect on *NR3C1* activity may be indirect. Some authors suggest that this variant may affect the *NR3C1* promoter by selectively acting on either repressor or enhancer sites [21].

This study presents some limitations. We included only patients with severe COVID-19 because this study was conducted in an intensive care unit, so we could not compare with less severe forms of the disease. Despite these limitations, we report the relevance of *SFTPB* and *NR3C1* genetic variants in the severity of COVID-19. The conducted intragroup study among patients with severe COVID-19 pneumonia allowed to determine the influence of the investigated genes on the disease course and may help personalize a new approach to treatment in the future.

5. Conclusions

We conclude that the results confirm the hypothesis of the influence of the *SFTPB* (rs11130866) and *NR3C1* (rs41423247) gene variants on therapy, course, and severity of the disease in patients with COVID-19. Of course, these results require further study, analysis, and larger, complex, systematic research.

Ethical approval

Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki, as revised in 2013), and was approved by the Ethics Committee of the Ukrainian Medical Stomatological Academy (protocol No. 188 of November 25, 2020). Informed consent was obtained from all individuals included in this study.

Conflict of Interest

The authors declare no competing interests.

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