

Monika Paskudzka^{1,2}, Aleksandra Kumorek³, Aleksandra Kijak³, Marcin Śmiarowski³, Dagmara Kogut², Milena Matecka-Giełdowska^{1,2}, Olga Ciepiela^{1,2}

¹Department of Laboratory Medicine, Medical University of Warsaw, Warsaw, Poland

²Central Laboratory, Central Teaching Hospital of University Clinical Centre of Medical University of Warsaw, Warsaw, Poland

³Students Scientific Group of Laboratory Medicine, Medical University of Warsaw, Warsaw, Poland

Rh negativity seems to predispose to a milder COVID-19 course

Corresponding author:

Monika Paskudzka
Department of Laboratory Medicine
Medical University of Warsaw
1a Banacha St.,
02-097 Warsaw, Poland
e-mail: monika.paskudzka@wum.edu.pl

ABSTRACT

Introduction: Infection with the SARS-CoV-2 virus can lead to the development of COVID-19. Currently, more than 700 million people worldwide have been diagnosed with COVID-19, of which nearly 7 million have died from the severe course of the disease. Recent reports suggest that patients with blood group A are most at risk of developing COVID-19, and people with natural anti-A antibodies (especially those with blood type 0) have a milder course of the disease.

This study aimed to assess the humoral response to infection with SARS-CoV-2 depending on the patient's blood type.

Material and methods: The study group consisted of 147 patients with confirmed previous COVID-19 (convalescents) and 147 individuals who declared no previous infection with SARS-CoV-2. All enrolled subjects were blood donors registered at the Regional Blood Centre. The concentration of SARS-CoV-2 anti-nucleocapsid antibodies was determined in the serum of the patients using the Elecsys Anti-SARS-CoV-2 test. The blood group was determined by a manual method using anti-A, anti-B, and anti-D monoclonal sera and A, B, and 0 standard red blood cells (RBC).

Results and conclusions: Based on anti-SARS-CoV-2 detection 68 people who denied contact with SARS-CoV-2 had previous asymptomatic infection. Blood type distribution differed between the asymptomatic convalescents and the declared convalescents, $p = 0.0013$. People with ARh-, BRh+, BRh-, and 0Rh- blood type were more often asymptotically infected. Moreover, the Rh- subjects more often didn't know about the previous infection than those with Rh+, $p = 0.0012$. It seems that subjects with Rh- blood type have a significantly milder course of disease than Rh+.

Keywords: Blood group, COVID-19, antibodies, convalescents, Rh factor

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Introduction

COVID-19 caused by the SARS-CoV-2 virus is responsible for a worldwide pandemic, which broke out in 2019. According to official information since March 2020, over 6.5 million Polish people have been diagnosed with coronavirus infection and over 110 thousand died from the severe course of the disease [1]. Coronavirus infections are usually associated with upper respiratory tract infections, which present symptoms like fever, headache, and cough. However, some patients can develop lower respiratory tract infections including severe pneumonia and dyspnoea

[2]. SARS-CoV-2 infection may have different disease manifestations, from asymptomatic infection and influenza-like illness to severe complications including ARDS (acute respiratory distress syndrome) and death [3, 4]. During the pandemic, researchers tried to assess protective and risk factors for COVID-19 severity and mortality, which could help to prevent disease spreading and limit the number of patients with severe course of the disease. Based on current evidence, demographic factors like older age, male sex, and ethnicity/race (African-American and Hispanic populations) are related to a higher risk of SARS-CoV-2 infection. In addition, elevated expression of ACE2 (angiotensin converting

enzyme-2) and TMPRSS2 (transmembrane serine protease 2) as well as changes in several laboratory indices such as an increase in biochemical markers of inflammation (C-reactive protein, procalcitonine), pro-inflammatory cytokines, and white blood cell count were reported as risk factors for COVID-19 progression [5, 6]. On the other hand, observations made during the pandemic prove that children have decreased susceptibility to SARS-CoV-2 infection as well as people following a healthy, balanced diet rich in microelements and vitamins such as vitamin D [7, 8].

Recent reports suggest an association between ABO blood types and the risk of SARS-CoV-2 infection as well as the severity of the disease. According to the work of Ellinghauset et al. patients with blood type A are at a higher risk of developing COVID-19 than patients with other blood types [9]. Moreover, blood type O shows a protective effect as compared with the other blood groups. The protective effect of blood type O may result from the presence of anti-A antibodies, which can block interactions between the S protein located on the coronavirus surface and angiotensin-converting enzyme-2 (ACE2), the main receptor for the virus on human cells [10]. ABO blood group types have been associated with other infectious diseases such as tuberculosis, malaria, and cholera [11]. Conversely, the Rh blood type system plays an important role in haemolytic disease of the foetus and newborn (HDFN) but its association with a higher risk of developing any viral or bacterial infection needs deeper analysis [12].

This study aimed to assess if there is any association between blood type and COVID-19 course in Polish blood donors.

Material and methods

Study group

In the study, 294 subjects 18 to 65 years old, both men and women were enrolled. They were volunteer blood donors at Warsaw's Blood Centre recruited from August 2021 to April 2022. All subjects were tested for SARS-CoV-2 infection and received a negative PCR result on the day of admission. Among all enrolled individuals, 147 declared previous mild to moderate SARS-CoV-2 infection at least 6 months before blood donation (the minimal period required between the disease and blood donation, which was confirmed by a positive PCR test result). The mild disease was defined as a lack of symptoms of lower respiratory disease (shortness of breath dyspnoea and abnormal

chest imaging) and oxygen saturation measured by pulse oximetry (SpO_2) $\geq 94\%$. Moderate COVID-19 was defined as symptoms of lower respiratory disease with $\text{SpO}_2 \geq 94\%$. Another 147 individuals declared no previous SARS-CoV-2 infection. In the next step, all the subjects were tested for antibodies against the SARS-CoV-2 N (Elecsys® Anti-SARS-CoV-2, Roche) protein in their blood. The Elecsys® Anti-SARS-CoV-2 test is a double-antigen sandwich electrochemiluminescence immunoassay (ECLIA) for the *in vitro* qualitative detection of antibodies to SARS-CoV-2 nucleocapsid protein (NCP), which uses recombinant NCP. The test was performed using a Cobas e801 analyser (Roche). The cutoff value is automatically calculated by the analyser based on the measurement of the calibrator signal. Results are expressed as a cutoff index (COI; sample signal/cutoff). Assay results ≥ 1.0 COI were interpreted as positive and < 1.0 COI as negative. Based on the anti-N SARS-CoV-2 antibody evaluation, among the 147 subjects who declared no contact with SARS-CoV-2 and no signs of respiratory tract infection, 68 had a positive result for these antibodies and were eventually classified as asymptomatic convalescents. Thus, the study group (convalescents) consisted of 215 subjects, and the control group (non-infected) consisted of 79 subjects. All the participants were 18-65 years old; specific data including age and sex, as well as physical characteristics (body weight, blood pressure, etc.), due to full anonymity, were not provided. The study was approved by the Bioethical Committee of the Medical University of Warsaw (AKBE/136/2021, date of approval 6.09.2021). Informed consent was obtained from all subjects involved in the study when donating blood to the Regional Blood Centre.

Blood typing

All blood samples were anonymized before being included in the study. For that reason, additional basing blood typing was performed on the day of enrolment. From every individual blood was collected for clot and centrifuged for 15 minutes at 2500 g. RBCs were suspended in saline and serum was isolated for further studies. Monoclonal antibodies anti-A (Millipore, LOT:TLJ2002D), anti-B (Millipore, LOT: TNC2101A), and anti-D (Millipore, LOT: GGC1901C) were used for A, B, and RhD antigens evaluation on RBC. RBC of A, B, and O groups (from packed RBC stored at local blood bank) were used for antibodies anti-A and anti-B assessment in patients' serum. Each blood type (ABO) was identified based on the result of the antigens and antibodies assessment. Rh positivity was defined

as strong agglutination of patients' RBC with anti-D monoclonal antibody in a test tube reaction, and Rh negativity as no agglutination of patients' RBC with anti-D monoclonal antibody.

Statistical analysis

The statistical analysis was performed with GraphPad Prism 9 software. The Chi-square test or Fisher exact test were used for the statistical analysis of the results. The probability value of $p < 0.05$ was considered statistically significant.

Results

ABO groups among convalescents and non-infected subjects

Comparison of blood types in convalescents ($n = 215$) and non-infected subjects ($n = 79$) showed similar frequency of all blood types in study groups as in the general Polish population. Surprisingly, non-infected subjects were less frequent A type and more frequent B type than the general Polish population [13]. However, statistical analysis did not show significant differences between both groups ($p = 0.5735$).

ABO and Rh blood types among convalescents and non-infected subjects

Comparison of blood types in convalescents ($n = 215$) and non-infected subjects ($n = 79$) showed similar frequency in study groups as in the general Polish population. Surprisingly, non-infected subjects

were less frequently A Rh positive than the general Polish population and convalescents were more frequently O Rh negative than the general Polish population [13]. However, statistical analysis did not show significant differences between both groups ($p = 0.8985$) (Tab. 1, Fig. 1).

Rh factor among convalescents and non-infected subjects

Comparison of Rh factor in convalescents ($n = 215$) and non-infected subjects ($n = 79$) showed similar frequency between both groups ($p = 0.8734$) (Tab. 2).

ABO groups among declared convalescents and asymptomatic convalescents

Comparison of blood types in symptomatic (declared) ($n = 147$) and asymptomatic convalescents ($n = 68$) showed some variations compared to the general Polish population. Surprisingly, asymptomatic convalescent were less frequent AB type and more frequent A type than the general Polish population [13]. However, statistical analysis did not show significant differences between both groups ($p = 0.12$) (Tab. 3).

ABO and Rh blood types among declared convalescents and asymptomatic convalescents

Comparison of blood types in symptomatic (declared) convalescents ($n = 147$) and asymptomatic convalescent ($n = 68$) showed some interesting deviation from the frequency of all blood types in the general Polish population. Surprisingly, asymptomatic convalescents were more frequently A Rh negative

Table 1. Blood types among study (convalescents) and control (non-infected) groups compared to blood types in the general Polish population. Analysis was performed using the Chi-square test

Blood type	Convalescents (%)	Non-infected (%)	General Polish population*
A Rh+	68 (31.6%)	19 (24.1%)	32%
A Rh-	14 (6.5%)	6 (7.6%)	6%
B Rh+	29 (13.5%)	14 (17.7%)	15%
B Rh-	9 (4.2%)	5 (6.3%)	2%
O Rh+	61 (28.4%)	23 (29.1%)	31%
O Rh-	20 (9.3%)	6 (7.6%)	6%
AB Rh+	11 (5.1%)	5 (6.3%)	7%
AB Rh-	3 (1.4%)	1 (1.3%)	1%

* — data from the Regional Centre for Blood Donation in Warsaw

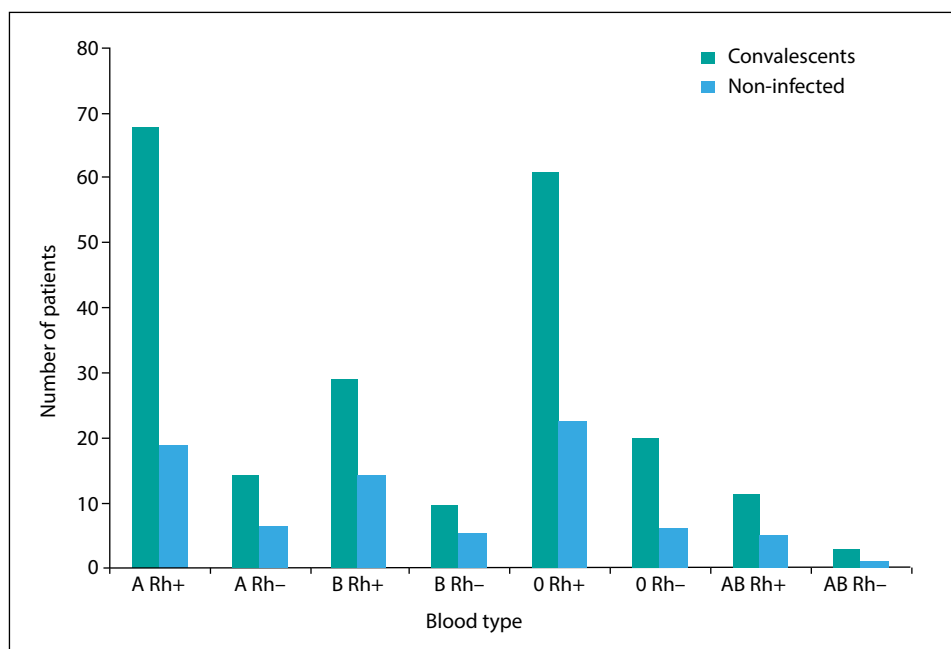


Figure 1. Blood types among study (convalescent) and control (non-infected) groups

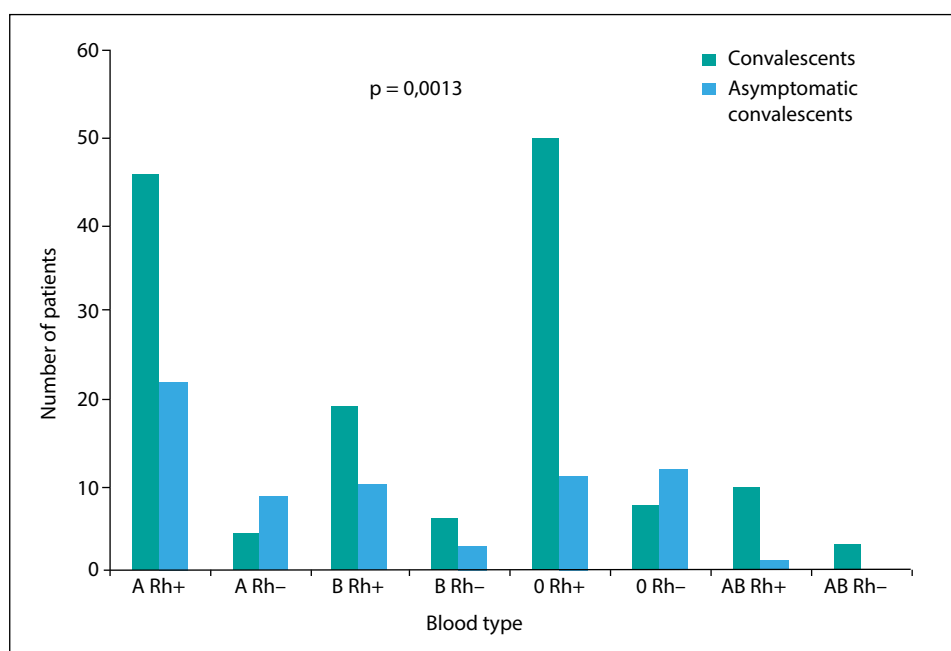


Figure 2. Blood types among symptomatic convalescent and asymptomatic convalescents

Table 2. Blood Rh factor among study (convalescent) and control (non-infected) groups compared to blood types in the general Polish population [13]

Rh factor	Convalescents	Non-infected	General Polish population*
Rh+	169 (78.6%)	61 (77.2%)	85
Rh-	46 (21.4%)	18 (22.8%)	15
Total	215	79	

*Data from the Regional Centre for Blood Donation in Warsaw. Analysis was performed using the Fisher exact test

Table 3. Blood types among symptomatic (declared) convalescents (n = 147) and asymptomatic convalescent (n = 68) groups compared to blood types in the general Polish population

Blood type	Convalescents	Asymptomatic convalescents	General Polish population*
A	51 (34.7%)	31 (45.6%)	38%
B	25 (17%)	13 (19.1%)	17%
AB	13 (8.8%)	1 (1.5%)	8%
O	58 (39.5)	23 (33.8%)	38%
Total	147	68	

*Data from the Regional Centre for Blood Donation in Warsaw. Analysis was performed using the Chi-square test

Table 4. Blood types among symptomatic convalescents and asymptomatic convalescents compared to blood types in the general Polish population

Blood type	Convalescents (%)	Asymptomatic convalescents	General Polish population*
A Rh+	46 (31.3%)	22 (32.4%)	32%
A Rh-	5 (3.4%)	9 (13.2%)	6%
B Rh+	19 (12.9%)	10 (14.7%)	15%
B Rh-	6 (4.1%)	3 (4.4%)	2%
O Rh+	50 (34%)	11 (16.2%)	31%
O Rh-	8 (5.5%)	12 (17.6%)	6%
AB Rh+	10 (6.8%)	1 (1.5%)	7%
AB Rh-	3 (2%)	0 (0%)	1%

*Data from the Regional Centre for Blood Donation in Warsaw. Analysis was performed using the Chi-square test

Table 5. Blood Rh factor among declared convalescents and asymptomatic convalescents groups compared to blood types in the general Polish population

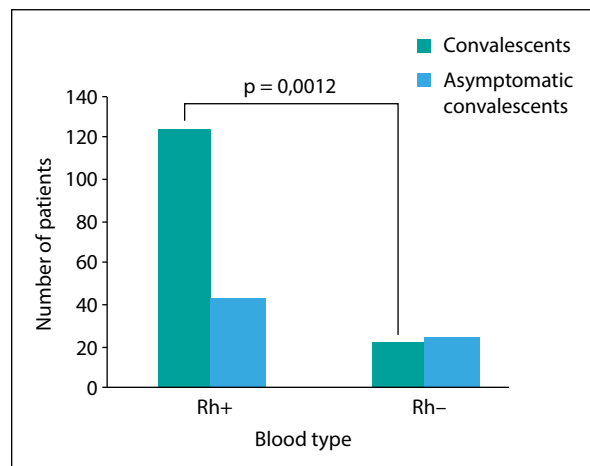
	Convalescents	Asymptomatic convalescents	General Polish population*
Rh+	125 (85%)	44 (64.7%)	85%
Rh-	22 (15%)	24 (35.3%)	15%
Total	147	68	

*Data from the Regional Centre for Blood Donation in Warsaw. Analysis was performed using the Fisher exact test

and O Rh negative and less frequently O Rh positive and AB Rh positive than the general Polish population [13]. Statistical analysis showed significant differences between both groups ($p = 0.0013$) (Tab. 4, Fig. 2).

Rh factor among declared convalescents and asymptomatic convalescents

Comparison of Rh factor in symptomatic convalescents (n = 147) and asymptomatic convalescents (n = 68) showed significant differences ($p = 0.0012$), with an odd ratio of 3.099 (95% confidence interval of 1.581 to 6.075) (Tab. 5, Fig. 3). Moreover, among asymptomatic convalescents frequency of Rh factor was substantially different from data of general Polish population [13], with more frequent Rh negativity.

**Figure 3.** Rh factor among symptomatic convalescent and asymptomatic convalescents

Discussion

This study aimed to assess if ABO and Rh D blood type are associated with a milder course of COVID-19 in the Polish population and found that individuals without Rh D factor are less susceptible to symptomatic course of SARS-CoV-2 infection than those Rh positive.

It is well-known that ABO and Rh groups show different frequencies among different populations and ethnic groups. In the Polish population, the most frequent blood group is A Rh+ (32%) and O Rh+ (31%) while AB Rh- group is the rarest (about 1%) [13]. Similar frequencies of blood types were observed in the included subjects (non-infected and convalescents), thus it can be stated that the study group well reflected the population.

Recent studies suggest that blood group A may be associated with a higher risk of developing COVID-19 and the severe course of the disease while the presence of anti-A antibodies (especially in people with O blood group) is considered a protective factor for SARS-CoV-2 virus infection. Zhao et al. [14] in their study from Wuhan and Shenzhen showed significantly higher risk for SARS-CoV-2 infection in patients with blood type A and lower risk in patients with blood type O. A study performed on 419 subjects with COVID-19 from Libya also showed, that patients with A-type blood more often had a severe course of the disease than patients with type O blood [15]. The meta-analysis performed by Balaouras et al. [16] and a study by Moslemi et al. [17] that included over 650 thousand blood donors also confirm this observation. Subjects included in the study were healthy blood donors, at least 6 months after confirmation of COVID-19 (if declared convalescent). Similar research was performed by Damiani et al. [18], who included blood donors positive for anti-SARS-CoV-2 IgG antibodies. In their study the seroprevalence was more frequent in A and AB groups, however, it has to be underlined that they did not divide the study group into symptomatic and asymptomatic convalescent and that in some of the COVID-19 convalescents the antibodies could be already at such a small titer, that could be undetectable. Direct comparison of studies from different world regions should be made very carefully, mainly because of different blood type distributions in different populations. In the Polish population, the predominant blood types are A and O. Statistical analysis did not show any difference between the rate of morbidity with COVID-19 between those two groups. There was also no difference in Rh factor between patients who were and were not infected. This could be explained by the relatively small study group (294 individuals included), however the size of the study group did not significantly

deviate from Nigerian [19] or Brazilian [20] studies. The present results, however, are in line with the Bhandari et al. study, which did not show any association between ABO/Rh blood groups and COVID-19 mortality and susceptibility. In addition, Bhandari et al. [21] work takes into consideration other confounding factors such as age and sex. Similar results were provided by Latz et al. [22], who showed no association between blood groups as an independent risk factor for COVID-19 severity. Moreover, subjects with blood group A were not at higher risk of testing positive for COVID-19, but B type and AB were.

Despite not showing any significant association between ABO and Rh blood types and COVID-19 susceptibility, some interesting findings were received when comparing blood type frequency in symptomatic and asymptomatic subjects. The first stage of the following study included the participants based on their declaration of previous disease. This declaration is based on SARS-CoV-2 RT-PCR positive results or lack of any symptoms of the disease and no positive viral test result. The second stage was to assess the presence of anti-N SARS-CoV-2 antibodies in all individuals sera, to confirm the lack of infection in a group of declared non-infected subjects. This analysis excluded 68 individuals (among 147) who tested positive for these antibodies from the "non-infected" group. Since the patients had not reported any symptoms of infection, they were classified as asymptomatic convalescents. This study found that asymptomatic convalescents were more frequently A Rh negative and O Rh negative and less frequently O Rh positive and AB Rh positive than the general Polish population. These observations do not confirm blood type A to be a risk factor for severe course of COVID-19 or the protective role of anti-A antibodies in SARS-CoV-2 infection. Nevertheless, when considering the Rh factor, it turned out that those subjects with A Rh negative and O Rh negative blood types significantly more often were asymptomatic than symptomatic COVID-19 convalescents. Studying the frequency further of Rh blood type in symptomatic and asymptomatic convalescents, statistically significant differences between Rh positive and Rh negative patients in included convalescents were discovered. These results show that asymptomatic convalescents were more likely Rh negative than symptomatic patients, which may suggest Rh negativity is associated with a milder course of COVID-19 disease. Similarly, in a large study from Ontario, Canada researchers showed that Rh-negative patients had a lower risk of severe COVID-19 disease or death, which supports the present findings [23]. On the contrary, other researchers did not find any association between the Rh factor and COVID-19 morbidity

[16, 24, 25], but they included only those positive for COVID-19 and healthy individuals, without extracting the group of asymptomatic subjects. The present results find some confirmation in a report presented by Khder Mustafa et al. [26] who showed that those individuals of Rh-negative blood type are at lower risk of COVID-19, but still, they included only subjects with symptomatic infection tested positive for SARS-CoV-2 presence. Thus, the present observation is highly pioneering.

At this place, the question must be asked, how does lack of D antigen from the Rh blood type system play a protective role against symptomatic SARS-CoV-2 infection? A study from Jahrsdoerfer et al. [27] sheds some light on this possible phenomenon. They found that Rh-negative convalescents have significantly lower titers of SARS-CoV-2 neutralizing antibodies. It may be explained in two ways: Rh-negative subjects produce fewer antibodies against the virus or a lower titer of antibodies is necessary to limit the infection in Rh-D-negative individuals. Rh D factor was previously reported as one, that may have an important role in susceptibility to viral infections. Rh negativity was found as a possible protective feature against parvovirus B19 [28], and one of the theories explaining this phenomenon is that the Rh D antigen is associated with the binding of the virus to the red blood cell surface. Sugrue et al. [29] found that Rh-negative males respond strongly to influenza A virus by activating IFN- γ pathway genes and suggest that this mechanism may be responsible for lower susceptibility to viral infections, including SARS-CoV-2. This finding seems to be the most convincing, however further studies will be necessary to confirm this thesis with regard to SARS-CoV-2 infection.

Conclusions

In conclusion, the study did not confirm the protective role of blood group 0 in SARS-CoV-2 infection or COVID-19 severity as well as a higher risk of infection in patients with blood group A in the Polish population. However, it did show that patients with Rh-negative blood type may present a milder course of COVID-19 disease. Despite convincing results, some limitations of this study can be identified. Firstly, the size of the sample is relatively small. Secondly, the study group consisted of volunteer blood donors at Warsaw's Blood Centre and no additional information about age or gender was provided. However, the authors still believe that the presented results provide new input in a worldwide discussion regarding factors that made people more or less susceptible to SARS-CoV-2 infection.

Article information

Data availability statement: *The data presented in this study are available on request from the corresponding author.*

Ethics statement: *The study was approved by the Bioethical Committee of the Medical University of Warsaw (protocol code AKBE/136/2021, date of approval 6.09.2021).*

Author contributions: *Conceptualisation — O.C.; methodology — M.P., M.M.-G., D.K., O.C.; software — M.P., O.C.; validation — M.P., M.M.-G., O.C.; formal analysis — M.P., A.K., A.K., M.Ś., D.K., M.M.-G.; investigation — M.P., A.K., A.K., M.Ś., D.K., M.M.-G., O.C.; resources — M.P., M.M.-G., D.K., O.C.; data curation — M.P., O.C.; writing — original draft preparation — M.P., O.C.; writing — review and editing — A.K., A.K., M.Ś., D.K., M.M.-G.; visualization — O.C.; supervision — O.C.; project administration — M.M.-G., O.C.; funding acquisition — O.C. All authors have read and agreed to the published version of the manuscript.*

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Conflict of interest: *None.*

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