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Distribution, Severity and Mortality of COVID-19 in Relation to Blood Group and Rh Factor

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Abstract

BACKGROUND: During the Covid-19 pandemic, a number of investigators reported that in patients with different blood groups the virus had a disparate influence regarding the severity and the outcome of the infection.

AIM: The goal of our study was to determine if there is an association between blood groups and Rh factor regarding the severity and mortality from COVID-19 in our analyzed patient population.

METHODS: We conducted a retrospective observational study of 250 patients with COVID-19 disease, treated as ambulatory or hospitalized patients in several health facilities during a time period of one year, from November 2020 till November 2021.

RESULTS: The majority of patients in our cohort were confirmed to have blood group A - 115 (46%), followed by blood group O - 67 (26.8%) patients, 45 (18%) patients were with blood group B, and 23 (9.2%) patients were with blood group AB. The data generated from our analysis revealed a higher than standard prevalence of SARS-CoV-2 infections among individuals with blood group A. Also, the analysis shows that patients with blood group A experience a more severe clinical course of the disease, as well as higher mortality, compared to patients with other blood groups. There is a substantial difference between Rh positive and Rh negative patients in terms of the severity of the disease. Rh positive patients more frequently experience a severe clinical course, confirmed by statistical significance, but the Rh factor does not affect the outcome of the infection.

CONCLUSIONS: The results from our analysis showed that patients with blood group A manifest a more severe clinical course of the disease and a higher mortality rate compared to patients with other blood groups, but statistical significance between them could not be documented.

Introduction

Corona virus disease (COVID-19) represents a public health risk of global proportions that causes substantial morbidity and a considerable burden of mortality that has overwhelmed health systems worldwide [1]. It has been recognized that SARS-CoV-2 virus attacks the organs and tissues of the human body through the ACE receptors located on the cell surface [2]. While a large proportion of infected patients express no symptoms, some patients experience moderate symptoms and in some patients the disease may progress to pneumonia, hypoxemia, severe respiratory distress syndrome and multiple organ failure, potentially leading to death. No physiological biomarker can accurately predict COVID-19 susceptibility, but ABO blood group has been reported to be correlated to the propensity of the SARS-CoV-2 infection.

It is thought that blood group antigens play a direct role in the infection through various mechanisms. On a molecular level, they can serve as receptors and co-receptors for pathogens and can also facilitate intracellular uptake of viral particles. Clinically, blood

groups have been linked to bacterial, parasitic, viral infections and various non-infectious diseases [3], [4], [5], [6], [7], [8].

The blood groups are defined by the presence of specific carbohydrate sugars on the surface of blood cells. The ABO antigens were later found in several tissues and secretions, such as saliva, intestinal mucosa and the respiratory tract epithelium. It is assumed that the influence of ABO blood groups regarding the susceptibility towards and the severity of COVID-19 infections are regulated by mechanisms of interaction between ABH antigens and sialoside glycans present on the host cell receptors [9].

During the most immense peaks of the pandemic, genotype analyses were performed in large patient populations with severe SARS-Cov-2 infections, revealing specific genomic loci coinciding with the ABO group locus. Statistical analyses confirmed existence of a higher risk of infections in individuals of the A blood group, as well as a protective role of the O blood group [10].

Meta-analyses were also performed, aimed at establishing associations between ABO phenotypes and various mechanisms of organ and system failures caused by the SARS-Cov-2 infection. Demographics were considered as possible means of preventing outbreaks within environments containing populations with different ratio coefficients regarding the blood groups [11].

The issue of different ratios and frequencies of blood groups in different populations, especially in different geographical locations, has been analyzed and observations have been associated with variations in overall susceptibility of a nation, region, or even continent towards certain types of infections, sometimes even diseases in general [12]. Therefore, when attempting to establish a specific link between a medical observation and an individuals' blood group, a certain degree, level of a demographic and population statistic overview is warranted.

The reports and derived conclusions regarding the role of ABO blood groups in the pathogenesis of COVID-19 infections are still controversial. Initial studies have observed no association between ABO blood groups and SARS-CoV-2 infection [13]. However, a considerable number of later studies showed that ABO polymorphism was associated with susceptibility to SARS-Cov-2 infection, indicating that blood type A and Rh positivity were risk factors regarding the severity of COVID-19 infection as well as the consecutive rate of mortality [14], [15].

Since the issues are not defined on a general level, our study was aimed at determining if there is an association between blood groups and the Rh factor in our patient population, when analyzed against the prevalence, as well as the severity and clinical outcomes of COVID-19 infections. The establishment of a correlation between blood types and the course of COVID-19 infections would be our contribution to the challenging problem and within a global assessment framework could lead to the development of clinically applicable strategies, guiding disease management and treatment in eventual future outbreaks.

Methods

We conducted a retrospective observational study of 250 patients with COVID-19 disease treated as outpatients or as hospitalized cases in several health facilities in the Republic of North Macedonia: Health Center Makedonski Brod, General Hospital Prilep, Health Center Shtip, General Hospital Kocani and the modular hospital at the University Clinic for infectious diseases and febrile conditions in Skopje, during a time period of one year, from November 2020 till November 2021. All patients had positive SARS-CoV-2 RT-PCR swabs and were older than 18 years. For all patients included in the study we collected data related to demographic characteristics, date of SARS-CoV-2 positive test, ABO blood group and Rh type, as well as medical data characterizing the severity of the COVID-19 infection.

We classified the patients according to the CDC classification of disease severity into three groups: 1. mild illness (fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell), 2. moderate illness (patients with evidence of lower respiratory disease during clinical assessment or imaging, with SpO_2 ≥94% on room air at sea level), treated as outpatients and 3. severe illness (patients with SpO_2 <94% on room air at sea level, PaO_2/FiO_2 <300 mm Hg, a respiratory rate >30 breaths/min, or lung infiltrates >50%) which required hospitalization. None of the patients included in the study were previously vaccinated.

ABO blood group and Rh type were determined either at initial clinical evaluation or were extracted from the system of electronic data for the respective patient. ABO blood groups and Rh types were determined at the Institute for transfusion medicine of the Republic of North Macedonia.

The frequencies of the blood groups among our patients are subject of the analysis in various comparator modalities, matched against the population standard. The frequency of the ABO phenotype in the Republic of North Macedonia is as follows: O blood group 33%, A 41%, B 18% and AB 8% of the population, as reported and documented by the national statistics in 2021 [16].

Statistical analysis of the data was performed using the statistical program SPSS 23.0. Categorical (attributive) variables are shown with absolute and relative values. The non-parametric Chi-square test was used to compare the categorical variables. $P<0.05$ was considered statistically significant. The data are presented in tables and graphics.

Results

Patient characteristics

Among the 250 subjects included in the study, 115 were male and 135 were female. The average patients' age was 51.3 years and 143 of them were older than 50 years (Table 1).

Table 1: Demographic characteristics of the patients

Parameter	n (%)
Gender	
female	115 (46)
male	135 (54)
Age group	
<49	107 (42.8)
≥50	143 (57.2)
Age/years (mean ± SD)	51.3 ± 16.4

Blood group distribution among patients

The majority of our patients were identified as having blood group A – 115, followed by blood group O - 67 patients, 45 patients were with blood group B, and 23 patients had blood group AB. (Figure 1) Compared to the standard distribution of blood groups in the general population, the analysis shows a misbalance, which is mainly due to the contrasted presence of individuals with blood group A (higher) and O (lower).

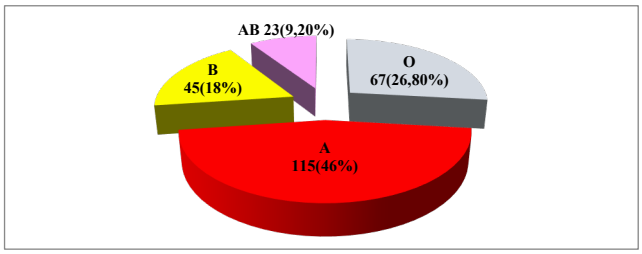

Figure 1: Proportion of patients according to blood group

The blood groups were proportionally distributed across different gender and age groups (p>0.05).

Among the patients that acquired a COVID infection, those having blood group B maintained the proportion size comparable to the general population. Patients with blood group O were represented in a smaller proportion than in the general population, delineating a possible lower susceptibility to the viral agent.

Table 2: The blood group of the patients in relation to gender and age

Variable		Blood group					p-value
		n	O n (%)	A n (%)	B n (%)	AB n (%)	
gender	female	115	29 (25.22)	55 (47.83)	20 (17.39)	11 (9.56)	X^2=0.4
	male	135	38 (28.15)	60 (44.44)	25 (18.52)	12 (11.1)	p=0.9
Age group	<49	107	29 (27.1)	46 (42.99)	20 (18.69)	12 (11.21)	X^2=1.2
	≥50	143	38 (26.57)	69 (48.25)	25 (17.48)	11 (7.69)	p=0.74
Standard distribution (national data)			33%	41%	18%	8%	

X^2, (Chi-square test).

In accordance with the reported higher susceptibility regarding patients with blood group A, our patients were represented with a margin from 3-7% higher than the ordinary, and those with blood group AB were within a range of 1.5-3% above the standard (Table 2). The most noticeable proportion increase is among the elderly patients with blood group A.

The blood group of the patients in relation to the clinical course severity and outcome

There is an easily detectable trend of increase in the severity of the disease in the patient population having blood group A, and partially (moderate severity) for patients with group AB. For patients with blood group O and B there is a stable or clearly declining trend regarding severity. This undoubtedly confirms the greater gravity of the consequences of the COVID infection among patients bearing the A blood group antigen. The proportion of fatal outcomes also confirms this connection, since patients with blood group A constitute for more than half of the fatalities, while patients with the other blood groups retain a positive or stable ratio between surviving and deceased patients (Table 3).

Table 3: The blood group of the patients in relation to the clinical course severity and mortality

Variable		Blood group					p-value
		n	O n (%)	A n (%)	B n (%)	AB n (%)	
Clinical course	Mild	103	32 (31.07)	42 (40.78)	20 (19.42)	9 (8.73)	X^2=4.2
	Moderate	43	9 (20.93)	20 (46.51)	8 (18.6)	6 (13.95)	p=0.65
	Severe	104	26 (25)	53 (50.96)	17 (16.35)	8 (7.69)	
Outcome	Survived	223	61 (27.35)	100 (44.84)	40 (17.94)	22 (9.87)	X^2=1.8
	Deceased	27	6 (22.22)	15 (55.56)	5 (18.52)	1 (3.7)	p=0.61

X^2, (Chi-square test).

The Rh factor in patients in relation to the disease severity and mortality

Patients with positive and negative Rh factor differed significantly in terms of the severity of the clinical course of the disease, as well as regarding the outcome (p=0.0021). Almost all patients with a severe disease course were with a positive Rh-factor. As expected, all of the fatal outcomes are noted in the Rh-positive patient population, while among Rh-negative patients no fatalities were observed (Table 4).

Table 4: The Rh factor in patients in relation to the disease severity and mortality

Variables		Rh factor				p-value
		n	positive n (%)		negative n (%)	
Clinical picture	Mild	103	91 (88.35)		12 (11.65)	X^2=12.3
	Moderate	43	35 (81.4)		8 (18.6)	**p=0.0021
	Severe	104	102 (98.08)		2 (1.92)	
Outcome	Survived	223	201 (90.13)		22 (9.87)	X^2=2.9
	Deceased	27	27 (100)		0	p=0.087

X^2, (Chi-square test).

Discussion

There are many studies which have investigated the connection between COVID-19 and blood types, and they all convey a variable level of differences in their assessments [17], [18]. Almost 20

years ago, Cheng and associates have noted the association between blood groups and susceptibility to SARS-CoV-1 infections, stating that individuals of blood type O are at lower risk of acquiring the disease [19].

Further trials revealed a protective effect of anti-A antibodies against intracellular uptake of SARS-CoV-1 [20].

These observations have led researchers to investigate whether an association exists between ABO polymorphism and susceptibility to SARS-CoV-2 infection.

In our study the majority of COVID-19 patients were with blood group A. They developed a more severe clinical course of the disease, and the mortality was higher in this blood type, compared to other types, as well as to the standard blood group distribution. In the study of Zhao and associates there was a similar observation as in our cohort of patients. Namely, their study showed that the majority of COVID-19 patients had blood type A and the highest affinity for bonding with the SARS-CoV-2 virus, contrary to patients with blood type O who had the lowest bonding affinity [14]. As reported before, the highest prevalence of a severe clinical disease course in our study is noted among individuals with blood group A. This blood type is also accountable for the highest proportion of fatal outcomes. On the other hand, individuals with blood type O and B (to some extent) bear a lower risk burden for developing a severe disease course, as well as for progressing to a fatal outcome, while those with the AB blood type are with a relatively "standard" prevalence, which substantially corresponds to the findings of Matzhold and associates [21].

Among relevant medical literature exploring and evaluating different databases, most reports confirm a significantly increased risk of infection in individuals with blood group A and a low risk in those with blood group O [18], [22], [23].

There are many hypotheses which can explain our findings and the most constant among them state that the presence or absence of any antigen of the ABO system is responsible for a different degree of susceptibility, also confirming that comorbidities are substantially more frequent in patients with antigen A (A blood group), while the absence of antigens (O blood group) is associated with a lower thrombotic and cardiovascular risk [24], [25].

Initial attempts to explain the observations connecting blood groups with the intensity of SARS-CoV-2 infections, were unable to document a direct molecular relationship between the ABO system antigens and the virus that could explain the true mechanism responsible for the susceptibility of individuals with different blood groups. Natural anti-A and anti-B antibodies from the ABO system were labeled as capable of interfering with the S protein of the SARS-CoV-2 virus and with the angiotensin-converting enzyme 2 on the host cell receptors. The presence of high plasma concentrations of antibodies in the O blood group was presumed to confer greater protection to these patients [26].

Many similar reports to our study, although documenting discrepancies regarding the severity of the disease and the outcome of the infection analyzed against different blood types and Rh-factors, did not succeed in establishing a statistical significance regarding the clinical outcomes of COVID-19 infections and the different ABO blood groups [27[, [28], [29].

More recently, Wu et al. documented that structural and sequence analogies exist within the receptor-binding domain of the surface spike protein of the SARS-CoV-2 virus and a certain carbohydrate-binding protein family called galectins, which accounts for the similarities in glycan-binding preferences. Galectins manifest a considerable affinity towards ABO(H) antigens and engage them. In particular, a specific one, labeled galectin-4C, expresses affinity towards blood group A structures found also on respiratory epithelial cells. On the contrary, no affinity was documented against the H antigen, present in blood group O, therefore concluding that a greater susceptibility exists for blood group A cells, than O cells, to be infected with the SARS-CoV-2 virus, and providing a molecular background for that observation [30]. Although on different levels, the study confirmed that the affinity persists among different strains of the virus.

In the study of Young and associates it was shown that Rh negative patients have a lower risk for acquiring COVID infection, but the Rh factor had no influence on the outcome of the disease. Our study showed similar findings, namely that the Rh positive patients in our cohort presented with a more severe clinical course compared to Rh negative patients, but this finding did not achieve statistical significance regarding the disease outcomes [17]. Our analysis, similar to the studies of Ray et al. and Zietz et al., found that subjects with Rh-negative blood type were at lower risk of viral infection, severe illness course, and mortality following a SARS CoV-2 infection [31], [32], [33]. Nevertheless, the results of these studies were affected by some limitations, such as selection bias for inpatient hospital population due to limited testing and moderate sample size. Greater sample sizes and meta-analyses are required in order to evaluate and elaborate the essence of these observations with more substantial precision.

Conclusions

The results from our analysis showed that patients with blood group A manifest a more severe clinical course of the disease and a higher mortality rate compared to patients with other blood groups, but statistical significance between them could not be documented. There is a substantial difference between

Rh positive and Rh negative patients in terms of the severity of the disease. Rh positive patients often have a more severe clinical course, which is corroborated by statistical significance, but the Rh factor does not affect the outcome of the infection. Many studies report that blood type A might predispose to increased susceptibility of infection with SARS-CoV-2, and type O and Rh-negative blood groups might be protective. Although this appears to be an emerging trend, the impact of blood type on clinical outcomes remains ambiguous, since outcomes also largely depend on the capacities of healthcare systems. The possible limitations of our study were its observational approach and the relatively small number of patients. The size of the cohort is the sole reason for not being able to verify that the correlations we investigated do actually bear statistical significance, since analyses performed on small sample sizes require a greater margin of difference in order to conclusively and beyond doubt prove causality. This becomes most obvious in the segment of disease outcomes among Rh-positive and negative patients, where the numerical difference is absolute, but statistical assessment cannot confirm significance. Nevertheless, we do believe that the numerical observations are very obvious and consistent, justifying our conclusions and incentives. Further studies and more data, assessed on sizeable larger and representative population samples, are required in order to provide a generally applicable and definite proof to the thesis that there is a genuine connection between the ABO and Rh blood type on one side, and the susceptibility, severity and clinical outcome of the disease in patients with a COVID-19 infection on the other. Should such a correlation be confirmed with undisputable statistical significance, the observations should be used in shaping the system of preventive measures, as well as in guiding therapeutic procedures during possible future epidemic threats, with evolved strains of the same virus or with entirely new pathogens, if a similar relationship is investigated and eventually observed.

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