Original article

Prevalence of ABO and RhD blood group phenotypes in the Croatian population and in patients with severe COVID-19 in Croatia

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Background - The aim of this study was to determine the distribution of ABO and RhD blood group phenotypes in the general population in the Republic of Croatia and among hospitalized patients with severe COVID-19.

Materials and methods - Data on ABO and RhD blood groups of all blood donors in Croatia (who donated blood during the period 2015-2020) and patients and pregnant women tested at the Croatian Institute of Transfusion Medicine during the 2-year period, 2019-2020, were obtained from the e-Delphyn blood bank information system. A total of 614,673 results were analyzed in this group. The other group consisted of 780 COVID-19 patients hospitalized with severe COVID-19. Data are presented as total number and percentages and a comparison of proportions test was performed.

Results - The most frequent ABO phenotype in the general population is A (38%), followed by O (37%), B (18%) and AB (7%). RhD positive individuals accounted for 81% of the general population and RhD negative for the other 19%. Among COVID-19 patients, phenotype A was the most frequent (42%), followed by phenotypes O (32%), B (17%) and AB (9%). Thus blood group A was significantly more common among COVID-19 patients than among the general population, whereas blood group O was significantly less frequent.

Discussion - This study provides the first official results of the distribution of ABO and RhD blood group phenotypes in the general population in Croatia. Moreover, this study confirms other researchers' observations about the predominance of the A blood group phenotype among COVID-19 patients.

Keywords: *ABO phenotype, RhD phenotype, COVID-19.*

INTRODUCTION

In addition to the role which ABO and Rh blood group systems play in transfusion therapy and transplantation, knowing the distribution of their antigens is of great importance in population studies and for estimates of migration effects, but also for the blood banks' planning of blood component supply[1,2].

It is a well-known fact that people with certain blood groups are more susceptible than others to certain diseases. Thus, there is a correlation between the ABO system and infectious diseases, and an increased susceptibility to individual bacteria,

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viruses and parasites for certain blood group antigens acting as receptors has been noticed[3]. Furthermore, the association between the ABO system and the frequency and/or severity of certain infectious diseases can also be affected by the presence of isohemagglutinins, which develop as a result of an early immune response to glycan structures from the environment, which have the same structure as various micro-organisms[4].

This paper reports the findings of a cross-sectional study of the ABO and RhD blood group systems in the Croatian population, the first for more than 40 years. In the meantime, our country underwent many migratory flows during the war and the post-war period, as well as recently, due to the entry of the Republic of Croatia in the European Union.

The aim of this study was to determine the distribution of the four main phenotypes of the ABO system (A, B, O and AB) and two RhD systems (positive/negative) in the Croatian population.

The second aim was to compare the distribution of individual ABO system phenotypes in the general population with the distribution of phenotypes in a group of patients who had severe COVID-19.

MATERIALS AND METHODS

The analysis was carried out on data recorded in e-Delphyn, the national information system designed for transfusion medicine. The group analyzed consisted of blood donors from the whole of Croatia, as well as patients and pregnant women who came to the Croatian Institute of Transfusion Medicine (CITM) for blood group determination as part of pre-operative treatment or during pregnancy. The data analyzed came from all individuals who donated blood in Croatia between January 1, 2015 and December 31, 2020 (all 568,559 donors), as well as all patients and pregnant women who had their ABO and RhD type determined in 2019 and 2020 (n=46,114). The Croatian transfusion service collects annually, on average, 192,000 units of blood and blood components from around 90,000 individuals, and the CITM carries out, on average, around 30,000 blood grouping of patients and pregnant women. This means that some subjects included in the study were tested repeatedly during the study period (especially in the blood donor group), but not more than once a year. The distribution of ABO blood groups among blood donors throughout the period analyzed was more or less constant and shown in **Figure 1**.

The decision to include patients and pregnant women in this study was aimed at avoiding possible erroneous conclusions, given that occasional shortages of blood components result in invitations to donors of certain blood groups (especially RhD negative ones) to donate blood.

The other group analyzed consisted of 780 patients with severe coronarvirus disease 2019 (COVID-19) who were treated in the "Dubrava" University Hospital and the "Dr. Fran Mihaljević" University Hospital for Infectious Diseases in the period between March 30, 2020 and February 1, 2021. This group was analyzed so as to assess the distribution of blood groups in the population of COVID-19 patients.

Data are presented as total number and percentages. A comparison of proportions test based on the χ-squared test was performed. A p value <0.05 was considered statistically significant. The statistical analysis was performed using MedCalc version 19.7.4.

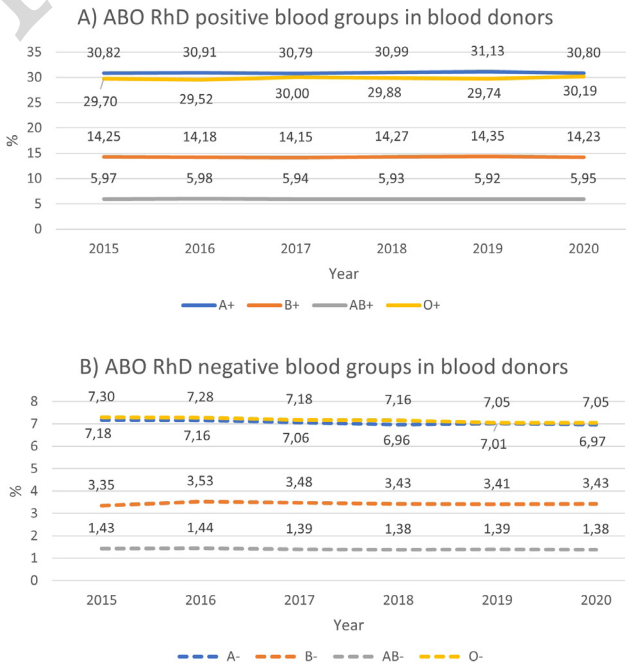


Figure 1 - Distribution of ABO and RhD blood groups in blood donors in Croatia from 2015 to 2020
(A) ABO RhD positive blood groups; (B) ABO RhD negative blood groups.

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RESULTS

According to the results obtained, the most frequent ABO phenotype in the Croatian population is blood group A (38.04%), followed by O (36.85%) and B (17.77%), while AB is the least frequent (7.34%). An overall analysis of the RhD system showed 497,682 (80.97 %) RhD positive individuals and 116,991 (19.03%) RhD negative ones.

In absolute numbers (373,173), blood types A positive and O positive account for more than 60% (60.7%) of the subjects, while subjects with the AB negative blood type are the rarest (1.4%). The comparison of the percentage of individual ABO blood types among the group of blood donors and the group of patients and pregnant women showed a significantly higher proportion of blood type O (both RhD positive and negative) in blood donors and higher proportions of blood types A positive, B positive and B negative in patients and pregnant women. The greatest difference in distribution was within the O negative blood group (**Table I**). The analysis of the total proportion of RhD negative phenotype in both groups showed a higher percentage (19.07%) among blood donors than among patients and pregnant women (18.53%).

The ABO blood group phenotypes of patients with severe COVID-19 were analyzed. Among the total of 780 subjects with COVID-19, the distribution of ABO blood group phenotypes was very similar in two hospitals that treated patients with severe COVID-19, and was, on average: A - 43 %, O - 31 %, B - 17 % and AB - 9 % (**Table II**).

When the distribution of ABO blood group phenotypes in COVID-19 patients from both hospitals was compared with the distribution in the general population (blood donors, pregnant women and patients) in the Republic of Croatia, the result was a statistically significantly higher frequency of the A blood group phenotype in COVID-19 patients (χ^2=6.752, p=0.009) and a significantly lower frequency of the O blood group phenotype in COVID-19 patients compared to the general population (χ^2=9.44, p=0.002) (**Table III**).

Table I - *Distribution of ABO phenotypes in the general population*

ABO and RhD blood group	Blood donors		Patients and pregnant women		Comparison of proportions test		Total	%	ABO phenotype	%
	N.	%	N.	%	p	χ^2				
A+	175,721	30.91	14,672	31.82	<0.001	16.52	190,393	30.98	A 233,793	38.04
A–	40,137	7.06	3,263	7.08	0.872	0.026	43,400	7.06		
O+	169,621	29.83	13,159	28.54	<0.001	33.974	182,780	29.74	O 226,500	36.85
O–	40,780	7.17	2,940	6.38	<0.001	40.303	43,720	7.11		
B+	80,950	14.24	7,030	15.24	<0.001	34.775	87,980	14.31	B 109,234	17.77
B–	19,555	3.44	1,699	3.68	0.007	7.359	21,254	3.46		
AB+	33,822	5.95	2,707	5.87	0.485	0.488	36,529	5.94	AB 45,146	7.34
AB–	7,973	1.40	644	1.40	0.86	0.031	8,617	1.40		
Total	568,559	100	46,114	100			614,673	100	614,673	100

Table II - *Distribution of ABO blood group phenotypes in two hospitals that treated only COVID-19 patients*

ABO blood group phenotype	"Dubrava" University Hospital		"Dr. Fran Mihaljević" University Hospital for Infectious Diseases		Total	
	N.	%	N.	%	N.	%
A	173	42.51	159	42.63	332	42.56
B	70	17.20	64	17.16	134	17.18
O	125	30.71	121	32.44	246	31.54
AB	39	9.58	29	7.77	68	8.72
Total	407	100	373	100	780	100

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Table III - *Comparison of proportions test between distribution of blood group phenotypes in the general population and hospitalized COVID-19 patients*

ABO blood group phenotype	General population*		COVID-19†		χ^2	p	95% CI
	N.	%	N.	%			
A	233,793	38.04	332	42.56	6.752	0.009	1.09-8.02 %
B	109,234	17.77	134	17.18	0.186	0.667	−2.22-3.08 %
O	226,500	36.85	246	31.54	9.44	0.002	1.96-8.48 %
AB	45,146	7.34	68	8.72	2.18	0.14	−0.40-3.57 %
Total	614,673	100	780	100			

*Voluntary blood donors in Croatia, patients and pregnant women who had their blood type determined in the Croatian Institute for Transfusion Medicine in the period 2015-2020. †Patients treated for severe COVID-19 in the "Dubrava" University Hospital and the University Hospital for Infectious Diseases. 95% CI: 95% confidence interval.

DISCUSSION

Blood group is determined by genes inherited from an indivual's parents. Thousands of years of evolution have led to the division of the ABO system, which is clinically the most important blood group system, into four basic groups: A, B, O and AB. The distribution of individual blood groups is not the same in all parts of the world. It varies according to the population, but there are also variants within certain subpopulations. For example, blood groups A and O are distributed almost equally in Europe, while blood group B is much less frequent. Among the Native American population, as many as 88% of them have blood group O, only 12% of them have blood group A, and blood groups B and AB are not present at all[1]. The distribution of blood group frequencies throughout the population of the United Kingdom shows correlations wtih historical population migrations and invasions, thus enabling the movements of the Vikings, Danes, Anglo-Saxons, Norsemen and Celts to be tracked. Human migrations throughout history have resulted in the prevalence of certain blood groups in certain geographical areas and continents. For example, blood group B is less frequent in Europe and America than blood groups A and O, while it is much more frequent among the Asian population. Theories of evolutionary biologists on different ages of certain blood groups are also interesting[5].

According to historical data which have never been published officially, the majority of people in Croatia have blood group A (42%), followed by O (34%) and B (18%), while blood group AB is the least frequent (only 6%). The same sources indicated that 85% of the population were RhD positive, while the remaining 15% were RhD negative. These data are over 40 years old and were based on a smaller sample of blood donors who donated blood in the CITM at that time. Data from our study show some differences compared to old data. Possible reasons for this difference between the two analyzed periods are a smaller number of samples from the first study and the fact that no national information system, which enables comprehensive data analysis from the entire Croatian territory, existed at the time of that study. Population migrations have probably also had an impact on the change in blood type distribution among the population.

According to the results of an extensive study carried out on a large sample, the distribution of the ABO blood group system in our country is very similar to its distribution in Middle Europe. In Croatia, 38% of individuals have an A blood group phenotype and 37% have an O phenotype, so the distribution of these phenotypes is similar, as in other European countries. With 18% and 7% of individuals having B and AB phenotypes, respectively, Croatia is more similar to South-Eastern Europe than to Western Europe. The slightly higher proportion of blood type O (both RhD positive and negative) in blood donors and higher proportion of A positive, B positive and B negative blood types in patients and pregnant women (at the expense of O) can be explained by the greater motivation of people with O blood type to donate blood ("universal donors"). The RhD positive phenotype was found in 81% of the overall group of subjects, while the remaining 19% were RhD negative. A higher proportion of the RhD negative blood type in the blood donor group can also be explained by the frequent invitations of the

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RhD negative population to donate blood (especially O negative). However, the difference was only 0.54%.

More than 50% of the world's population have blood type O, primarily people living in South America, North America and Africa, as well as in parts of Western Europe; this blood type is less present in Eastern Europe. More than 20% of the world's population are carriers of blood type A, which is predominant among Australian Aborigines and Scandinavians. Blood type B is much less common, with an average prevalence of only 16%. This percentage is higher among the Asian population (more than 25%) and Jews, while it is extremely low in North and South America and Australia[6,7]. Blood group AB is evolutionarily the youngest group, and is most frequent among the Japanese, Chinese, Koreans, Hindus in India and the Tatars, while it is very rare in other populations (≤5%) and entirely absent from some, such as Aboriginal people, Māori, Malays, Navajo Indians, Peruvian Indians, Nicobarese, and Sudanese[8].

An increased frequency of some diseases in people with certain blood groups has long been a focus of attention of many researchers. There is a correlation between the ABO system and infectious diseases, as well as a proven susceptibility of individual bacteria, viruses and parasites to bind to erythrocytes with certain blood group antigens acting as receptors. For example, people with blood group O are relatively more resistant to a severe type of malaria[3]. It is assumed that oligosaccharide components making the difference in the composition of the ABO blood group system in certain parts of the world were the cause of epidemics of infectious diseases in the past[4].

The pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) triggered considerable research, including research on viral susceptibility of people with certain blood types. Numerous papers have been published on the binding of viruses to polysaccharide antigens of the ABO system and a comparison of the relationship between individual ABO blood group phenotypes and susceptibility to diseases. Zhao and colleagues were among the first to publish a paper containing results of a comparison between the distribution of individual blood types among 1,775 patients with COVID-19 from Wuhan hospital and a control group of healthy people, which showed a statistically significantly larger number of people with blood group A among the patients and a higher mortality rate among them[9]. At the same time, their colleagues (Li *et al*.) published similar results, together with analyses of co-morbidity and other factors, and they consolidated the results of blood type distribution from three hospitals in Wuhan. They concluded that people with the A phenotype are more susceptible to the disease and are more likely to develop pneumonia and die, and highlighted that people with blood group O are the least susceptible, but they should nevertheless be cautious. This could be explained by the fact that O blood group individuals have plasma von Willebrand factor and factor VIII levels 25% lower than those of non-O individuals, which puts them at a lower risk of the thromboembolic complications typical of COVID-19[10].

Turkish researchers also came to a similar conclusion, although based on a small sample of patients (n=186)[11]. Leaf and colleagues analyzed 561 patients in the USA and concluded that the A phenotype was the most frequent among COVID-19 patients, but no effects on severe clinical symptoms or fatal outcomes were observed[12]. Their colleagues analyzed twice as many subjects and observed a higher rate of COVID-19 in patients with A and B phenotypes, again without any influence of blood group phenotype on clinical severity of the disease or mortality[13]. Among the Iranian population, those with the AB phenotype were the most susceptible to the disease[14], while in Canada people with A and AB phenotypes were most susceptible[15]. None of the studies identified any differences in clinical presentation and mortality that could be attributed to the ABO phenotype. Barnkob analyzed 7,422 Danish patients and concluded that patients with blood group O were significantly less susceptible to COVID-19[16]. A group of Spanish researchers also reported that, together with older age, people with the blood group A phenotype were more susceptible to SARS-CoV-2 infection and had more severe COVID-19; the group A individuals also had increased mortality[17]. Franchini and co-authors concluded in a systematic review of 21 published studies that O blood group individuals have a lower infection rate than non-O individuals, but that there is no evidence to suggest an effect of O blood group on disease severity in infected patients[18].

Our results, which were obtained by analyzing 780 hospitalized patients with a severe clinical presentation of COVID-19, lead to similar conclusions. Compared with the

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distribution of ABO blood group phenotypes in the general population, we have recorded a statistically significantly greater frequency of the A phenotype in hospitalized COVID-19 patients who had a severe clinical presentation (χ^2 = 6.752, p=0.009). Results of the comparison of proportions test show a significantly greater distribution of blood group O in the general population (χ^2=9.44, p=0.002).

CONCLUSIONS

This study was carried out on a large number of subjects and presents the first official description of the distribution of ABO and RhD blood group phenotypes in the general population in the Republic of Croatia. The findings differ from the published data that have been used until now, and that are based on unpublished results of a study performed more than 40 years ago. Given that voluntary blood donors, as a representative sample of a healthy population, are being used in numerous studies as a control group, the results of our study will serve as a reliable reference for future research on the relationship between blood groups and different diseases/states. This study is also significant because it confirms other authors' observations about the predominance of the A blood group phenotype among patients with severe COVID-19.

AUTHORSHIP CONTRIBUTIONS

All Authors contributed to the design of the study, and to the acquisition, analysis, and interpretation of data.

The Authors declare no conflicts of interest.

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