Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Letter to the Editor

DNA genotyping of the ABO gene showed a significant association of the A-group (A1/A2 variants) with severe COVID-19.

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Letter

The coronavirus disease 2019 (COVID-19) is caused by the new beta-coronavirus SARS-CoV-2. The virus has spread worldwide causing a pandemic with millions of infected. The adverse outcome is associated with advanced age and pre-existing conditions such as hypertension and cardiovascular disease. There is a heterogeneous host response to SARS-CoV-2 infection ranging from asymptomatic to severe pneumonia that requires hospitalization and admission in the Intensive Care Unit (ICU). The host genetic background might influence the outcome and the risk of developing severe COVID-19. The first genome-wide association study (GWAs) with patients (n=1,980) and controls (n=2,205) found a significant association with a single nucleotide polymorphism (SNP rs657152) in the *ABO* blood group locus [1]. This variant is in linkage disequilibrium with rs8176719 -/G**, the** main genetic determinant of blood group O (homozygotes for the G-deletion). The GWAs suggested that the O group might be associated with a reduced risk for COVID-19 (Odds Ratio, OR=0.65;95% confidence interval, CI= 0.53-0.79).

Antibodies against A, B or both are naturally present in the serum of persons who don't express the corresponding antigen. The ABO gene maps to chromosome 9 and encodes a single glycosyltransferase with a null activity in the O group. The current evidence indicates that the blood group might be associated with the risk of being infected by the SARS-CoV-2, and also with the risk of developing severe COVID-19 [2, 3]. An important limitation of the case-control studies was the lack of information about the control's age. The conclusion of an association between ABO and COVID-19 could thus be inaccurate if controls were not age matched with patients [4]. In a study of COVID-19 from the first first epidemic wave (March-June 2020) in Spain we did not find significant association between the rs8176719 deletion-genotype (O group) and disease severity [5]. We sought to investigate the association between other common ABO gene variants and COVID-19, and for this purpose we studied patients from the first and second waves (September-December 2020).

We studied 566 patients who required hospitalization due to COVID-19 (mean age 64.57 years, range 24-95; 65% male). All them were Caucasian from the region of Asturias (Northern Spain). The study was approved by the Ethics Committee of Asturias and informed consent was obtained from all the patients. All the patients were positive for the SARS-CoV-2 in a naso-pharyngeal PCR test. A total of 236 patients required admission in the ICU based on the Guidelines for the Management of Community Acquired Pneumonia in Adults. These patients needed intubation and mechanical ventilation or vasoactive drugs due to shock. In 35% (N=198) of the patients the blood ABO-serotype was known (recorded in the clinical history). A total of 300 healthy individuals with a similar age range (mean age 68.84, range 60-88; 60% male) were recruited as population controls. They were included in the study with the only purpose of determining the ABO genotype frequencies in our population. The statistical analysis was performed with the R-software, with logistic-regression tests to compare frequencies between the groups. Hypertension was significantly associated with COVID-19 hospitalization (patients vs. controls, OR=1.66, 96% CI=1.24-2.22), and with the risk of severe-ICU (OR=2.37, 1.68-3.33). Male sex was also a risk factor for severity (OR=1.63, 1.14-2.33) (Table 1)

We designated a protocol for genotyping two ABO variants, rs8176719 (**c.259insG, p.Thr87AspfsTer107),** and rs8176746 (c.793C>A, p.Leu265Met). Homozygotes for the rs8176719 del-G were classified as O-group, while carriers of the G-allele were classified as AA. AO, AB, or BO based on the rs8176746 genotype (**suppl table**). The rs8176719 and rs8176746 genotypes were visualised by polymerase-chain reaction (PCR) followed by restriction enzyme digestion with *KpnI* or *NlaIII* (**suppl. figure**). The method was validated by sequencing PCR fragments representing the three genotypes. Patients and controls who were genotyped as group A-carriers (AA, AO, AB) were Sanger sequenced to determine the rs1053878 C/T (p.Pro155Leu) and rs41302905 A/G (p.Gly267Arg). These variants corresponded to the A1/A2 and *O.02 subtypes. **(suppl. figure).**

Other authors involved in this work: Elías Cuesta-Llavona[1,5], Laura Amado-Rodríguez[2,5,7,8], Inés Lopez-Alonso[2,5,7,8], Tamara Hermida[3], Ana I. Enriquez[3], Cristina Hernandez-González[3], Victoria Alvarez[1,5], Belén Alonso[1,5], Sara Iglesias[1,5]

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The rs8176719, rs8176746 and rs41302905 determine the primary classification of the ABO genotype. We did not find significant differences between total patients and controls (table 1). The A-group was significantly more frequent in the ICU compared to non-ICU cases (OR=1.55, 95%CI=1.10-2.16). As previously reported, O-group frequency was less frequent in the ICU patients, that could suggest a protective effect, but the difference was non-significant. Although there was a lower frequency of B-carriers in the UCI-group an association between the B-group and COVID-19 could not be concluded due to the low number of patients with this group. The rs1053878 SNP differentiated the A1/A2 variants. We did not find significant differences for the A1/A2 genotypes between the groups (table 1).

It can be argued that the determination of the blood group based on our genotyping approach might not represent the true serotype frequencies. The main cause of genotype-serotype discrepancies should be the wrong classification as non-O group. We think this is unlikely because we determined the two most common O-alleles, that represent more than 95% of the O-alleles. Moreover, in 35% of the patients the serotype was known and only one O-group was genotyped as AO. In our controls the blood-type frequencies according to the genotype were almost identical to the serotype from >47,000 individuals aged 65 and older (records from the Haematology Service).

Male sex, age, hypertension, and the A-group were associated with severe-ICU. We performed a multivariate logistic regression with these variables, and the A-blood remained significantly increased in the ICU-cohort (OR=1.47, 95%CI=1.04-2.08) (**suppl. table**). This was in agreement with the no significant difference between ABO-frequencies in hypertensives vs. normotensives (**suppl. table**).

Previous studies have reported that O-blood group would protect from COVID-19. Some of the studies compared patients with younger healthy controls. Some authors have pointed to the importance of proper selection of age-matched controls. For instance, group O are frequently recruited as preferred blood donors and this would result in inaccurate conclusions about the risk of developing COVID-19 when blood donors are recruited as population controls. One study followed 1,769 crew-members confined in the French Navy nuclear aircraft carrier Charles de Gaulle, where a COVID-19 outbreak occurred in April 2020. The authors showed that ABO blood groups were not associated with the risk of SARS-CoV-2 infection among young adults, although no conclusion about the risk of hospitalization due to COVID-19 could be concluded from this study [6].

It is possible that differences in blood group antigen expression can influence the host susceptibility to viral infection [7]. The SARS-COV that caused acute severe respiratory syndrome in 2003 also binds to ACE-2, and anti-A antibodies would specifically inhibit the interaction between the viral spike (S) protein and ACE-2. Thus, anti-A antibodies in individuals might block the interaction of the S-protein with ACE-2 reducing the viral infection [8]. Individuals with blood group A would not produce anti-A antibodies and might thus be more vulnerable to SARS-CoV compared to individuals without A [9,10]. This would explain why people in group O, young and healthy, are infected less than those in group A, due to the ability to interfere with the binding of the virus to its ACE-2 receptor. However, once the infection has been contracted this mechanism would not explain the clinical severity and worse outcome of group A patients. It has been reported that compared to O-group, the A-group is associated with increased odds of major adverse cardiovascular events in COVID-19 [11]. In the GWAs study the blood group A was also associated with increased risk of respiratory failure while blood group O was protective [1]. The biological mechanism that explains the effect of ABO blood groups in COVID-19 is unclear. However, the A-group has been associated with increased levels of von Willebrand and factor VIII and this could increase the risk of arterial and venous thrombotic events [12,13]. The genetic variation in the ABO locus might also contribute to adverse outcomes by affecting the serum levels of inflammatory markers [14].

Finally, two COVID-19 waves have occurred since the SARS-CoV-2 pandemic originated. In response to the first wave we adopted severe restrictions in mobility and social contact, that resulted in almost no hospitalizations in the months of July-August, with no patient requiring ICU-admission. Currently (November 2020), we are suffering a severe second wave that put hospitals and ICUs close to the collapse. The reported data from the second wave suggested a demographic shift toward a younger population with fewer comorbidities, with less severe patients at admission and a lower rate of severe cases. The differences between the two waves may be attributed to several factors, such as shorter time between disease onset and hospital admission, comorbidities, improvement of the treatment, and differences in patient background. We compared the ABO frequencies between patients from the two pandemic waves. We observed higher A-frequencies among the ICU cases in the two waves, with significant difference in the second wave (Figure 1).

The main limitation of our study was the lack of data about a correlation between the blood group and a biological marker (such as inflammatory or coagulation) that could explain the association with

Table 1
Main characteristics of the COVID-19 patients and population controls. Severe-ICU cases were those in need of critical care support, including high-flow oxygen, positive-pressure ventilation or vasoactive drugs. A1/A2 frequencies relative to the A and AB groups.
AV= adjusting variable; ns=non-significant (p>0.05)

	COVIDN=566	ControlsN=300	**p-value**	ICUCOVIDN=236	Non-ICUCOVIDN=330	**p-value**
Male %	367 (65%)	180 (60%)	AV	168 (71%)	199 (60%)	<0.01
Mean Age	64.57	68.84	AV	66.54	65.63	ns
Age range	24-95	60-88	AV	28-80	24-95	ns
Hypertension	261 (45%)	102 (34%)	<0.001	138 (58%)	123 (37%)	<0.001
Genotype						
Blood group						
A	**257 (0.45)**	**141 (0.47)**	**ns**	**122 (0.52)**	**135 (0.41)**	0.01*
A1O	150 (0.58)	86 (0.61)		70 (0.57)	80 (0.57)	
A2O	41 (0.16)	29 (0.21)		18 (0.15)	23 (0.16)	
A1A1	40 (0.16)	14 (0.10)		20 (0.16)	20 (0.14)	
A1A2	21 (0.08)	10 (0.07)		11 (0.09)	10 (0.07)	
A2A2	5 (0.02)	2 (0.01)		3 (0.02)	2 (0.01)	
B	**37 (0.07)**	**22 (0.07)**		**9 (0.04)**	**28 (0.08)**	
AB	**27 (0.05)**	**15 (0.05)**		**8 (0.03)**	**19 (0.06)**	
A1B	24 (0.88)	12 (0.80)		8 (0.03)	16 (0.04)	
A2B	3 (0.12)	3 (0.20)		0	3 (0.01)	
O	**245 (0.43)**	**122 (0.41)**		**97 (0.41)**	**148 (0.45)**	

* Group A genotypes (AA+AO) were significantly more common in the ICU compared to non-ICU patients (p=0.01, OR=1.55, 95%CI=1.10-2.16).

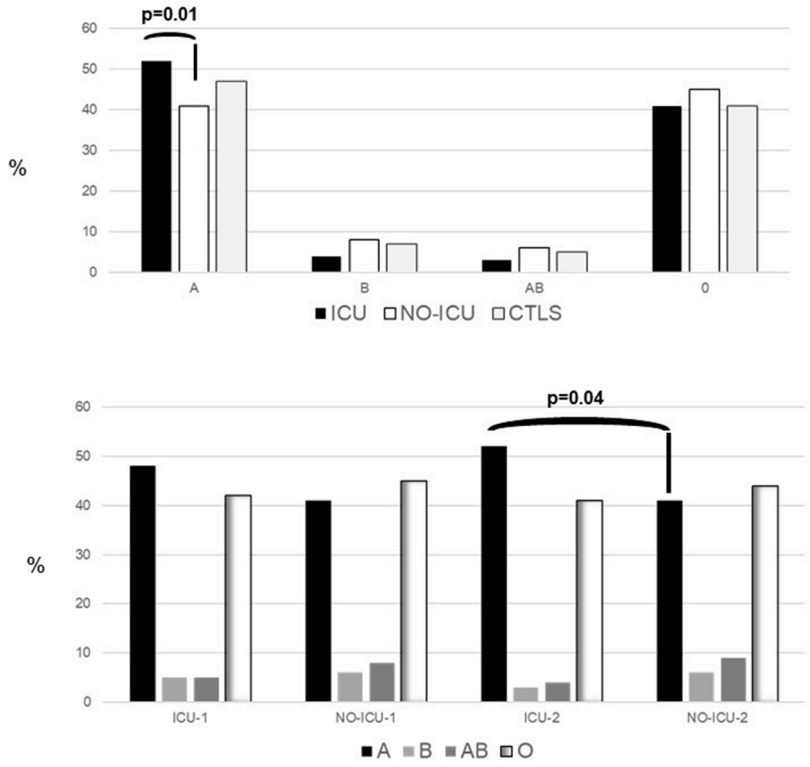

Figure 1. Frequency of the different ABO-genotypes in severe-ICU, non-ICU, and controls (above). The A-group was significantly increased in the ICU patients compared to non-ICU. Below, frequency of the ABO-genotypes in the two patient categories in the first and second pandemic 2020 waves. The A-group frequency was higher in the ICU patients in the two waves.

disease severity. This limitation is shared with most of the study that examined the ABO-groups in COVID-19.

In conclusion, our blood-group ABO classification based on DNA genotypes showed that the A-group was a significant risk factor for developing a severe form of COVID-19 with ICU-admission. Compared with healthy population controls, patients with COVID-19 requiring hospitalization showed no significantly different ABO-genotype frequencies.

Contributorship

All the authors contributed to this work by recruiting the patients and performing the genetic and statistical analysis. All the authors approved the submission of this Letter.

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Competing interests

None of the authors have competing interests related to this work.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2021.02.016.

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