TRANSFUSION

Lewis and ABO histo-blood types and the secretor status of patients hospitalized with COVID-19 implicate a role for ABO antibodies in susceptibility to infection with SARS-CoV-2

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets the respiratory and gastric epithelium, causing coronavirus disease 2019 (COVID-19). Tissue antigen expression variations influence host susceptibility to many infections. This study aimed to investigate the closely linked Lewis (*FUT3*) and ABO histo-blood types, including secretor (*FUT2*) status, to infections with SARS-CoV-2 and the corresponding severity of COVID-19.

Study Design and Methods: Patients (Caucasians, $n = 338$) were genotyped for *ABO*, *FUT3*, and *FUT2*, and compared to a reference population of blood donors ($n = 250,298$). The association between blood types and severity of COVID-19 was addressed by dividing patients into four categories: hospitalized individuals in general wards, patients admitted to the intensive care unit with and without intubation, and deceased patients. Comorbidities were considered in subsequent analyses.

Results: Patients with blood type Lewis (a−b−) or O were significantly less likely to be hospitalized (odds ratio [OR] 0.669, confidence interval [CI] 0.446–0.971, OR 0.710, CI 0.556–0.900, respectively), while type AB was significantly more prevalent in the patient cohort (OR 1.519, CI 1.014–2.203). The proportions of secretors/nonsecretors, and Lewis a+ or Lewis b+ types were consistent between patients and controls. The analyzed blood groups were not associated with the clinical outcome as defined.

Discussion: Blood types Lewis (a−b−) and O were found to be protective factors, whereas the group AB is suggested to be a risk factor for COVID-19. The antigens investigated may not be prognostic for disease severity, but a role for ABO isoagglutinins in SARS-CoV-2 infections is strongly suggested.

KEYWORDS
ABO, COVID-19, host–pathogen interaction, Lewis, secretor

1 | INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) targets the respiratory mucosa, and it can infect and replicate in the gastric and intestinal epithelium, causing coronavirus disease 2019 (COVID-19).[1,2] This emerging infectious disease is usually characterized by an acute respiratory infection, with symptoms varying from mild to severe, including pneumonia, respiratory failure, coagulopathy, multiple organ failure, and death.[3,4] COVID-19 is a complex and multifactorial disease, where inherited predispositions together with existing comorbidities and acquired risk factors are likely to influence the severity of the disease.

Differences in blood group antigen expression can increase or decrease host susceptibility to many infections.[5] Pathogens including viruses, bacteria, and eukaryotic parasites carry lectins on their surfaces, which can bind to glycan structures such as blood group antigens on the host cell surface as a first step in the infection process. Blood group antigen structures may be involved in the binding of their toxins, facilitating invasion and colonization or evasion of host clearance mechanisms. They can also serve as false receptors, preventing pathogen binding to target tissues. In addition, it was reported that microorganisms can stimulate antibodies against blood group antigens, including ABO and Lewis.[5,6] Molecular mimicry can modify the innate immune response to infections, when directed against enveloped viruses that express blood group-like carbohydrate antigens.[7]

The carbohydrate histo-blood group antigens Lewis and ABO are widely expressed in many tissues, including respiratory and gastric mucosa, endothelium, kidney, and heart.[8] The Lewis enzyme (fucosyltransferase 3), encoded by *FUT3*, is responsible for the last step in the biosynthesis of Lewis antigens.[9] The immunodominant glycan structures defining the ABO antigens are synthesized by glycosyltransferases, encoded by the *ABO* gene.[10] The expression of soluble ABO(H) antigens in secretory epithelium is regulated by Fucosyltransferse 2 (Fut 2), encoded by the *FUT2* gene.[11] The activity of fucosyltransferase 2 (Fut 2) is also required for the production of Lewis b (Le b) antigen, and reflected by the Le (a−b+) phenotype. In contrast, the Le (a+b−) phenotype is found in ABH nonsecretors (18%–22%).[8,11]

The naturally occurring anti-A and anti-B isoagglutinins have a high titer and avidity, activate complement, and consist of mainly IgM and to a lesser extent IgG. Different titers of these antibodies may be the result of environmental rather than hereditary factors.[12–14]

The soluble ABH and Le b antigens, which are found in the respiratory and gastric mucosa of secretors, may influence the mechanisms of SARS-CoV-2 invasion to the target tissues. The interaction between *ABO*, *FUT2*, and *FUT3* impacts the amount of soluble ABH and Lewis antigens present[15] and thus may increase the risk for a severe course of the disease.

Lewis antigens were identified as Helicobacter pylori virulence factors, enabling bacterial adherence to and invasion of gastric epithelial cells.[16–19] A relationship between ABO/Lewis phenotype, gastric ulcers, chronic *H. pylori* infections, and urinary tract infections has been discussed.[20–24] ABO blood types have been reported to affect susceptibility to several infections,[25–28] and an additional role for ABO glycans and glycosyltransferases in inflammatory vascular diseases, cardiovascular diseases, and acute respiratory distress syndrome has been suggested.[29–37]

European and American studies support preliminary investigations in China regarding an association between the ABO blood group system and SARS-CoV-2 infections.[38–47] The secretor phenotype was suggested to possibly moderate disease progression, especially among type A carriers.[47] A contribution of the Lewis blood type to infection with SARS-CoV-2 has not yet been studied.

This is the first study investigating the closely linked Lewis and ABO blood group systems and blood group secretor type simultaneously, relative to the severity of COVID-19 in hospitalized patients. As recently discussed,[48] our results strongly implicate a possible role for the ABO isoagglutinins in the process of infection with SARS-CoV-2.

2 | STUDY DESIGN AND METHODS

2.1 | Patients

This retrospective study included 338 Caucasian patients, over the age of 18, admitted to the Department of Internal Medicine at the University Hospital Graz and to Landeskrankenhaus Graz II, with a diagnosis of COVID-19, between March and May 2020 during the first wave of coronavirus infection in Austria. All study participants tested positive for SARS-CoV-2 RNA by real-time PCR (qPCR). After extraction using the EMAG® platform (bioMérieux S.A., Marcy l'Etoile, France), nucleic acids were amplified using the RIDA® GENE SARS-CoV-2 (r-biopharm, Darmstadt, Germany) with the LightCycler® 480 II (Roche Molecular Diagnostics, Rotkreuz, Switzerland). Additionally, the Cobas® SARS-CoV-2 test (Roche Molecular Systems, Branchburg, NJ) was applied on the Cobas® 6800/8800 system (Roche Molecular Diagnostics).[49]

The diagnosis of COVID-19 was established based on the national guidelines published by the Austrian Ministry of Health.[50] Admission referred to COVID-19-related hospitalization, and mortality was defined as all-cause mortality in SARS-CoV-2 infected patients.[51,52]

Demographic information and concurrent diagnoses, based on the International Classification of Diseases (ICD-10), were obtained from the electronic health records of patients.

The study was approved by the local Ethics Committee at the Medical University of Graz (32-436 ex 19/20).

2.2 | Genetic analysis

Nasal- and pharyngeal swab specimens of the 338 patients were used for the extraction of human DNA with the Qiamp DNA Micro Kit (Qiagen, GmbH, Germany).

The ABO region, containing exons 6 and 7, was amplified using the primer pair ABO_Ex6_F (5′-GCCTCTCTCC ATGTGCAGTA-3′),[53] and ABO_Ex7_R (5′-CCTAGGCTT CAGTTACTCAC-3′). Sanger sequencing was done with ABO_In6_223R (5′-GCCTCTGGAGAAGGAGCT-3′) and ABO_Ex7_F1 (5′-CATCGCTGGGAAGAGGATGAAGTG-3′) as recently described.[54]

The single nucleotide polymorphisms (SNPs) *c.261delG* (rs1556058284), *c.297A>G* (rs8176720), *c.467C>T* (rs1053878), *c.526C>G* (rs7853989), *c.646T>A* (rs8176740), *c.681G>A* (rs8176742), *c.703G>A* (rs8176743), *c.771C>T* (rs8176745), *c.796C>A* (rs8176746), *c.802G>A* (rs41302905), *c.803G>C* (rs8176747), *c.829G>A* (rs8176748), *c.930G>*A (rs8176749), and *c.1061delC* (rs56392308) were analyzed to discriminate the presence of *ABO*A1, A2, B.O1, O.01, O.02,* or *O.03* alleles according to Olsson et al.[55] and the ISBT database.

The *FUT2* gene was investigated by restriction fragment length polymorphism analysis using recombinant AvaII (R0153S, New England Biolabs, Frankfurt, Germany) for detecting the *c.428G>A* (rs601338) inactivating SNP.[56] The presence of this variant, which is the most common *FUT2* null allele in Caucasians (47%–49.5%),[57] was used to determine secretor status. Homozygosity of c.*428A* results in the nonsecretor phenotype.

The coding region of *FUT3* was analyzed using Sanger Sequencing. The detected SNPs were assigned to the corresponding functional or nonfunctional alleles as reported before.[58]

The Lewis types were inferred from the results of *FUT2* and *FUT3* genotyping. If at least one functional *FUT3* allele was present, secretors were predicted to have the Lewis type Le b+, whereas nonsecretors were predicted to have type Le a+. Homozygous nonfunctional *FUT3* genotypes were defined as Le (a−b−), regardless the *FUT2* genotype detected. Using this method, it was not possible to predict the Le (a+b+) phenotype as a separate proportion. However, the frequency of the Le (a+b+) phenotype as observed in our blood donors is very low (0.4%), and therefore was considered to be negligible for the purposes of our analysis. The reliability of this approach was previously validated, and confirmed by consistent results for serologic and genetic Lewis typing as reported before.[58]

2.3 | Blood donors

Data about the ABO and Lewis blood groups of Styrian blood donors ($n = 250{,}298$) were used to calculate the blood type distributions of healthy controls. Repeat donors' data were excluded. A selection bias due to selective retention of blood group O individuals in our blood donor campaign can be ruled out. Serological ABO typing was performed on the Olympus automated blood group testing system (Olympus PK7300, Beckman Coulter, Hamburg, Germany). Lewis blood groups were determined with standard serologic and gel matrix techniques (MicroTyping System, Bio-Rad).

The secretor status of randomly selected blood donors ($n = 480$) was defined by *FUT2* genotyping, as described earlier for the patients. Genomic DNA was prepared from peripheral blood leucocytes from the donors' blood by silica-magnetic particle technology using a DNA purification kit and a biorobot system (EZ1 DSP DNA and EZ1 Advanced XL, respectively, Qiagen GmbH, Germany).

2.4 | Statistical analysis

The COVID-19 cohort was classified into four categories reflecting symptom severity: patients hospitalized in the general ward (1); patients who needed admission to the intensive care unit (ICU) without (2) and with intubation (3); and deceased patients (4). A broader outcome classification that distinguished between surviving and deceased patients was included.

The distribution of blood types in hospitalized COVID-19 patients was compared to that of Styrian blood donors ($n = 250{,}298$ for ABO, $n = 7241$ for Lewis) by means of chi-squared tests, along with odds ratios (ORs) with 95% confidence intervals (CI).

The association between blood groups and symptom severity was investigated with chi-squared tests or by Fisher's exact test as appropriate. Group differences in continuous variables were conducted with Kruskal–Wallis tests. To test the contribution of blood types to patients' outcomes (deceased vs. recovered), a logistic regression, adjusting for potential confounders, was performed. These confounders comprised age, sex, and predictors with $p < .02$ in the univariable analysis (malignant neoplasm, diabetes mellitus, hypertension, and

acute kidney injury/chronic kidney disease). Adjusted ORs with 95% CI were generated. A p value of ≤.05 was considered significant. All statistical analyses were conducted using R version 4.0.2 (https://www.r-project.org).

3 | RESULTS

3.1 | Characteristics of the COVID-19 patients

Of the 338 individuals, more females ($n = 187$) were hospitalized than males ($n = 151$). The females (median age of 80.0 [IQR = 69.0–88.0]) were significantly older than the males (median age of 74.0 [IQR = 64.5–80.0]), $p < .001$. A higher proportion of males (70.4%, $n = 19$) than females (29.6%, $n = 8$), $p = .004$, underwent treatment in the ICU. Furthermore, males had higher rates of malignant neoplasm (12.6%, $n = 19$) than females (5.9%,

$n = 11$), $p = .031$, diabetes mellitus (males: 20.5%, $n = 31$; females: 12.3%, $n = 23$), $p = .040$, and gastrointestinal diseases (males: 19.9%, $n = 30$; females: 9.6%, $n = 18$), $p = .007$. The mortality rate among male patients was 26.5% ($n = 40$), versus 20.3% ($n = 38$) of female patients, which was not significantly different, $p = .181$.

In Table 1, the demographics and comorbidities of the patients in relation to the four categories reflecting symptom severity are outlined.

3.2 | Lewis and ABO types and frequencies of secretors/nonsecretors

Of the pool of 338 hospitalized patients, DNA quality issues prevented the successful analysis of two ABO and two *FUT3* genotypes, leaving a sample size of 336 patients for ABO- and Lewis-related analyses. The data set for secretor status was complete ($n = 338$).

TABLE 1 Demographics and patient comorbidities presented by symptom severity

	Total (N = 338)	General ward (N = 233)	ICU no intubation (N = 10)	ICU with intubation (N = 17)	Deceased (N = 78)	p
Sex						.008[a]
Male	151 (44.7%)	92 (39.5%)	8 (80.0%)	11 (64.7%)	40 (51.3%)	
Female	187 (55.3%)	141 (60.5%)	2 (20.0%)	6 (35.3%)	38 (48.7%)	
Age (years)						<.001[b]
Median	77.0	76.0	60.0	67.0	82.5	
Min–Max	23.0–100.0	23.0–99.0	36.0–77.0	51.0–79.0	53.0–100.0	
Hospitalization (days)						<.001[b]
Median	11.0	12.0	11.0	28.0	8.5	
Min–Max	1.0–133.0	1.0–133.0	6.0–30.0	11.0–100.0	1.0–90.0	
Concurrent conditions						
COPD	27 (8.0%)	17 (7.3%)	3 (30.0%)	1 (5.9%)	6 (7.7%)	.123[a]
Malignant neoplasm	30 (8.9%)	23 (9.9%)	0 (0.0%)	3 (17.6%)	4 (5.1%)	.254[a]
AKI/CKD	70 (20.7%)	42 (18.0%)	0 (0.0%)	2 (11.8%)	26 (33.3%)	.009[a]
Diabetes mellitus	54 (16.0%)	32 (13.7%)	1 (10.0%)	2 (11.8%)	19 (24.4%)	.161[a]
Coronary artery disease	14 (4.1%)	7 (3.0%)	1 (10.0%)	2 (11.8%)	4 (5.1%)	.128[a]
Deep vein thrombosis	6 (1.8%)	4 (1.7%)	0 (0.0%)	1 (5.9%)	1 (1.3%)	.498[a]
Gastrointestinal diseases	48 (14.2%)	33 (14.2%)	1 (10.0%)	5 (29.4%)	9 (11.5%)	.289[a]
Hypertensive diseases	173 (51.2%)	127 (54.5%)	4 (40.0%)	8 (47.1%)	34 (43.6%)	.324[a]
Infectious and parasitic diseases (excluding Coronavirus)	12 (3.6%)	7 (3.0%)	2 (20.0%)	0 (0.0%)	3 (3.8%)	.109[a]

Note: Demographic data and underlying chronic diseases of the patients, in relation to the 4 categories of symptom severity.

Abbreviations: AKI, acute kidney injury; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit.

[a]The association of symptom severity with demographic and clinical information was tested by means of Fisher's exact tests for categorical variables.

[b]The association of symptom severity with demographic and clinical information was tested by means of Kruskal–Wallis rank sum tests for continuous variables. The proportion of males/females, age, days of hospitalization, as well as the proportion of patients with acute kidney injury/chronic kidney disease, differed significantly among the four groups.

TABLE 2 Frequencies of blood types in healthy blood donors versus hospitalized patients and their associations with COVID-19

Blood type	Blood donors in % (N)	COVID-19 patients in % (N)	p	OR	95% CI
O	37.4 (93,579)	29.8 (100)	.008[a]	0.710	0.556–0.900
A	42.7 (106,861)	44.9 (151)		1.095	0.878–1.366
B	13.7 (34,164)	16.1 (54)		1.211	0.888–1.626
AB	6.3 (15,694)	9.2 (31)		1.519	1.014–2.203
Secretor	82.3 (395)	84.0 (284)	.404[a]	1.132	0.767–1.679
Nonsecretor	17.7 (85)	16.0 (54)			
Le a+	14.8 (1070)	14.6 (49)	.083[a]	0.985	0.707–1.347
Le b+	71.6 (5186)	75.9 (255)		1.247	0.962–1.630
Le (a−b−)	13.6 (985)	9.5 (32)		0.669	0.446–0.971

Note: The proportions of the ABO and Lewis blood types, as well as the frequencies of secretors and nonsecretors in COVID-19 patients are compared against the population of blood donors. The odds ratios of having a specific blood type against the other blood types in patients are reported. Secretors were compared to Nonsecretors. Le a+ were compared to Non-Le a+, Le b+ were compared to Non-Le b+, and Le (a−b−) were tested versus Le a+ and Le b+ types.
Abbreviations: CI, confidence interval; N, number of individuals typed for a specific ABO type in the cohort of blood donors and the patients; OR, odds ratio.
[a]p-Values refer to chi-squared tests.

TABLE 3 Blood types in relation to the severity of COVID-19

Blood type	General ward (N = 233)	ICU without intubation (N = 10)	ICU with intubation (N = 17)	Deceased (N = 78)	p
ABO					.925[a]
O	68 (29.4%)	5 (50.0%)	5 (29.4%)	22 (28.2%)	
A	105 (45.5%)	3 (30.0%)	6 (35.3%)	37 (47.4%)	
B	37 (16.0%)	1 (10.0%)	4 (23.5%)	12 (15.4%)	
AB	21 (9.1%)	1 (10.0%)	2 (11.8%)	7 (9.0%)	
Secretor status					.801[a]
Secretor	192 (82.4%)	9 (90.0%)	15 (88.2%)	68 (87.2%)	
Nonsecretor	41 (17.6%)	1 (10.0%)	2 (11.8%)	10 (12.8%)	
Lewis					.917[a]
Le a+	36 (15.6%)	1 (10.0%)	2 (11.8%)	10 (12.8%)	
Le b+	170 (73.6%)	8 (80.0%)	14 (82.4%)	63 (80.8%)	
Le (a−b−)	25 (10.8%)	1 (10.0%)	1 (5.9%)	5 (6.4%)	

Note: The investigated blood types are indicated in relation to the four hospitalized patient categories reflecting symptom severity.
Abbreviation: ICU, Intensive care unit.
[a]Fisher's exact test.

Table 2 indicates the proportions of the Lewis and ABO blood types, as well as the frequencies of secretors/nonsecretors in the reference sample of blood donors, as compared to the cohort of COVID-19 patients. The frequencies of the different blood types observed in the blood donors are largely in line with data reported among Europeans or Caucasian individuals.[8,59]

No significant differences were found in the proportion of secretors/nonsecretors and Lewis types between the patients and the blood donors. However, the Le (a−b−) type was present more frequently in the blood donors than in the patients (13.6% vs. 9.5%). The distribution of ABO blood types in the COVID-19 patients differed significantly from the distribution observed in the blood donors (p = .008).

Additionally, the cooperative interaction of ABO blood type with secretor status was analyzed by comparing the proportion of secretors versus nonsecretors in each ABO group in patients versus blood donors. No significant differences in the distributions were found (A secretor/A nonsecretor, p = .312; B secretor/B nonsecretor, p = .999; AB secretor/AB nonsecretor, p = .996; O secretor/O nonsecretor, p = .816).

TABLE 4 Blood types and the risk for the outcomes "deceased" or "recovered"

Blood type	Deceased (N = 78)	Recovered (N = 260)	OR univariable	CI	p	AOR multivarable	95% CI	p
A	37 (47.4%)	114 (44.2%)	Reference					
AB	7 (9.0%)	24 (9.3%)	0.89	0.33–2.14	.805	1.12	0.36–3.20	.840
B	12 (15.4%)	42 (16.3%)	0.87	0.40–1.79	.719	0.77	0.32–1.81	.562
O	22 (28.2%)	78 (30.2%)	0.86	0.47–1.56	.627	1.28	0.64–2.54	.486
Secretor	68 (87.2%)	216 (83.1%)	Reference					
Nonsecretor	10 (12.8%)	44 (16.9%)	0.75	0.34–1.53	.453	0.71	0.29–1.6	.421
Le b+	63 (80.8%)	192 (74.4%)	Reference					
Le a+	10 (12.8%)	39 (15.1%)	0.80	0.36–1.65	.565	0.73	0.30–1.66	.469
Le (a−b−)	5 (6.4%)	27 (10.5%)	0.56	0.19–1.41	.260	0.64	0.20–1.74	.408

Note: The ABO types AB, B, and O were tested compared to blood group A. Nonsecretors were compared to secretors, and patients with the Le (a−b−) and Le a+ types were compared to individuals positive for Le b. Multivariable analyses were conducted, adjusting for sex, age, and concurrent diagnoses (cancer, diabetes mellitus, hypertension, and acute kidney injury/chronic kidney disease).
Abbreviations: AOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.

3.3 | Association of blood types with COVID-19

The odds ratio of patients in the COVID-19 study group with Lewis type Le (a−b−) was significantly lower, than those with other Lewis types (Le a+ and Le b+), as shown in Table 2. The blood type O was detected significantly less often in the COVID-19 patients, as compared to the other ABO types (A, B, AB), and the odds ratio of patients with blood type AB was significantly higher.

The proportions of blood types did not differ significantly in the four categories of hospitalized patients (Table 3). Both univariable and multivariable analyses revealed no significant contribution of the blood types Lewis, ABO, or secretor/nonsecretor status to the outcome "deceased" versus "recovered" (Table 4).

4 | DISCUSSION

This study investigated the contribution of Lewis, ABO, and secretor type to COVID-19 outcomes in hospitalized Caucasian patients.

Consistent with the sex ratio of people infected with SARS-CoV-2 as reported by the Austrian Federal Office for Safety in Health Care,[60] more females than males needed inpatient treatment in this study. This may be explained by the higher percentage of females in the 60-and-over age group in the Austrian population. However, more males than females were admitted to the ICU, which can be explained by the comorbidities from which predominantly the males suffered. Consistent with the clinical characteristics of COVID-19 cohorts investigated in Italy, Spain, and the USA, the patients predominantly

suffered from comorbidities such as hypertension (51.2%) and diabetes mellitus (16.0%). There were higher rates of AKI/CKD (20.7%), whereas CAD was present less frequently (4.1%), compared to recently reported studies.[43,44,61–64] The deceased patients (23.01%) were older (median age = 82.5), than the survivors, regardless of sex, and had a higher incidence of AKI/CKD (33.3%) and diabetes mellitus (24%).

Our results revealed a significantly different frequency of ABO blood types in the patients, compared to the frequencies observed in a healthy control group. Proportions of the Lewis blood types and frequencies of secretors and nonsecretors did not differ significantly in our COVID-19 sample group, as compared to the healthy population. None of our analyses predicted a certain blood type to be a risk factor for a severe course of the disease.

4.1 | Association between the Lewis type and COVID-19

The Le (a−b−) type appeared to be at least a mitigating factor against hospitalization with COVID-19. It may therefore be speculated that the presence of certain fucosylated glycosphingolipids in Le (a+b−) and Le (a−b+) phenotypes may also play a role in the progression of this infectious disease. Mucins, secreted by epithelial cells, are highly glycosylated proteins, and are suggested to be substrates for active Fut 3, which is expressed in secretory mucosa. The composition of bound and mobile mucins, including the presence or absence of a certain type of glycan, may influence the binding affinities or the persistence of infectious organisms in secretory tissues.[65,66]
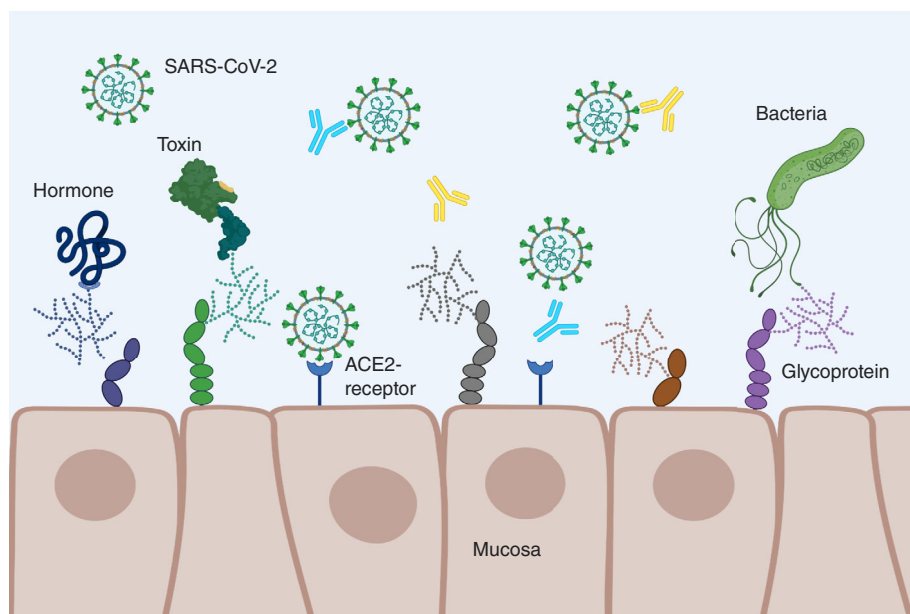
FIGURE 1 Schematic illustration of mucosa cells with glycosylated structures and receptor–ligand interactions. Hormones, antibodies, as well as viruses and bacteria or their toxins, interact with oligosaccharide epitopes (glycoproteins) that serve as receptors or co-receptors. The suggested interference of ABO antibodies with SARS-CoV-2 targeting of host cells via angiotensin-converting enzyme 2 (ACE-2) receptor is indicated. Made with Biorender.com

The frequency of possessing an inactive Lewis gene responsible for the Le (a−b−) blood type is much higher among African Americans than among Europeans and Caucasian Americans (22%–29% vs. 4%–11%). However, the Le (a+b+) phenotype is very rare (0% to <1%) in European and Caucasian Americans, it has an incidence of 6%–25% and 27% in Taiwan Chinese and Hong Kong Chinese, respectively.[8,67,68] A possible impact of the Lewis carbohydrate structures may merit further investigation, especially with regard to their prevalence in African Americans and Chinese.

This study confirms previous evidence reporting a protective effect of ABO blood group O, and a higher risk for COVID-19 associated with blood type AB.[43,47,69,70] Our data are different from studies also reporting risk associations for the blood types A[38,40,42,45] or B.[43] As well, the ABO types were not associated with higher odds of suffering from more severe COVID-19 symptoms in our study.[45,47,70] Our data are in line with previous results, regarding disease severity, confirming no association between the ABO type and the risk of intubation or of a fatal outcome of COVID-19 infection among hospitalized individuals.[43,71] Likewise, the ABO blood type of critically ill patients with COVID-19 was not related to 28-day mortality in another study performed.[44] Our results do not confirm the protective effect of nonsecretor phenotype as observed by Valenti et al.[47]

Divergent observations regarding associations of ABO blood types may be explained by different blood group distributions observed in different geographic regions or ethnicities.[8,72,73]

Recently, adhesion of the SARS-CoV-2 receptor binding domain to A antigen on a solid phase glycan microarray was demonstrated in vitro, indicating a possible contribution of ABO(H) antigens to the infection.[74] The lower incidence of individuals with ABO blood type O, accompanied by a higher incidence of individuals with blood type AB in our cohort of patients, suggests that the variable susceptibility to the infection with SARS-CoV-2 could also be related to an interference caused by circulating ABO antibodies (Figure 1). The lack of any significant differences regarding the interactions of the ABO and secretor types in the cohort of patients compared to the blood donors, strengthens our theory that emphasis should not be put on the ABH antigenic structures, but rather on the isoagglutinins.

SARS-CoV-2 is an enveloped virus. It targets host cells via interaction of the viral adhesion glycoprotein, SARS-CoV spike (S) protein, with the angiotensin-converting enzyme 2 (ACE-2), which serves as its cellular receptor.[75,76] The S protein consists of a complex glycan structure, which was reported to be capable of supporting ABH epitopes in an in vitro study investigating infections with SARS-CoV in 2008.[77] The S protein, experimentally modified to express A antigen, was effectively blocked by high titer (>1:256) monoclonal anti-A and human anti-A. Inhibitory effects of anti-B were not investigated in their experiments. As the virus targets respiratory and gastrointestinal mucosa, it was suggested that human isolates express ABH antigens on the S protein and host envelope glycosphingolipids by utilizing the host's enzymes and post-translational glycosylation machineries.[77–79] Thus, the ability of ABO antibodies to decrease the risk of initial infection may depend on ABO incompatibility between the infected and exposed individuals. However, the presence of ABO antibodies may at least delay the

spread of the virus among people, particularly when an individual is exposed to aerosols with only a low viral load. The rates of infection with SARS-CoV-2 throughout the population may be further influenced by the titer of ABO isoagglutinins and the incidence of blood group O in the affected population or region.

To conclude, our findings suggest that Lewis antigens contribute to infection with SARS-CoV-2. Furthermore, we confirm, ABO blood group O, with obligatory anti-A and anti-B antibodies present, to be protective against COVID-19. The blood type AB, lacking those isoagglutinins, is suggested to increase the risk of infection. There are therefore strong indications that ABO antibodies affect susceptibility to COVID-19, and should be the subject of further research. Sophisticated in vitro studies investigating the mechanism of virus–host cell interactions should be included.

4.2 | Limitations of the study

Some limitations of the present study should be kept in mind. Our sample size was restricted to patients hospitalized with COVID-19. Future studies should take into account the whole cohort of individuals tested positive for SARS-CoV-2 to investigate the relationship between the disease and blood group types. The sample size of 338 patients is relatively small compared to other studies on this topic.[43–45,47,70] As this is a retrospective observational study, we cannot rule out the fact that unmeasured confounding factors may have influenced the outcome.

CONFLICT OF INTEREST
The authors have disclosed no conflicts of interest.

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