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A large cohort study of the effects of Lewis, ABO, 13 other blood groups, and secretor status on COVID-19 susceptibility, severity, and long COVID-19

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Abstract

Background: Previous studies have reported Blood type O to confer a lower risk of SARS-CoV-2 infection, while secretor status and other blood groups have been suspected to have a similar effect as well.

Study design and methods: To determine whether any other blood groups influence testing positive for SARS-CoV-2, COVID-19 severity, or prolonged COVID-19, we used a large cohort of 650,156 Danish blood donors with varying available data for secretor status and blood groups ABO, Rh, Colton, Duffy, Diego, Dombrock, Kell, Kidd, Knops, Lewis, Lutheran, MNS, P1PK, Vel, and Yt.

Of these, 36,068 tested positive for SARS-CoV-2 whereas 614,088 tested negative between 2020-02-17 and 2021-08-04. Associations between infection and blood groups were assessed using logistic regression models with sex and age as covariates.

Results: The Lewis blood group antigen Le^a displayed strongly reduced SARS-CoV-2 susceptibility OR 0.85 CI[0.79–0.93] $p < .001$. Compared to blood type O, the blood types B, A, and AB were found more susceptible toward infection with ORs 1.1 CI[1.06–1.14] $p < .001$, 1.17 CI[1.14–1.2] $p < .001$, and 1.2 CI[1.14–1.26] $p < .001$, respectively. No susceptibility associations were found for the other 13 blood groups investigated. There was no association

Abbreviations: CI, Confidence Interval; COVID-19, Coronavirus disease 2019; DBDS, The Danish Blood Donor Study; fdr, False discovery rate; Long COVID-19, Symptoms persistingthreemonths after infection; MiBa, Danish Microbiology Database.; N, Number; OR, Odds ratio; RBC, Red blood cell; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; SCANDAT, SCANdinavian Donation And Transfusion database.

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between any blood groups and COVID-19 hospitalization or long COVID-19. No secretor status associations were found.

Discussion: This study uncovers a new association to reduced SARS-CoV-2 susceptibility for Lewis type Le[a] and confirms the previous link to blood group O. The new association to Le[a] could be explained by a link between mucosal microbiome and SARS-CoV-2.

KEYWORDS

ABO, blood antigen, blood groups, blood systems, COVID hospitalization, COVID severity, COVID susceptibility, COVID-19, Diego, Dombrock, Duffy, FUT2, FUT3, Kell, Kidd, Knops, Lewis, long COVID symptoms, long COVID-19, Lutheran, MNS, P1PK, Rh, SARS-CoV-2, secretor, Vel, Yt

1 | INTRODUCTION

Early in the SARS-CoV-2 pandemic, a link was reported between ABO blood type and infection susceptibility.[1] Evidence for this link has been mounting as size and quality of data have grown,[2–5] although a clear explanation for the mechanism behind this link has yet to be definitively established. One of the several explanations that has been put forward is a potential interaction between anti-A and/or anti-B antibodies and SARS-CoV-2 viral products.[6,7] This would explain why most studies conclude that ABO type O, with presence of both anti-A and anti-B antibodies in the blood, confers the lowest infection susceptibility.

Recent interest in SARS-CoV-2 and blood groups associations has moved beyond susceptibility, to focus on disease severity and symptom duration. While the ABO blood group continues to be the main focus of such investigations, some studies have ventured beyond and also looked at secretor status[8–10] and some of the many other recognized blood group systems, such as Rh[5,11–15] and Lewis.[10]

However, limitations of these previous studies include, for example, matching patient cases with blood donor controls[4,10] and small cohort sizes.[13,14,16,17] In addition, so far, few studies have looked at the effects of the many existing blood groups beyond ABO and Rh.

With access to data on sex, age, and SARS-CoV-2 tests results for 650,156 blood donors: Data on COVID-19 related hospitalization: Nearly complete blood type data available for ABO and RhD: Partial availability of blood type data for selected antigens from 13 other blood group systems, and secretor status; The present study aimed to address some of these shortcomings and investigated the influence of blood groups on SARS-CoV-2 susceptibility, COVID-19 hospitalization, and long COVID-19.

2 | MATERIALS AND METHODS

The nationwide and complete SCANDAT[18] cohort of Danish blood donors included 650,156 donors with at least one SARS-CoV-2 real-time polymerase chain reaction (RT-PCR) or antigen test registered in the Danish Microbiology Database (MiBa).[19] Sex, age, serological ABO, and RhD blood type data were available for almost all donors in the cohort. Data in other blood groups were only available for varying proportions of the donors (Table S1).

We identified 36,068 SARS-CoV-2 cases, defined as a blood donor with any positive RT-PCR or antigen test and 614,088 controls, defined as any blood donor with only negative test result entries in the MiBa dataset, covering the period between 2020-02-17 and 2021-08-04.

RT-PCR and antigen quick tests were a cornerstone in the Danish strategy to keep COVID-19 in check. Testing is free of charge and for the unvaccinated, a negative test result grants a temporary COVID-19 passport, which in periods has been necessary for certain social activities, like restaurant visits, attending events, etc. If an antigen quick test is positive, a PCR test is encouraged to confirm COVID-19, and upon confirmation, self-isolation is recommended. During 2020–2021, where the Danish vaccination program was slowly being rolled out, the Danish population was subject to frequent antigen and RT-PCR testing.

2.1 | Definitions of severe and long COVID-19

The MiBa dataset also included information on infection-related hospitalizations, defined as either not hospitalized, hospitalized for short duration of less than two

TABLE 1 Cohort demographic data

	COVID-(M)	COVID-(F)	COVID + (M)	COVID + (F)	Hospitalized (M)	Hospitalized (F)	Long-COVID (M)	Long-COVID (F)
Total	261,002 (44.6%)	323,778 (55.4%)	14,685 (43.5%)	19,089 (56.5%)	694 (55.6%)	554 (44.4%)	154 (35.4%)	281 (64.6%)
Median age [min:max]	52 [18:100]	47 [18:99]	46 [18:98]	42 [18:99]	63 [22:94]	54 [20:95]	50.5 [21:75]	48 [19:79]
ABO (A)	109,086 (41.8%)	133,932 (41.4%)	6620 (45.1%)	8393 (44%)	336 (48.4%)	243 (43.9%)	61 (39.6%)	123 (43.8%)
ABO (AB)	12,470 (4.8%)	15,838 (4.9%)	763 (5.2%)	1003 (5.3%)	46 (6.6%)	43 (7.8%)	8 (5.2%)	16 (5.7%)
ABO (B)	28,787 (11%)	36,651 (11.3%)	1621 (11%)	2180 (11.4%)	65 (9.4%)	57 (10.3%)	17 (11%)	26 (9.3%)
ABO (O)	110,659 (42.4%)	137,357 (42.4%)	5681 (38.7%)	7513 (39.4%)	247 (35.6%)	211 (38.1%)	68 (44.2%)	116 (41.3%)
Lewis (Lea-)	39,649 (80.8%)	40,313 (81.2%)	1563 (83.0%)	1681 (83.5%)	99 (82.5%)	62 (80.5%)	19	24 (77.4%)
Lewis (Lea+)	9429 (19.2%)	9357 (18.8%)	320 (17.0%)	331 (16.5%)	21 (17.5%)	15 (19.5%)	<5	7 (22.6%)
Lewis (Leb-)	9212 (26.8%)	8208 (26.8%)	303 (25.1%)	299 (27.0%)	26 (23.6%)	16 (26.2%)	<5	8 (32.0%)
Lewis (Leb+)	25,217 (73.2%)	22,413 (73.2%)	904 (74.9%)	807 (73.0%)	84 (76.4%)	45 (73.8%)	16	17 (68.0%)
Rh (D-)	45,923 (17.6%)	62,978 (19.4%)	2597 (17.7%)	3578 (18.7%)	131 (18.9%)	106 (19.1%)	29 (18.8%)	50 (17.8%)
Rh (D+)	215,216 (82.4%)	260,984 (80.6%)	12,086 (82.3%)	15,515 (81.3%)	563 (81.1%)	448 (80.9%)	125 (81.2%)	231 (82.2%)
Secretor (Se-)	9177 (20.0%)	9045 (19.8%)	561 (19.5%)	571 (19.4%)	20 (25.6%)	13 (23.6%)	20 (18.5%)	44 (22.6%)
Secretor (Se+)	36,782 (80.0%)	36,668 (80.2%)	2314 (80.5%)	2376 (80.6%)	58 (74.4%)	42 (76.4%)	88 (81.5%)	151 (77.4%)

Note: Demographic stats for the 618,554 donors in the cohort. Blood group is named first, then the antigen in question, and lastly positive (+) or negative (−) status for each antigen. Males (M) and females (F) have separate columns, as does COVID-19 status (COVID+/COVID−), COVID-19-related hospitalization (hospitalized), and long COVID-19 symptoms (long COVID-19).

weeks, hospitalized for more than two weeks, or hospitalized at intensive care unit (ICU) requiring respiratory support. Based on these additional registrations, 1317 *severe COVID-19* cases were identified, defined as any who were treated at a hospital for their infection, and 32,209 *severe COVID-19* controls, who were infected, but not treated at a hospital.

A total of 135,326 of the Danish blood donors had previously consented to participate in the Danish Blood Donor Study (DBDS).[20] A subgroup of 80,000 active DBDS donors was invited to fill out an online questionnaire on COVID-19 between 2020-10-30 and 2021-01-19. A total of 32,837 donors (41%) responded to the questionnaires. The questionnaire included items on specific COVID-19-related symptoms both at time of infection and, if persistent, at the time of filling out the questionnaire. These included, fever, chills, runny or stuffy nose, decreased sense of smell or taste, sneezing, sore throat, cough, shortness of breath, headache, muscle and joint pain, chest pain, tiredness and exhaustion, memory problems / difficulty concentrating, lack of appetite, colored sputum / mucus, reddened watery eyes, nausea, vomiting, diarrhea, and stomach pain. Based on the questionnaires, we identified 1578 COVID-19 cases. Of these, 441 were *long COVID-19* cases, defined as any respondent with a positive RT-PCR test who reported having any of the aforementioned COVID-19-related symptoms more than three months after a positive RT-PCR result.

2.2 | Blood group data

Blood group data were mainly sourced from a large dataset of serological blood type tests retrieved from electronic blood bank systems. In addition, some blood type data was generated using genetic data obtained using Infinium Global Screening Array from Illumina, with subsequent imputation at deCODE genetics, Reykjavik, Iceland, using North European reference sequence panel. Genetic data were available for ~100,000 DBDS participants.

All sourced blood type data, except for ABO, were stored in binary variables, as either positive (1) or negative (0), for a given RBC antigen. This binary encoding was used in the logistic regression models.

The ABO system blood types are categorized as A, B, AB, or O. ABO logistic regression models were performed using two alternate encodings of this data. First, we compared type O versus either A, B, or AB. However, since the prevailing theory on the mechanism of ABO and COVID-19 susceptibility implicates ABO antibodies interacting with the virus, an alternate encoding was used based on presence of the ABO antibodies like anti-A, anti-B, or anti-A + anti-B in the blood. These were encoded as binary variables indicating present (1) or absent (0).

Similar to most other antigen data, the Lewis blood group system was determined serologically for the Le^a and Le^b antigens separately and combined into the Lewis phenotypes Le(a+b-), Le(a-b+), and Le(a-b-). The Lewis blood group logistic models were first based on being either positive (1) or negative (0) for a given Lewis phenotype. This encoding was chosen due to a previous study[10] finding an association between having Lewis phenotype Le(a-b-) and lower COVID-19 severity. However, separate Le^a and Le^b RBC antigen models were also analyzed because there were more serological test results available for Le^a than Le^b (102,000 vs 67,000) to increase the statistical power.

Secretor status has known associations with susceptibility to infectious agents, including certain viruses.[21,22] Given prior viral associations, secretor status has been suspected to influence ABO COVID-19 susceptibility in other studies.[8,9] To verify these findings, logistic models were made accounting for a potential interaction between secretor status and the effect of ABO types on COVID-19 susceptibility.

TABLE 2 Sex & age COVID-19 associations

Model	COVID-19 association	N	N-diagnosis(+)	N-diagnosis(−)	OR (95% CI)	P-value
Age	susceptibility	650,156	36,068	614,088	0.977 (0.9766–0.9780)	~0
Sex (M/F)	susceptibility	650,156	36,068	614,088	1.044 (1.022–1.067)	9.365 E−05
Age	severity	33,526	1317	32,209	1.066 (1.062–1.070)	1.374 E−240
Sex (M/F)	severity	33,526	1317	32,209	1.356 (1.209–1.520)	1.840 E−07
Age	length	1578	441	1137	1.019 (1.010–1.027)	9.906 E−06
Sex (M/F)	length	1578	441	1137	0.669 (0.530–0.842)	6.532 E−04

Note: Cohort tally and results from the logistic regression model exploring the effect of sex and age on COVID-19 susceptibility, severity, and symptom length (above or below three months). N-diagnosis tallies the number of persons in the cohort with (+), or without (−) a given diagnosis. The diagnosis in question is denoted in the COVID-19 association column.

TABLE 3 ABO blood group COVID-19 associations

Model	COVID-19 association	N	N-phenotype (+)	N-phenotype (−)	N-diagnosis (+)	N-diagnosis (−)	OR (95% CI)	*p*-value
A versus O	Susceptibility	618,554			33,774	584,780	1.168 (1.140–1.197)	1.29 E−36
B versus O	Susceptibility	618,554			33,774	584,780	1.100 (1.060–1.141)	5.42 E−07
AB versus O	Susceptibility	618,554			33,774	584,780	1.196 (1.136–1.259)	7.85 E−12
A versus O	Severity	31,415			1248	30,167	1.069 (0.940–1.216)	.308
B versus O	*Severity*	31,415			1248	30,167	0.921 (0.745–1.130)	.438
AB versus O	*Severity*	31,415			1248	30,167	1382 (1.08–1.749)	8.43 E−3
A versus O	Length	1561			435	1126	0.957 (0.759–1.220)	.723
B versus O	Length	1561			435	1126	1.023 (0.683–1.511)	.910
AB versus O	Length	1561			435	1126	0.961 (0.572–1.570)	.878
Secretor (−)<−>A versus O	Susceptibility	97,494			5822	91,672	0.939 (0.812–1.084)	.388
Secretor (−)<−>B versus O	Susceptibility	97,494			5822	91,672	0.870 (0.683–1.100)	.250
Secretor (−) <−> AB versus O	Susceptibility	97,494			5822	91,672	0.705 (0.487–0.998)	.055
anti A & anti B	Susceptibility	618,554	261,245	357,309	33,774	584,780	0.864 (0.845–0.884)	5.09 E−37
anti A	Susceptibility	618,554	330,478	288,076	33,774	584,780	0.871 (0.852–0.891)	1.52 E−34
anti B	Susceptibility	618,554	519,250	99,304	33,774	584,780	0.960 (0.932–0.989)	6.61 E−03
anti A & anti B	Severity	31,415	12,229	19,186	1248	30,167	0.935 (0.828–1.054)	.273
anti A	Severity	31,415	15,775	15,640	1248	30,167	0.891 (0.793–1.001)	.052
anti B	Severity	31,415	26,201	5214	1248	30,167	0.968 (0.831–1.133)	.683
anti A & anti B	Length	1561	650	911	435	1126	1.033 (0.823–1.295)	.780
anti A	Length	1561	801	760	435	1126	1.049 (0.839–1.312)	.676
anti B	Length	1561	1320	241	435	1126	0.978 (0.721–1.340)	.888

Note: Cohort tally and results from the logistic regression model exploring the effect of ABO blood group, and ABO antibodies on COVID-19 susceptibility, severity, and symptom length. Model interaction (<−>) between ABO blood group COVID-19 susceptibility and secretor status are also included. N-diagnosis tallies the number of persons in the cohort with (+) or without (−) the given diagnosis. The diagnosis in question is denoted in the COVID-19 association column. N-phenotype tallies the number of persons with (+) or without (−) a given phenotype. The phenotype in question is denoted in the first column.

TABLE 4 Lewis blood group COVID-19 associations

Model	COVID-19 association	N	N-phenotype (+)	N-phenotype (−)	N-diagnosis (+)	N-diagnosis (−)	OR (95% CI)	*p*-value
Le(a-b−)	Susceptibility	62,833	5035	57,798	2137	60,696	1.066 (0.911–1.241)	.415
Le(a + b−)	Susceptibility	62,833	12,068	50,765	2137	60,696	0.880 (0.785–0.985)	2.86 E-02
Le(a-b+)	Susceptibility	62,833	45,730	17,103	2137	60,696	1.075 (0.975–1.187)	.151
Le^a	Susceptibility	102,643	19,437	83,206	3895	98,748	0.858 (0.787–0.935)	4.85 e−04
Le^b	Susceptibility	67,363	49,341	18,022	2313	65,050	1.047 (0.953–1.151)	.346
Le(a-b−)	Severity	2008	173	1835	157	1851	0.966 (0.503–1.715)	.910
Le(a + b−)	Severity	2008	345	1663	157	1851	0.933 (0.581–1.446)	.763
Le(a-b+)	Severity	2008	1490	518	157	1851	1.068 (0.730–1.595)	.740
Le^a	Severity	3652	602	3050	197	3455	1.065 (0.712–1.553)	.752
Le^b	Severity	2171	1608	563	171	2000	1.098 (0.760–1.614)	.626
Le(a-b−)	Length	106	9	97	40	66		
Le(a + b−)	Length	106	22	84	40	66	1.052 (0.383–2.781)	.920
Le(a-b+)	Length	106	75	31	40	66	2.187 (0.866–5.932)	.108
Le^a	Length	175	25	150	53	122	1.477 (0.591–3.565)	.390
Le^b	Length	115	82	33	43	72	1.978 (0.812–5.121)	.144

Note: Cohort tally and results from the logistic regression model exploring the effect of Lewis blood group on COVID-19 susceptibility, severity, and symptom length. Two models are used: one measuring the effect of each of the three Lewis phenotypes Le(a + b−) Le(a-b+) Le(a-b−), and one measuring the effect of the two Lewis antigens Le^a and Le^b. N-diagnosis tallies the number of persons in the cohort with (+) or without (−) the given diagnosis. The diagnosis in question is denoted in the COVID-19 association column. N-phenotype tallies the number of persons with (+) or without (−) a given phenotype. The phenotype in question is denoted in the first column.

All secretor status data in our study was genetically derived using variant rs601338,[23] while all Lewis, ABO, and RhD data were based on serological tests. The remaining blood groups were a mix of both genetic and serological sources (Table S2).

3 | STATISTICS

All data analysis was performed using the glm function in R v4.0.0. Logistic regression models were used in all statistical analyses reporting odds ratios (ORs) with 95% confidence intervals (CI). All logistic regression model results are reported with having a positive phenotype as the dependent/outcome variable. All logistic regression models are adjusted for sex and age.

p-values for models in blood groups with prior published COVID-19 associations (ABO, secretor, Lewis, RhD) were not adjusted for multiple testing, as the tests were assessed as confirming prior findings. For the remaining analyses, the false discovery rate was set using the Benjamini & Hochberg procedure (fdr).[13]

4 | RESULTS

The demographic data are presented in Table 1. SARS-CoV-2 cases were on average five years younger than the controls and the hospitalized SARS-CoV-2 cases were on average eight years older than those that were not hospitalized. The OR of infection susceptibility with increasing age was 0.98 CI[0.977–0.978] $p < 2\,e{-}16$, for each year increase in age. Infection severity and long COVID-19 were both strongly associated with each year of increasing age, with ORs of 1.07 CI[1.06.-1.07] $p = 1.37\,e{-}240$ and 1.02 CI[1.01–1.03] $p = 9.91\,e{-}06$, respectively. There was a strong association with sex, with males having a higher susceptibility OR of 1.04 CI[1.02–1.07] $p = 9.36\,e{-}05$, a higher severity OR of 1.36 CI[1.2–1.52] $p = 1.84\,e{-}07$, and a lower long COVID-19 OR of 0.67 CI[0.53–0.84] $p = 6.53\,e{-}04$. (Table 2).

A difference in ABO blood type ratios was seen between SARS-CoV-2 cases and controls with the latter having approximately 4% higher occurrence of blood type O, and lower occurrence of the other ABO blood types (Table 1). Individuals with blood types B, A, and AB were found more susceptible to SARS-CoV-2 with ORs of 1.10 CI[1.06–1.14] $p = 5.42\,e{-}07$, 1.17 CI[1.14–1.20] $p = 1.29\,e{-}36$, and 1.20 CI[1.14–1.26] $p = 7.85\,e{-}12$, respectively, when compared to blood type O. ABO antibody-specific models revealed strong SARS-CoV-2 protective effects for presence of both anti-A + anti-B in blood, only anti-A in blood or only anti-B in blood, with ORs 0.86 CI[0.84–0.88] $p = 5.85\,e{-}37$, 0.87 CI[0.85–0.89] $1.52\,e{-}34$, and 0.95 CI[0.93–0.99] $p = 6.61\,e{-}03$, respectively (Table 3). We performed a sensitivity analysis revealing that the significance of the finding could be eliminated ($p > .05$) by reducing the ABO cohort size down below 1.2% (~7000 donors); however, the ORs remain higher for A, B, and AB compared to O.

We found no evidence for an interaction between genetically determined secretor status and higher infection susceptibility of ABO blood types A, B, or AB, $p > .05$ (Table 3).

An association was found between SARS-CoV-2 positives and the non-secretor Lewis phenotype Le(a+b−) with OR 0.88 CI[0.78–0.99] $p = 0.029$, whereas phenotypes Le(a-b+) and Le(a-b−) were not associated, $p > .05$. The Lewis Le^a antigen-specific model resulted in a stronger association with OR 0.85 CI[0.79–0.93] $p = 4.85\,e{-}04$, while the Le^b RBC antigen model was insignificant $p > .05$ (Table 4). No infection severity associations were found for Lewis phenotypes Le(a+b-), Le(a-b+), and Le(a-b−), and results did not change when using the Lewis RBC antigen-specific models, $p > .05$. Furthermore, no association between Lewis types Le(a+b−) and Le(a-b+) and long COVID-19 were found. There was insufficient data for a Le(a-b−) model. The Lewis antigen-specific models did not shift the results (Table 4).

Secretor status by itself was neither associated with infection susceptibility, disease severity, nor long COVID-19, $p > .05$ (Table 5).

TABLE 5 Secretor status COVID-19 associations

Model	COVID-19 association	N	N-phenotype (+)	N-phenotype (−)	N-diagnosis (+)	N-diagnosis (−)	OR (95% CI)	*p*-value
Secretor	susceptibility	97,494	78,140	19,354	5822	91,672	1.035 (0.968–1.107)	.318
Secretor	Severity	5430	4379	1051	133	5297	0.680 (0.458–1.033)	.14 E−02
Secretor	Length	1069	862	207	303	766	1.140 (0.813–1.586)	.441

Note: Cohort tally and results from the logistic regression model exploring the effect secretor status on COVID-19 susceptibility, severity, and symptom length. N-diagnosis tallies the number of persons in the cohort with (+) or without (−) the given diagnosis. The diagnosis in question is denoted in the COVID-19 association column. N-phenotype tallies the number of persons with (+) or without (−) a given phenotype. The phenotype in question is denoted in the first column.

After adjusting for multiple testing, no blood group antigens or phenotypes beyond ABO and Lewis gave any significant associations to any of the outcomes tested (Tables S3 and S4).

5 | DISCUSSION

This large nationwide Danish study on SARS-CoV-2 and blood groups confirmed previous reports of type O being least susceptible to infection. This study further reports a new association suggesting that those who have the Le^a blood group antigen are also less susceptible to infection. Since the presence of Le^a antigen signals a non-secretor phenotype, this association is well compatible with previous reports suggesting increased disease severity for secretors[8] on one hand and decreased susceptibility to disease for non-secretors, at least for blood group A.[9]

In accordance with previous large studies, we found support for type AB having the highest OR for infection susceptibility.[2,5] However, AB is the rarest ABO type with a 5% prevalence in the Danish population. This is reflected in a wider confidence interval for AB compared to the more common type A, which has a high prevalence of 42% in the Danish population.

There have been conflicting reports regarding ABO blood type and SARS-CoV-2 susceptibility. One study has pointed to AB being least susceptible,[24] while most studies seem to agree that type O is least susceptible to infection.[2,3,5–9,11,13,15] Conflicting results may well reflect the small sample sizes for the majority of published studies or because of the setting of the studies. Some studies have much higher exposure risk and inoculum sizes than others.[25,26] Thus, as previous studies have speculated, the protective effect of ABO-antibodies on SARS-CoV-2 susceptibility could be eliminated with increasing exposure.[27]

Another source of conflicting results could be due to cohort mixing, such as case–control studies where cases are COVID-19 patients from the general population, while the controls are blood donors.[10,4] Blood bank cohorts are known to have different blood type ratios when compared to the general population. This is a known phenomenon due to blood banks having biases toward certain blood types, deemed more useful for transfusions, giving rise to a slight overrepresentation of blood donors with the desired blood types.[28]

In regards to the mechanism behind the association, the theory of ABO antigens facilitating SARS-CoV-2 entry is contented.[29] When considering the alternate theory of ABO antibodies interacting with the SARS-CoV-2 virus,[7,26] the results of the ABO antibody centric models make more sense. The hypothesis is that anti-A has a stronger protective effect, providing subsequent lower susceptibility, when compared to anti-B. Supporting this notion, having both anti-A and anti-B showed the lowest susceptibility in our data, benefiting from a hypothesized two-way antibody attack on the virus. A third theory currently under investigation relates to iron levels, which have been linked to ABO blood type,[30] and play an important role in the immune system.[31]

A potential confounder is admixture of blood donors of other ethnicities than Danish (approximately 4% of the donors) who might have different blood type ratios than the Danish population. In addition, a higher infection rate has been reported among these, which we confirmed by observing a 1.8% higher incidence of infection among donors with a birth country other than Denmark in our cohort. The majority of our foreign donors are European, mainly from Germany and Scandinavia. To verify that non-European donors did not bias the results, we removed persons born outside Europe (1.8%) and repeated the analysis. This did not impact the *p*-values, or ORs of the associations (Table S5).

Secretor status is determined by the *FUT2* gene, where individuals with at least one functional allele, called secretors, produce the α1-2-fucosyltransferase 2 enzyme. This enzyme has a crucial role to play in determining if ABH antigens are present in body fluid of the individual. Furthermore, Lewis phenotypes are determined by both the *FUT2* and *FUT3* genes. Having at least one functional *FUT3* allele leads to the production of an α1-3/4-fucosyltransferase which synthesizes the Le^a and Le^b antigens from type 1 (Le^c) precursor and H type 1 (Le^d) glycolipids, respectively. In the absence of a functional *FUT2*-encoded enzyme, the *FUT3*-encoded enzyme will convert almost all precursor into Le^a and result in the Le(a + b−) non-secretor phenotype, while its presence will result in the Le(a-b+) secretor phenotype. The absence of a functional *FUT3* allele on the other hand results in the Le(a-b−) phenotype regardless of the *FUT2*-derived enzyme.

Judging from our results, there seem to be a protective effect associated with having the Le^a blood group antigen. This is not related to Lewis antibodies interacting with SARS-CoV-2, in a similar manner to the one supposed in the ABO blood group, since Lewis antibodies are irregular, rare, and almost exclusively found in Le(a-b−) individuals,[32] which are also the rarest phenotype (8%). In our models, Le(a-b−) individuals did not have a different infection susceptibility, which also does not support involvement of Lewis antibodies.

In the Lewis blood group, the Le^a and Le^b antigens are mostly mutually exclusive. Therefore, there is a chance that Le^b is actually conferring a higher infection susceptibility, but remains insignificant in our model because we have fewer Le^b antigen test results. In that

case, the protective effect of Le^a in our model might just be due to Le^a excluding Le^b. If we enrich our data with hypothetical antigen tests where each positive Le^a test would generate a negative Le^b test and vice versa, and rerun the Le^a and Le^b models, then both will indeed become statistically significant, with opposing ORs as expected. This would indeed mimic the studies which have indicated non-secretors to be protected and secretors to be worse off.[8,9] However, we choose to base our Lewis results on actual serological test data, so the enriched data models aren't included in this study.

The mutually exclusive nature of Le^a and Le^b makes it difficult to conclude which antigen is directly responsible for our SARS-CoV-2 infection susceptibility findings, or if they merely signal a certain secretor or non-secretor status.

However, the secretor model does not report such an association. This could be due to 8% of our cohort having Lewis phenotype Le(a-b-), for whom secretor status is not a factor. These 8% could reduce the link between Lewis phenotype and secretor status enough to require a much larger cohort to reach above the significance threshold. It is worth noting that, although not significant, the secretor susceptibility model does report an elevated OR for secretors, which is what we would expect. Since both the secretor and Lewis models have large cohorts, the fact that Lewis is significant, but secretor is not, hints that susceptibility might be more directly linked to Lewis phenotype, and not secretor status. While other published studies have reported links between SARS-CoV-2 infection and secretor status,[8–10] our cohort size for both Lewis and secretor status dwarfs them all by a substantial degree.

It is worth noting that there are known cases of viruses and bacteria using Lewis antigens as receptors for cell invasion, with resulting higher susceptibility to the disease in question. Lewis antigens aren't produced by the red blood cells themselves, but rather secreted by epithelial cells, and subsequently absorbed passively onto the RBC surface. The specific binding of norovirus virus-like particles to ABO and Lewis antigens has been demonstrated.[33] Norovirus uses secreted Lewis antigens for attachment to human epithelial cells.[34–36] This favors a theory of Le^b perhaps acting as a mediator for SARS-CoV-2 infection in a similar manner. In addition, both Lewis and secretor status associate to microbiota of the mucosal surfaces which could explain the association to infection susceptibility.[37,38] The present results provide sufficient evidence of a potential link between SARS-CoV-2 and the Lewis blood group to warrant future investigations exploring the association in greater detail and testing these theories experimentally.

We did not find any other associations between blood groups and SARS-CoV-2. This questions the validity of previous findings of an association to the Rh blood group.[11,12,14,15]

Two COVID-19 severity results worth discussing are blood type O vs AB, which was significant ($p = .008$), and secretor status, which was close to being significant ($p = .06$). Blood type AB is a rare phenotype (5%), which reduces confidence in that result. It's also worth noting that type A and B, which are more common phenotypes, do not report significant results for COVID-19 severity. Some meta-analysis studies report a potential link between COVID-19 severity and ABO phenotype,[6] while others do not.[2] The secretor/severity cohort is both small, and has an OR of 0.68, which is in conflict with other studies.[8,9] Given the above facts, we do not feel confident in interpreting these findings either way.

5.1 | Strength and limitations

This study benefits greatly from having access to a large, national cohort. The SARS-CoV-2 test results were registered by a centralized authority, which circumvents potential confounding biases. The entire cohort, being composed of blood donors, ensures a degree of homogeneity among cases and controls, thereby circumventing other potential confounders including, for example, undetected SARS-CoV-2 cases.[2] We have adjusted for the most common confounders in the analysis.

There are arguments to be made in favor of including other covariates often used in such association studies, and one such covariate is Charlson comorbidity score.[39] However, it could be argued that while the inclusion of a Charlson score might be advantageous in studies of normal background population cohorts, it is not likely to make much of a difference for a blood donor study. Blood donors are known to be healthier than the background population, in a phenomenon called the healthy donor effect.[40] In an earlier published study,[41] only 3.5% of 37,808 DBDS participants were found to have a Charlson score above 0.

The fact that blood donors are known to be healthier than the background population, likely makes obtaining sufficient statistical strength for severity and long COVID-19 studies more difficult. As such, while we can be reasonably assured that our findings regarding severity and long COVID-19 are internally consistent within blood donors, the higher healthiness of such a cohort might cast doubts on the external validity of these results when applied to the general population. This could potentially be why we could not replicate the findings of other smaller studies.[8,9,13,42] However, the differences in our findings might also be due to different thresholds for

admitting patients to hospitals, and in the way we define COVID-19 severity.

5.2 | Conclusion

In this study, we found strong associations between ABO and Lewis blood groups and SARS-CoV-2 susceptibility, whereas the other investigated blood group systems did not display such an association. Larger studies of these blood groups might yet find other SARS-CoV-2 associations; however, these are expected to be small given the large sample sizes in our study.

In our long COVID-19 and disease severity models, the cohort sizes were comparatively small and could warrant bigger cohort studies in the future for a more definite conclusion regarding links to COVID-19. This is especially true for the Lewis blood group and secretor status.

AUTHOR CONTRBUTIONS

This study was conceived, the data analysis was performed, and the initial paper draft was written by the corresponding author C. Moslemi. Martin L Olsson, Sisse R Ostrowski, Rune Larsen, Susanne Sækmose, and Ole B Pedersen have contributed with expert knowledge, advice, and direction during the progress and writing of the study. Susanne Sækmose, Thorsten Brodersen, Maria Didriksen, Henrik Hjalgrim, Karina Banasik, Kaspar R Nielsen, Mie T Bruun, Joseph Dowsett, Kathrine A Kasperen, Susan Mikkelsen, Thomas F Hansen, Henrik Ullum, and Christian Erikstrup have established the blood donor cohorts, collected the data, and established the infrastructure used in this study. Additionally, all coauthors have contributed to the final editing phase of the paper writing process with corrections, feedback, and suggestions.

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CONFLICT OF INTEREST

Martin L Olsson and his spouse are inventors on patents about Vel blood group genotyping and own 50% each of the shares in BLUsang AB, an incorporated consulting firm which receives royalties for said patents. They are both co-authors of AABB books and members of the Transfusion editorial board. Maria Didriksen received consultant fee for helping with a study tracking COVID-19 infection among Falck Health Care Workers, which has no connection to the present study. All other authors declare no conflict of interest.

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REFERENCES

1. Zhao J, Yang Y, Huang H, et al. Relationship between the ABO blood group and the COVID-19 susceptibility. medRxiv. 2020;19:54–63. https://doi.org/10.1101/2020.03.11.20031096
2. Gutiérrez-Valencia M, Leache L, Librero J, Jericó C, Enguita Germán M, García-Erce JA. ABO blood group and risk of COVID-19 infection and complications: a systematic review and meta-analysis. Transfusion. 2021;62:493–505. https://doi.org/10.1111/trf.16748
3. Zhang Y, Garner R, Salehi S, La Rocca M, Duncan D. Association between ABO blood types and coronavirus disease 2019 (COVID-19), genetic associations, and underlying molecular mechanisms: a literature review of 23 studies. Ann Hematol. 2021;100:1123–32.
4. Ellinghaus D, Degenhardt F, Bujanda L, et al. Genomewide association study of severe Covid-19 with respiratory failure. N Engl J Med. 2020;383:1522–34. https://doi.org/10.1056/NEJMoa2020283
5. Barnkob MB, Pottegård A, Støvring H, Haunstrup TM, Homburg K, Larsen R, et al. Reduced prevalence of SARS-CoV-2 infection in ABO blood group O. Blood Adv. 2020;4:4990–3.
6. Goel R, Bloch EM, Pirenne F, al-Riyami AZ, Crowe E, Dau L, et al. ABO blood group and COVID-19: a review on behalf of the ISBT COVID-19 working group. Vox Sang. 2021;116:849–61.
7. Yamamoto F, Yamamoto M, Muñiz-Diaz E. Blood group ABO polymorphism inhibits SARS-CoV-2 infection and affects COVID-19 progression. Vox Sang. 2020;116:15–7. https://doi.org/10.1111/vox.13004
8. Valenti L, Villa S, Baselli G, Temporiti R, Bandera A, Scudeller L, et al. Association of ABO blood group and secretor phenotype with severe COVID-19. Transfusion. 2020;60:3067–70.
9. Mankelow TJ, Singleton BK, Moura PL, Stevens-Hernandez CJ, Cogan NM, Gyorffy G, et al. Blood group type a secretors are associated with a higher risk of COVID-19 cardiovascular disease complications. EJHaem. 2021;2:175–87.
10. Matzhold EM, Berghold A, Bemelmans MKB, Banfi C, Stelzl E, Kessler HH, et al. 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. Transfusion. 2021;61:2736–45.

11. Donskov SI, Bulanov AY, Simarova IB, Belyakova VV, Mayorova OA, Kravtsova EA, et al. AB0 and rhesus blood groups as a risk factor for ARVI COVID-19. Klin Lab Diagn. 2021;66:661–5.
12. Anderson JL, May HT, Knight S, Bair TL, Horne BD, Knowlton KU. Association of Rhesus factor blood type with risk of SARS-CoV-2 infection and COVID-19 severity. Br J Haematol. 2022;197:573–5.
13. Behboudi E, Hamidi V, Gholizadeh F, Grala EM, Ghelmani Y, Nakhaie M, et al. Association between ABO blood groups and rhesus antigen and susceptibility to COVID-19 in the Yazd hospital. New Microbes New Infect. 2021;44:100934.
14. Kerbage A, Haddad SF, Nasr L, Riachy A, Mekhael E, Nassim N, et al. Impact of ABO and rhesus blood groups on COVID-19 susceptibility and severity: a case-control study. J Med Virol. 2021;94:1162–6. https://doi.org/10.1002/jmv.27444
15. Ray JG, Schull MJ, Vermeulen MJ, Park AL. Association between ABO and Rh blood groups and SARS-CoV-2 infection or severe COVID-19 illness: a population-based cohort study. Ann Intern Med. 2021;174:308–15.
16. Hermel DJ, Spierling Bagsic SR, Costantini CL, Mason JR, Gahvari ZJ, Saven A. ABO phenotype and clinical correlates of COVID-19 severity in hospitalized patients. Future Sci OA. 2021;7, FSO735. DOI: 10.3389/fmicb.2021.743048
17. Greco S, Fabbri N, Bella A, Bonsi B, Violi A, Fortunato V, et al. COVID-19 and blood groups: a six-months observational study in Ferrara, Italy. Hematol Rep. 2021;13:9177.
18. Edgren G, Rostgaard K, Vasan SK, Wikman A, Norda R, Pedersen OB, et al. The new Scandinavian donations and transfusions database (SCANDAT2): a blood safety resource with added versatility. Transfusion. 2015;55:1600–6.
19. Schønning K, Dessau RB, Jensen TG, Thorsen NM, Wiuff C, Nielsen L, et al. Electronic reporting of diagnostic laboratory test results from all healthcare sectors is a cornerstone of national preparedness and control of COVID-19 in Denmark. APMIS. 2021;129:438–51.
20. Pedersen OB, Erikstrup C, Kotzé SR, Sørensen E, Petersen MS, Grau K, et al. The Danish blood donor study: a large, prospective cohort and biobank for medical research. Vox Sang. 2012;102:271.
21. Kindberg E, Hejdeman B, Bratt G, Wahren B, Lindblom B, Hinkula J, et al. A nonsense mutation (428G-->a) in the fucosyltransferase FUT2 gene affects the progression of HIV-1 infection. AIDS. 2006;20:685–9.
22. Rydell GE, Kindberg E, Larson G, Svensson L. Susceptibility to winter vomiting disease: a sweet matter. Rev Med Virol. 2011;21:370–82.
23. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB. Sequence and expression of a candidate for the human secretor blood group alpha(1,2)fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem. 1995;270:4640–9.
24. Kabrah SM, Kabrah AM, Flemban AF, Abuzerr S. Systematic review and meta-analysis of the susceptibility of ABO blood group to COVID-19 infection. Transfus Apher Sci. 2021;60:103169.
25. Boudin L, Janvier F, Bylicki O, Dutasta F. ABO blood groups are not associated with the risk of acquiring SARS-CoV-2 infection in young adults. Haematologica. 2020;105:2841–3.
26. Ellis PJI. Modelling suggests ABO histo-incompatibility may substantially reduce SARS-CoV-2 transmission. Epidemics. 2021;35:100446.
27. Pendu JL, Breiman A, Rocher J, Dion M, Ruvoën-Clouet N. ABO blood types and COVID-19: spurious, anecdotal, or truly important relationships? A reasoned review of available data. Viruses. 2021;13:160.
28. Golding J, Northstone K, Miller LL, Davey Smith G, Pembrey M. Differences between blood donors and a population sample: implications for case-control studies. Int J Epidemiol. 2013;42:1145–56.
29. Schetelig J, Baldauf H, Wendler S, Heidenreich F, Real R, Kolditz M, et al. Blood group a epitopes do not facilitate entry of SARS-CoV-2. J Intern Med. 2021;290:223–6. https://doi.org/10.1111/joim.13256
30. Rigas AS, Berkfors AA, Pedersen OB, Sørensen E, Nielsen KR, Larsen MH, et al. Reduced ferritin levels in individuals with non-O blood group: results from the Danish blood donor study. Transfusion. 2017;57:2914–9.
31. Haschka D, Hoffmann A, Weiss G. Iron in immune cell function and host defense. Semin Cell Dev Biol. 2021;115:27–36.
32. Cohn CS, Delaney M, Johnson ST. Katz LM. Technical Manual. 2020:aaBB.
33. Nilsson J, Rydell GE, Le Pendu J, Larson G. Norwalk virus-like particles bind specifically to a, H and difucosylated Lewis but not to B histo-blood group active glycosphingolipids. Glycoconj J. 2009;26:1171–80.
34. Lozniewski A, Haristoy X, Rasko DA, Hatier Ŕ, Plénat Ţ, Taylor DE, et al. Influence of Lewis antigen expression by helicobacter pylori on bacterial internalization by gastric epithelial cells. Infect Immun. 2003;71:2902–6.
35. Ruvoën N, Le Pendu J. Genetic susceptibility to norovirus infection. Pathol Biol (Paris). 2013;61:28–35.
36. Schwartz S, Vergoulidou M, Schreier E, Loddenkemper C, Reinwald M, Schmidt-Hieber M, et al. Norovirus gastroenteritis causes severe and lethal complications after chemotherapy and hematopoietic stem cell transplantation. Blood. 2011;117:5850–6.
37. Jabczyk M, Nowak J, Hudzik B, Zubelewicz-Szkodzińska B. Microbiota and its impact on the immune system in COVID-19-a narrative review. J Clin Med. 2021;10:4537.
38. de Oliveira GLV, Oliveira CNS, Pinzan CF, de Salis LVV, Cardoso CR d B. Microbiota modulation of the gut-lung Axis in COVID-19. Front Immunol. 2021;12:635471.
39. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40:373–83.
40. Atsma F, Veldhuizen I, Verbeek A, de Kort W, de Vegt F. Healthy donor effect: its magnitude in health research among blood donors. Transfusion. 2011;51:1820–8.
41. Kaspersen KA, Pedersen OB, Petersen MS, Hjalgrim H, Rostgaard K, Møller BK, et al. Obesity and risk of infection: results from the Danish blood donor study. Epidemiology. 2015;26:580–9.

42. Niavarani A, Poustchi H, Shayanrad A, Sharafkhah M, Mohammadi Z, Mansour-Ghanaei F, et al. Dynamics of the COVID-19 clinical findings and the serologic response. Front Microbiol. 2021;12. DOI: 10.3389/fmicb.2021.743048

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