DONOR INFECTIOUS DISEASE TESTING

TRANSFUSION

Trends and risk factors of SARS-CoV-2 infection in asymptomatic blood donors

Luca Valenti[1,2] | Serena Pelusi[2] | Alessandro Cherubini[2] |
Cristiana Bianco[2] | Luisa Ronzoni[2] | Sara Uceda Renteria[3] |
Elena Coluccio[2] | Alessandra Berzuini[2] | Angela Lombardi[2] |
Leonardo Terranova[4] | Francesco Malvestiti[1] | Giuseppe Lamorte[2] |
Elisa Erba[2] | Massimo Oggioni[3] | Ferruccio Ceriotti[3] | Daniele Prati[2]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milano, Italy

[2]Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, Milano, Italy

[3]Clinical Laboratory, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, Milano, Italy

[4]Respiratory Diseases, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, Milano, Italy

Correspondence
Luca Valenti, Department of Pathophysiology and Transplantation, Università degli Studi di Milano Precision Medicine, Milano, Italy.
Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via F Sforza 35, 20122, Milan, Italy.
Email: luca.valenti@unimi.it

Daniele Prati, Department of Transfusion Medicine and Hematology, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, via F Sforza 35, 20122, Milan, Italy.
Email: daniele.prati@policlinico.mi.it

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Abstract

Background: A large proportion of SARS-CoV-2-infected individuals does not develop severe symptoms. Serological tests help in evaluating the spread of infection and disease immunization. The aim of this study was to prospectively examine the trends and risk factors of SARS-CoV-2 infection in blood donors.

Study design and methods: We screened 8798 asymptomatic donors presenting in Milan from July 2020 to February 2021 (10,680 presentations) before the vaccination campaign for anti-nucleoprotein (NP) antibodies, and for anti-spike receptor-binding domain (RBD) antibodies and nasopharyngeal swab PCR in those who tested positive.

Results: The prevalence of anti-NP+/RBD+ tests increased progressively with time up to ~15% ($p < .0001$), preceded by a peak of PCR+ tests. Anti-RBD titers were higher in anti-NP IgG+/IgM+ than in IgG+/IgM− individuals and in those with a history of infection ($p < .0001$); of these 197/630 (31.2%) displayed high titers (>80 AU/ml). Anti-RBD titers declined during follow-up, depending on baseline titers ($p < .0001$) and time ($p = .025$). Risk factors for seroconversion were a later presentation date and non-O ABO blood group ($p < .001$). A positive PCR was detected in 0.7% of participants in the absence of SARS-CoV-2 viremia.

Abbreviations: BMI, body mass index; LFIA, lateral flow immunoassay; NP, nucleoprotein; NPS, nasopharyngeal swab; RBD, receptor binding domain

Conclusions: During the second wave of SARS-CoV-2 infection in Northern Italy, we detected an increase in seroprevalence in healthy blood donors from ~4% to ~15%, with a trend paralleling that observed in the general population. Seroconversion was more frequent in carriers of non-O blood groups. The persistence of anti-RBD antibodies was short-lived.

KEYWORDS

ABO blood group, anemia, COVID-19, epidemiology, ferritin

1 | INTRODUCTION

Since the beginning of the pandemic SARS-CoV-2, the virus that causes COVID-19 disease[1] has caused more than 203 million confirmed cases and 4.3 million deaths.[2] Italy was one of the first countries to be severely involved, and >6% of the population has been diagnosed with SARS-CoV-2 infection by reference molecular tests, with an overall 3% mortality rate.[3] Respiratory failure is the most frequent severe manifestation of COVID-19 and in Northern Italy was associated with a ~ 26% mortality rate in critically ill patients.[4] However, some individuals develop an asymptomatic or mildly symptomatic infection,[5] and there is still little agreement about epidemiological trends and extent of disease transmission in these subjects. Depending on the population considered, the estimate of asymptomatic infections ranges approximately from 13 to 31%.[5–7] These subjects can transmit SARS-CoV-2 infection, although overall evidence suggests that they are responsible for fewer secondary infections than people with symptoms.[6,8]

The COVID-19 pandemic had a major impact on the supply and use of blood transfusions.[9] Serological testing against SARS-CoV-2 plays a major role in evaluating the spread of COVID-19 and the extent of disease immunization, and may also help to collect hyperimmune plasma donations, which may have therapeutic application in selected cases.[9] We recently retrospectively examined trends in the prevalence of SARS-CoV-2 antibody reactivity among healthy blood donors during the initial outbreak of COVID-19 in Milan.[10] Briefly, we showed that SARS-CoV-2 infection was already circulating in Milan at the outbreak start. By the end of April 2020, 2.4%–9.0% of healthy adults had evidence of seroconversion.[10]

The aim of this study was to prospectively examine the trends and risk factors of SARS-CoV-2 infection in a cohort of asymptomatic individuals during the second COVID-19 wave in Milan, from July 2020 to March 2021, before the beginning of the mass vaccination campaign in Italy. Secondary outcomes were the uptake rate of donations from individuals with high titers of neutralizing antibodies and blood safety.

2 | MATERIALS AND METHODS

2.1 | Study cohort

Participants were asymptomatic repeat blood donors (age 18–70 years), enrolled within the prospective section second phase of the Fondazione COVID-19 Donors Study (CoDS), presenting for blood donation at the Milan Blood Center.[10] The study flowchart is presented in Figure 1. We considered 8798 individuals, who were evaluated at least once during the prospective phase of the project from July 1, 2020 to February 23, 2021, covering the second wave of SARS-

FIGURE 1 Study flowchart. CoDS, Fondazione IRCCS Ca′ Granda COVID-19 Donors Study; NPS, nasopharyngeal swab; RBD, receptor-binding domain
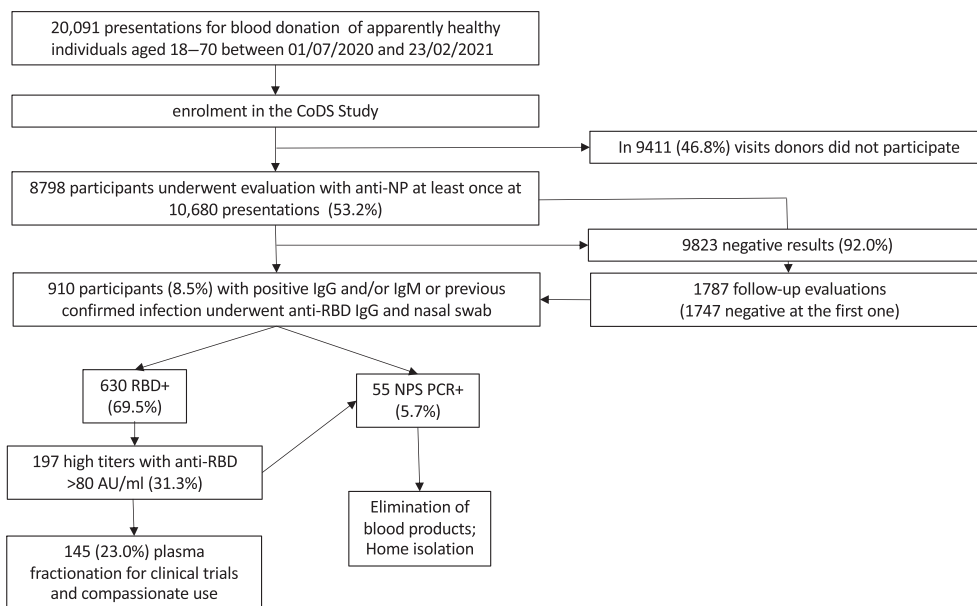

TABLE 1 Demographic and clinical features of 8798 participants to the CoDS study, who presented between July 1, 2020 to February 23, 2021, stratified by the number of evaluations

	One		Two		Three		
	N/mean	%/SD	*N*/mean	%/SD	*N*/mean	%/SD	*p*-value
N=	7031	80.0%	1652	18.7%	115	1.3%	
Sex, F	2821	40.1%	284	17.2%	4	3.5%	<.0001
Age, years	42.4	13.5	46.0	12.6	48.3	10.8	<.0001
BMI, kg/m^2	24.3	3.4	25.0	3.2	26.1	3.2	<.0001
Hypertension, yes	440	6.3%	143	8.7%	18	15.6%	<.0001
Residence, Milan	4432	63.0%	1035	62.6%	73	63.5%	.82
Blood group, non-O	3670	51.8%	907	56.3%	58	47.9%	.47
COVID-19 history, yes	28	0.4%	10	0.6%	1	0.8%	.20
Time after enrolment, weeks	0	0	17.8	4.7	24.5	2.0	<.0001

Note: p-values were determined at ordinal regression models. *N* or means and % or SD were reported for categorical and continuous variables, respectively.

CoV-2 infection outbreak in Italy. Their clinical features are presented in Table 1.

In addition to standard criteria, to qualify for a blood donation the candidates should have not been infected by SARS-CoV-2, have symptoms possibly related to infection, or have been in close/unprotected contact with infected individuals in the preceding 14 days.[10] The minimum interdonation interval was 12 weeks.

The study protocol complies with the Declaration of Helsinki and was approved by the Ethical Committee of the Fondazione IRCCS Ca′ Granda ("COVID-19 Donors Study," CoDS), n.334-2020 on April 3, 2020, and all participants signed a written informed consent to the CoDS study.

2.2 | Analytical procedures

The presence of anti-nucleoprotein (NP) IgG and IgM antibodies was evaluated in whole blood samples by the anti-NP rapid lateral flow immunoassay (LFIA, COVID-19 IgG/IgM Rapid Test, PrimaLab, CH).[10] Reactivity was measured semiquantitatively by automated optical densitometry (Igloo™ + Dx Car, Experiment X Germany GmbH, DE); IgG values >50% and IgM values >10% of a reference positive control were considered positive. This threshold has 100% sensitivity and 94% specificity to detect anti-IgG antibodies in 20 patients at the time at hospital discharge versus 100 historical controls. Patients with positive tests, and those with previous confirmed infection, underwent evaluation of anti-Spike S1/S2

antigens of receptor-binding domain (RBD) IgG antibodies by a chemiluminescence immunoassay (LIAISON® SARS-CoV-2 S1/S2 IgG assay, LFIA Diasorin, Italy).[11,12] The titers of these antibodies correlated with the serum neutralizing activity titer against SARS-CoV-2.[11,12] Anti-RBP titers >12 AU/ml were considered positive, whereas those with titers >80 AU/ml ("high titers") were selected for fractionation of plasma, in line with indications of the local health authorities. For the present analysis, samples showing combined reactivity for both anti-NP and anti-RBD tests (anti-NP+/RBD+) were considered true positive. Participants who tested positive to the rapid anti-NP tests were screened for the presence of upper respiratory airways SARS-CoV-2 RNA on nasopharyngeal swabs (NPS) by the Alinity m-SARS-CoV-2 assay on Alinity (Abbott Molecular, Rome, Italy), Allplex SARS-CoV-2 Assay (Seegene, Seoul, South Korea), and GeneFinder COVID-19 Plus RealAmp kit on ELITe InGenius platform (ELITechGroup, Torino, Italy). Determination of viremia was performed by the SARS-CoV-2 ELITe MGB Kit on ELITe InGenius platform on plasma samples of the subset of donors with positive NPS since January 2021; sensitivity was 100 copies/ml.

2.3 | Statistical analysis

For descriptive statistics, continuous traits were summarized as mean $\pm$ SD, whereas highly skewed variables were summarized as medians and interquartile range. Categorical variables were shown as percentages. Analyses were performed by fitting data to generalized linear models or logistic regression models to examine binary traits and were adjusted for main confounders. In particular, the variable-associated changes in anti-RBD antibodies between different evaluations normalized by the time elapsed were evaluated by multivariable generalized linear models. Logistic regression was performed to examine the independent predictors of testing positive for anti-NP+/RBD+ and NPS+ evaluated cross-sectionally at the time of presentation in the whole cohort. The impact of clinical features on seroconversion to anti-NP+/RBD+ status in the subset participants who were not reactive at baseline evaluation but presented multiple times during the study period ($n = 1747$), thereby allowing a time-to-event analysis, was assessed by Kaplan–Meier curves and log-rank test, whereas the independent predictors of seroconversion were evaluated by multivariate Cox regression proportional hazard models. Observations of participants who did not seroconverted during the study period were censored at the time of the last study presentation. p-values <.05 (two-tailed) were considered statistically significant.

Results were reported according to the STROBE guidelines. Statistical analysis was carried out using the JMP Pro 16.0 Statistical Analysis Software (SAS Institute, Cary, NC) and R statistical analysis software version 3.5.2 (http://www.R-project.org/).

3 | RESULTS

3.1 | Clinical features of the study cohort and seroprevalence trends

Participants were predominantly young individuals (<35 years) or middle-aged men with a relatively low rate of well-controlled metabolic comorbidities and without organ damage (Table 1). Of these, 7031 (80.0%) presented once, 1652 (18.7%) twice, and 115 (1.3%) thrice, for a total of 10,680 donations. Age, the frequency of male sex, BMI, and prevalence of hypertension were higher in those who were evaluated multiple times ($p < .0001$). The serological pattern of anti-NP antibodies according to the presentation order is shown in Figure 2A.

During the study time frame, there was a trend for an increase in the prevalence of anti-NP IgG+ positive tests, especially in association with anti-RBD+, which was more marked after the beginning of the "second wave" of the pandemic followed by the implementation of social distancing measures (Figure 2B). The rise in the prevalence of positive serological tests was preceded by a sharp peak of positive nasal swab PCR tests, which were first observed following the return from summer holidays in September 2020 (Figure 2B).

The prevalence of anti-NP+/RBD+ (true positive) tests increased progressively during the study ($p < .0001$, Figure 2C).

3.2 | Seroconversion rate

Among 1747 participants who were not classified as true positives at baseline and who underwent a second evaluation, 114 (6.5%) seroconverted to anti-NP+/RBD+ at the first follow-up presentation, and among the 111 who were still negative after the second evaluation and underwent a third one, 6 (5.4%) seroconverted to anti-NP+/RBD+. The incidence rate of seroconversion to anti-NP+/RBD+ is shown in Figure 2D; by 24 weeks after the inclusion in the study, 10.4% of at-risk participants seroconverted.

3.3 | Relationship of anti-NP pattern with anti-RBD titers and ongoing infection

The relationship between anti-NP serological pattern, anti-RBD titers, and detection of active infection by NPS
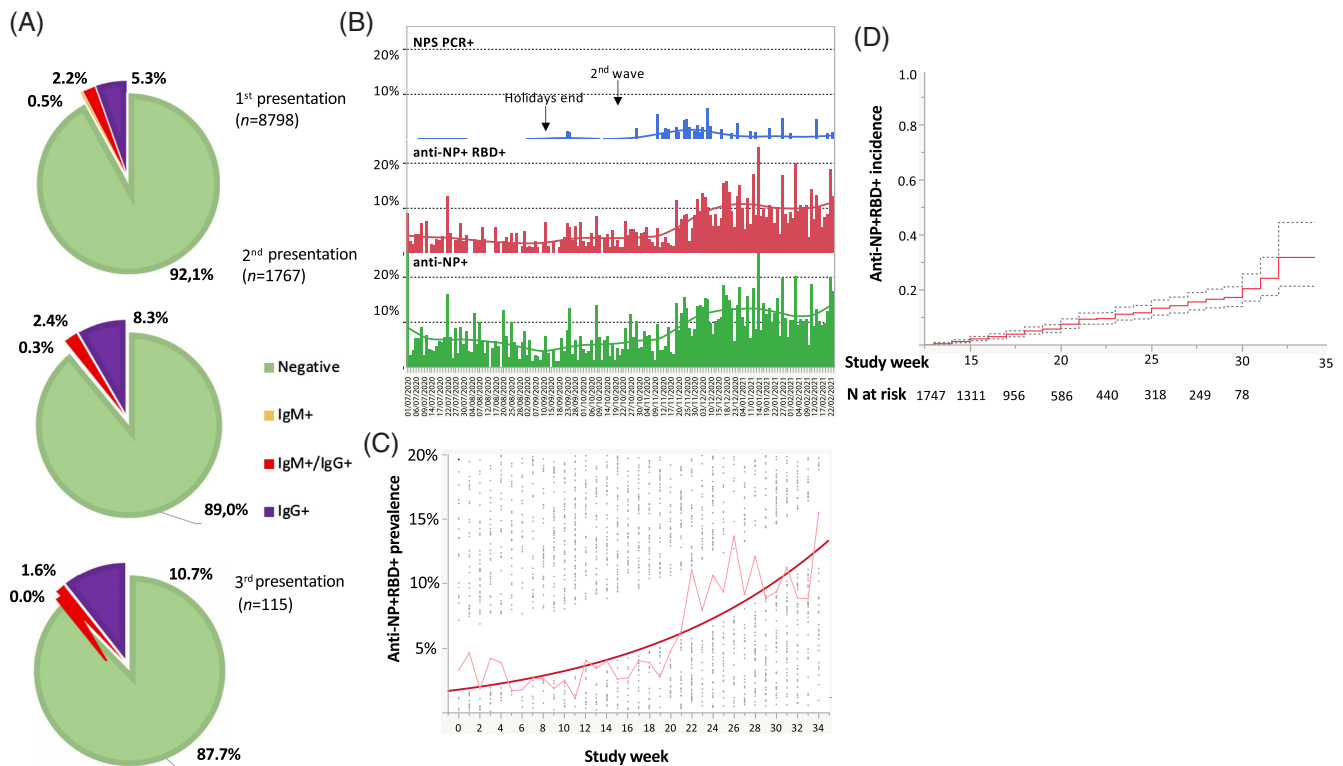
FIGURE 2 Prevalence of SARS-CoV-2 infection. (A) Serological pattern of SARS-CoV-2 anti-NP in participants stratified by the study presentation time. $p < .0001$ for the increasing prevalence of IgG+ with presentation times; (B) histograms and kernel density smoothing lines show the frequency of anti-nucleocapsid (anti-NP)-positive (+), and of anti-spike receptor-binding domain (anti-RBD+ and nasopharyngeal swab (NPS) PCR+ tests in anti-NP+ by evaluation date, as a fraction of all participants tested; (C) predicted percentages of true anti-NP+/RBD + calculated with a logistic regression model (red curve); the rate curve is shown in pink. Dots indicate participants with positive (bottom) and negative (above) tests; (D) cumulative incidence estimates of seroconversion to anti-NP+/RBD+ in 1747 susceptible participants after the first evaluation with available follow-up. The 95% CI is shown by dashed lines [Color figure can be viewed at wileyonlinelibrary.com]

swab molecular tests is reported in Figure 3. Anti-RBD titers were highest in those with IgG+/IgM+ tests, followed by those with IgG+. Anti-RBD titers were also detectable at high levels in a fraction of those with a history of previous infection, irrespective of the presence of anti-NP antibodies, but these were negligible in those with isolated IgM+ (upper panel and quantiles distribution, $p < .0001$). The prevalence of anti-RBD+ and of high titers followed the same pattern.

We detected a PCR+ NPS in 55 participants (0.7%). Active infection was only detected in participants with IgM+/IgG+ or IgG+ anti-NP pattern ($p < .0001$), with a highest probability in those with IgG+/IgM+ versus IgG+ alone (OR 1.87, 95% CI 1.06–3.27; $p = .025$). On the other hand, there was no significant association between anti-RBD titers and detection of active infection (P=NS).

In the subset of individuals where plasma samples were collected at the time of detection of positive NPS swabs ($n = 18$), SARS-CoV-2 viremia was not detectable (<100 copies/ml).

3.4 | Evolution of antibody titers

In participants with follow-up evaluations, the probability of testing anti-NP+/RBD+positive was higher in those who were already positive at a previous evaluation. Remarkably, however, reactivity was maintained in only about one half of them (35/73, 47.9% vs. 121/1861, 6.5% in previous positive vs. previous negative, respectively; $p < .0001$). Even in patients who confirmed anti-NP+/RBD+, anti-RBD titers declined at the time of the second evaluation (mean difference -35.4 ± 7.2 AU, $p = 6*10^{-6}$ at paired t-test). After adjustment for age and sex, higher baseline titers (estimate -0.65 ± 0.11 per baseline unit; $p = 7*10^{-8}$) and longer duration of follow-up (estimate -3.1 ± 1.3 per week; $p = .025$) were independent predictors of anti-RBD titers decline.

3.5 | Predictors of infection

The independent predictors of confirmed serological positivity (anti-NP+/RBP+) are shown in Table 2, left panel.
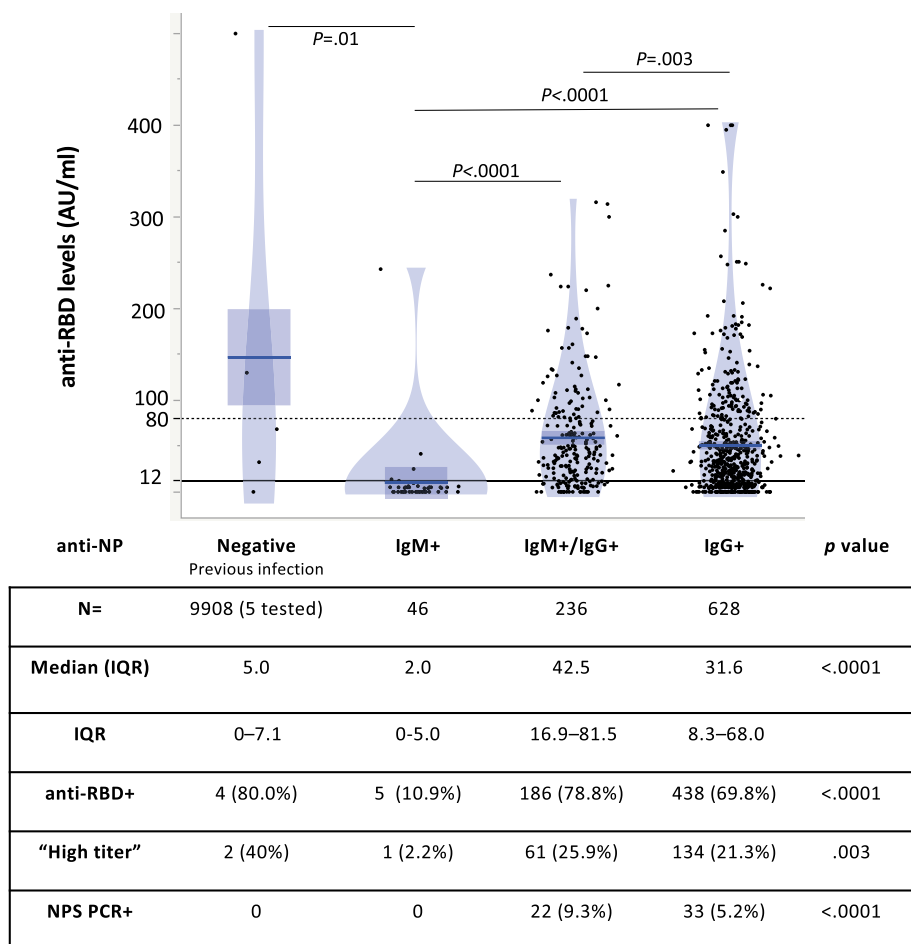
FIGURE 3 Impact of anti-NP serological pattern on anti-RBD and risk of infection. IQR, Interquartile range; NPS, nasopharyngeal swab; RBD, receptor-binding domain. *p*-values at logistic regression models are reported [Color figure can be viewed at wileyonlinelibrary.com]

anti-NP	Negative Previous infection	IgM+	IgM+/IgG+	IgG+	p value
N=	9908 (5 tested)	46	236	628	
Median (IQR)	5.0	2.0	42.5	31.6	<.0001
IQR	0–7.1	0-5.0	16.9–81.5	8.3–68.0	
anti-RBD+	4 (80.0%)	5 (10.9%)	186 (78.8%)	438 (69.8%)	<.0001
"High titer"	2 (40%)	1 (2.2%)	61 (25.9%)	134 (21.3%)	.003
NPS PCR+	0	0	22 (9.3%)	33 (5.2%)	<.0001

TABLE 2 Independent predictors of the risk of infection (positive serology anti-NP+/RBD+ or active infection with positive molecular test) analyzed cross-sectionally during 10,680 presentations between July 1, 2020 and February 23, 2021

	Positive serology (*n* = 910)			Active infection (*n* = 55)		
	OR	95% CI	*p*-value	OR	95% CI	*p*-value
Sex, female	1.06	0.88–1.28	.52	1.06	0.59–1.91	.84
Age, 10 years	1.07	1.00–1.14	.032	0.99	0.97–1.01	.43
BMI, kg/m^2	1.03	1.01–1.06	.009	1.06	0.98–1.13	.16
COVID-19 history, yes	25.1	13.2–47.8	$6*10^{-19}$	-	-	-
Study week, *n*	1.07	1.06–1.08	$7*10^{-40}$	1.08	1.04–1.11	$1*10^{-5}$

Note: *p*-values were determined at logistic regression models adjusted for the variables reported in the table and for the presentation number. We included in the final model demographic and anthropometric features and independent variables associated with positive serology at univariate analysis with *p* < .1.

The probability of testing positive at serology increased with time and was higher in older donors and those with a positive history of infection and higher BMI (*p* < .05), irrespective of ABO blood group (*p* > .5).

The independent predictors of the positive NPS molecular test prior to screening are shown in Table 2, right panel. Only a presentation at later time during the outbreak (*p* < .0001) was significantly associated with NPS reactivity. We did not observe a differential risk according to blood group (*p* > .5), whereas the use of ACE inhibitors was associated with a nonsignificant trend towards increased risk (*p* = .1).

The independent predictors of seroconversion to anti-NP+/RBD+ in participants who were negative at baseline are shown in Table 3. Seroconversion was associated with evaluation at an earlier time and non-O ABO blood

TABLE 3 Independent predictors of seroconversion to anti-NP+/RBD+ status in the subset of 1747 participants who were negative at baseline and underwent at least one follow-up examination

Term	HR	Lower 95%	Upper 95%	*p*-value
Age, 10 years	0.98	0.94	1.02	.39
COVID-19 history, yes	1.03	0.98	1.08	.36
Blood group, non-O	1.42	1.32	1.54	.00045
Study week of enrolment, n	1.05	1.04	1.06	.00076

Note: At Cox regression proportional hazard models; SE, standard error. We included in the final model demographic and anthropometric features and independent variables associated with positive serology at univariate analysis with *p* < .1.

group (*p* < .001 for all), whereas there was no impact of sex and BMI (*p* > .8).

4 | DISCUSSION

In this study, we examined the SARS-CoV-2 infection trends, as estimated by combined positivity to serological tests and positive NPS molecular test, in healthy blood donors during the second COVID-19 wave in Italy. In line with previous results obtained in a subset of the same cohort during the first wave of infections,[10] we observed that the seroprevalence trends mirrored those of confirmed infections and hospitalizations in the same geographical area. In particular, the estimated prevalence of exposure to SARS-CoV-2 in blood donors based on serological tests was ~4% at the beginning of July 2020, consistent with our previous reports at the end of the first wave,[10] but higher than that in blood donors evaluated in other areas of Italy, which were less affected by the first COVID-19 outbreak.[13] The prevalence of confirmed infections remained substantially stable during the summer. However, it began to rise during November 2020 concomitantly with the second wave of COVID-19 cases, reaching at least a 15% prevalence at the end of February 2021, as estimated by a logistic regression model, which has inherent limitations. These data provide an updated estimate of the population exposure to SARS-CoV-2 in the Milan area before the beginning of the vaccination campaign. Remarkably, similar trends for the prevalence of anti-NP and anti-Spike antibodies were recently reported in a large study evaluating blood donors representative of the US population, where the overall infection-induced seroprevalence estimate increased from 3.5% in July 2020 to 20.2% in May 2021.[14]

The rise in seroprevalence was preceded by an increase in the fraction of donors with evidence of active SARS-CoV-2 replication in the upper airways, which were not detected during the summer, rising up to ~5% of daily participants, at the peak that occurred in mid-December 2020. Occasional NPS PCR+ tests were then observed until the end of the study. This is notable

because to qualify for blood donation participants had to report the lack of symptoms consistent with COVID-19 and unprotected contacts with infected individuals during the 2 weeks preceding the donation. Thus, these criteria cannot rule out infection during periods of intense viral circulation.

We could make other interesting observations related to serological testing. The first one is that, in anti-NP IgG + donors, anti-NP IgM+ reactivity increased the probability of finding viral nucleic acids in the upper airways by 87% and was associated with higher anti-RBD titers, but on the other hand has to be probably interpreted as a false positive in IgG− donors. The second one is that we could confirm a significant decline in the titer of anti-RBD antibodies correlating with anti-SARS-CoV-2 neutralizing activity[11,12] with time. This decline was steeper in those with higher baseline levels and time-dependent. Remarkably, ~50% of those who tested anti-RBD+ became negative after a median follow-up of 18 weeks, suggesting that the duration of antibody-dependent immunity after mild infection is not only characterized by the development of lower antibody titers, but is also short-lived in non-vaccinated individuals.[15] These data also suggest that the combined anti-NP+/RBD+ status may underestimate the true prevalence of previous exposure to SARS-CoV-2 infection in the population, where most infections are not severe. However, the decline in anti-RBD titers observed in the present study may be assay-specific.

Expectedly, we observed that the main risk factor of SARS-CoV-2 infection was represented by the phase of the epidemic when the participants presented for evaluation. In addition, older and overweight donors as well as those with a previous confirmed SARS-CoV-2 infection had higher probability of testing anti-NP+/RBD+. Of note, among those who were negative at a previous donation, being carriers of non-O blood groups was the main risk factor for seroconversion, which most frequently occurred in the absence of clinical symptoms. These data are in agreement with those indicating that the *ABO* gene is a main inherited determinant of the interindividual susceptibility to SARS-CoV-2 infection,[16–18] as now confirmed by large international collaborative efforts.[19]

Although independent validation is still required, our results suggest that the *ABO* gene influences susceptibility to SARS-CoV-2 infection even in asymptomatic individuals.

Concerning the secondary outcomes relevant for transfusion medicine, the program allowed the collection of a large number of whole blood donations from donors with high titers of anti-RBD antibodies. The plasma units recovered from these donations could be used in clinical trials and for selected therapeutic indications. However, the enthusiasm for convalescent plasma treatment has been tempered by the predominantly negative results of randomized controlled trials in patients with severe COVID-19 and by the rapid development of therapeutic monoclonal antibodies and effective vaccines.[20,21] Blood donations of ~0.7% participants, who underwent home isolation according to the local regulations, had to be discarded due to the evidence of viral replication, although it was reassuring that we did not detect SARS-CoV-2 viremia in a subset of samples tested. This study has limitations, which include the voluntary participation in the study of an already selected population of young and apparently healthy European individuals, therefore not fully representative of the general population and of other epidemiological settings, the lack of possibility of testing all participants for the presence of anti-RBD antibodies, and to determine the viral strain in NPS PCR+ donors due to the average low levels of viral titers. Furthermore, the impact of continuous predictors of seroconversion was modeled under the assumption of linearity. Notwithstanding, we provided a large prospective and systematic evaluation of SARS-CoV-2 infection rates in blood donors, which mirrored the trends in the general population, and we were able to estimate the consequences on blood management programs.

In conclusion, during the second wave of SARS-CoV-2 infection in Northern Italy, we detected an increase in seroprevalence in healthy blood donors from ~4% to ~15%. The rise in seroprevalence was preceded by a peak of asymptomatic infections in participants with anti-NP IgG+, in particular when also IgM+, and in anti-RBD+ antibodies, paralleling the trends in the general population. Seroconversion was more frequent in carriers of non-O blood groups. However, the persistence of anti-RBD antibodies was short-lived, with a time-dependent decrease in the titers, which felt under the positive threshold after 16 weeks in about half of those who were retested. These results may help to refine blood management strategies to cope with the pandemic.

ORCID
Luca Valenti https://orcid.org/0000-0001-8909-0345
Sara Uceda Renteria https://orcid.org/0000-0002-2027-8478
Massimo Oggioni https://orcid.org/0000-0003-2709-6965
Daniele Prati https://orcid.org/0000-0002-2281-7498

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