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Original article

Seroprevalence of SARS-CoV-2 in blood donors from the Lodi Red Zone and adjacent Lodi metropolitan and suburban area

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ABSTRACT

Objectives: To define the seroprevalence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in blood donors (referred to the first lockdown area (Lodi Red-Zone) of the Lombardy region and in a contiguous area that was not included in the first lockdown); to define the agreement between a commercial serological assay and a reference microneutralization assay; and to evaluate the persistence of SARS-CoV-2 neutralizing antibodies in a cohort of blood donors.

Methods: Blood donors referred to the first lockdown area in Lombardy Region and the neighbouring area were analysed for SARS-CoV-2 IgG-specific antibodies during the period 18 March to 24 June 2020. Serum samples were analysed using both a chemiluminescent immunoassay (LIAISON® SARS-CoV-2 S1/S2 IgG, DiaSorin) for the quantitative characterization of SARS-CoV-2 anti-S1 and anti-S2 IgG antibodies and a neutralizing antibodies (NT-Abs) assay.

Results: In the period from 18 March to 24 June, 1922 blood donors were tested for the presence of SARS-CoV-2 IgG showing a prevalence of 378/1922 (19.7%). A subgroup of 1139 blood donors were tested in parallel with a SARS-CoV-2 IgG assay and a microneutralization assay showing a prevalence of 22.2% and 21.6%, respectively. SARS-CoV-2 IgG quantification was correlated with NT-Abs titres. In 78.2% of participants the NT-Abs titre was maintained, but in 15.8% it decreased by one four-fold dilution and in 6.0% it increased by one four-fold dilution.

Conclusions: The duration of immunity of SARS-CoV-2 is crucial for the course of the pandemic and for this reason the monitoring of NT Abs is important. Despite a stable NT-Abs titre being observed in the majority of blood donors, our findings need to be validated in a long-term period of follow up.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) originated in Wuhan, China, in December 2019; it has caused more than 72 million cases worldwide with approximately 1.6 million deaths, at the time of writing (14 December 2020) [1]. Recently,

antibody detection has been considered as a central point in epidemiological studies and to evaluate population-level control programmes [2].

Results of serological assays may differ according to the type of protein used as antigen. A degree of cross-reactivity is shown in assays that use whole virions and nucleocapsid protein as target for antibody detection [3]. In contrast, the spike protein (S2) seems to be more specific, limiting cross-reactivity with other coronavirus strains [4]. In addition, SARS-CoV-2 neutralizing antibodies (NT Abs), mostly recognizing the S2 protein, play an important role in

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virus clearance and are pivotal for the screening of potential donors for convalescent plasma therapy [5,6].

Based on our preliminary findings, the plasma of recovered patients with a neutralization titre equal to or higher than 1:160 seems to have an impact as a coronavirus disease 2019 (COVID-19) therapy in individuals with severe disease and in pregnant women [5,7]. However, the duration and kinetics of NT Abs is still unknown. These data would be of great significance for the control of COVID-19, for treatment and for future vaccine evaluation.

The aims of our study were (a) to define the seroprevalence of SARS-CoV-2 in blood donors referred to the first lockdown area (Lodi Red Zone) of the Lombardy region and in a contiguous area that was not included in the first lockdown in the period 18 March to 24 June using a commercial assay for SARS-CoV-2 S1/S2 antigens; (b) to define the agreement of commercial serological assay with a reference microneutralization assay; and (c) to evaluate the persistence of SARS-CoV-2 NT Abs in a cohort of blood donors.

Materials and methods

Study population and design

Briefly, 1922 out of about 2500 blood donors (approximately 77%) referred to local blood donor associations of the first lockdown area in Lombardy Region (Lodi Red Zone), including ten municipalities (Bertonico, Casalpusterlengo, Castelgerundo, Castiglione d'Adda, Codogno, Fombio, Maleo, San Fiorano, Somaglia, Terranova dei Passerini) and the neighbouring area, including Lodi metropolitan and suburban area, agreed to participate in the study and were analysed for SARS-CoV-2 IgG-specific antibodies during the period 18 March to 24 June 2020.

The study was approved by the Institutional Review Board of the Fondazione IRCCS Policlinico San Matteo (protocols P-20200035863 and P-20200027987).

Quantitative SARS-CoV-2 S1/S2 IgG and NT-Abs measurement

Serum samples were analysed using a chemiluminescent immunoassay (CLIA) (LIAISON® SARS-CoV-2 S1/S2 IgG; DiaSorin, Saluggia (VC), Italy) for the quantitative characterization of SARS-CoV-2 anti-S1 and anti-S2 IgG antibodies, according to the manufacturer's instructions. Results were given as AU/mL and a cut-off of 15 AU/mL was considered for definition of positive samples. Results ranging from 12 to 15 AU/mL were considered borderline or weak positive and IgG titres <12 AU/mL were given as a negative result. Reagents were kindly provided by the manufacturer free of charge. A titre of NT Abs against SARS-CoV-2 was defined as previously described [8,9]. Briefly, 50 µL of sample from each patient, starting from 1:10 in a serial four-fold dilution series, was added to two wells of a flat-bottom tissue-culture microtitre plate (COSTAR, Corning Incorporated, Corning, NY, USA), mixed with an equal volume of 100 TCID₅₀ of a SARS-CoV-2 strain isolated from a symptomatic patient, previously titrated and incubated at 33°C in 5% CO₂. All dilutions were made in Eagle's minimum essential medium with addition of 1% penicillin, streptomycin and glutamine and 5 γ/mL of trypsin. After 1 hour of incubation at 33°C in 5% CO₂, VERO E6 cells (VERO C1008 (Vero 76, cloneE6, Vero E6); ATCC® CRL-1586™) were added to each well. After 48 hours of incubation at 33°C in 5% CO₂, wells were stained with Gram's crystal violet solution (Merck KGaA, Darmstadt, Germany) plus 5% formaldehyde 40% m/v (Carlo ErbaSpA, Arese, Italy) for 30 minutes. Microtitre plates were then washed in running water. Wells were scored to evaluate the degree of cytopathic effect compared with the virus

control. Blue staining of wells indicated the presence of NT Abs. Neutralizing titre was the maximum dilution with the reduction of 90% of cytopathic effect. A positive titre was equal to or greater than 1:10. Positive and negative controls were included in all test runs.

Data analysis and statistics

Frequency and percentage of SARS-CoV-2 S1/S2 immunoglobulin-positive blood donors in the Lodi Red Zone as well as adjacent Lodi metropolitan and suburban area (from now on referred to as 'Lodi province') were determined. Moreover, in a subset of blood donors, data from the SARS-CoV-2 S1/S2 IgG assay were compared with those obtained from a microneutralization assay. Finally, kinetics of SARS-CoV-2 NT Abs was defined in a group of participants. Specifically, data were shown as median and interquartile range (IQR) or frequency, on the basis of the variable type. Mann–Whitney *U* test and Fisher's exact test were used for quantitative and qualitative variables, respectively. All the tests were two-tailed and a *p* value lower than 0.05 was considered significant. Analyses were performed using GRAPHPAD PRISM 8.3.0 (GraphPad, La Jolla, CA, USA).

Results

Prevalence of SARS-CoV-2 IgG and NT-Abs positivity in the Lodi province, Lombardy

We observed that 378/1922 (19.7%) blood donors of Lodi province were positive for SARS-CoV-2 S1/S2 IgG; the other 1544/1922 (80.3%) tested negative for SARS-CoV-2 S1/S2 IgG. Overall, 1139/1922 (59.3%) blood donors were tested in parallel for SARS-CoV-2 S1/S2 IgG and SARS-CoV-2 NT Abs to define the agreement between the two methods.

It was shown that 253/1139 (22.2%) blood donors were positive for S1/S2 IgG. Of them, 208/253 (82.2%) were also positive for SARS-CoV-2 NT Abs (NT-Abs titre ≥1:10) whereas the remaining 45/253 (17.8%) were negative for SARS-CoV-2 NT Abs (NT-Abs titre <1:10). Overall, 17/45 (37.8%) blood donors negative for SARS-CoV-2 NT Abs showed low levels of S1/S2 IgG (<20 AU/mL), 24/45 (53.3%) were positive for medium levels of S1/S2 IgG (20–80 AU/mL) and only 4/45 (8.9%) showed a high level of S1/S2 IgG (>80 AU/mL).

In contrast, 886/1139 (77.8%) were negative for S1/S2 IgG: of them, 847/886 (95.6%) were confirmed as negative by the microneutralization assay (NT-Abs titre <1:10) whereas the remaining 39/886 (4.4%) tested positive for SARS-CoV-2 NT Abs (NT-Abs titre ≥1:10) (Table 1). Of note, 36/39 (92.3%) of the discordant samples showed a low titre of SARS-CoV-2 NT Abs, ranging from 1:10 to 1:20. Hence, considering the positivity of NT assay only, as many as 247/1139 (21.6%) blood donors showed evidence of SARS-CoV-2 infection.

Table 1

Agreement between SARS-CoV-2 NT Abs and SARS-CoV-2 S1/S2 IgG in 1139 blood donors

SARS-CoV-2 NT Abs	SARS-CoV-2 S1/S2 IgG		
	Positive	Negative	Total
Positive	208	39	247
Negative	45	847	892
Total	253	886	1139

Abbreviations: NT Abs, neutralizing antibodies; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SARS-CoV-2 S1/S2 IgG level was related to SARS-CoV-2 NT-Abs titre

We analysed the S1/S2 IgG levels in 247 SARS-CoV-2 NT-Abs positive individuals, stratified according to NT-Abs titres. In detail, 126/247 (51.0%) showed a low titre of SARS-CoV-2 NT Abs (NT-Abs titre 1:10–1:20), 96/247 (38.9%) tested positive for SARS-CoV-2 NT Abs at medium titre (NT-Abs titre 1:40–1:80) and the remaining 25/247 (10.1%) showed high levels of SARS-CoV-2 NT-Abs (NT-Abs titre >1:160). The median level of S1/S2 IgG was 18.2 (IQR 9.78–31.33) AU/mL in the group of blood donors with low titre of SARS-CoV-2 NT Abs, but it reached the median levels of 57.6 (IQR 40.0–86.3) AU/mL and 82.2 (40.50–128.5) AU/mL in those participants with medium and high SARS-CoV-2 NT-Abs titres, respectively (Fig. 1).

Moreover, although the detection rate of SARS-CoV-2 S1/S2 IgG assay was 71.4% in the group of individuals with low NT-Abs levels (only 90/126 tested positive for SARS-CoV-2 S1/S2 IgG), this rose to 97.9% (94/96) and 100% (25/25) in the groups of participants with medium and high SARS-CoV-2 NT-Abs titres, respectively.

SARS-CoV-2 NT-Abs titre kinetics in COVID-19-positive individuals

In 133/247 (53.8%) individuals with a detectable SARS-CoV-2 NT-Abs titre, the stability of SARS-CoV-2 NT Abs was evaluated over time. In detail, the SARS-CoV-2 NT-Abs titre was evaluated at time of enrolment and after a median of 20 days (IQR 14–41.5 days). Briefly, at time of enrolment we observed that most individuals showed NT-Abs titres of 1:40 (56/133; 42.1%) or 1:20 (27/133; 20.3%), whereas 24/133 (18.1%) had a titre of 1:80 and 18/133 (13.5%) showed a titre of 1:160. Finally, only 3/133 had a titre of 1:10 and 3/133 and 2/133 had NT-Abs titres of 1:320 and 1:640, respectively. At the time of follow up, SARS-CoV-2 NT-Abs titres were distributed as follows: 21/133 (15.8%) 1:10, 26/133 (19.5%) 1:20, 45/133 (33.8%) 1:40, 13/133 (9.9%) 1:80, 26/133 (19.5%) 1:160 and 2/133 (1.5%) 1:320; none showed a titre of 1:640 or higher. Interestingly, none of the 133 participants were negative for SARS-CoV-2 NT-Abs at the time of follow up (Fig. 2).

Overall, in 104/133 (78.2%) participants, SARS-CoV-2 NT-Abs titre was maintained, whereas in 21/133 (15.8%) blood donors NT-

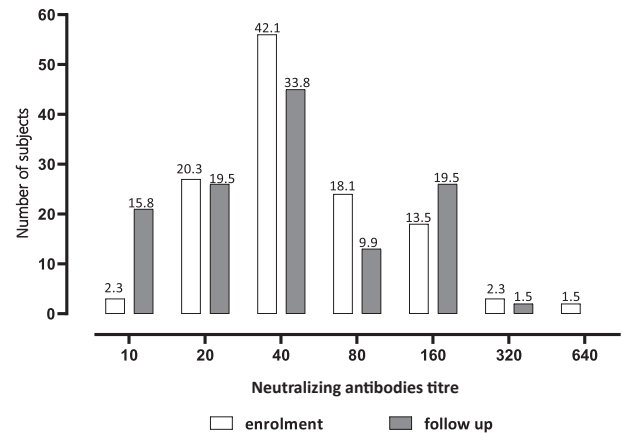


Fig. 2. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibodies (NT-Abs) titre distribution at enrolment (white boxes) and follow up (grey boxes). For each histogram, the percentage of participants is included.

Abs titre decreased by one four-fold dilution and in 8/133 (6.0%) the NT-Abs titre increased by one four-fold dilution.

Individual kinetics of SARS-CoV-2 NT-Abs titres between enrolment and follow up are shown in the [Supplementary material \(Fig. S1\)](#).

Discussion

In the present study we found that nearly 20% of asymptomatic or paucisymptomatic blood donors referred to the initial Lodi Red Zone and the contiguous area from March to June 2020 tested positive for SARS-CoV-2 S1/S2 IgG. Moreover, we observed a high level of agreement between the LIAISON CLIA detecting SARS-CoV-2 S1/S2 and the SARS-CoV-2 NT assay. Finally, in a small subset of donors we were able to monitor SARS-CoV-2 NT Abs over time, finding a stable SARS-CoV-2 NT-Abs titre in about 78% of donors.

Overall, the results of this follow-up study confirm and extend our previous observation on a smaller group of blood donors [9], indicating that in the first hot-spot of SARS-CoV-2 in Lombardy and the adjacent area less than one-quarter of resident blood donors experienced the infection. In addition, we observed a high level of agreement between results obtained by a commercial assay detecting SARS-CoV-2 S1/S2 and our home-made micro-neutralization assay, as already reported [4,10]. This finding supports the hypothesis that SARS-CoV-2 S1/S2 IgG quantification could present a possible alternative for estimating SARS-CoV-2 NT Abs for laboratories not provided with P3 biosafety facilities. Despite SARS-CoV-2 S1/S2 IgG assay reporting a lower positive detection rate in individuals with low SARS-CoV-2 NT-Abs levels, we observed that 45/253 (17.8%) blood donors who would have been considered as seronegative by SARS-CoV-2 NT assay, were identified as seropositive if tested by S1/S2 LIAISON CLIA.

A decrease in the detection rate of the LIAISON CLIA was also observed by other authors, albeit in a smaller sample subset [11]. To date, a number of commercial assays, both spike-based and nucleocapsid-based, for the quantification of SARS-CoV-2 IgG are available. Based on recent findings, a high level of agreement was reported between spike-based ELISA and CLIA [11,12] as well as between spike-based and nucleocapsid-based tests [13].

Finally, the duration of immunity of SARS-CoV-2 represents a critical issue for the course of the pandemic and monitoring of NT Abs, especially in individuals with mild symptoms that develop lower titres, is crucial. Previous studies indicated that individuals who had been infected with common human coronavirus strains

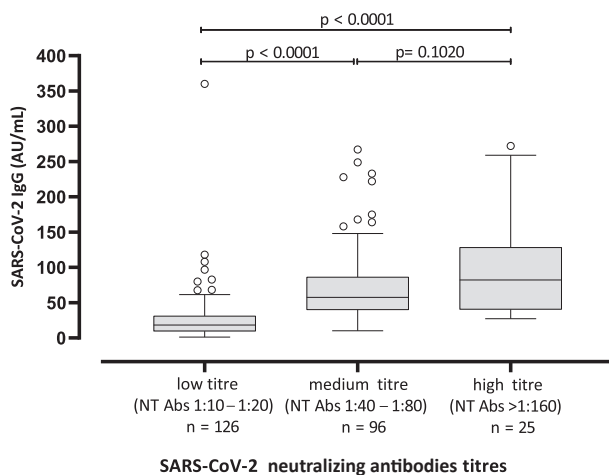


Fig. 1. Distribution of S1/S2 IgG concentration based on the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) neutralizing antibodies (NT-Abs) levels is shown. SARS-CoV-2 NT-Abs positive individuals were divided into three groups based on the level of NT-Abs titre: low titre (NT-Abs 1:10–1:20), medium titre (NT-Abs 1:40–1:80) a high titre (NT-Abs >1:160). Median S1/S2 IgG level was measured in all the three groups and the p value for each comparison is given.

(229E, OC43, HKU and NL63) are protected from reinfection for some years [14] or show mild symptoms [15]. As infection with SARS-CoV-1 induces NT Abs that persist for several years [15], it can be hypothesized that SARS-CoV-2 NT Abs could also have a protective role.

In SARS-CoV-1 infection some authors found detectable levels of NT Abs at 36 months after symptom onset [16]. It is not known if the level of NT Abs to SARS-CoV-2 may last over time. In our study, a stable NT-Abs titre was observed in the majority of blood donors with <16% of them showing a decrease in NT-Abs titre. It could be speculated that the decline of NT Abs observed might also be associated with the decline or disappearance of SARS-CoV-2-specific IgM rather than a real ‘waning immunity’. Moreover, the duration of the interval between first sampling and follow up was not associated with a decline in terms of NT-Abs titres.

There are some limitations to this research. First, the sensitivity of the S1/S2 IgG assay was lower in those participants with a low SARS-CoV-2 NT-Abs titre. This revealed that, despite the high level of agreement between the two assays, the lower detection rate of the SARS-CoV-2 S1/S2 IgG assay might affect the perception of results in those areas with low SARS-CoV-2 seroprevalence. Moreover, the follow up of SARS-CoV-2 NT-Abs titre over time was performed in only a small portion of the study cohort (133/1922; 6.9%), which did not reflect the population at large; moreover a short observation time was considered. The results obtained might not be representative enough to draw clear conclusions, so further analyses are required to confirm and strengthen these findings.

In conclusion, these findings highlight that even in such highly affected area the vast majority of individuals might still be susceptible to infection. A caveat of this evidence is to implement strict surveillance measures to avoid further outbreaks. The monitoring of SARS-CoV-2 NT-Abs level should be implemented over time in order to characterize the duration of the SARS-CoV-2-specific humoral response.

Authors' contributions

FB conceived and design the study. IC, EP, AS and FB analysed data, wrote the manuscript and generated the table and figures. GC, AS, AB, GF, AC, RDM, EVN, RM, ADN and AF collected laboratory and clinical data. All the authors critically reviewed and approved the final version of the manuscript.

Transparency declaration

The authors declare no conflict of interest.

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CoV-2 S1/S2 IgG reagents were kindly provided by DiaSorin (Saluggia, VC, Italy) free of charge.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.cmi.2021.01.030>.

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