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Low Prevalence of Antibodies to SARS-CoV-2 and Undetectable Viral Load in Seropositive Blood Donors from South-Eastern Italy

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Keywords
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Abstract
Recently, a significant cluster of pneumonia caused by a novel betacoronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) was described initially in China and then spread throughout the world. Like other coronaviridae, the viral transmission occurs mainly through droplets. In addition, the virus has been detected in different clinical specimens, suggesting a potential transmission by other routes, including blood transfusion. However, the potential risk of transmission of SARS-CoV-2 via blood products is still unclear. The aim of our study was to investigate the prevalence of antibodies against SARS-CoV-2 among blood donors from South-Eastern Italy. Moreover, in the seropositive donors, we searched for the presence of the virus in nasopharyngeal swabs and in plasma samples. Overall, 1,797 blood donors from the Apulia region were tested for anti-SARS-CoV-2 antibodies, using a commercially available assay. Only 18/1,797 donors (1.0%) tested positive for anti-SARS-CoV-2 antibodies; in none of them SARS-CoV-2 viral RNA was detected in nasopharyngeal swabs and in plasma samples. Our results indicate that most of the blood donors in Apulia remained uninfected during this wave of the pandemic; further, none had detectable virus both in nasopharyngeal swabs and in blood samples. The risk to carry and transmit the virus by healthy and asymptomatic blood donors is probably very low.

Introduction

Recently, a cluster of pneumonia cases of unknown etiology was reported in Wuhan, Hubei Province of China, in late December 2019 [1, 2]. The causative agent, the

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severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), belongs to the beta genus of the coronaviridiae family. In a few months, the coronavirus disease (COVID-19) pandemic spread throughout the world, causing 14,537,975 infections and approximately 607,389 deaths as of July 2020 [3].

Pandemic SARS-COV-2 is transmitted by virus-infected individuals, by contact and by respiratory secretions. Other potential transmissions might take place by saliva, urine, feces, blood, and seminal fluid[4–6].

Available studies do not allow, to date, to draw definitive conclusion regarding the presence of the virus in blood, questioning the risk of transmission of SARS-CoV-2 via blood products and blood derivatives [7–11]. The purpose of the present study was to evaluate the prevalence of antibodies to SARS-CoV-2 among blood donors from Southern-Eastern Italy and the presence of the virus in nasopharyngeal swabs and blood in the seropositive subjects.

Materials and Methods

A total of 1,797 blood donors, who had presented for blood donation at the University Hospital in Foggia from March to June 2020, were studied. All the blood donors were offered pre- and posttest counseling, and informed consent was obtained. Criteria for exclusion of donors were (1) age <18 years or >65 years; (2) body weight <50 kg; (3) hemoglobin value <12.5 g/dL for females and 13.5 g/dL for males; (4) history of jaundice; (5) sickle cell disease; (6) hypertension; (7) current fever; or (8) reported previously diagnosed SARS-CoV-2 infection. All were asked for symptoms of COVID-19.

Blood donors were subjected to all routine blood analysis and tested for antibodies to SARS-CoV-2. Seropositive subjects were recalled and tested, for viral RNA in nasopharyngeal swabs and blood, the day after.

We used 2 different commercially available kits based on the immunometric technique to study the presence of SARS-CoV-2-specific antibodies (Ortho Clinical diagnostic Great Britain): the VITROS anti-SARS-CoV-2 total Ig (IgG, IgM, and IgA) and the VITROS anti-SARS-CoV-2 IgG. Detailed information is found in the brochure of the kit and brief, and the technique involves a 2-stage reaction. In the first stage, antibodies to SARS-CoV-2 present in the sample bind with SARS-CoV-2 spike protein S1 antigen coated; in the second stage, HRP-labeled murine monoclonal anti-human IgG antibodies are added in the conjugate reagent. The conjugate binds to the antibody portion of the antigen-antibody complex. A reagent containing luminogenic substrates is added to the wells catalyzing the oxidation of the luminol derivative, producing light. The light signals are read by the system. The amount of HRP conjugate bound is indicative of the amount of SARS-CoV-2 total Ig antibody present. Results are automatically calculated by the VITROS Immunodiagnostic and VITROS integrated systems. Samples positive for total Ig were analyzed by using, with the same modalities, the VITROS anti-SARS-CoV-2 IgG.

Further, anti-SARS-CoV-2 IgM was also analyzed by using a chemiluminescent analytical assay commercially available (New Industries Biomedical Engineering Co., Ltd [Snibe], 94 Shenzhen, China) performed according to the manufacturer's instructions as reported elsewhere [12]. For SARS-CoV-2 RNA detection in nasopharyngeal swabs, viral RNA was extracted within 2 h from sample collection using the STARMag 96 × 4 Universal Cartridge kit with the Microlab NIMBUS IVD instrument according to the manufacturer's instructions (Seegene Inc. Seoul, Korea). Indeed, viral RNA from plasma was extracted using the Qiamp viral RNA mini kit following the manufacturer's instructions (Qiagen GmbH, Qiagen Strasse 1, 40724 Hilden, Germany). Amplification and detection of target genes (N, E, and RdRP) were performed using the commercially available kit Allplex TM 2019-nCoV Assay (Seegene Inc. Seoul, Korea) with the CFX96TM instrument (Bio-Rad, Hercules, CA, USA). The producer declared limit of detection of the Allplex™ 2019-nCoV Assay, from respiratory samples (including nasopharyngeal swab), of 1,250 copies/mL. Result interpretation was performed by using the Seegene Viewer software.

Statistical Analysis

A χ^2 test was used to investigate the relationship between the presence of anti-SARS-COV-2 and gender or age group. A p value <0.05 was considered as significant. Data were also analyzed by a "2 × 2" contingency table and Fisher's exact test.

Results

Between March and June 2020, we enrolled 1,797 blood donors, mean age 33 years (SD = 10.24), with demographic distribution characteristics shown in Tables 1 and 2. One-thousand three-hundred and thirty-eight (74.5%) of the 1,797 blood donors were male and 459 (25.5%) were female. The highest number of male and female blood donors were found to be 36–55 years old.

The presence of anti-SARS-CoV-2 antibodies was detected in 18/1,797 blood donors (1.0%). Of these, 17 donors were anti-SARS-CoV-2 IgG positive; one was found to have both anti-SARS-CoV-2 IgG and IgM.

No differences in seroprevalence were observed in the different months studied (April, May, or June). No SARS-CoV-2 RNA was found neither in nasopharyngeal swabs nor in blood specimens.

Furthermore, we also evaluated the presence of SARS-CoV-2 RNA in plasma samples, and no circulating virus was observed in any plasma samples. No significant correlations were found between the presence of anti-SARS-CoV-2, the age, and the sex of the donors enrolled into the study ($p \geq 0.5$).

Table 1. Anti-SARS-CoV-2 antibodies in blood donors according to sex and age

Blood donors	Age, years	*N* (%)	Anti-SARS-CoV-2 positive	
			N	%
Males		1,338 (74.5)	13	1.0
	18–25	151	2	1.3
	26–35	277	7	2.5
	36–45	282	1	0.4
	46–55	407	2	0.5
	56–65	207	1	0.5
	>65	14	0	0.0
Females		459 (25.5)	5	1.1
	18–25	74	1	1.4
	26–35	93	1	1.1
	36–45	104	1	1.0
	46–55	136	0	0.0
	56–65	49	2	4.0
	>65	3	0	0.0
Total		1,797	18	1.0

Table 2. Anti-SARS-CoV-2 antibodies in blood donors according to sex and age

Blood donors	Age, years	*N* (%)	Anti-SARS-CoV-2 positive	
			N	%
Males	Total	1,338 (74.5)	13	1.0
	18–35	428	9	2.1
	36–	910	4	0.4
Females	Total	459 (25.5)	5	1.1
	18–35	167	2	1.2
	36–	292	3	1.0
Total		1,797	18	1.0

2 × 2 contingency table and Fischer's test: 2-tailed *p* value equals 0.3260.

Discussion

The results presented here show a low seroprevalence of anti-SARS-CoV-2 antibodies (1.0%) in blood donors from Apulia, a region with a low prevalence of COVID-19 (4,500 confirmed cases over 4 months in a population of 4,000,000 inhabitants). Our results are in line with other studies in which a low seroprevalence in blood donors was found in countries with low incidence of SARS-CoV-2 infection, such as Germany (0.8%) and Jordan (0.0%) [13, 14]. On the contrary, a higher rate of anti-SARS-CoV-2 antibodies has been reported in studies conducted in regions with high incidence of infection. In fact, in Lombardy, the most affected region in Italy, the seroprevalence in blood donors was 5.2%, a number that is justified by the wide circulation of the virus in that area during the pandemic [15]. Similarly, higher rates were reported in Brazil (3.3%) and in Wuhan, China (2.3%) [16, 17].

Testing for virus-related antibodies in blood donors can thus help to understand the extent of infection in the community and to identify individuals, both symptomatic and asymptomatic, who have been infected with SARS-CoV-2. In a study by Stringhini and collaborators [18], the population of Geneva remained uninfected during the wave of the pandemic, despite the high prevalence of COVID-19 infection in the region.

Thus, suggesting that whether the presence of IgG antibodies is associated with immunity, the epidemic is far from coming to an end (herd immunity) [18]. Our estimates may not be accurately reflecting the seroprevalence in the general population because donors blood represent a minority and have higher level of awareness to health issues, still they give an important trend.

Interestingly, none of the 18 positive donors for anti-SARS-CoV-2 antibodies had positive PCR test results neither in nasopharyngeal swabs nor in blood samples. Limited data have shown that viral RNA could be detected in plasma or serum from COVID-19 patients, suggesting that infection sometimes may be systemic [7–9], but other studies did not confirm these observations [10, 11]. If a viremic phase exists during the asymptomatic phase, transfusions might contribute to an ever-widening pool of infection in the population.

In the blood transfusion setting, Chinese researchers found SARS-CoV-2 RNA in 4 out of 4,995 blood donations from asymptomatic blood donors. However, no transmission cases were linked to the administration of blood products derived from these subjects [19]. The risk of transmission through blood transfusion might be most likely related to the extent of virus circulation in the general population in a given geographical area and to the presence of clinical symptoms or detectable virus.

This study has certain limitations: (a) the absence of viral RNA in the samples tested at the time of donation does not exclude a viremic phase before or after; (b) no archival material was available for the subjects tested. As well, sequential samples are still not available (although we are collecting them at present).

In conclusion, our results indicate that most of the blood donors in Apulia remained uninfected during this wave of the pandemic, and further, none had detectable virus both in nasopharyngeal swabs and in blood samples. This observation suggests that healthy and asymptomatic blood donors should not be considered at risk to carry and transmit the virus through blood products. Further studies are needed to draw definitive conclusions regarding this public health relevant issue.

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Statement of Ethics

The subjects enrolled in the study have signed a written informed consent form. The study protocol was approved by the Department of Clinical and Experimental Medicine Internal Ethical Board, University of Foggia, Foggia, Italy.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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