

# Analysis of current SARS-CoV-2 infection in a large population of blood donors evidenced that RNAemia is rare in plasma

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## Abstract

**Background:** Transmission of SARS-CoV-2 by asymptomatic individuals and by blood transfusion are important issues to understand to control the viral spread. In this work, we estimated the current SARS-CoV-2 infection rate in blood donors from Belo Horizonte, Brazil.

**Study design and methods:** Saliva and blood samples were collected from 4103 blood donors from June 15 to September 30, 2020. Saliva samples were tested by real-time RT-PCR for SARS-CoV-2 in mini-pools of four samples. Individual samples were tested for positive or inconclusive pools, and positive donors had their plasma tested.

**Results:** Twenty-seven (0.66%) blood donors were positive for SARS-CoV-2 in their saliva, but their plasma was negative, except for one, who presented a high viral load in saliva and nasopharyngeal samples and RNAemia in the plasma close to the limit of detection. Fourteen (56%) positive blood donors reported mild symptoms related to COVID-19 after donation, but the viral load levels were not statistically different between symptomatic and asymptomatic individuals.

**Discussion:** Despite the measures taken by Blood Centers to avoid blood donors with SARS-CoV-2 infection, asymptomatic or presymptomatic carriers are able to donate. The risk of the virus transmission by transfusion seems to be negligible since plasma RNAemia was seen at a very low level in only one (3.7%) of the positive donors, but other studies must be performed to confirm this finding.

## KEYWORDS

asymptomatic carriers, blood donors, COVID-19, RT-PCR, saliva, SARS-CoV-2

## 1 | INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) emerged in November 2019 in Wuhan, China and expanded worldwide.<sup>1</sup> SARS-CoV-2 was declared a public health emergency of international concern on January 30, 2020.<sup>2</sup> As of February 28, 2021, coronavirus disease 2019 (COVID-19) has caused over 113.4 million cases and 2,520,653 deaths globally.<sup>3</sup>

Since the beginning of the pandemic, health systems around the world have been discussing the risk of transmission of SARS-CoV-2 by transfusion. Experience with outbreaks of other respiratory coronaviruses has shown a low transmission risk by this route. Still, studies have been proposed to elucidate the risk of transmission of SARS-CoV-2 by transfusion of blood components and to evaluate the applicability of common infection control strategies in blood banks, such as nucleic acid tests<sup>4,5</sup> and inactivation of pathogens.<sup>6</sup>

Blood donation by individuals infected by SARS-CoV-2 is possible considering that they can remain asymptomatic or may be asymptomatic at the time of donation. The detection of viral RNA in the blood of patients<sup>7-9</sup> and blood donors<sup>10,11</sup> raised concerns about the safety of blood transfusion in areas with active virus transmission. Otherwise, the failure to isolate the virus from positive PCR blood samples<sup>4,12</sup> and the demonstration that the presence of viral RNA in the donor's blood does not seem to be a common event<sup>10</sup> raise doubts about the possibility of transfusion transmission.

Since most of the current knowledge about the presence of SARS-CoV-2 in blood donors comes from small and heterogeneous studies, there is a need for more conclusive studies on this issue, including large longitudinal donor testing studies using molecular assays to assess the prevalence and incidence of SARS-CoV-2 RNA in blood donors and blood components.<sup>13</sup>

In this manuscript, we report the prevalence of current SARS-CoV-2 infection among blood donors in a large Brazilian blood center, using RT-PCR to test their saliva, nasopharyngeal swabs, and blood samples collected during the COVID-19 first wave in Brazil. Additionally, their viral loads and the SARS-CoV-2 positive donors' data are presented.

## 2 | MATERIALS AND METHODS

### 2.1 | Study population

This study enrolled eligible blood donors at a Brazilian blood center (Fundação HEMOMINAS, Minas Gerais, Brazil) from June 15 to September 30, 2020. Absence of

COVID-19 symptoms and no high-risk contacts during the 30 days before blood donation were requirements for their blood to be accepted. All participants signed a written informed consent form. The Institutional Ethics Committee approved this study.

### 2.2 | Sample collection and processing

After confirming that the recruited blood donors had not eaten or drunk anything in the 30 min before sample collection, they were asked to accumulate saliva for a few seconds. Saliva samples were self-collected in an 80 mL sterile cup until the bottom was filled (approximately 1–2 mL). Nasopharyngeal samples were randomly collected from some blood donors using rayon-tipped swabs to assess the agreement of the RT-PCR results between these samples and the saliva. To minimize the impact on the routine of clinical screening of donors, only one swab collection per day was scheduled. After collection, the swabs were stored in a tube containing Dulbecco's minimal essential medium (DMEM) (LGC Biotecnologia, Cotia, Brazil). Saliva and nasopharyngeal swab samples were stored in a thermal box at 15°C and processed up to 3 h after collection. Blood samples were collected in a tube containing EDTA from all participants. Saliva (1 mL) was transferred to a microtube and centrifuged at 3000g for 2 min. Mini-pools of 200 µL saliva were prepared using 50 µL each from the supernatant of four saliva samples. Samples from the swabs were vortexed vigorously for 15 s before being transferred to a microtube. Blood samples were centrifuged at 3000g for 10 min at 18°C and the plasma was immediately aliquoted. All samples were frozen at –80°C until the RNA purification.

### 2.3 | Detection of SARS-CoV-2

Total RNA was extracted from the 200 µL saliva pools, plasma, and nasopharyngeal swab samples using a Bio Gene Viral DNA/RNA Extraction kit (Quibasa, Belo Horizonte, Brazil) in accordance with manufacturer's protocol. RNA was eluted by adding 50 µL of RNase free deionized water. SARS-CoV-2 RNA was detected by RT-PCR using US CDC real-time RT-PCR primer/probe sets for 2019-nCoV\_N1 and 2019-nCoV\_N2 and the human RNase P (RP) as a control (IDT, Coralville, IA, USA). The reverse transcription and amplification of each target was performed in separate tubes using 5 µL of RNA and RT-PCR iTaq universal Probes One-Step Kit (Bio-Rad) according to the manufacturer's recommendations and the following cycle conditions: 10 min at 50°C and 2 min at 95°C followed by 45 cycles of 15 s at 95°C

and 1 min at 55°C when using an ABI Prism 7300 Sequence Detector System (Applied Biosystems, Foster City, CA, USA) or followed by 45 cycles of 3 s at 95°C and 30 s at 55°C when using an ABI Prism 7500 Fast Sequence Detector System (Applied Biosystems).

Samples were considered positive when both N1 and N2 targets were detected at Ct < 40 and RP at Ct ≤ 38, and inconclusive when just one viral target was amplified. Samples that were positive or inconclusive among the mini-pools were individually submitted to RNA purification and tested by the same RT-PCR protocol. Plasma samples from positive blood donors were also tested individually by RT-PCR.

Viral loads of the positive individual samples were quantified using a serial 10-fold dilution of plasmid carrying the genome sequence of SARS-CoV-2 (2019-nCoV\_N\_Positive Control, IDT) to obtain a standard curve with a known number of target copies. For this quantification, only amplification of N1 was performed.

## 2.4 | Assessment of the blood donors' profile

Blood donors with positive SARS-CoV-2 RT-PCR results were contacted by telephone at least 20 days after donation. They answered a questionnaire regarding demographic, behavioral data, and information regarding their clinical status and the development of COVID-19 symptoms. Data from all donors (sex, age, address, serological

status, ABO blood group, and rate of return donors) were collected from the blood center records.

## 2.5 | Statistical analysis

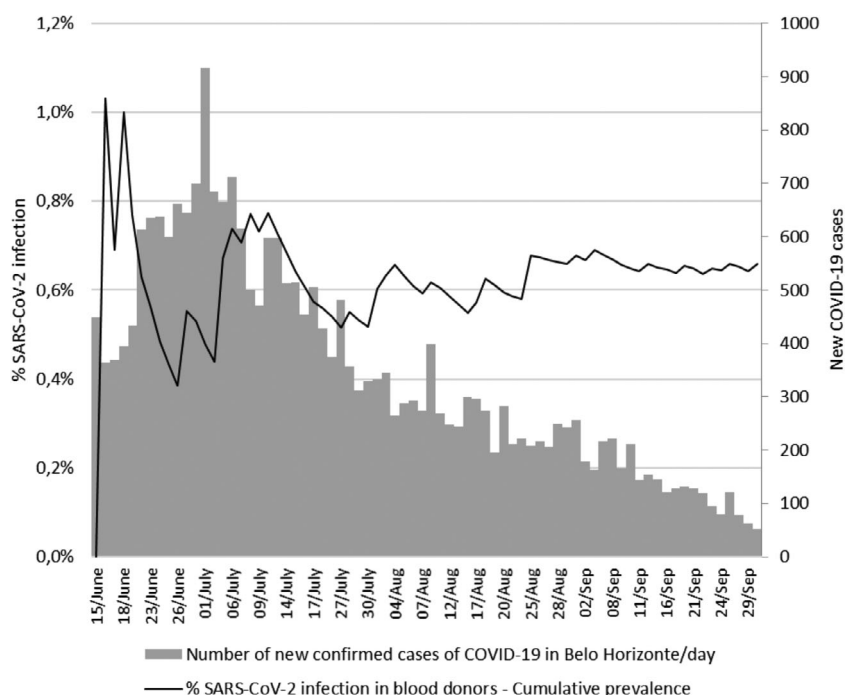
The number of events and their respective frequencies were calculated for the categorical variables. Comparisons were made using Fisher's exact test. Medians with interquartile range (IQR) were calculated for the continuous variables and the comparisons were performed by double-sided Mann-Whitney tests. The differences were considered statistically significant when  $p < 0.05$ .

## 3 | RESULTS

This study included 4103 blood donors at a Brazilian blood center (Hemocentro de Belo Horizonte, Fundação HEMOMINAS) from June 15 to September 30, 2020. In addition to the saliva collection, nasopharyngeal swabs were randomly collected from 67 blood donors. The RT-PCR results of the saliva and swab samples were completely concordant, identifying one donor positive for SARS-CoV-2 RNA.

SARS-CoV-2 RNA was detected in 27 saliva samples from the blood donors. The cumulative virus prevalence was 0.66% (95% CI 0.45–0.96), which remained stable through August and September, despite a decline in new confirmed cases of COVID-19 in the city (Figure 1). The

**FIGURE 1** Frequency of blood donors with current infection of SARS-CoV-2 detected by RT-PCR. An average of 55 samples/day were collected from June 15 to September 30 (74 days), totaling 4103 blood donors tested. At the end of the analyzed period, the cumulative prevalence of infection was 0.66%. The gray bars show the number of new confirmed cases of COVID-19 in Belo Horizonte by date of onset of symptoms during the study period



median age of the positive donors was 29 years old and 15 (55.6%) of them were women.

SARS-CoV-2 viral load in saliva from positive blood donors was quantified and the median was  $7.6 \times 10^4$  copies/ml. Eight (29.6%) individuals presented with a high viral load in their saliva ( $>10^6$  copies/ml), but the plasma samples were negative for seven of them. The only blood donor (1/27, 3.7%) whose plasma sample was also positive, but with very low viral load (43 copies/ml), had elevated levels of RNAemia in the nasopharyngeal swab ( $2.27 \times 10^7$  copies/ml) and in the saliva ( $1.82 \times 10^6$  copies/ml) (Table 2).

We compared the characteristics of blood donors that were negative and positive for SARS-CoV-2, and there was no statistical difference between the two groups regarding age, sex, frequency of ABO and Rh blood groups, or positive serological tests for blood-borne infections (Table 1). The motivation for donation was also similar between the groups, but the frequency of SARS-CoV-2 infection was significantly higher among donors donating blood for the first time ( $p = 0.009$ ; OR = 2.999, 95% CI 1.405–6.401).

Detailed data of the SARS-CoV-2-positive blood donors are summarized in Table 2. We were unable to contact two donors and had to complete the questionnaire for one donor. The post donation questionnaires revealed that 56% (14/25) positive blood donors reported mild symptoms related to COVID-19 after blood donation. The most prevalent symptoms were fatigue (9/14, 64.3%) and anosmia and/or ageusia (8/14, 57.1%). The development of COVID-19 symptoms occurred at a median of 2 days after blood donation (IQR, 1–3 days). Among the positive blood donors, 45.8% (11/24) sought medical attention and five of them (45.5%) received drug treatment (i.e., azithromycin, ivermectin, prednisone, dipyrrone, chloroquine, and paracetamol), including one who was asymptomatic. Use of public transport and participation in social events in the 15 days prior to donation were reported by 45.8% (11/24) and 8.3% (2/24) positive donors, respectively. Twelve positive donors (50%) remained in face-to-face work in diverse occupations, some of them requiring intense public contact. Constant use of masks was reported by the majority of individuals (19/24, 79.2%).

Characteristics	SARS-CoV-2 infection status		p value
	Negative	Positive	
Number of blood donors (%)	4076 (99.34)	27 (0.66)	—
Age in years, median (IQR)	33 (26–42)	29 (24–37)	.089
Sex, male, (%)	1976 (48.5)	12 (44.4)	.823
ABO blood group (%)			
A	1408 (34.5)	9 (33.3)	.999
B	366 (9.0)	2 (7.4)	.999
AB	130 (3.2)	1 (3.7)	.999
O	2172 (53.3)	15 (55.6)	.971
Rh-positive (%)	3390 (83.1)	24 (88.9)	.622
Positive serological tests (%) <sup>a</sup>	64 (1.57)	1 (3.70)	.702
Syphilis	32 (0.78)	1 (3.70)	.393
HBV	21 (0.51)	0 (0.00)	—
HCV	4 (0.10)	0 (0.00)	—
HIV	4 (0.10)	0 (0.00)	—
HTLV	2 (0.05)	0 (0.00)	—
Chagas disease	2 (0.05)	0 (0.00)	—
First-time donors (%)	964 (23.6)	13 (48.1)	<b>.009</b>
Type of donation (%)			
Invited	178 (4.4)	1 (3.8)	.999
Spontaneous	2601 (63.8)	14 (51.8)	.277
Replacement	1297 (31.8)	12 (44.4)	.234

**TABLE 1** Characteristics of blood donors with negative and positive SARS-CoV-2 RT-PCR test

Note: Significant *p*-values are marked in bold.

Abbreviation: IQR, Interquartile range.

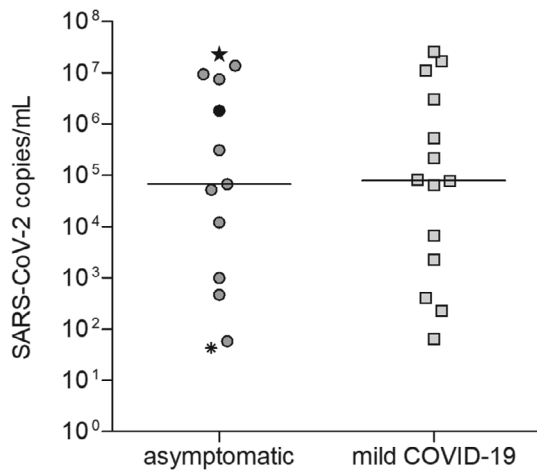
<sup>a</sup>One blood donor was positive for syphilis and HBV serological biomarkers.

TABLE 2 Detailed data of blood donors with present SARS-CoV-2 infection detected by RT-PCR in saliva samples

Donor number	Sex	Age	Blood type	Repeat donor	Viral load (copies/mL)	COVID-19 symptoms	Days post-donation until symptoms	Treatment	Pre-sential work	Occupation	Constant mask use reported	Use of public transport
1	M	43	A	Yes	$1.11 \times 10^7$	DPN; FTG; FVR; HDC; STH	3	No	Yes	Penitentiary employee	Yes	No
2	F	24	O	No	$2.15 \times 10^5$	FTG; FVR; MLG; OGD	1	No	No	Student	Yes	No
3	F	31	O	No	$3.11 \times 10^5$	None	NA	No	No	Cooker	Yes	Yes
4	F	20	O	Yes	$5.29 \times 10^5$	DPN; OGD; STH	3	No	Yes	Nursing technician	Yes	No
5	F	43	A	No	$4.05 \times 10^2$	CGH; FTG; OGD	1	No	Yes	Office assistant	Yes	Yes
6	F	24	O	No	$2.30 \times 10^2$	NVT; OGD	1	No	Yes	Penitentiary employee	Yes	No
7	F	23	O	No	$8.18 \times 10^4$	HDC; OGD	1	No	No	Store attendant	Yes	Yes
8	F	27	A	Yes	58.00	None	NA	No	Yes	Office assistant	Yes	No
9	M	24	O	Yes	$7.61 \times 10^4$	FTG; MLG; OGD	2	No	Yes	Hotel receptionist	No	No
10 <sup>a</sup>	M	45	B	Yes	$1.82 \times 10^6$	None	NA	Azithromycin, ivermectin, prednisone	No	Hospital pharmacy clerk	Yes	No
11	M	25	A	No	$1.67 \times 10^7$	FTG; FVR	1	No	No	Self-employed	Yes	No
12	F	43	O	Yes	$4.70 \times 10^2$	None	NA	No	No	Biologist	Yes	No
13	F	24	A	Yes	$2.25 \times 10^3$	DRH; FVR; HDC; MLG; OGD	6	Azithromycin	Yes	Office assistant	Yes	Yes
14	M	29	A	No	$9.87 \times 10^2$	None	NA	No	Yes	Driver	Yes	Yes
15	F	37	A	Yes	$2.57 \times 10^7$	DPN; FTG	9	Azithromycin, chloroquine	Not working	Housewife	No	Yes
16	F	41	O	Yes	$9.40 \times 10^6$	None	NA	No	Yes	Saleswoman	Yes	Yes
17	F	25	A	No	$6.42 \times 10^4$	CGH; FTG; FVR; MLG; OGD	2	Azithromycin, dipyrrone	Not working	Self-employed	No	No
18	F	35	O	No	$6.63 \times 10^3$	DRH; HDC	1	No	Not working	Housewife	No	Yes
19	F	22	O	No	64.00	CGH; FTG; MLG; TPH	2	No	No	Receptionist	Yes	Yes
20	M	29	O	Yes	$3.02 \times 10^6$	CGH; DPN; DRH; FTG; HDC	2	Paracetamol	Yes	Delivery biker	Yes	No
21	M	29	O	No	$7.48 \times 10^6$	None	NA	No	Yes	Bricklayer	No	No
22	M	25	A	No	$1.38 \times 10^7$	None	NA	No	No	Military army	Yes	No
23	M	29	O	No	$2.35 \times 10^2$	UNK	UNK	UNK	UNK	Military army	UNK	UNK
24	M	41	O	Yes	$5.24 \times 10^4$	None	UNK	UNK	UNK	Railroader	UNK	UNK
25	M	33	AB	Yes	$6.70 \times 10^4$	None	NA	No	Yes	Mechanical	Yes	Yes
26	F	34	O	Yes	$1.10 \times 10^5$	UNK	UNK	UNK	UNK	Waitress	UNK	UNK
27	M	27	B	Yes	$1.20 \times 10^4$	None	NA	No	No	Student	Yes	Yes

Abbreviations: CGH, cough; DPN, dyspnea; DRH, Diarrhea; FTG, fatigue; FVR, fever; HDC, headache; MLG, myalgia; NA, Non applicable; NVT, Nausea and vomiting; OGD, olfactory and gustatory disorders; anosmia and/or ageusia; STH, sore throat; TPH, throat phlegm.

<sup>a</sup>Patient with detectable SARS-CoV-2 RNA in saliva, nasopharyngeal swab ( $2.27 \times 10^7$  copies/ml), and plasma ( $43$  copies/ml) samples. UNK: unknown.



**FIGURE 2** SARS-CoV-2 viral load in saliva samples from blood donors who remained asymptomatic or developed mild COVID-19 post-donation. The symbols ■ and \* indicate respectively swab and plasma samples from the same blood donor (black circle), respectively. The difference of viral load level between the groups was not significant ( $p = .947$ , Mann-Whitney  $U$ -test)

The median SARS-CoV-2 load in individuals who remained asymptomatic was  $6.70 \times 10^4$  copies/ml and it was  $7.89 \times 10^4$  copies/ml in those who developed mild symptoms of COVID-19. Viral load levels in these two groups were not statistically different ( $p = .947$ ) (Figure 2).

## 4 | DISCUSSION

The pandemic of SARS-CoV-2 had a great impact on the entire society, and the containment of infection transmission is the primary measure to fight the pandemic. For hemotherapy, the impact of the pandemic could be felt in the fall of blood donor's attendance.<sup>14</sup> In addition, there is concern about whether or not SARS-CoV-2 can be transmitted by transfusion. To our knowledge, this is the first study to analyze a large population of blood donors by RT-PCR testing of saliva samples. We chose to test mostly saliva instead of nasopharyngeal swabs due to the scarcity of materials for swab collection, since saliva allows easy noninvasive self-collection. Previous studies also have shown equivalent or even better sensitivity of the RT-PCR when using saliva samples for SARS-CoV-2 RNA detection.<sup>15-17</sup> We also chose to perform the test in mini-pools, which was advantageous for saving materials and time without a loss of sensitivity, being ideal for use in populations with a low prevalence of infection, such as blood donors.<sup>18-20</sup>

Blood donors represent a strictly controlled population for the absence of symptoms of infection. Despite this, 27 of 4103 donors (0.66%; 95% CI 0.45-0.96)

evaluated in this study tested positive on the RT-PCR for SARS-CoV-2 in saliva (Figure 1), which proves that asymptomatic or presymptomatic individuals with COVID-19 are donating blood. This situation is occurring despite the use by Blood Centers of specific questions about the symptoms of COVID-19 during the screening of blood donors to decrease the risk of blood donation by infected individuals. This finding is relevant to the concern about the possibility of transmission of SARS-CoV-2 by blood transfusion. However, it was very important to note that among all positive donors in the SARS-CoV-2 RT-PCR test on their saliva, only one was also positive in plasma, confirming that RNAemia in plasma is not a common event in asymptomatic or presymptomatic individuals. Furthermore, the viral load in the SARS-CoV-2 positive plasma sample was very low, in agreement with findings observed in French blood donors,<sup>4</sup> indicating a weak likelihood of virus transfusion transmission. Despite these results, the Blood Center opted to discard all components produced with blood from all donors positive on the SARS-CoV-2 RT-PCR test of saliva.

It was interesting to note that despite the continued drop in new cases of COVID-19 in the city of Belo Horizonte from the second half of July, the prevalence of current infection of SARS-CoV-2 in blood donors remained stable (Figure 1). Although a small proportion of donors ( $n = 254$ ; 6.19%) do not live in the metropolitan area of Belo Horizonte, this result suggests that SARS-CoV-2 infection was underestimated in the general population of the city. This is not surprising, since practically only individuals strongly suspected of having COVID-19 were being tested, especially among groups at high risk.

The first studies that showed that SARS-CoV-2 could be detected in plasma analyzed symptomatic individuals. In Wuhan, China, the first 41 patients with COVID-19 were tested for SARS-CoV-2 RT-PCR in plasma and six (14.6%) presented RNAemia. The median viral load was low (cycle threshold value of 35.1) and there was no significant difference between patients submitted or not to intensive care.<sup>7</sup> Another Chinese study reported that SARS-CoV-2 RNA in plasma was detected in 10.5% (6/57) of patients with COVID-19, and all of them progressed to severe symptoms, suggesting that viral RNA in blood is a strong indicator of disease severity. A German study tested 77 whole blood, plasma, and serum samples from 15 symptomatic patients infected with SARS-CoV-2 and also three asymptomatic individuals. The authors showed that RNAemia was detected only in one plasma sample (5.6%) from an individual who had severe COVID-19.<sup>21</sup> These studies indicated a relatively low frequency of virus RNA in plasma, even in symptomatic patients.

The investigation of SARS-CoV-2 in asymptomatic individuals eligible for blood donation has been reported

in previous studies. A study conducted in January 2020 in Wuhan, China, using individual or mini-pool plasma samples showed the presence of viral RNA in four (0.05%) out of 7425 blood donations. On the other hand, another study carried out in Hubei, China, between February and April 2020 evaluated a total of 98,342 donations in 12 blood establishments by testing plasma samples individually or in mini-pools, and verified that all donations were negative for SARS-CoV-2 RNA.<sup>10</sup> However, since these studies did not test SARS-CoV-2 in samples that are considered more suitable for the detection of the virus, such as nasopharyngeal swab or saliva, they are unable to describe the rate of current SARS-CoV-2 infection among donors. However, in our study, we could define the rate of plasma RNAemia in individuals infected by SARS-CoV-2, and we confirmed that it was low (3.7%). Since the presence of SARS-CoV-2 RNA in blood does not mean the presence of virions able to survive the blood product preparation and storage conditions and still remain infectious until transfusion, and considering the low frequencies and low viral loads detected in plasma from asymptomatic individuals, we can suppose that the risk of SARS-CoV-2 transfusion transmission must be negligible. This is strongly supported by a study that showed a failure to isolate the virus from two plasma units with RNAemia confirmed by RT-PCR assays, indicating that the plasma was not infectious in cell culture.<sup>4</sup>

In this study, the viral load levels for SARS-CoV-2 did not show significant differences between individuals who remained asymptomatic and those who developed mild symptoms of COVID-19 (Figure 2). However, since the peak of viral load seems to occur in the early stages of infection, often before the onset of symptoms,<sup>22</sup> this result suggests that the level of viral load does not appear to be an indicator that can predict whether the carrier will remain asymptomatic or will develop symptoms of the disease. Previous studies also found that viral loads were similar in asymptomatic and presymptomatic individuals.<sup>23,24</sup>

It is important to note that we found four asymptomatic and four presymptomatic individuals with a high viral load ( $>10^6$  copies/ml) and only one had a low level of RNAemia in plasma, close to the detection limit. Thus, although the risk of transfusion transmission could be negligible, these individuals can be potential transmitters of the virus by other routes. This result alerts to the potential for virus transmission by individuals without symptoms<sup>25,26</sup> and reinforces the need to take measures to avoid transmission, even in places that adopt measures to detect suspected cases of COVID-19, such as with temperature checks.

Comparing some characteristics between the groups of blood donors with or without SARS-CoV-2 infection

(Table 1), the unique significant difference was a higher frequency of individuals donating blood for the first time in the SARS-CoV-2 positive group (OR = 2.999,  $p = .009$ ). There was also a trend ( $p = .086$ ) that SARS-CoV-2 positive donors were younger than negative donors. In the Blood Center of Belo Horizonte, about 70% of blood donors return to make another donation, and this rate increased this year, possibly because of active recruitment of repeat donors.<sup>14</sup> Therefore, the higher frequency of repeat donors participating in the present study is in agreement with this rate. We verified that first-time blood donors were significantly younger than repeat donors (median 27 vs. 36 years old,  $p < 0.001$ ; data not shown), and we believe that these first-time donors were more likely to have SARS-CoV-2 infection because they were younger. Young people tend to consider themselves less vulnerable to COVID-19, and are thus less careful about measures to prevent infection.

The risk factors for being infected by SARS-CoV-2 seemed to be diverse among the positive blood donors, but the use of public transport and professional activities seem to be relevant (Table 2). Previous contact with known individuals with COVID-19 cannot be considered important because this was a cause of deferral for donation. However, 50% (11/22) individuals living with others reported that other residents had COVID-19, including three donors who remained asymptomatic. The majority of individuals (19/24, 79.2%) reported the constant use of a mask. This safety accessory became obligatory in Belo Horizonte city since April 22, but many people do not use them properly.

Any SARS-CoV-2 RT-PCR positive result was communicated directly to donors by phone immediately after obtaining the RT-PCR result. On average, the time between sample collection and communication of the positive RT-PCR result was 5 days. After receiving a test with a positive result for SARS-CoV-2, with guidance to remain in social isolation, the vast majority (20/24, 83.3%) followed this recommendation. This result indicates that expanding testing to detect asymptomatic people is important to decrease the rate of viral transmission through social isolation.

A little more than half (56%) of positive blood donors developed mild symptoms related to COVID-19 about 2 days after blood donation. Fatigue, anosmia, and/or ageusia were the most prevalent symptoms, in agreement with previous studies.<sup>27,28</sup> Other influenza-like symptoms were also observed, as expected. As the symptoms were mild, most blood donors did not use medications to treat their infection. A study that investigated clinical characteristics in 172 South Korean patients with mild COVID-19 described that cough (40.1%), hyposmia (39.5%), and sputum (39.5%) were the most common

symptoms.<sup>29</sup> However, they observed that fever (>37.5°C) occurred in only 20 (11.6%) individuals. In the present study, fever was reported by five (35.7%) blood donors who developed mild COVID-19, and none of them presented sputum. A previous study analyzed 417 mild-to-moderate COVID-19 patients from 12 European hospitals and reported that 85.6% and 88.0% of patients developed olfactory and gustatory dysfunctions, respectively.<sup>28</sup> These data point out that sudden anosmia or ageusia should be recognized as important symptoms to suspect COVID-19, even in the absence of influenza-like symptoms.

In conclusion, we proved that a low frequency of blood donors were infected by SARS-CoV-2 at the time of donation, and that RNAemia in plasma is not a common event in asymptomatic and presymptomatic individuals. These findings suggest that the risk of SARS-CoV-2 transfusion transmission may be negligible, but studies concerning the presence of virions in blood components and retrovigilance must be performed to confirm this statement.

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## CONFLICT OF INTEREST

All authors declare no competing financial interests.


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