TRANSFUSION

Risk of transmission of severe acute respiratory syndrome coronavirus 2 by transfusion: A literature review

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel human coronavirus responsible for coronavirus disease 2019 (COVID-19). The emergence of this virus in Wuhan, China, at the end of 2019 and its worldwide spread to reach the pandemic stage has raised concerns about the possible risk that it might be transmissible by transfusion. This theoretical risk is further supported by reports of the detection of viral RNA in the blood of some infected individuals. To further address this risk, a thorough *PubMed* literature search was performed to systematically identify studies reporting data on the detection of SARS-CoV-2 RNA in blood or its components. Complementary searches were done to identify articles reporting data on the in vitro infectivity of blood components. At least 23 articles presenting data on the detection of SARS-CoV-2 RNA in blood, plasma, or serum were identified. Of these, three studies reported on blood donors with COVID-19 infection identified after donation, and no cases of transfusion transmission were identified. A few studies mentioned results of in vitro infectivity assays of blood components in permissive cell lines, none of which were able to detect infectious virus in blood or its components. Complementary searches have identified reports demonstrating that the correlation between the presence of viral RNA in a biologic sample and infectivity requires a minimal RNA load, which is rarely, if ever, observed in blood components. Overall, the available evidence suggests that the risk of transmission of SARS-CoV-2 by transfusion remains theoretical.

K E Y W O R D S
blood, COVID-19, infectivity, RNAemia, SARS-CoV-2, Vero cell lines

1 | INTRODUCTION

In January 2020, Chinese health authorities reported several cases of a new acute respiratory illness arising in December 2019 in the city of Wuhan, Hubei province.[1–4] Symptoms of this novel illness are typical of respiratory infections of viral origin; fever, fatigue, myalgia, and dry cough are commonly observed.[2–5] Although most

patients experience mild to moderate symptoms and recover within a few days, some 20% of identified patients exhibit more severe forms of the disease requiring prolonged hospitalization and, in some cases, acute care and ventilation.[6] The exact mortality rate is difficult to assess, as varying proportions of asymptomatic or presymptomatic cases have been reported, and broad serosurveys to understand the true burden of disease have been hampered by a variety of logistic and scientific issues.[7] However, the detailed study of Wu and McGoogan, reporting on 72 314 suspected cases from the Chinese Center for Disease Control and Prevention, including 44 672 confirmed cases, provides an estimated case fatality rate of 2.3%.[6]

Simultaneously to the primary reports of cases of coronavirus disease 2019 (COVID-19), a virus was isolated from bronchoalveolar lavage fluids of affected patients. Characterization of the virus and elucidation of the nucleotide sequence of its genome identified an enveloped, nonsegmented positive single-stranded RNA virus, a novel member of the betacoronavirus family, subfamily *Orthocoronaviridae*. This new virus, SARS-CoV-2, shares 79.6% genomic sequence identity with severe acute respiratory syndrome coronavirus (SARS-CoV).[2,8] The latter is a coronavirus that was responsible for an outbreak of a severe acute respiratory syndrome that affected several countries in 2003. That outbreak was successfully managed through strict confinement of infected individuals and quarantine of their contacts. During that outbreak, there was no evidence of transfusion transmission.[9] Conversely, SARS-CoV-2 rapidly spread on a broad scale as a result of air travel and the relative ease by which the virus is transmitted by respiratory droplets from coughing and sneezing. On 11 March 2020, the World Health Organization officially declared that COVID-19, the disease caused by SARS-CoV-2 infection, had reached the pandemic level.[10] As of 30 June 2020, more than 10.4 million cases of SARS-CoV-2 infection, and more than 509 000 deaths from COVID-19, have been reported worldwide.[11–13]

The emergence of this novel infectious agent has forced blood component suppliers to raise their level of awareness and to quickly assess the potential risk to blood safety. This article aims to evaluate the available evidence on the theoretical risk of SARS-CoV-2 transmission by transfusion, including attempts at determining infectiousness of blood components.

2 | SEARCH STRATEGY

The PubMed public biomedical literature database (https://pubmed.ncbi.nlm.nih.gov/) was searched for references that pertain to the risk of transmission of COVID-19/SARS-CoV-2 by transfusion. More specifically, PubMed was interrogated with a series of queries aimed at identifying references that relate to COVID-19/SARS-CoV-2 and the detection of viral genomic material in blood, plasma, or serum. As this enveloped virus would not be expected to survive the fractionation process, key words associated with purified plasma products were not included in the search. Queries were built from a basic search script recommended by PubMed to provide a broad coverage of the COVID-19/SARS-CoV-2 literature:

$$((\text{wuhan[All Fields] AND (``coronavirus''[MeSH Terms]}$$
$$\text{OR``coronavirus''[All Fields])) AND 2019/12[PDAT]}$$
$$: 2030[\text{PDAT}]) \text{ OR } 2019 - n\text{CoV[All Fields] OR 2019nCoV}$$
$$[\text{All Fields}] \text{ OR COVID} - 19[\text{All Fields}] \text{ OR SARS} - \text{CoV}$$
$$- 2[\text{All Fields}]$$

From this core script, queries focused on the detection of viral genomic material in blood were built. Queries and their respective search results are shown in Table 1. Titles and abstracts from the nonoverlapping 734 references from Searches 2 and 5 (equivalent to Search #4) were examined. From this screen, 23 references reporting any data or stating any information on the detection of SARS-CoV-2 genomic material in human blood, plasma, or serum were selected (Table 2).

While examining the above 734 references, some references pertaining to in vitro or animal models of SARS-CoV-2 infectivity were intercepted and saved. Additional searches specifically targeting the in vitro infectivity of blood, plasma, or serum samples were performed to complete the list of references on that topic.

Additional searches were performed to identify references pertaining to COVID-19/SARS-CoV-2 and infection of endothelial cells. A nonexhaustive, restricted list of relevant references were selected and are discussed in the text.

3 | DETECTION OF SARS-CoV-2 GENOMIC MATERIAL IN BLOOD

An exhaustive search strategy led to the identification of 23 references reporting data on the detection of SARS-CoV-2 genomic material in blood components (Table 2).[4,8,14–34] As correctly pointed out by Huang et al,[4] the presence of SARS-CoV-2 genomic material in the blood of asymptomatic/presymptomatic individuals or patients with COVID-19 should be referred to as

TABLE 1 PubMed search strategy

Search number	Query	Result (number of hits)
1	(((wuhan[All Fields] AND ("coronavirus"[MeSH Terms] OR "coronavirus"[All Fields])) AND 2019/12[PDAT]: 2030[PDAT]) OR 2019-nCoV[All Fields] OR 2019nCoV[All Fields] OR COVID-19[All Fields] OR SARS-CoV-2[All Fields]) AND transfusion	142
2	(((wuhan[All Fields] AND ("coronavirus"[MeSH Terms] OR "coronavirus"[All Fields])) AND 2019/12[PDAT]: 2030[PDAT]) OR 2019-nCoV[All Fields] OR 2019nCoV[All Fields] OR COVID-19[All Fields] OR SARS-CoV-2[All Fields]) AND (transfusion OR "detection in blood"[All Fields] OR "blood detection"[All Fields] OR "detection in plasma"[All fields] OR "plasma detection"[All Fields] OR "genome detection" [All Fields])	154
3	#1 NOT #2	0
4	(((wuhan[All Fields] AND ("coronavirus"[MeSH Terms] OR "coronavirus"[All Fields])) AND 2019/12[PDAT]: 2030[PDAT]) OR 2019-nCoV[All Fields] OR 2019nCoV[All Fields] OR COVID-19[All Fields] OR SARS-CoV-2[All Fields]) AND ((transfusion OR ((detect*[TIAB] OR identifi*[TIAB]) AND (blood[TIAB] OR plasma[TIAB] OR serum[TIAB] OR sera[TIAB] OR genome*[TIAB]) OR "viral load*"[TIAB])))	734
5	#4 NOT #2	580
6	#5 NOT #4	0

Note: Searches were performed on 17 June 2020.

RNAemia, as opposed to viremia, which refers to the presence of intact, infectious virions in blood. We adhere to this terminology throughout the text.

Several observations can be made from the data summarized in Table 2. First, RNAemia, when present, is close to the limit of detection of real-time reverse transcription quantitative polymerase chain reaction (rRT-qPCR), with cycle threshold (Ct) values well above 30 in the vast majority of cases. Second, RNAemia tends to be associated with more severe disease.[18,20,26,28,30,32] Third, 18 of the 23 identified studies report on cases of patients diagnosed with COVID-19. Even when RNA testing is done on whole blood, plasma, or serum from a preselected cohort of patients with COVID-19, the prevalence of RNAemia is generally low. In this context, the article of Zheng et al[28] is an exception, with 39 of 96 hospitalized patients with COVID-19 that were RNAemic.

Fourth, three studies bear particular relevance to the field of transfusion safety. Kwon et al[23] and Cho et al[34] report on cases of patients transfused with blood components from donors who were subsequently found to be infected by SARS-CoV-2. Notably, none of the donations implicated in Kwon et al's case reports were RNAemic, whereas Cho et al did not test whether the implicated donation was RNAemic. None of the transfused patients from these two case reports developed COVID-19. Chang et al[24] report the most exhaustive screening study of an unselected, presumably healthy cohort of blood donors from a COVID-19–endemic region (Wuhan, China). A total of 2430 donations were screened for SARS-CoV-2 RNAemia. In addition, 4995 repository samples from previous donations were screened. Furthermore, an unspecified number of telephone follow-ups to screen newly symptomatic donors were done. Collectively, these procedures allowed for the detection of four RNAemia-positive donors. None of the RNAemic blood components had been transfused, and thus all these components were recalled. Collectively, these three studies suggest that some individuals who are eligible to donate blood are infected with SARS-CoV-2 but are asymptomatic/presymptomatic. Furthermore, the data suggest that very few of these asymptomatic/presymptomatic individuals are RNAemic.

Fifth, when present, RNAemia appears to occur early in the course of the infection, and the duration of RNAemia is relatively short. In support of this observation, Zhou et al[8] were unable to detect RNAemia in blood samples from five patients with COVID-19 collected from 18 to 29 days after symptom onset. Additionally, the longitudinal case series of Kim et al,[17] Lescure et al,[20] Wölfel et al,[22] and Kim et al[33] support the notion that RNAemia tends to peak within 10 days of symptom onset and to decline thereafter.

Finally, only 2 of the 23 identified studies explicitly state that attempts were made to grow the SARS-CoV-2 in culture in the presence of permissive cell lines.[17,33] However, Kim et al[17] did not specify the tissue origin (respiratory tract or blood) of samples that were tested in an infectivity assay. Interestingly, all samples analyzed by Kim et al[33] were from symptomatic patients with COVID-19, which are more likely to yield higher RNAemia, and would possibly increase the chances of detecting infectivity in blood. Yet both studies failed at

TABLE 2 Studies reporting results on the detection of SARS-CoV-2 RNA in the blood, plasma, or serum of suspected COVID-19 patients and screened healthy individuals, and on in vitro infectivity assays of RNAemia-positive blood, plasma, or serum samples

Reference	Timing of blood sample collection[a]	Type of sample analyzed	RNAemia-positive individuals/total number of COVID-19+ individuals tested	Details regarding assay results and/or Ct cutoff values	In vitro infectivity assay results
Chan et al[14]	Single positive sample collected 6 d after symptom onset	Serum and plasma	1/7 (serum sample)	Ct of single positive sample: 40	ND
Holshue et al[15]	4- and 7 d after symptom onset	Serum	0/1 (single case report)	NS	...
Zhou et al[8]	From 18 to 29 d after symptom onset	Blood	0/5	NS	...
Huang et al[4]	Median of 11 d after symptom onset	Plasma	6/41	Median Ct of positive samples: 35.1 (IQR = 34.7-35.1)	ND
Zhang et al[16]	NS	WB and serum	6/15 (WB) 3/15 (serum; also positive with WB)	Ct range of positive WB samples: 30.3-32.1 Ct range of positive serum samples: 24.3-34.5	ND
Kim et al[17]	Patient 1 serum samples positive on Days 6, 8, 12, and 13 after symptom onset Patient 2: One plasma sample positive on Day 17 after symptom onset	Serum and plasma	2/2 (longitudinal case series)	Patient 1 Ct range of positive samples: 28.76-39.61 Patient 2 Ct of single positive plasma sample: 26.97 (Ct cutoff: ≤37; LOD: 2.69 copies/μL)	Viral replication in culture unsuccessful (unspecified sample origin)
Chen et al[18]	Positive samples collected 6-12 d after symptom onset	Serum	6/57	Ct range of positive samples: 32-41	ND
Wang et al[19]	During hospitalization	Blood	3/205 (3/307 samples tested)	Mean Ct of positive samples: 34.6 Range: 34.1-35.4 (Ct cutoff: <40)	ND
Lescure et al[20]	Positive samples collected on Days 7, 8, 9, and 12 after symptom onset	WB, plasma, and serum	1/5 (plasma and WB) (case series)	Ct range of positive samples: 35.8-38.4	ND
Yu et al[21]	NS	Plasma	0/4 samples (unclear whether from four different patients or repeat samples from some patients)	Ct cutoff: ≤38	...
Wölfel et al[22]	From 3 to 21 d after symptom onset	Serum	0/9[b]	NS	...

(Continues)

TABLE 2 (Continued)

Reference	Timing of blood sample collection[a]	Type of sample analyzed	RNAemia-positive individuals/total number of COVID-19+ individuals tested	Details regarding assay results and/or Ct cutoff values	In vitro infectivity assay results
Kwon et al[23]	Postdonation COVID-19 diagnosis	WB repository samples	0/6	NS	...
Chang et al[24]	Donor 1 (asymptomatic): Screened by blood center; Donor 2 (asymptomatic): Retrospective sample testing; Donors 3 and 4: Episodes of fever ascertained by postdonation telephone follow-ups	Plasma	4/2430 blood donations	Ct cutoffs: ≤42 for one genomic region, and ≤45 for a second genomic region; LOD: 10 copies/mL in 1.6 mL plasma	ND
Huang et al[25]	Positive samples collected 10-12 d after symptom onset	Plasma (2 positive samples) and serum (three positive samples)	1/16 ICU patients	Ct range for the single positive patient: 30.10-37.57; Ct cutoff: < 40	ND
Chen et al[26]	Upon admission to the hospital	Serum	5 (all in critical condition)/48	Ct range for the five positive patients: 34.58-39.01; Ct cutoff: <40	ND
Wu et al[27]	NS	Blood	4/132	NS	ND
Zheng et al[28]	Positive patients (39) collected from 1 to 4 wk after symptom onset among 96 hospitalized COVID-19 patients	Serum	39/96	Ct cutoff: ≤38.0	ND
Chan et al[29]	Positive samples collected from 4 to 13 d after symptom onset	Plasma	10/87	Mean of positive samples: 7.86×10^3 copies/mL	ND
COVID-19 Investigation Team[30]	Positive samples from single positive patient collected on Days 9, 11, and 13 after symptom onset	Serum	1/11	Ct range for the single positive patient: 36.3-36.8	ND
Peng et al[31]	Samples from the two positive patients collected 3 d after symptom onset	Blood	2/9	RNA concentration for the two positive patients: 8.04 and 91.1 copies/mL	ND

TABLE 2 (Continued)

Reference	Timing of blood sample collection[a]	Type of sample analyzed	RNAemia-positive individuals/total number of COVID-19+ individuals tested	Details regarding assay results and/or Ct cutoff values	In vitro infectivity assay results
Corman et al[32]	Patients with severe symptoms, during inpatient treatment	Blood, serum, and plasma	1 (ARDS)/18	RNA concentration for the single positive patient: 179 copies/mL, detected in only one of eight serum/plasma samples	ND
Kim et al[33]	From one to 10 d post symptom onset	Serum	6/74 patients tested (8-9/323 samples tested)[c]	127-1210 copies/μL[d]	No viral replication in culture
Cho et al[34]	ND	...	...	...	ND

Note: References are presented in ascending order of online publication date.

Abbreviations: ARDS, acute respiratory distress syndrome; Ct, cycle threshold; LOD, limit of detection; ND, not done; NS, not specified; WB, whole blood.

[a]For those studies that detected RNAemia, this column shows the timing of collection of positive samples. For those studies that did not detect RNAemia, this column shows the entire range of times when blood samples were collected.

[b]There is ambiguity regarding the total number of samples tested. The text (p. 466 of the article) mentions that a total of 31 samples were tested; Figure 1A (p. 466) suggests that a total of 51 serum samples were tested.

[c]There is ambiguity regarding the total number of SARS-CoV-2 RNA-positive serum samples. The text (p. 114) states that nine serum samples were positive; the data of Figure 1 (p. 116) indicate that eight serum samples were positive.

[d]There is an ambiguity regarding the mean RNA concentrations in SARS-CoV-2-positive serum samples. The Abstract (p. 112) mentions a concentration of 1210 ± 1861 copies/μL in positive samples, whereas the Results and Discussion section (p. 114) mentions a concentration of 127 copies/μL in positive samples. In any event, these concentrations seem relatively high.

detecting infection of a permissive cell line, suggesting that for SARS-CoV-2, RNAemia does not indicate the presence of infectious virions. This observation is consistent with what is known of human coronaviruses,[35] and respiratory viruses in general.

4 | INFECTIVITY OF BLOOD COMPONENTS AND OTHER NONRESPIRATORY SAMPLES

The viral infectivity of a biologic sample, including blood, plasma, or serum, can be determined in vitro with cells that are known to be susceptible to infection. In such a cellular model, infection results in either detectable cytopathic effects, cell lysis, intracellular replication of the virus and production of viral particles in the culture supernatant, or a combination of these manifestations. Infectivity can also be demonstrated in a susceptible animal model, in which viral infection will result in signs and symptoms similar to those observed in humans.

Several cell lines can support SARS-CoV-2 replication.[36–39] In fact, any cell line that expresses the cognate angiotensin-converting enzyme 2 and capable of sustaining replication of the virus can be used to assess infectivity.[40] Among the most commonly used cell lines are Vero and its derivatives. Originally derived from African green monkey kidney epithelial cells, the Vero cell line is broadly used for the study of human respiratory viruses. This in vitro model permitted confirmation of the infectivity of respiratory samples collected from suspected COVID-19 cases.[2,8,22] Other cell lines (Huh7, Calu3, Caco-2) have also been shown to be permissive for SARS-CoV-2 replication.[37] Various animal models of infection have also been identified and characterized.[41–43]

As stated earlier, attempts at detecting infectivity in blood have been so far unsuccessful. In fact, infectivity in biologic samples outside of the respiratory tract has not been demonstrated. Although infectivity can be detected in respiratory samples, a minimal RNA load, in terms of equivalent RNA copy number, appears to be necessary for in vitro infection of cell lines to occur. The data of La Scola et al[44] suggest that individuals whose respiratory samples yield Ct values above 34 are no longer contagious. Bullard et al's[45] results support the idea that the quantitative criterion for infectivity could be even higher: their data suggest that the infectivity of samples with Ct values greater than 24 might be below the limit of detection of an in vitro infectivity assay. The recent article by Huang et al[46] is consistent with these observations. Given that RNAemic blood samples generally give Ct values in the high 30s, the above reports on the relationship between RNAemia in respiratory samples and infectivity

are consistent with the idea that blood is unlikely to be an infectious source of SARS-CoV-2.

5 | SARS-CoV-2 INFECTION OF ENDOTHELIAL CELLS AND T CELLS

Aside from a respiratory infection, SARS-CoV-2 appears to induce systemic effects, which likely contribute to the pathological mechanisms observed in the most severe cases of infection.[3,4] Furthermore, some reports have suggested that the SARS-CoV-2 virus could infect endothelial cells lining the interior of blood vessels,[47–50] raising the possibility that infectious virions might be present in the circulation. However, some of these findings have been challenged.[51] Furthermore, these findings are based on case reports of patients with COVID-19 or postmortem analysis of deceased patients with COVID-19, and the presumed detection of SARS-CoV-2 virions was performed by electron microscopy and immunohistochemistry, which are prone to artifacts and misinterpretations.[51] In addition, two of these articles report on deceased patients that had comorbidities that were directly involved with the organ origin of the suspected observation of SARS-CoV-2 virions, namely, the kidney of a patient who had undergone a kidney transplant[47] and the brain of a patient with Parkinson disease.[50] SARS-CoV-2 RNA has also been detected in 5 of 104 endomyocardial biopsy samples from patients exhibiting signs of myocarditis or unexplained cardiac failure, suggesting that the virus might leach into the myocardium. However, Escher et al[52] did not report the detection of virions by electron microscopy or immunohistochemistry, nor were they able to demonstrate that the RNA, detected after 33 or more cycles of rRT-qPCR, was infectious. Thus, this observation could be a bystander detection or leaching/contamination of the biopsy sample with lung tissue.

There is some evidence that SARS-CoV-2 can infect human primary CD4+ T cells in culture and drive the expression of viral proteins in these cells. However, the relevance of these infections is not known, as these infections did not appear to be productive in terms of live viral particles. It is also expected that the burden of infected T cells, if it were to occur in vivo, would be substantially reduced through leukoreduction.[53]

6 | CONCLUSIONS

To this day, there has not been a single reported case of transmission of a respiratory virus by transfusion. Accordingly, the long historical track record on the mode of transmission of respiratory viruses predicts that SARS-CoV-2

would not be transmissible by transfusion. So far, this hypothesis appears to be true, as there has been no documented case of transfusion-transmitted SARS-CoV-2.

As stated by Katz,[54] given that some asymptomatic/presymptomatic individuals appear to be infectious (through their respiratory secretions), and that some of these individuals must have donated blood since the beginning of the pandemic, if indeed SARS-CoV-2 was hematogenous, then it is likely that some cases of transmission by transfusion would have been identified among transfused patients on a worldwide scale. Furthermore, RNAemia is generally associated with a more severe disease course; accordingly, the majority of RNAemic individuals are not healthy enough to donate blood, which further reduces the theoretical risk of transmission by transfusion. The fact that epidemiologic investigations and contact tracing indicate that new COVID-19 cases are generally related to close contacts with infected individuals and that no cases have been linked to transfusion is reassuring from a blood safety standpoint.

CONFLICT OF INTEREST
The authors declare no potential conflict of interest.

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