RISK OF TRANSMISSION OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS-2 BY TRANSFUSION: A LITERATURE REVIEW

Jean-François Leblanc[1], Marc Germain[1], Gilles Delage[2], Sheila O'Brien[3], Steven J. Drews[4], Antoine Lewin[2,5]

[1]*Medical Affairs and Innovation, Héma-Québec, Québec, Québec, Canada*

[2]*Medical Affairs and Innovation, Héma-Québec, Saint-Laurent, Québec, Canada*

[3]*Canadian Blood Services, Ottawa, Ontario, Canada*

[4]*Canadian Blood Services, Edmonton, Alberta, Canada*

[5]*Faculty of Medicine and Health Sciences, University of Sherbrooke, Sherbrooke, Québec, Canada*

Corresponding author: Antoine Lewin

Full postal address: Héma-Québec

4045, boulevard Côte-Vertu

Montréal (Québec) H4R 2W7

Canada

Phone number: 514-832-5000 ext. 3490, or toll-free 1-888-666-4362, ext. 3490

Fax number: 514-832-1025

Email: Antoine.Lewin@hema-quebec.qc.ca

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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 post-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 biological sample and infectivity requires a minimal RNA load, which is rarely, if at all observed, in blood components. Overall, the available evidence suggests that the risk of transmission of SARS-CoV-2 by transfusion remains theoretical.

Key words: COVID-19, SARS-CoV-2, RNAaemia, infectivity, blood, Vero cell lines

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 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, non-segmented positive single-stranded RNA virus, a novel member of the betacoronavirus family, subfamily *Orthocoronaviridae*. This new virus, referred to by the acronym 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 which 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 March 11, 2020, the World Health

Organization (WHO) officially declared that COVID-19, the disease acronym caused by SARS-CoV-2 infection, had reached the pandemic level.[10] As of June 30, 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.

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:

((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]

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 non-overlapping 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 non-exhaustive, restricted list of relevant references were selected and are discussed in the text.

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 COVID-19 patients should be referred to as RNAaemia, as opposed to viremia, which refers to the presence of intact, infectious virions in blood. We shall adhere to this terminology throughout the text.

Several observations can be made from the data summarized in Table 2. First, RNAaemia, when present, is close to the limit of detection of rRT-qPCR, with cycle threshold (Ct) values well above 30 in the vast

majority of cases. Second, RNAaemia 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 COVID-19 patients, the prevalence of RNAaemia is generally low. In this context, the article of Zheng et al. (2020)[28] is an exception, with 39 out of 96 hospitalized COVID-19 patients that were RNAaemic.

Fourth, three studies bear particular relevance to the field of transfusion safety. Kwon et al. (2020)[23] and Cho et al. (2020)[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 RNAaemic, wherease Cho et al. did not test whether the implicated donation was RNAaemic or not. None of the transfused patients from these two case reports developed COVID-19. Chang et al. (2020)[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 2,430 donations were screened for SARS-CoV-2 RNAaemia. In addition, 4,995 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 RNAaemia-positive donors. None of the RNAaemic 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 RNAaemic.

Fifth, when present, RNAaemia appears to occur early in the course of the infection, and the duration of RNAaemia is relatively short. In support of this observation, Zhou et al. (2020)[8] were unable to detect RNAaemia in blood samples from five COVID-19 patients collected from 18 to 29 days post symptom

onset. Additionally, the longitudinal case series of Kim et al. (2020a),[17] Lescure et al. (2020),[20] Wölfel et al. (2020)[22] and Kim et al. (2020b)[33] support the notion that RNAaemia tends to peak within 10 days of symptom onset and to decline thereafter.

Finally, only two 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. (2020a) 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. (2020b) were from symptomatic COVID-19 patients, which are more likely to yield higher RNAaemia, and would possibly increase the chances of detecting infectivity in blood. Yet, both studies failed at detecting infection of a permissive cell line, suggesting that for SARS-CoV-2, RNAaemia 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.

INFECTIVITY OF BLOOD COMPONENTS AND OTHER NON-RESPIRATORY SAMPLES

The viral infectivity of a biological sample, including blood, plasma, or serum, can be determined *in vitro* using 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 which expresses the cognate angiotensin-converting enzyme 2 (ACE2) 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 biological 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. (2020)[44] suggest that individuals whose respiratory samples yield Ct values above 34 are no longer contagious. Bullard et al.'s 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 > 24 might be below the limit of detection of an *in vitro* infectivity assay.[45] The recent article by Huang et al. (2020c) is consistent with these observations.[46] Given that RNAaemic blood samples generally give Ct values in the high 30's, the above reports on the relationship between RNAaemia in respiratory samples and infectivity are consistent with the idea that blood is unlikely to be an infectious source of SARS-CoV-2.

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 COVID-19 patients or post-mortem analysis of deceased COVID-19 patients, 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 kidney transplant patient[47] and the brain of a Parkinson's disease patient.[50] SARS-CoV-2 RNA has also been detected in five out of 104 endomyocardial biopsy samples from patients exhibiting signs of myocarditis or unexplained cardiac failure, suggesting that the virus might leach into the myocardium.[52] However, Escher et al. did not report the detection of virions by electron microscopy or immunohistochemistry, nor were they able to demonstrate that the RNA, detected after $\geq$ 33 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]

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 (2020),[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, RNAaemia is generally associated with a more severe disease course; accordingly, the majority of RNAaemic individuals are not healthy enough to donate blood, which further reduces the theoretical risk of transmission by transfusion. The fact that epidemiological 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.

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TABLES

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

Searches were performed on 2020/06/17.

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 RNAaemia-positive blood, plasma, or serum samples.

Reference	Timing of blood sample collection*	Type of sample analyzed	RNAaemia-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. (2020a)[14]	Single positive sample collected 6 days post symptom onset	Serum and plasma	1/7 (serum sample)	Ct of single positive sample: 40	ND†
Holshue et al. (2020)[15]	4- and 7-days post symptom onset	Serum	0/1 (single case report)	NS†	-
Zhou et al. (2020)[8]	From 18 to 29 days post symptom onset	Blood	0/5	NS	-
Huang et al. (2020a)[4]	Median of 11 days post symptom onset	Plasma	6/41	Median Ct of positive samples: 35.1 (IQR = 34.7-35.1)	ND
Zhang et al. (2020)[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

Reference	Timing of blood sample collection*	Type of sample analyzed	RNAaemia-positive individuals/ Total number of COVID-19+ individuals tested	Details regarding assay results and/or Ct† cutoff values	*In vitro* infectivity assay results
Kim et al. (2020a)[17]	Patient 1 serum samples positive on days 6, 8, 12, 13 post symptom onset Patient 2: one plasma sample positive on day 17 post 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. (2020a)[18]	Positive samples collected 6-12 days post symptom onset	Serum	6/57	Ct range of positive samples: 32-41	ND
Wang et al. (2020)[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. (2020)[20]	Positive samples collected on days 7, 8, 9, and 12 post 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. (2020a)[21]	NS	Plasma	0/4 samples (unclear whether from four different patients or repeat samples from some patients)	Ct cutoff: ≤ 38	-
Wölfel et al. (2020)[22]	From 3 to 21 days post symptom onset	Serum	0/9‡	NS	-

Reference	Timing of blood sample collection*	Type of sample analyzed	RNAaemia-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. (2020)[23]	(post-donation COVID-19 diagnosis)	WB repository samples	0/6	NS	-
Chang et al. (2020)[24]	Donor 1 (asymptomatic): screened by blood center Donor 2 (asymptomatic): retrospective sample testing Donors 3 and 4: episodes of fever ascertained by post-donation telephone follow-ups	Plasma	4/2,430 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. (2020b)[25]	Positive samples collected 10-12 days post symptom onset	Plasma (2 positive samples) and serum (3 positive samples)	1/16 ICU patients	Ct range for the single positive patient: 30.10-37.57 Ct cutoff: < 40	ND
Chen et al. (2020b)[26]	Upon admission to the hospital	Serum	5 (all in critical condition)/48	Ct range for the 5 positive patients: 34.58-39.01 Ct cutoff: < 40	ND
Wu et al. (2020)[27]	NS	Blood	4/132	NS	ND

Reference	Timing of blood sample collection*	Type of sample analyzed	RNAaemia-positive individuals/ Total number of COVID-19+ individuals tested	Details regarding assay results and/or Ct† cutoff values	*In vitro* infectivity assay results
Zheng et al. (2020)[28]	Positive patients (39) collected from 1 to 4 weeks post symptom onset among 96 hospitalized COVID-19 patients	Serum	39/96	Ct cutoff: ≤ 38.0	ND
Chan et al. (2020b)[29]	Positive samples collected from 4 to 13 days post symptom onset	Plasma	10/87	Mean of positive samples: 7.86×10^3 copies/mL	ND
COVID-19 Investigation Team (2020)[30]	Positive samples from single positive patient collected on days 9, 11, and 13 post symptom onset	Serum	1/11	Ct range for the single positive patient: 36.3-36.8	ND
Peng et al. (2020)[31]	Samples from the two positive patients collected 3 days post symptom onset	Blood	2/9	RNA concentration for the two positive patients: 8.04 and 91.1 copies/mL	ND
Corman et al. (2020)[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 8 serum/plasma samples	ND
Kim et al. (2020b)[33]	From one to 10 days post symptom onset	Serum	6/74 patients tested (8-9/323 samples tested)§	127-1,210 copies/µL‖	No viral replication in culture

Reference	Timing of blood sample collection*	Type of sample analyzed	RNAaemia-positive individuals/ Total number of COVID-19+ individuals tested	Details regarding assay results and/or Ct† cutoff values	*In vitro* infectivity assay results
Cho et al. (2020)[34]	ND	-	-	-	ND

References are presented in ascending order of online publication date.

*For those studies which detected RNAaemia, this column shows the timing of collection of positive samples. For those studies which did not detect RNAaemia, this column shows the entire range of times when blood samples were collected.

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

‡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; Fig. 1a (p. 466) suggests that a total of 51 serum samples were tested.

§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 Fig. 1 (p. 116) indicate that eight serum samples were positive.

‖There is an ambiguity regarding the mean RNA concentratios in SARS-CoV-2-positive serum samples. The Abstract (p. 112) mentions a concentration of 1,210 ± 1,861 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.