specificity of the assay. The primer and probe sequences, used by the four laboratories, are listed in Table 2. Among the six samples tested, only one was found to be weakly positive for the E gene (36.1 cycle threshold; cycle threshold of a positive result was ≤40) by two of the four testing centers (Table 1). This was a plasma donation dedicated to fractionation. This discrepancy among the testing laboratories was related to the volume of plasma used to isolate the viral RNA. Indeed, by using a larger (up to 280 μL vs 140 μL) volume of plasma during the viral RNA isolation, these two laboratories were able to get a weak positive result by PCR. The infectivity of the SARS-CoV-2 found in this donation was evaluated using Vero E6 cell line[5] and was found to be noninfectious. A plasma donation given 6 days earlier by this same donor was also recalled, tested, and found to be negative for SARS-CoV-2 RNA. Interestingly, for the donor who had mild symptoms at the time of her donation, this latter was tested negative for the presence of SARS-CoV-2 RNA. All six donors were also tested for the presence of antibodies against SARS-CoV-2 and all were negative, confirming that they were in the early phase of infection. These results are consistent with those reported by Chang and colleagues from China,[4] where only four donors out of more than 7000 were found to be weakly positive for SARS-CoV-2 RNA in their blood.

In conclusion, our data indicate that SARS-CoV-2, like other respiratory viruses such as SARS-CoV and MERS-CoV, is present in very limited amount if not totally absent in blood products donated shortly before the onset of symptoms in infected individuals. Consequently, the risk of transmission of COVID-19 by blood transfusion, if it exists, would appear to be negligible.

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

Lionel Loubaki[1,2]
Pierre Gantner[3]
Amélie Pagliuzza[3]
Hugues Fausther-Bovendo[4]
Gary Kobinger[4]
Nicolas Chomont[3]
Marc Germain[1]

[1]*Héma-Québec, Affaires médicales et Innovation, Québec, Québec, Canada*
[2]*Department of Biochemistry, Microbiology and Bioinformatics, Laval University, Québec, Québec, Canada*
[3]*Centre de recherche du CHUM, Axe Immunopathologie, Montréal, Québec, Canada*
[4]*Centre de recherche du CHU de Québec-Université Laval, Axe maladies infectieuses et immunitaires, Québec, Québec, Canada*

Correspondence
Lionel Loubaki, Héma-Québec,Affaires Médicales et Innovation, 1070 avenue des Sciences-de-la-Vie, Québec, QC G1V 5C3, Canada.
Email: lionel.loubaki@hema-quebec.qc.ca
DOI 10.1111/trf.16155

ORCID

Lionel Loubaki https://orcid.org/0000-0002-4744-5387

REFERENCES

1. World Health Organization. Report of the WHO-China joint Mission on coronavirus disease 2019 (COVID-19). Geneva, Switzerland: WHO, 2020. https://www.who.int/docs/default-source/coronaviruse/who-china-joint-mission-on-covid-19-final-report.pdf. Accessed 30 June 2020.
2. Zou L, Ruan F, Huang M, et al. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med. 2020; 382(12):1177–1179.
3. Kwon S, Kim E, Jung YS, Jang JS, Cho N. Post-donation COVID-19 identification in blood donors. Vox Sang. 2020. https://doi.org/10.1111/vox.12925.
4. Chang L, Zhao L, Gong H, Wang L, Wang L. Severe acute respiratory syndrome coronavirus 2 RNA detected in blood donations. Emerg Infect Dis. 2020;26:1631–1633.
5. Ogando NS, Dalebout TJ, Zevenhoven-Dobbe JC, et al. SARS-coronavirus-2 replication in Vero E6 cells: replication kinetics, rapid adaptation and cytopathology. J Gen Virol. 2020;101: 925–940.

Decrease in serum antibodies to SARS-CoV-2 in convalescent plasma donors over time

To the Editor

Convalescent plasma, collected from donors who have previously recovered from viral illnesses, has been used to treat patients with a variety of emerging infectious diseases. This product has become more prominent during the current COVID-19 pandemic, including with the recent emergency use authorization from the Food and Drug Administration, but the longevity of

SARS-CoV-2 antibodies in donated plasma is unknown. Historically, convalescent plasma has been used to reduce mortality in a number of illnesses, such as SARS, H5N1, H1N1, Ebola, and others.[1,2] A limited number of prospective trials have been published or are pre-publication regarding treatment of COVID-19 with convalescent plasma, many of which are small but report conflicting data spanning from no benefit to statistical significance in specific patient groups but not overall, and the uncertainty of effectiveness is reflected in a large review as well.[3–5] Post-infection antibody titers vary and may remain constant or wax and wane over time independent of sex and age.[6,7] Our institution collected convalescent plasma throughout the current COVID-19 pandemic, and recorded data points including the Signal-to-Cutoff ratio (S/C ratio) of antibodies to SARS-CoV-2 in these units. This letter aims to describe trends in the S/C ratio observed in donor units over the past 4 months to ultimately improve donor collection practices and yield units with sufficient antibody to qualify as convalescent plasma.

Plasma donors self-referred to our donor center by completing an online form, advertised on the blood center website, and were eligible to donate 14 days after resolution of symptoms. This form asked for demographic data points and for information regarding their COVID-19 disease history, and donors were collected if they had laboratory evidence of positive PCR for SARS-CoV-2 virus or antibodies against the virus, regardless of disease severity or presence of symptoms. After passing a routine donation screen, donor plasma was collected by apheresis and subsequently tested for antibodies via the EuroImmun SARS-CoV-2 IgG ELISA (Lubeck, Germany) for semiquantitative analysis of the presence of antibodies against the S1 domain of the spike protein of SARS-CoV-2. Donors were allowed to donate plasma up to once a week for 4 weeks, then asked to space further donations to every 4 weeks thereafter. A 1:320 dilution was performed at each donation and this reactivity was used to distinguish suitable convalescent plasma units, as used in one clinical trial of treatment with convalescent plasma as compared to standard plasma.[3] Donor plasma was not used as convalescent plasma if it was not positive at the 1:320 dilution, and those donors were asked not to donate further convalescent plasma.

This institution overall had 262 individual donors, of which 38 qualified for analysis. Donors were included in this data set if they successfully completed at least three convalescent plasma donations over a minimum of 30 days. This was set to allow for enough data points over time to establish a trend in S/C ratios. Donors included had an age range of 19-75 years at time of first donation, with an average age of 48.2 years (median: 51 years). The last day of symptoms is marked as day 0, which was self-reported from donors on their recruitment form. While the donor forms did not allow for assessment of severity of symptoms, all donors did have some degree of symptoms and none reported an asymptomatic disease. Over the course of this data set, two donors became ineligible to donate due to low S/C ratio values, at 96 and 133 days. These donors were 41 and 48 years old, respectively. All donor plasma showed a decrease in the antibody S/C ratio from day 1 to their most recent donation, which varies from 64 to 139 days (Figure 1). Collected products overall

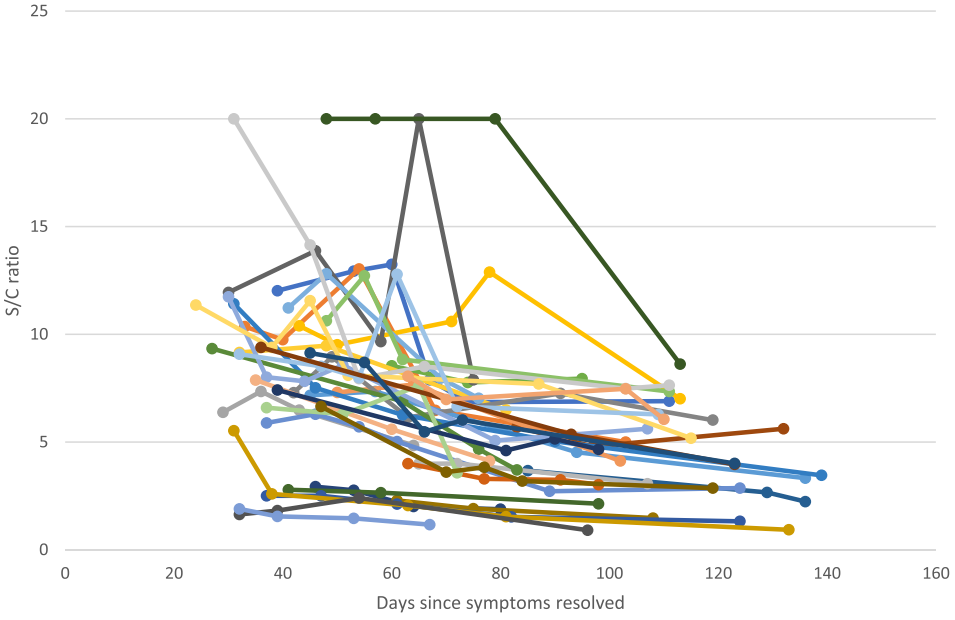

FIGURE 1 Change in S/C ratio over time for each individual donor. Each data point represents the S/C ratio calculated from an individual convalescent plasma donation, and each color represents an individual donor followed longitudinally. Each timepoint was calculated using the day of donation and the donor's self-reported last day of symptoms [Color figure can be viewed at wileyonlinelibrary.com]

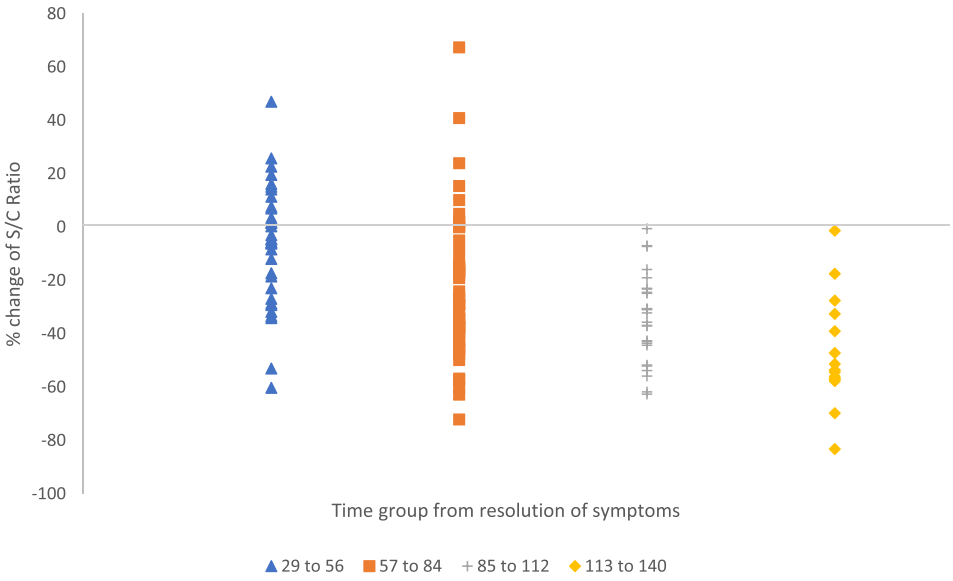

FIGURE 2 Percent change in S/C ratio as grouped by uniform time periods since resolution of symptoms. Each 28-day period includes all convalescent plasma units donated that fall within a specific time interval. The percent change was calculated from the donor's baseline S/C ratio value as defined as the first unit of convalescent plasma donated. The days included in each time group were calculated from the last day of donor symptoms [Color figure can be viewed at wileyonlinelibrary.com]

showed a decrease in S/C ratio in all time periods and more significant decreases over time. Specifically, for products collected on days 29 to 56 days, 57% of products showed a decrease in S/C ratio from that donor's baseline, as compared to 78% of products with a decrease from 57 to 84 days and 100% of products with a decrease from 85 to 112 days and 113 to 140 days (Figure 2). The average S/C ratio decreased over time for each additional month after symptoms resolved. Donors had an average S/C ratio of 10.3 from days 1 to 28, and this decreased to 8.1 for days 29 to 56, 6.5 for days 57 to 84, 5.1 for days 85 to 112, and 4 for days 113 to 140. In the group that donated at 4 months or later post-recovery (113 days-140 days), these donors had an average decrease in S/C ratio from baseline of 47% (mean 53.6%).

Overall, this data set suggests that antibody S/C ratios decrease over time, and most pronounced at 12 weeks or later using the EuroImmun assay. This assay has been shown to be comparable in sensitivity and specificity to other major antibody detection assays, including the Ortho-Clinical Diagnostics assay.[8] Many donors also showed stable or increasing levels during the first eight to 11 weeks after donation. Based on our data, donor centers may yield more convalescent plasma units if each collection is optimized to maximize volume collected and donors are collected more frequently closer to their disease recovery, ideally within 4 to 8 weeks, as opposed to many collections over a long period of time. This would facilitate increased antibody titers in collected plasma and reduce collections of plasma that is unable to be used as convalescent plasma. We believe our findings will aid other collection centers in the development of convalescent plasma collection algorithms to optimize SARS-CoV-2 antibody titers.

CONFLICT OF INTEREST

The authors disclose no conflicts of interest.

Kristina Prus
Caroline R. Alquist
Jose A. Cancelas
David Oh

Hoxworth Blood Center, University of Cincinnati, Cincinnati, Ohio

Correspondence
Kristina Prus, Hoxworth Blood Center, University of Cincinnati, Cincinnati, OH, USA.
Email: pruska@ucmail.uc.edu
DOI 10.1111/trf.16172

ORCID

Jose A. Cancelas https://orcid.org/0000-0002-1291-7233

REFERENCES

1. Winkler AM, Koepsell SA. The use of convalescent plasma to treat emerging infectious diseases: Focus on Ebola virus disease. Curr Opin Hematol. 2015;22(6):521–526. https://doi.org/10.1097/MOH.0000000000000191.
2. Hung IF, To KK, Lee CK, et al. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. Clin Infect Dis. 2011;52(4):447–456. https://doi.org/10.1093/cid/ciq106.
3. Liu STH, Lin H, Baine I, et al. Convalescent plasma treatment of severe COVID-19: A propensity score–matched control study. Nat Med. 2020. https://doi.org/10.1038/s41591-020-1088-9. Epub ahead of print. PMID: 32934372.
4. Li L, Zhang W, Hu Y, et al. Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: A randomized clinical trial. JAMA.

2020;324(5):460–470. https://doi.org/10.1001/jama.2020.10044. Erratum in: JAMA. 2020 Aug 4;324(5):519.
5. Piechotta V, Chai KL, Valk SJ, et al. Convalescent plasma or hyperimmune immunoglobulin for people with COVID-19: A living systematic review. Cochrane Database Syst Rev. 2020;7(7): CD013600. https://doi.org/10.1002/14651858.CD013600.pub2.
6. Hsu JP, Zhao X, Chen MI, et al. Rate of decline of antibody titers to pandemic influenza A (H1N1–2009) by hemagglutination inhibition and virus microneutralization assays in a cohort of seroconverting adults in Singapore. BMC Infect Dis. 2014;14: 414. https://doi.org/10.1186/1471-2334-14-414.
7. Khalenkov A, He Y, Reed JL, et al. Characterization of source plasma from self-identified vaccinated or convalescent donors during the 2009 H1N1 pandemic. Transfusion. 2018;58(5): 1108–1116. https://doi.org/10.1111/trf.14530.
8. Theel ES, Harring J, Hilgart H, Granger D. Performance characteristics of four high-throughput immunoassays for detection of IgG antibodies against SARS-CoV-2. J Clin Microbiol. 2020;58(8):e01243–e01220. https://doi.org/10.1128/JCM.01243-20.

Lack of association between SNPsrs8176719 (O blood group) and COVID-19: Data from Spanish age matched patients and controls

To the Editor,

The ABO blood groups have been associated with the risk of COVID-19.[1,2] A study involving 265 patients and 3694 controls from the Wuhan area found a significantly lower frequency of the O group among the patients (25.7 vs 33.8).[1] An important limitation of these studies was the lack of information about the control's age. A genome-wide association study (GWAs) based on patients (n = 1980) and controls (n = 2205) from Italy and Spain found a significant association with a single nucleotide polymorphism (SNP rs657152) in the *ABO* blood group locus (OR = 0.65; 95% CI, 0.53-0.79).[3] This SNP is in almost complete linkage disequilibrium (LD; D′ = 0.996, r^2 = 0.97) with rs8176719 (c.259-1_259insG, p.Thr87AspfsTer107) (Supplementary files). The rs8176719 deletion is the main determinant of group O, allele *ABO*O.01.01* (International Society of blood transfusion, ISBT; www.isbtweb.org).[4,5]

In the COVID-19 GWAs the controls were significantly younger than the patients (interquartile ranges 56-75 and 33-59, respectively). According to some authors, the blood group O might be associated with a decreased mortality, mainly due to a protective effect against cardiovascular disease.[6,7] The conclusion of an association between ABO and COVID-19 could thus be inaccurate if controls were not age matched with patients.

We studied 318 patients who required hospitalization due to COVID-19 (mean age 63.37 years, range 24-95; 63% male) and 350 healthy controls (mean age 68.84, range 60-88; 55% male). All participants were Caucasian from the region of Asturias (Northern Spain). The study was approved by the Ethics Committee of Asturias and informed consent was obtained from all the patients. The rs8176719 genotypes were determined by polymerase-chain reaction (PCR) followed by restriction enzyme digestion with *KpnI*. The method was validated by sequencing PCR fragments representing the three genotypes (Supplementary Figures). The deletion allele is the main determinant of the O group (homozygotes for this variant).[4]

Genotype frequencies for the rs8176719 did not differ from the Hardy-Weinberg equilibrium in patients and controls. We found no significantly different allele and genotype frequencies between patients and controls (Table 1). Moreover, frequencies did not differ between severe (n = 122) and non-severe COVID-19 cases (n = 196). This was in agreement with a recent prospective study that concluded that blood type was not associated with risk of intubation or death in patients with COVID-19.[8]

We did not determine the rs8176719 frequencies in younger controls, and thus we cannot evaluate age-related differences. However, the reported deletion frequency among Spanish was 0.64, compared to 0.59 among our controls (www.ensembl.org), (Supplementary files). This could explain the observed differences comparing COVID-19 patients with younger population controls.

Dzik et al. examined ABO types among SARS-CoV-2 infected patients (n = 957) at two Hospitals in Boston.[9] The O group frequencies were 46.6% and 48.6% among non-COVID and COVID-19 patients, respectively. The authors highlighted the importance that reference populations used to compare ABO distributions must be properly selected. For instance, it is well known that group O persons are recruited as preferred blood donors and this would result in