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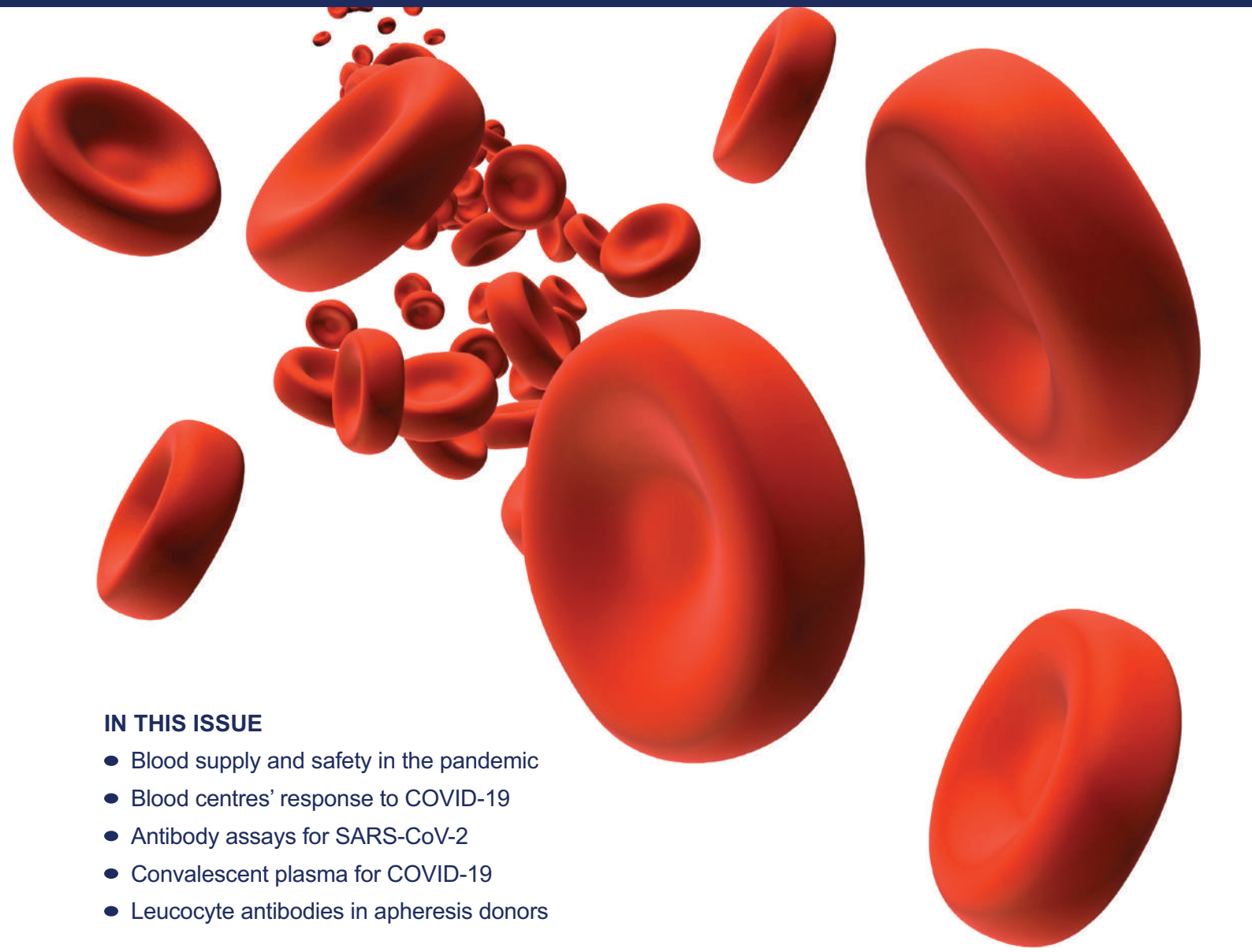

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EDITORIAL

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The need for and challenges of comparing SARS-CoV-2 antibody assays

Since its appearance in Wuhan, China, in late December 2019, the novel coronavirus SARS-CoV-2 has rapidly spread, causing over 1.2 million deaths worldwide as of early November 2020.[1] Currently, no targeted therapy is available for the associated disease COVID-19, but several options are being investigated, including COVID-19 convalescent plasma (CCP). Its use relies on the principle of passive immunity.

One of the largest experiences comes from the Mayo Clinic expanded access program (EAP) in the United States.[2] The goal was to increase CCP availability for adults in the early phase of the pandemic. Starting in March 2020, convalescent donors were qualified by PCR-based evidence of SARS-CoV-2 infection and a sufficient recovery time interval; antibody testing was not widely available. Several different antibody assays have since obtained regulatory approval in the United States.[3] Retrospective analysis of the EAP data showed that CPP transfusion was safe. Higher CCP antibody levels were associated with improved mortality when a population subset (3082 patients) was analysed. The safety data and suggestion of a dose-response contributed to the Food and Drug Administration issuing an emergency use authorization (EUA) for CCP on August 23, 2020. Of note, an expert panel did comment on limitations from the EAP.[4] In the European Union (EU), CPP treatments have occurred predominantly in randomised clinical trials except for specific compassionate use programmes.[5]

The challenge of determining antibody levels stems from the complexity of antibody responses. For instance, in some patients, the T-cell response may dominate explaining why some recovered donors have few antibodies.[6] Antibodies that prevent viral entry into host cells are called neutralising antibodies and are considered the most effective. The gold standard method for determination of neutralising antibodies is by plaque reduction (PRNT) in infected host cell cultures but requires live SARS-CoV-2 virus. These assays must be performed in high containment biosafety level 3 (BSL 3) laboratories. Alternative assays using pseudovirus still require BSL 2 facilities. Despite being deployed in several EU countries for selecting CCP donors in ongoing trials, such assays are not widely available nor easy to scale up, especially if future needs increase. In contrast, immunoassays are more accessible and are compatible with BSL 1 laboratories but vary in their sensitivity for anti-SARS-CoV-2 immunoglobulins (IgG and/or IgM). Immunoassays that directly detect inhibition of (recombinant) SARS-CoV-2 proteins and host cell receptors are commercially available (eg, AcroBiosystems, Newark, DE) or under development and good correlation with virus neutralisation is suggested.[7,8]

Most clinical trials in the EU arbitrarily define a bottom threshold for including CCP based on neutralising antibody titre measurements in live virus assays. For instance, a threshold of 1:320 means that only CCP that inhibits 50% of SARS-CoV-2 viral activity at a 1:320 dilution in vitro will be included in trials. This threshold may however differ by trial design and donor availability because no robust scientific evidence is available to rationally justify a strict cut-off for the neutralising antibody titre. In addition, titres vary depending on the assay performance and a precise correlation with clinical efficacy is not proven. Depending on the assay used and the clinical protocol being followed, each programme can establish its own policy.[9] In the United States, the CCP EUA specifically calls for convalescent plasma to be assayed for antibodies using the Ortho Vitros IgG SARS-CoV-2.[10] Because many blood collection centres have already implemented other assays, it is very difficult logistically for them to change platforms. Furthermore, reliance on a single assay poses risks in case of supply chain constraints and critical reagent shortages. Therefore, correlations must be established between the various immunoassays and neutralising tests so the EUA can be amended to include other assays.

Harvala and colleagues described a comparison between a live virus (micro)neutralisation assay, a pseudovirus reporter neutralisation (RVPN) assay, and four different enzyme-linked immunoassays (ELISAs) targeting the SARS-CoV-2 spike protein.[11] The goal was to determine optimal immunoassay cut-off values corresponding to adequate neutralising antibody levels. A neutralising titre threshold of 1:100 was selected arbitrarily.[9] In this study, 43% of samples from 52 recovered donors in April 2020 exceeded 1:100, which has implications for the availability of convalescent plasma given that physicians will favour transfusing higher titre units. Blood centres need to encourage high titre donors to return but must also increase recruitment because antibody levels decrease over time.[12]

All ELISAs detected antibodies and the strongest correlation occurred with the EUROimmun IgG. Selecting a signal to cut-off of 9.1 successfully excluded 26 samples below the 1:100 neutralising antibody threshold. Lower signal to cut-offs increased the risk for false positives, that is, the possibility that the CCP contains insufficient neutralising antibodies. However, the EUROimmun reading of 9.1 only identified 65% of donors above the threshold,[11] illustrating the delicate balance between accepting CCP units with low neutralising antibodies and discarding units with sufficiently high levels. Of note, the positive and negative predictive values depend on seroprevalence, which can widely vary between locations,[13] and

whether convalescent donors are identified by population screening or must provide proof of past infection.

These findings mirror other recent reports.[14,15] Luchsinger and colleagues found that most of their CCP samples had modest antibody levels and that commercially available tests have varying accuracy in predicting neutralising antibody activity. Goodhue and colleagues suggested a two-step testing scheme in which samples below an immunoassay threshold undergo reflex neutralising antibody testing. Thus, CCP is qualified if either the immunoassay or neutralising antibody threshold is met.

To support wider use of CCP, equivalent antibody levels must be established between different assays. However, reports have shown that correlation is complicated by differences in donor responses and binding vs neutralising assays. Novel quick and reliable immunoassays that directly measure the presence of neutralising antibodies in a wide dynamic range need to be developed.

CONFLICT OF INTEREST

The authors declare no competing interests.

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ORIGINAL ARTICLE

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Deterrents in recruitment of COVID-19 convalescent plasma donors: Experience from a hospital-based blood centre in India

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Abstract

Introduction: Recruitment of Covid-19 convalescent plasma (CCP) donors may present as a challenge due to inexperience and differences in donor profile as compared to whole blood donation. Present study highlights the deterrents to recruiting CCP donors at a hospital based blood centre.

Materials and methods: Potential CCP donors were contacted individually by telephone and a group approach through camp organisers from May to July 2020. Recruitment challenges were noted and deferrals of these recruited donors during screening and medical examination was obtained and analysed.

Results: Total 1165 potential CCP donors were contacted. Around 47% donors were lost due to challenges related to information storage and retrieval. Fear of health, family pressure, and fear of a new procedure were major reason (27.2%) for unwillingness to donate. The main reasons for deferral among potential donors were multiparity (38%) and being overage/underage (31.6%). Finally, 468 donors were recruited including 408 by individual approach and 60 by a group approach. From these absence of detectable COVID-19 antibodies were found in 15.4%. Few donors (9.0%) were deferred as they had not completed 28 days post recovery.

Conclusion: The process of CCP donor recruitment differs from that of whole blood donation and requires an individualised approach with involvement of clinicians in the initial phases of the pandemic. A group approach targeting specific organisations could be adopted for a successful CCP collection program. There is a need to relook into some aspects of donor selection such as consideration of multiparous female donors and overage/underage donors after reviewing scientific evidence.

KEYWORDS
COVID-19 convalescent plasma (CCP) donors, deferrals, deterrents, donor motivation, donor recruitment

1 | INTRODUCTION

SARS-CoV-2 originated in Wuhan, China and subsequently spread globally with WHO declaring it as a pandemic. Its rapid spread and variable but higher mortality (2%–12%) and lack of any definitive cure lead to the trial of various treatment options.[1] The experience of use of convalescent plasma and its limited benefits in other diseases such as H_1N_1 influenza pandemic in 2009–2010, SARS-CoV-1 epidemic in 2003, MERS-CoV epidemic in 2012 and Ebola virus[2-5] disease, projected its use as one of the promising investigational therapies for COVID-19 disease. Though the effectiveness of COVID-19 convalescent plasma (CCP) was unknown, but its safety

profile and potential benefit led to its use as an off label therapy globally including India.[6]

India has a decentralised blood collection system with >3000 centres majority of which are hospital based blood centres. All blood centres operate independently with a provision to transport blood and blood components to other blood centres in case of shortage. Blood collection using cell separators is limited to only a few centres across major cities with plateletpheresis being the most common procedure. During the COVID-19 pandemic, there was a surge in demand of CCP across major cities in India as well, and blood centres were faced with a challenge to convert COVID-19 recovered patients into potential CCP donors. The limited number of recovered COVID-19 patients being pursued by multiple blood centres in the same city made the task of recruiting CCP donors challenging. The lack of donor selection guidelines for CCP donation added to the difficulties in recruiting these donors. The donors were selected following the screening criterion as per Drugs and Cosmetic Act & rule 1945, India and the interim guidance for donor selection in-view of COVID-19 pandemic given by National Blood transfusion council which were specific to whole blood donation.[7] A COVID-19 recovered patient was considered to be eligible for CCP donation when he has completed 28 days post discharge from COVID-19 treating facility or has completed 28-days in-home isolation after being reverse transcriptase-polymerase chain reaction positive and remained asymptomatic during this period.[8] Our experience may highlight some of the differences and challenges in recruiting these plasma donors in comparison to whole blood and platelet donors. This would also help formulate strategies to approach these donors and ease the process of plasma donation in pandemic situations in future. The present report is thus aimed to analyse the challenges in recruitment of potential convalescent plasma donors as well as to understand the reasons for deferral of plasma donors who were willing to donate convalescent plasma.

2 | MATERIAL AND METHODS

2.1 | Study setting

The study involved retrospective collection and analysis of data related to recruitment of CCP donor in a blood centre of tertiary care hospital in Northern India. The blood centre started CCP donor recruitment and collection from May 2020 in view of the progressing pandemic. Ethical clearance was obtained from Institutional Ethics Committee before collection and analysis of donor data.

2.2 | CCP donor recruitment process

A list of lab-confirmed COVID-19 patients treated and discharged from our hospital along with their contact details was requested from the team responsible for treatment of COVID patients. Patients who had completed 28 days post discharge were identified from the list and were contacted by two staff nurses who are trained to recruit blood donors. On making a contact, the blood centre staff introduced themselves and asked the potential donors a convenient time to talk. If the donor was willing to talk, the staff enquired about their health status and assessed their eligibility to donate. Then they were informed about the need and importance of CCP plasma and its potential application in treatment of COVID-19 patients and were asked for their willingness to donate. If they agreed to donate, they were explained the procedure of plasma collection in brief and the approximate time required for donation. All queries were resolved and basic information like age and parity (in case of female donors) was enquired telephonically before confirming the appointment at blood centre. They were then given an appointment at a suitable time as per their convenience for pre donation screening and health check-up at the blood centre.

If the donor was unwilling to donate, the reason for their unwillingness was asked and documented verbatim by the recruiter. Various deterrent and deferral reasons were coded from A to J and also divided into subcategories by one of the authors (Table 1).

These coding were reassessed and verified by another author. In case the potential donor could not be reached through telecommunication due to network related issues or phone being switched off or busy at the time, one more attempt was made to contact them after a period of 2 days. Only two attempts were made, and a negative response was documented. Information related to donors with an incorrect contact details were also documented.

On the day of appointment, the donor was asked to fill up a donor history questionnaire followed by a physical examination. An ethylene diamine tetraacetic acid sample was drawn from the donor and the donor was screened for the presence of SARS-COV-2 IgG antibodies using Architect i1000SR immunoassay analyser in addition to a complete blood count, Transfusion transmitted infection markers, blood group, antibody screen for unexpected red cell antibodies and

TABLE 1 Coding of deterrents and deferral reasons

Code	Deterrent/deferral reasons
A	Not responded to phone calls
B	Duration of recovery <28 days
C	Previous donation <3 months
D	Multiparous women
E	Donor out of town
F	Donor in containment zone
G	Donor unwilling to donate
	G1: Fear of health
	G2: Fear of procedure
	G3: Family pressure
	G4: Other commitment
	G5: No reason given
H	Donor not showed up to blood centre
I	Donor deferred due to other medical reasons
J	COVID-19 antibodies not detected in donor

total serum protein. Plasmapheresis procedure was performed on donors who fulfilled the eligibility criteria. If the donor was ineligible the reason for deferral were documented and coded. Donors were also recruited by a group approach where blood donation camp organisers and other big organisations were contacted to motivate their recovered personnel to donate CCP. Any voluntary and replacement CCP donors who reported directly to the blood centre were also screened and the same post-recruitment procedure as described above was followed.

2.3 | Study plan

The present study included collation and retrospective analysis of the information collected at the time of recruitment of potential CCP donors focusing on various deterrents as well as the reasons for deferral of plasma donors. The data related to these parameters was collected from the records, and entered into a spreadsheet (Excel, Microsoft office 365). Descriptive statistics was applied to describe the different parameters.

3 | RESULTS

3.1 | Selection of potentially recruitable CCP donors

During the study period, attempt to recruit CCP donors from a total of 1165 recovered COVID-19 patients was done using telephonic communication. Three-fourth (870/1165) of these recovered patients were reachable and one fourth (295/1165) of the patients could not be contacted due to various reasons Figure 1. Of the 74.6% donors who could be reached, 21.7% (253/1165) has their residence in other cities/states and had already left the city thus making them unsuitable

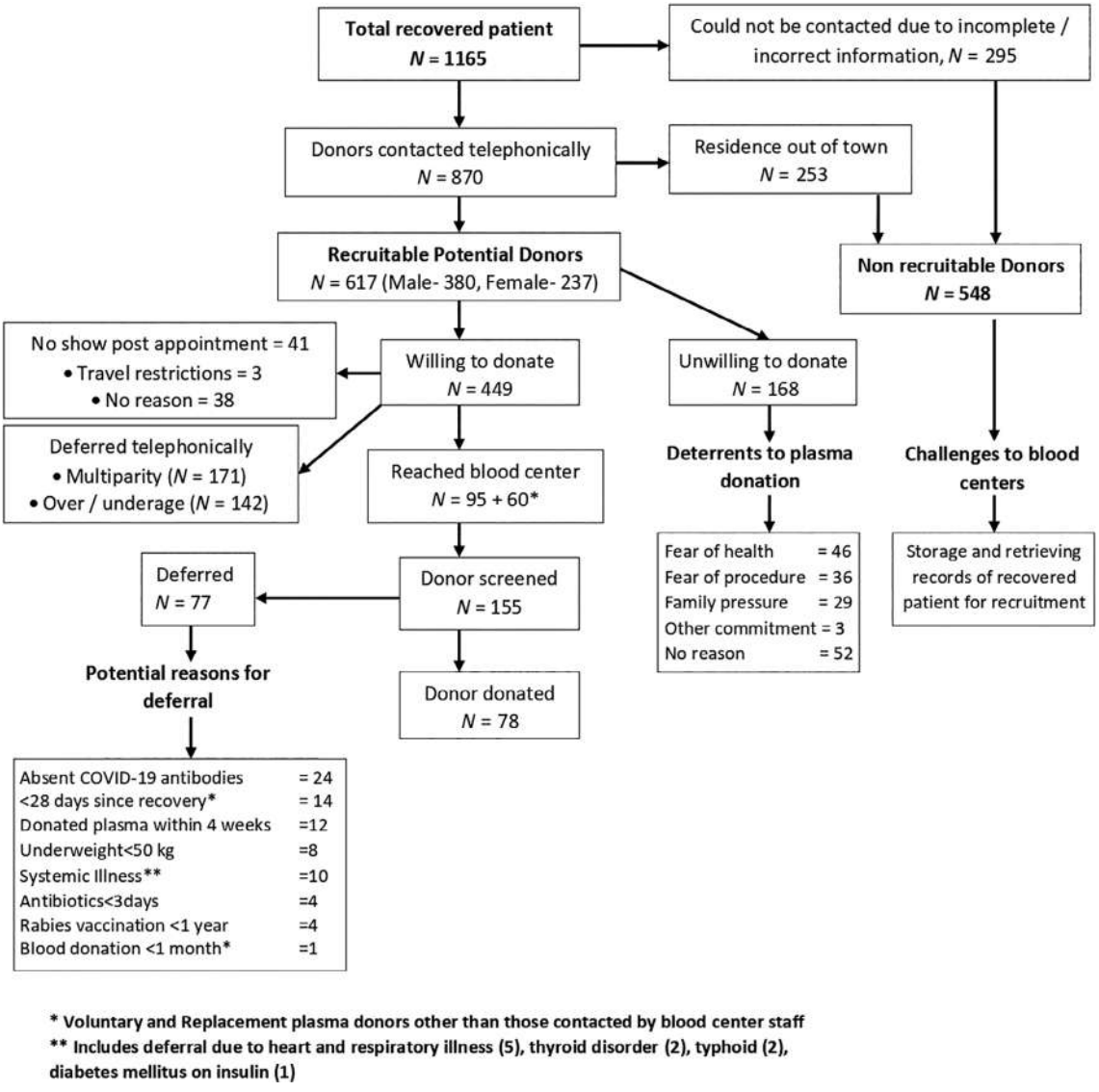

FIGURE 1 Flow showing process of recruitment of Covid-19 convalescent plasma donors with challenges, deterrents and reasons for deferral

to donate due to the lockdown. Among the remaining 617 potentially recruitable CCP donors 61.5% (380) were males and 38.4% (237) were females.

3.2 | Deterrents to CCP donation among study subjects

Among the 617 recruitable donors, 27.2% (168/617) expressed their unwillingness to donate plasma out of which 11 were females and 157 were males. Majority of these (30.9%, 52/168) did not gave any specific reason for refusal while 27.4% (46/168) expressed fear of health and weakness as a reason to not donate plasma. 17.3% (29/168) had family pressure to not donate and 2.9% (5/168) had already committed to someone else for plasma donation hence they refused (Figure 1).

Remaining 72.8% (449/617) donors were willing to donate plasma and were given an appointment for health check-up and screening for their eligibility. However, 9.1% (41/449) of these donors did not show up for their appointment. Of these, three donors could not come due to the lockdown restrictions in their areas.

3.3 | Reasons for donor deferral of CCP donors

The blood centre in total screened 468 potential plasma donors. These included 408 willing donors who reached the blood centre other than those who were screened and deferred telephonically due to either multiparity or age. These also included 60 voluntary and replacement donors who presented at the blood centre directly through group approach.

Of the total telephonically contacted willing donors 38% (171/449) and 31.6% (142/449) donors were deferred due to multiparity and overage (>60 years)/underage (<18 years) respectively.

Finally, 155 donors were screened with donor history questionnaire and out of them 34.1% (53/155) donors were deferred. The major reasons for deferral at this level included donors who have not completed 28 days post-discharge or end of home isolation (9.0% [14/155]) and underweight (5.1% [08/155]). These were the donors who showed up directly at the blood centre as voluntary and replacement donors other than contacted by blood bank staff. A proportion of these potential plasma donors (15.4%, 24/155) were deferred due to absence of COVID-19 antibodies after antibody testing on the day of presentation (Figure 1).

4 | DISCUSSION

With the start of COVID-19 pandemic a number of potential therapies were explored globally. One such therapy included CCP which was collected from the patients who have recovered from the illness and were supposed to be having protective antibodies against the illness. With publication of various reports describing benefit of using CCP in patients, the demand for the CCP increased exponentially.[9,10] However, the availability of the product was limited in the initial phases of the pandemic. Blood collection centres across the world were faced with an uphill task to motivate and recruit potential CCP donors to meet the increasing demand. Present study highlights the experience of a hospital-based blood centre from a resource limited country in recruiting potential plasma donors.

We observed that the process of motivation and recruitment of a CCP donor differs from that of a whole blood donor in terms of the available pool where entire population between the age group 18–65 years is eligible to donate in the latter, while CCP donor pool was limited to individuals between 18–60 years of age who have just recovered from a serious illness. In a country like ours, where the awareness of voluntary blood donation is limited, motivating recovered patients to donate is a big challenge especially in a pandemic situation and this demands adopting different strategies. Attempts to motivate potential CCP donors during the current pandemic were done by advertising the need and eligibility using print, electronic and social media though various government channels. We however suggest a more personalised approach wherein clinical staff involved in their care could be involved in recruitment. This could be a more practical approach in a country like ours where blood banking is decentralised with each hospital having its own blood collection and distribution. This is in contrast to the recruitment of CCP donors done during EBOLA outbreak in 2014 where "plasma mobile" systems were used for collection and peer educators were appointed to motivate and recruit Ebola virus disease survivors under supervision of medical doctors.[11]

We also observed that there was an apprehension among potential CCP donors regarding their health due to procedure. This emphasises the importance of adopting different strategy to motivate and recruit these donors when general public is facing a taboo of COVID-19 and is living in fear of getting infected while coming to donate in a hospital set up.[12,13] This calls for an approach where, in addition to motivating donors for CCP donation, efforts should also be made to inform them of the safety of the procedure. Such donors could also be motivated to donate whole blood instead of donating using apheresis as reported by Wong et al[14] but this approach would limit their chances of repeat donation to once every 3 months as per our country regulations.

While mass communication strategies or blood donation drives along with motivating factors, according to voluntary functions inventory[15] namely 'Value', 'Social', 'Esteem' and 'Understanding', have been our usual approach for recruiting whole blood donors, a targeted approach need to be adopted for CCP donors. We suggest that the process of motivation should begin at the time when the patient is recovering especially towards the time of discharge in collaboration with the clinical staff, directly involved with patient care in order to build confidence of the potential donor and gain their trust. In addition, COVID-19 survivors who have already donated CCP would prove to be more efficient recruiters.

It is also evident from the present study that around 50% potential donors were lost due to either inaccurate contact information or a

residence out of town similar to CCP recruitment attempt by Wong et al.[14] It may be challenging to maintain the patient information of all the patients to be contacted at a later date especially when the patient needs to be isolated in separate facilities. More than one mode of communication should be registered to avoid loss of donors due to inaccurate/incorrect information. Accurate and complete information of a recovered patient using various tools like a web-based survey will also facilitate triaging of these patients into eligible, soon to be eligible and ineligible donors and then coordinating them to appropriate blood centres near their residential location.[16] However, success of such approaches will need to be tested in technologically limited, resource poor countries. Explaining the eligibility criteria to donors during telecommunication may be a much more efficient way of recruiting CCP donors in countries like ours but would demand more time and manpower.

We also observed that there was a significant time duration (28 days in case of COVID-19) after discharge before a patient can donate and this also resulted in loss of donors in the absence of follow-up. Blood centres should develop mechanisms to contact these potential donors at specified intervals right after discharge so that the motivation to donate CCP is reinforced. This could also be achieved by reducing the eligibility to 14 days specifically for CCP donation as opposed to 28 days as is being followed by some European and American countries[17]when the donors are still motivated. This will also ensure that donation is done in a time period when antibody levels are sufficient. Moreover, unwilling donors on the 15th day can be counselled and motivated and contacted again on the 28th day thereby increasing chances of recruitment. In addition, a discharge advice by the clinician to follow-up with the blood centre after a specified time (e.g. 1 week after discharge) will help in retaining more donors. Facility of online registration and providing non-monetary incentives like masks, sanitizers and transport facility to these donors may also provide some confidence to these donors towards blood centres. This will also prevent black marketing of this precious product as was being reported from various countries.[18]

In initial phase of the pandemic lack of clarity regarding selection of CCP donors posed a major challenge. Deferral of multiparous female donors, which is not done routinely for whole blood donors, was also added to the already existing guidelines specifically for CCP donations. The rationale behind this was the evidence that plasma from multiparous female donors was earlier reported to be associated with TRALI due to the presence of anti-HLA and anti-HNA antibodies.[19] While this may not affect the whole blood donor pool in our country where only 3%–10% of blood donation is contributed by female blood,[20] but it had significant repercussions for recruitment of CCP donors where multiparous female donors formed around 40% of our recruitable donors and 72% of these willing female donors were deferred due to multiparity. Considering that less than one third of multiparous female donors have been shown to have anti-HLA or anti-HNA, these recommendations need a relook especially with such limited availability of CCP donors. While a number of countries have relaxed donor selection guidelines for blood donation in view of the decreased blood supply due to pandemic, such relaxation could be considered for CCP donor selection too. We lost around 10% of donors who were between age groups 60–65 years and were eligible to donate blood but not CCP as CCP donations could only be collected till 60 years of age in our country.

As the pandemic progressed and the number of eligible donors increased, we changed our recruitment strategy and approached various organisations where employees were working throughout, and chances of recruiting donors were good. We could conduct an in-house voluntary plasma donation camp with around 25 donors donating plasma on a single day. Group approach seems to be another way to recruit plasma donors during the pandemic. However, this approach may be adopted once the pandemic has progressed with sufficient number of recovered donors and may not work in the initial phases.

4.1 | Limitations

Our approach to recruit CCP donors was limited by the fact that due to limited trained manpower and time we could contact the donors only once. We could neither follow the donors who initially showed their willingness to donate CCP but did not reported to us on the scheduled day of appointment nor were able to ascertain the reasons for their no-show.

5 | CONCLUSION

Donor selection for a new component using a less known technique could be a challenging task especially in countries like ours where awareness for voluntary donation is less and the donor pool itself is limited. However, an individualised approach with involvement of clinical staff could be a feasible approach in the initial phases of the pandemic that is to be conveyed with utmost sensitivity and good will in order to gain their trust and altruistic affection for the ones in need. A group approach in later phases targeting specific organisations could be adopted. A pandemic poses a unique situation, and a flexible evolving system could be a key to a successful CCP collection program.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

Conceptualization of study, initial search of the literature, review the searched literature preparation of initial draft, analysis of the data and final preparation of manuscript: Yashaswi Dhiman. Conceptualization of study and final review of manuscript: Poonam Coshic. Conceptualization of study, analysis of data and review of manuscript: Hem Chandra Pandey. Data collection, preparation of initial draft, data analysis and bibliography: Basanta Khatiwada. Coding reassessment and donor recruitment and data collection: Jasmeet Singh. Donor recruitment and data collection: Vikas Mehta and Sanjay Gupta.

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ORIGINAL ARTICLE

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Emergency response to COVID-19 epidemic: One Chinese blood centre's experience

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Abstract

Objective: The COVID-19 epidemic has caused a significant global social and economic impact since December 2019. The objective of this study was to demonstrate the emergency response of a Chinese blood centre on maintaining both the safety and the sufficiency of blood supply during large, emerging, infectious epidemics.

Materials and Methods: Early on in the outbreak of COVID-19, the Chengdu Blood Center developed strategies and implemented a series of measures, including enhanced recruitment efforts, addition of new donation deferral criteria and notification after donation, optimisation of donor experience, development and implementation of a new coronavirus nucleic acid detection technology platform for blood screening and screening all donations for SARS-CoV-2 RNA to maximumly protect the safety of blood supply during a time of unclear risk.

Results: Starting on February 20, the immediate satisfaction rate of blood product orders in Chengdu city's clinical settings reached 100%, and there was no case of blood transfusion infection.

Conclusion: The recent experience during the outbreak of SARS-CoV-2 reminded us that improvement in the areas of national and international collaborative programmes for dealing with blood availability and safety concerns during early stages of a disaster and regional and national mechanisms for timely communication with the general public on behalf of blood services should help to better prepare us for future disasters.

KEYWORDS

blood center, COVID-19, emergency response

1 | INTRODUCTION

A novel beta-coronavirus infection, which was later named Corona Virus Disease 2019 (COVID-19), was first identified in Wuhan, Hubei province, China in December 2019 and has since rapidly spread worldwide.[1,2] Then, the World Health Organisation (WHO) announced the outbreak of COVID-19 in China as a Public Health Emergency of International Concern (PHEIC) on January 30.[3-5] On March 11, COVID-19 was announced as a pandemic by the WHO.[6]

By April 20 2020, according to Center for Disease Control and Prevention surveillance,[7] 2 314 621 cases of COVID-19 had been reported in 212 countries, territories or areas, including the United States, Spain, Italy, Germany, the United Kingdom, France, Turkey,

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Iran, China and so on. Although recent data indicate that COVID-19 has been sustainably controlled in China,[8] the infection is still being increasingly diagnosed internationally, imposing tremendous challenges on the entire healthcare system, as well as having a significant social and economic impact globally.

The issue of emergency disaster planning for blood collection services received attention after the September 11, 2001, terrorist attacks.[9-11] Large-scale emergency disaster events, either man-made or naturally occurring, present additional challenges to the blood collection system, which is already constantly under stress to maintain a safe and sufficient blood supply. The previous outbreak of severe acute respiratory syndrome (SARS) in Guangdong province, China, resulted in a significant negative impact on blood supply.[12,13] How to maintain both the safety and the sufficiency of blood supply during large emerging infectious epidemics, especially in the early stage when less data are available, is a challenge shared by blood services around the world.

The Chengdu Blood Center was founded in 1962 and is responsible for ensuring the sufficiency and safety of the clinical use of blood for more than 350 medical institutions in 22 districts in Chengdu, Sichuan Province. For many years, its blood collection and supply capacity has ranked among the top in China; 409 000 units of blood were collected in 2019, ranking fourth in China and second in subprovincial cities of China; the donation rate was 15 per 1000, and the 18 to 25 age group was the main population of blood donation. This paper introduces the measures taken by the Chengdu Blood Center (CBC) during the SARS-CoV-2 epidemic and hopes to provide information based on experience for all blood services.

2 | MATERIALS AND METHODS

Early on during the outbreak of COVID-19, the CBC developed strategies and implemented a series of measures in accordance with policies developed by the Sichuan provincial health commission and the recommendations of the Chinese Society of Blood Transfusion in order to ensure blood safety and balance blood collection and supply.[14] Details are shown in Figure 1.

SARS-CoV 2, the virus responsible for COVID-19, is a new pathogen for humans, and there are many unanswered or partially answered questions about it. On January 21, 2020, the first imported case of COVID-19 was reported in Sichuan Province. On the same day, the CBC held the first meeting of COVID-19 control to evaluate and prepare for the possible impact of the COVID-19 outbreak. The timing coincided with the Chinese Lunar New Year national holiday. CBC's leadership group for COVID-19 consists of several working groups that work separately but collaboratively to develop, implement and adjust CBC's emergency response plans. Serving as the provincial blood management centre, CBC established the Provincial Emergency Management Plan, collected data and assisted other blood centres in Sichuan Province through audio conferences.

The most immediate impact of COVID-19 was on blood availability. From January 2020, blood shortages began to occur in several parts of China. Multiple factors contributed to decreased blood availability, with the most significant factor being government-mandated extended closure of workplaces and all non-essential public places/services, as well as the call for people to stay at home to create social distancing. These measures greatly interrupted the pre-epidemic blood collection workflow, which predominantly relied on blood mobiles stationed at high-traffic public places and workplace blood drives.[15-21]

In addition to the social distancing measures, which restricted potential donors' mobility, many people also had concerns of contracting the infection by going to a blood centre to donate blood. At the same time, there were media programmes encouraging the public to optimise nutrition intake to enhance immunity. This message likely unconsciously created psychological barriers for people to give blood during the epidemic.

To overcome these challenges, the CBC developed a programme to maintain blood availability through enhanced recruitment efforts using media publicity, education and close coordination and cooperation between all stakeholders to achieve synergy and efficiency.

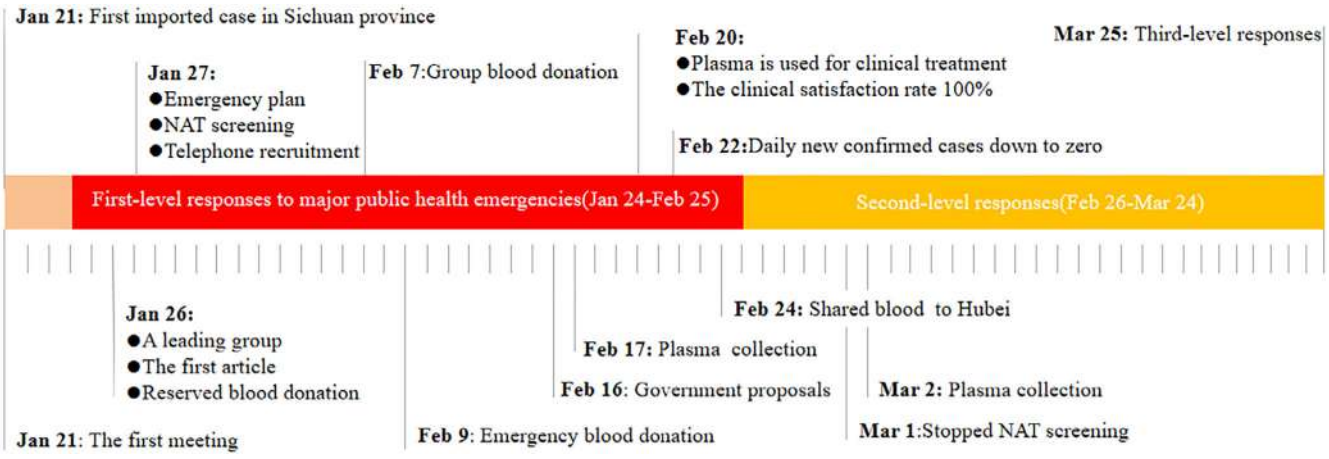

FIGURE 1 Timelines of emergency response of Chengdu Blood Center during the outbreak of COVID-19

2.1 | Media and publicity

1. Full advantage was taken of the Chengdu Blood Donation accounts with Sina Microblog and WeChat. These popular social media venues provide direct access to millions of users in the Chengdu area.
2. Collaborate with local TV stations and other media to widely publicise knowledge about voluntary blood donation, increase public's awareness of the need for blood and dispel people's doubts and worries by providing information about COVID-19. From January 21 to March 24, a total of 2221 blood donation-related articles were published in local media. Among them, "First level response, we are in action" published on January 26, 2020, was the first public report about COVID-19 by a Chinese blood service and has been read more than 40 000 times.

2.2 | Strengthen recruitment efforts

We enhanced our recruitment efforts through the use of short message service (SMS) by increasing both the number of messages and coverage area. Recruitment using mobile phone calling was also implemented. In addition, we worked closely with the city government to organise emergency group blood drives. The Sichuan Provincial Health Commission published a plea encouraging people to give blood. From January 21 to March 24, 2020, more than 670 000 recruitment-related short messages were distributed, and over 5200 recruitment phone calls were placed.

2.3 | Optimising the donor experience

A 24-hour hotline was set up to assist both individuals and groups with online donation appointment scheduling. Within a short time, functionality of the online scheduling system was improved so that donors could easily search for an appropriate donation time and location, as well as make the appointment online using mobile phones. Donations are scheduled in a manner to prevent crowding at the donation sites in order to protect donor and staff safety. Blood collection sites were set up and locations adjusted according to donation appointment volume to maximise the convenience and safety of donors.

Epidemiological data suggest that the main transmission route for SARS-CoV 2 is mostly through contact and droplet transmission; infected individuals with no or mild symptoms can spread the virus as well.[22-24] Protecting staff from contracting COVID-19 during the blood collection process is a priority for CBC. This is particularly critical for Chinese blood services because, typically, a region is only supported by one blood service, for example, the CBC is the only blood provider supporting Chengdu city with a population of 16 million. If staff member infection occurs and the infection spreads within a blood service, the resultant significant interruption of the blood service's functions may severely compromise an already tenuous blood supply situation. The CBC took the following measures to protect both donors and staff at donation sites:

2.4 | During blood collection

All staff members and donors were asked to wear protective face masks, and body temperature was checked before entering the collection site. Hand sanitisation was required before body temperature was taken. Reminders and barriers were set up to ensure safe physical distance between individuals in the waiting area and during the process of completing questionnaire, physical examination and blood collection.

2.5 | Disinfection and medical waste management

The CBC enforced strict adherence of standard operating procedures (SOPs) for site and equipment disinfection. Facility air ventilation was performed at least twice a day for not less than 30 minutes each time. Surfaces including seats, stairs, escalator handrails, workbenches, floors and instruments were wiped with chlorine disinfectant or 75% alcohol before and after work. Regular and enhanced disinfection of blood delivery vehicles was performed between each trip, especially the steering wheels, door handles and seats. The use of central air conditioning was minimised.

Retraining and reminders were provided to the staff to follow established procedures for handling medical waste. An emergency treatment plan was developed for blood, secretions or vomit from individuals diagnosed or suspected of having COVID-19.

2.6 | Maximising blood safety during a time of unclear risk

For any emerging infectious outbreak, one of the challenges is how to maximally protect the safety of blood supply even when only incomplete information is available. So far, there has not been direct evidence proving transfusion-transmitted infection of SARS-CoV 2. At the same time, some infected individuals, including asymptomatic blood donors, have been found to have detectable SARS-CoV 2 RNA in their blood.[25] A possibility for transmission of SARS-CoV 2 through transfusion cannot be completely eliminated.[26]

The CBC implemented the following measures to reduce the risk of potential transmission of SARS-CoV 2 through transfusion:

1. New deferral criteria: Any prospective donor with any of the following conditions were deferred for at least 28 days: a travel or residency history to the Hubei province; contact history with individuals with a travel history to the Hubei province; the donor or relatives experiencing symptoms including fever, dry cough or other clinical symptoms suggestive of COVID-19 infection.

2. All donors were instructed to notify the CBC within 28 days after donation if they or their relatives have symptoms of COVID-19, such as fever, cough, fatigue and shortness of breath, or had been quarantined. Print instructions were provided to all donors. If a blood donor after donation reports suspected symptoms, the CBC would 1, quarantine the associated blood components; 2, retrieve blood components from clinical facilities if they were not yet transfused; and 3, quarantine the staff members exposed to the donor. The CBC followed up with these donors with phone calls. If a donor was later suspected or diagnosed with COVID-19 and the related blood components have been transfused to patient (s), reports would be filed immediately with local health authorities.

3 | RESULTS

3.1 | Blood collection and supply

From January 21 to March 24, 2020, the CBC collected a total of 33 812 blood donations (58 810 units of blood products). During the first-level responses to major public health emergencies of Sichuan Province (January 24 to February 252 020), the unit of blood products and clinical orders suffered a 36.9% and 21.8% decline, respectively, compared with the same period in the Spring Festival of 2019, which showed a 17.2% and 12.6% decline during the second-level responses, respectively (February 26 to March 24, 2020). Starting from February 20, the immediate satisfaction rate of blood product orders (the units of the blood product distributed/the units of the blood product in the order*100% within 24 hours) in Chengdu city's clinical settings reached 100%. In addition, on February 24 and March 12, the CBC was able to export a total of 900 units of red blood cells to Hubei province, which is the most heavily affected Chinese region.

3.2 | Donors and staff safety

From January 21 to March 24, 2020, none of the donors and staff were infected with COVID-19. A total of 14 call-back cases were investigated, and no suspected or confirmed infection was found.

3.3 | Blood screening and plasma collection

The CBC made the early decision to screen all donations for SARS-CoV-2 RNA when waiting for additional blood safety information to be available. A new coronavirus nucleic acid detection technology platform for blood screening was developed and quickly implemented (manuscript submitted). Between 27 January and February 29, 2020, a total of 16 287 blood donor specimens were screened, and all results were negative.

3.4 | Convalescent plasma therapy

Convalescent plasma therapy was among the therapeutic methods listed in the fourth edition of the COVID-19 diagnosis and treatment guidelines issued by the Chinese National Health Commission.[27] Within 48 hours of the publication of the new guidelines, the CBC completed convalescent donor testing, plasma collection and preparation. As of March 24, a total of 2800 mL of plasma from eight recovered patients has been provided to hospitals and transfused into critically ill patients.

4 | DISCUSSION

In recent years, the CBC experienced the SARS epidemic (2003), Wenchuan Earthquake (2008) and Lushan Earthquake (2011). Through these public health and natural disaster crises, the CBC has accumulated experiences and developed a system for responding to such unforeseeable challenges.[28-30] However, a preparedness plan and response to serious public emergencies always faces unexpected challenges due to the unpredictable characteristics of the new crisis. We hope to share the CBC's experiences during the SARS-CoV-2 epidemic. Protecting the availability and safety of blood is a challenge for blood services around the globe.

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All authors participated in COVID-19 prevention and control work. XMF made significant contributions to supervising the COVID-19 prevention and control work and finalising the manuscript. PH, JXK, YL and XCL performed the emergency response, analysed the data and drafted the manuscript, and ML, MD, YWZ, HT, RL, JZ and YX performed the emergency response of blood supply. WL and JLG reviewed and revised the manuscript. HS contributed to reviewing and revising the manuscript. The work should be attributed to the Chengdu Blood Center.

CONFLICT OF INTEREST

The work has not been submitted elsewhere for publication, in whole or in part. All authors have read the manuscript and approved submission to your journal. There is no ethical/legal conflict involved in the article.

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ORIGINAL ARTICLE

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Impact of Covid-19 epidemic on the activities of a blood centre, transfusion support for infected patients and clinical outcomes

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Abstract

Objectives: We evaluated how the Severe Acute Respiratory disease from Coronavirus 2 (SARS-CoV-2) epidemic impacted transfusion services, transfusion support required by Covid-19 patients and their clinical outcome.

Background: In Italy, the first confirmed case of SARS-CoV-2 infection was registered on 21 February 2020. As of 20 April, about 250 000 cases were registered, 1143 of which were in the province of Pescara.

Methods: We compared transfusion services provided by the blood centre of Pescara between 1 March and 20 April 2019 and between 1 March and 20 April 2020. We assessed the number and type of blood components donated, those transfused in the various hospital departments and those transfused to Covid-19 patients.

Results: Compared to 2019, we documented a decrease of 32% in the number of donations. The number of transfusions increased by 139% in the infectious diseases department (IDD), dedicated to Covid-19 patients, and by 76% in the intensive care unit (ICU), whereas it markedly decreased in the other departments. Of 299 patients with Covid-19, 60 were transfused (20.1%). Transfused patients in the ICU were significantly younger than those in IDD and had a lower number of lymphocytes, lower post-transfusion increment of haemoglobin levels and higher D-dimer and C reactive protein values. Mortality rate was 60.7% among transfused patients in the ICU and 39.0% among those in the IDD (p = 0.02).

Conclusion: The Covid-19 epidemic had a profound impact on transfusion activities. The important blood demand for Covid-19 patients was satisfied because of the reduction in activities in other hospital wards. Covid-19-positive transfused patients showed a very poor prognosis.

KEYWORDS

Covid-19, mortality, transfusion efficacy, transfusion support

1 | INTRODUCTION

In Italy, the first confirmed case of Severe Acute Respiratory disease from Coronavirus 2 (SARS-CoV-2) infection was registered on 21 February 2020 in a young patient residing in the Lombardy region. As of 20 April, about 250 000 positive cases were registered, about 3000 of which were in the Abruzzo region and 1143 of which in the province of Pescara. As the number of cases increased, the national

blood centre, regional blood centres, hospitals and transfusion services responded with measures aimed at providing appropriate health care services.[1,2] This report describes how the Coronavirus Disease 2019 (Covid-19) pandemic impacted transfusion services. More specifically, we evaluated how the epidemic changed donor access and the number of transfusions performed compared to the same period of the previous year, the transfusion support required by Covid-19 patients and clinical outcomes.

2 | MATERIALS AND METHODS

The Civil Hospital of Pescara is a general hospital with 653 beds and includes, among others, departments of surgery, internal medicine, haematology-oncology, maternal and child health and emergency. The haematology ward is the largest in Italy, with 62 beds, and also includes a haematopoietic stem cell transplant unit. The haematology department represents the reference centre for the Abruzzo region, which has 1.3 million inhabitants, and provides inpatient and outpatient care for a large array of different haematological conditions, including acute and chronic leukaemia, lymphoma, multiple myeloma, myelodysplastic syndromes, congenital and acquired anaemia, haemophilia and other coagulation disorders. A multidisciplinary outpatient "anaemia clinic" for patient blood management is operated in the blood centre of the hospital, under the coordination of a transfusion medicine specialist.

The department of haematology also includes a blood centre for the collection, processing, qualification and distribution of blood components and is self-sufficient to meet the needs of the hospital. On average, about 20 000 transfusions per year are performed.

During the Covid-19 pandemic, our hospital faced an exponential increase in the demand for intensive care unit (ICU) beds even outside the conventional setting. Pescara Hospital was equipped with 10 ICU beds, of which 2 had negative pressure. The speed of the local epidemic required immediate operational planning to contain mortality and morbidity. The Crisis Unit defined a plan for a 180-bed Covid-19 hospitalisation area. The Covid-19 area was organised by intensity of care, and patients with worsening clinical conditions were placed in a sub-intensive area or in a critical care area. Within the Covid-19 area, a 22 pressure-negative beds section of the ICU was organised, with complete equipment except extracorporeal membrane oxygenation (ECMO). This section was placed next to the sub-intensive care area, managed by a multi-specialist internal medicine team, coordinated by the infection disease department (IDD) and equipped with monitoring and non-invasive ventilation. Stable patients were placed in the non-negative pressure rooms. Patient location was reassessed daily. Due to the growing demand for ICU beds, an additional intensive hospitalisation area was placed under negative pressure, including 10 beds. The system was progressively implemented and gradually downgraded in the period 07 March 2020–31 May 2020, when the areas were returned to the pre-COVID-19 destination.

The other activities of the hospital were also reorganised. During the first 10 days of March, indications were given by the Ministry of Health on the identification of Covid-19 hospitals, the definition of access routes to health facilities with access restrictions and closure of non-urgent outpatient activities. Blood donation was considered an urgent procedure. Elective surgeries have been cancelled starting from 13 March, with the exception of surgeries for cancer patients or high-specialty surgical interventions.

Haematological patients hospitalised in the ward and in the transplant unit were fully supported during the pandemic.

The aim of this study was to compare transfusion services provided by the Blood Centre of Pescara General Hospital between 1 March and 20 April 2019 and those provided between 1 March and 20 April 2020. We assessed the number and type of blood components donated and number and type of blood components transfused in the various hospital departments (onco-haematology, surgery, internal medicine, paediatrics, infectious diseases and intensive care). The number of transfused patients and transfusions/patient were compared between the two periods of observation. Patient Blood Management (PBM) consultancy activities for hospitalised and outpatient patients were also assessed.

Characteristics of patients with confirmed Covid-19 and admitted to the infectious diseases and intensive care wards were also analysed. We evaluated the number and type of transfused blood components in Covid-19 patients, along with transfusion threshold, transfusion efficacy, laboratory tests indicative of clinical status and outcome of transfused patients.

2.1 | Statistical methods

Continuous variables were summarised as median and interquartile range (IQR), whereas categorical variables were summarised as percentages. Between-group comparisons were based on the Mann-Whitney *U* test for continuous variables and the chi-squared test for categorical variables. All *p* values are two-sided, and values <0.05 were considered statistically significant.

3 | RESULTS

3.1 | Donations and blood components collected

Overall, 2136 donations were made between 1 March and 20 April 2020 for a total of 2293 blood components collected, of which 1448 were red blood cells (RBC), 431 platelet concentrates (PLT) and 685 fresh-frozen plasma (FFP). In the same period of 2019, 3143 donations were made, for a total of 3310 blood components collected, of which 2226 were RBC, 638 PLT and 850 FFP. Therefore, compared to 2019, we documented a decrease of 32% in the total number of donations. The number of donations and the number of blood components per week are given in Figure 1. Following an awareness campaign in early March, the number of donations in the third week of March was similar to that of 2019; however, in the following weeks, a substantial decrease was observed. The drop in

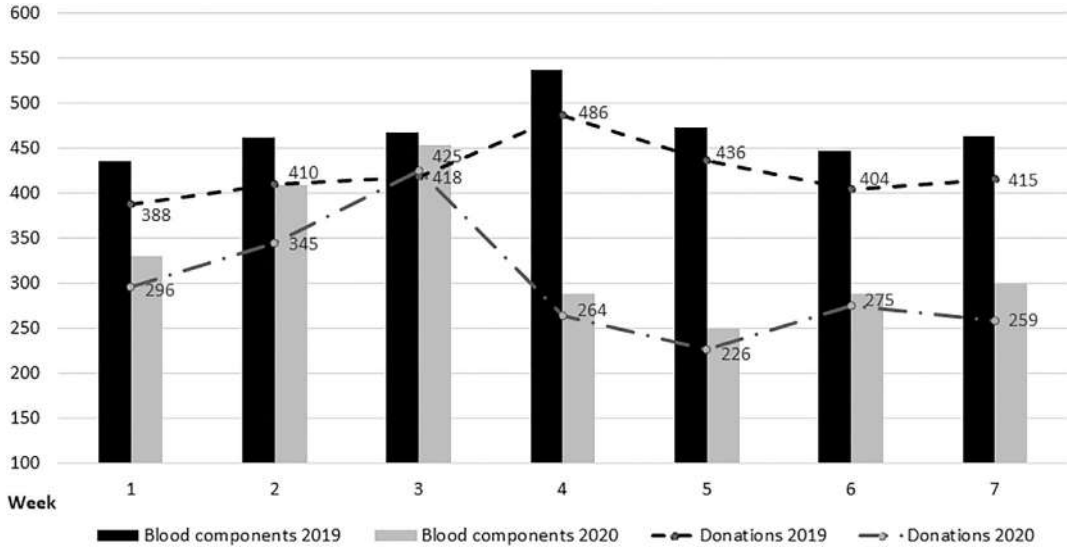

FIGURE 1 Number of weekly blood donations (dotted lines) and blood components (bars) between 1 March and 20 April: comparison between the years 2019 and 2020

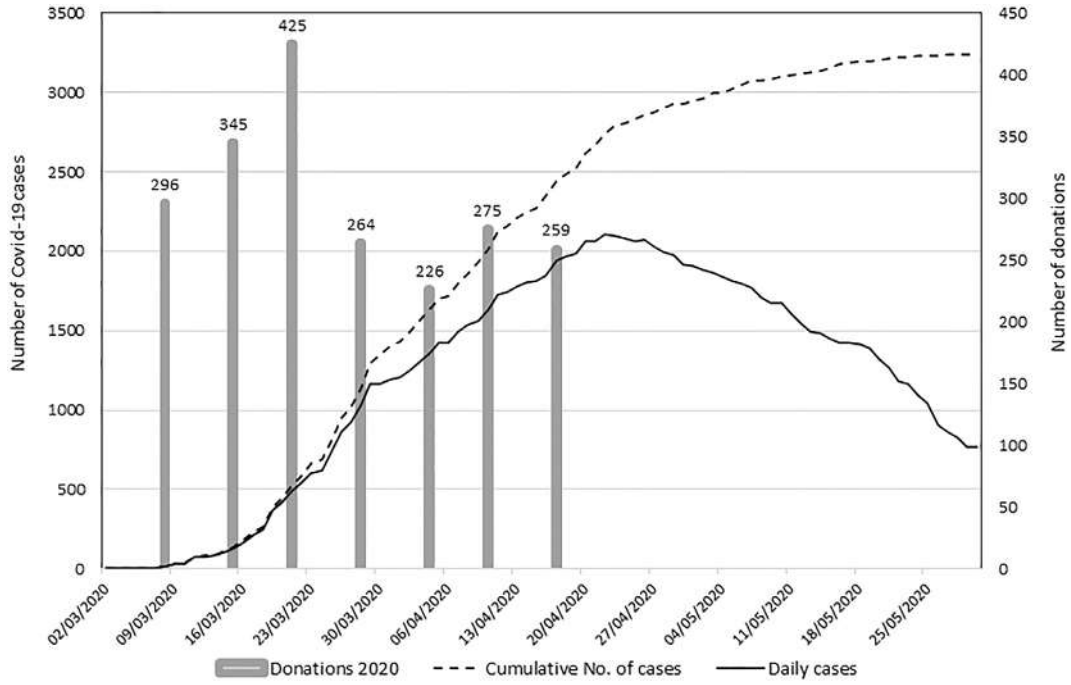

FIGURE 2 Number of weekly blood donations in relation to the number of daily new cases and cumulative number of cases of Covid-19 in Abruzzo region

number of donors occurred in parallel with the worsening of the pandemic (Figure 2).

3.2 | Patients and transfusions

Overall, 824 patients were transfused between 1 March and 30 April 2019, for a total of 3191 blood components infused. In the same period of 2020, 612 patients were transfused (−25.7%), for a total of 2341 components infused (−26.6%); 166 blood components were infused to 60 patients affected by Covid-19.

The number of blood components transfused in the different hospital departments in 2019 and 2020 is reported in Table 1. A marked reduction was documented in the number of transfusions performed in the surgery (−56.0%) and internal medicine departments (−48.7%). On the other hand, the number of transfusions increased by 139.1% in the infectious diseases department (IDD—entirely dedicated to Covid-19 patients) and by 76.4% in the ICU.

TABLE 1 Transfusions performed, overall and by hospital department: Comparison between 2019 and 2020

	1 March–20 April 2019	1 March–20 April 2020	2020–2019% change
No. of transfused patients	824	612	−25.7%
Total No. of transfusions	3191	2341	−26.6%
Onco-haematology	1667	1393	−16.4%
Surgery	812	357	−56.0%
Internal medicine	417	214	−48.7%
Emergency	144	98	−31.9%
Infectious diseases	46	110	+139.1%
Intensive care unit	89	157	+76.4%
Paediatrics	16	12	−25.0%

During the period evaluated, there was a 68.4% decrease in PBM consultancies (133 in 2019 vs. 42 in 2020), mainly due to the closure of the anaemia outpatient centre and the drastic reduction of the scheduled surgical interventions.

Overall, 299 patients were admitted to our hospital with confirmed Covid-19, 73 of whom were admitted to ICU and 226 admitted to IDD. The median age was 64 years (55–68) for patients in the ICU and 73.0 years (54.0–84.0) for those in IDD.

Of a total of 612 patients transfused during the period of observation, 60 were Covid-19-positive (9.8%), 36 of whom were men (60.0%) and 24 were women (40.0%), with a median age of 72.0 (64.3–82.7) years. Covid-19 patients were transfused with 154 RBC (79 in ICU and 75 in IDD), 10 PLT (5 in ICU and 5 in IDD) and 2 FFP (both in ICU). The proportion of patients transfused was 37.0% among those admitted to ICU (27 out 73) and 14.6% among those admitted to IDD (33 of 226).

The trigger for transfusion in Covid-19 patients was generally set at 8 g/L of haemoglobin for both patients admitted to the ICU and those in the IDD. However, the transfusion trigger was decided on the basis of multiple parameters, including PaO_2/FiO_2 ratio (severity of respiratory failure), presence of haemodynamic instability, sepsis/septic shock and increased lactate levels. The average number of units transfused was 2 (range 1–12) for patients admitted to ICU and 2 (range 1–12) for patients admitted to the IDD.

Table 2 demonstrates the number of patients transfused in the ICU and IDD in 2019 and those admitted for Covid-19 in 2020. Gender distribution (61.1% and 60.0% males in 2019 and 2020, respectively; $p = 0.91$), median age (71 [56–80] years in 2019 and 72 [64–83] in 2020; $p = 0.35$) and average number of transfusions per patient (2.0 [2.0–4.0] in 2019 and 2.0 [1.0–3.0] in 2020; $p = 0.07$) were similar in the two periods, but an increase in the number of transfusions (+22.9%), patients transfused (+66.7%) and number of RBC transfusions (+65.6%) was documented for patients with Covid-19.

TABLE 2 Blood components in transfused Covid-19 patients admitted to intensive care unit or infectious diseases department between 1 March and 20 April 2020 compared with transfused patients in the same departments between 1 March and 20 April 2019

Characteristics	1 March–20 April 2019	1 March–20 April 2020	2020–2019% change
No. of transfused patients	36	60	+66.7%
No. of transfusions	135	166	+22.9%
No. of RBC transfusions	93	154	+65.6%
No. of PLT transfusions	34	10	−70.6%
No. of FPC transfusions	8	2	−75.0%

3.3 | Transfusion threshold and efficacy

Patients with Covid-19 requiring RBC transfusions had an average haemoglobin level of 7.8 (7.5–8.2) g/dl, an average platelet count of 178 (78–239) $\times 10^9$/L and an average number of lymphocytes of 0.8 (0.5–1.2) $\times 10^3$/μl.

Average post-transfusion haemoglobin levels were 8.8 (8.1–9.4) g/dl, corresponding to a post-transfusion increment of 0.84 (0.39–1.40) g/dl. Four patients were transfused with a total of 10 units of PLT from buffy coat pool, with a median pre-transfusion count of 14 $\times 10^9$/L (9–23), a median post-transfusion count at 18–24 hours of 43 $\times 10^9$/L (28–48) and a median increment of 25 $\times 10^9$/L (range 7–34).

Two units of FFP were transfused to two patients with an international normalised ratio (INR) > 1.5.

Transfused patients admitted to the ICU were significantly younger than those admitted to the IDD, were more frequently of male gender, showed slightly higher pre-transfusion haemoglobin levels and a lower number of lymphocytes and had a significantly lower post-transfusion increment of haemoglobin levels (Table 3).

3.4 | Laboratory tests indicative of clinical status

At the time of transfusion, Covid-19 patients had a prothrombin time (PT) of 13.9 (12.7–16.7) s, activated partial thromboplastin time (APTT) of 30.2 (27.0–38.5) s, international normalised ratio (INR) of 1.17 (1.11–1.32), antithrombin 3 activity (AT3) of 68 (52–80) %, fibrinogen levels of 352 (218–550) mg/L and D-dimer levels of 3.6 (1.6–6.0) mg/L. Among the indices of inflammation and sepsis, levels of C-reactive protein (CRP) and procalcitonin (PCT) were 76.7 (35.2–183) mg/L and 0.55 (0.15–1.67) mg/L, respectively. The same parameters were compared between Covid-19 patients admitted to the ICU and those admitted to the IDD. Transfused patients in the ICU had significantly higher D-dimer and CRP levels, whereas no statistically significant differences were documented for the other parameters examined (Table 3).

TABLE 3 Laboratory characteristics of Covid-19 patients admitted to ICU or infectious diseases department

Characteristics	ICU	Infectious diseases dept.	*p* Value
Age	66.3 (59.0–71.2)	81.2 (71.5–88.2)	<0.0001
Gender: Male	70.0%	51.5%	0.18
ABO group			0.20
O	37.0%	51.5%	
A	55.6%	33.3%	
B/AB	7.4%	15.2%	
Pre-transfusion haemoglobin (g/L)	8.0 (7.7–8.4)	7.7 (7.2–8.0)	0.07
Platelets ($\times 10^9$/L)	155 (87–237)	184 (70–250)	0.44
Lymphocytes ($\times 10^3$/μl)	0.60 (0.50–1.10)	0.90 (0.60–1.20)	0.029
Post-transfusion increment of haemoglobin (g/L)	0.69 (0.29–1.19)	1.1 (0.50–1.60)	0.028
PT (s)	14.0 (12.7–19.5)	13.8 (12.8–16.1)	0.96
APTT (s)	33.2 (27.1–41.1)	29.4 (26.1–34.2)	0.10
INR	1.17 (1.11–1.32)	1.19 (1.07–1.33)	0.21
AT3 (%)	66.0 (50.3–78.8)	68.0 (54.0–88.0)	0.19
Fibrinogen (mg/L)	353 (223–554)	342 (213–522)	0.63
D-dimer (mg/L)	4.1 (2.1–6.3)	1.9 (1.2–4.9)	0.027
CRP (mg/L)	89.2 (41.7–195.5)	62.2 (12.3–133.0)	0.039
Procalcitonin (mg/L)	0.70 (0.23–1.85)	0.46 (0.10–0.97)	0.30

Abbreviation: ICU, intensive care unit.

3.5 | Outcome of transfused patients

Information on vital status was available for all Covid-19-positive, transfused patients. Overall, 27 patients died (45.0%); the mortality rate was significantly higher among patients admitted to the ICU (17 of 27 patients; 60.7%) compared to those admitted to the IDD (11 of 33 patients; 39.3%) (p = 0.02). Patients who died differed from those who survived in terms of number of lymphocytes (0.60×10^3/μl [0.37–1.10] vs. 0.95×10^3/μl [0.60–1.20]; p = 0.028) and PCT levels (1.03 [0.52–4.52] mg/L vs. 0.14 [0.07–0.46] mg/L; $p < 0.001$). No statistically significant differences emerged in terms of age, gender, ABO group, pre-transfusion haemoglobin, platelet count, post-transfusion haemoglobin increment, PT, PTT, INR, AT3, D-dimer and CRP levels.

4 | DISCUSSION

4.1 | Main findings

The Covid-19 pandemic has had a profound impact on many aspects of transfusion medicine, from donation to the availability of blood components to the need of managing transfusion needs of patients positive for Covid-19. The SARS-CoV-2 infection is in fact often associated with multifactorial anaemia, coagulation disorders and multi-organ failure in the most severe cases.[3]

The Italian National Blood Centre (Centro Nazionale Sangue [CNS]) promoted an awareness campaign on the importance of donation[1,2] through the media at the national and local levels, highlighting all the adopted measures to mitigate the risk to blood, donor and staff safety. Volunteers of the blood donor associations were involved in contacting donors by telephone or through automatic messaging systems and social media. Following the recommendations of CNS, a triage with a check of body temperature was implemented in our hospital, and donors with a recent history (last 2 weeks) of body temperature over 37.5°C, symptoms of respiratory infection (cough, dyspnoea, sore throat, rhinorrhoea) or having had contact with a suspected or confirmed case of Covid-19 were not allowed to donate. To comply with the requirement of social distancing, donors were advised to make prior appointments in order to avoid waiting times and long duration of stay at donation venues. Rigid post-donation measures were also adopted to collect additional information regarding possible cases of infection in people who had made a donation; in such cases, blood components not yet used were discarded, and a close monitoring of transfused patients was put in place.

Despite all these activities, we documented a decrease of 32% in the number of donations compared to the same period of 2019. Following the national and regional awareness campaigns in the first 2 weeks of March 2020, in the third week of March, the total number of donations was close to that registered in the previous year. However, in the following weeks, the number of donations decreased markedly, in conjunction with the worsening of the pandemic outbreak. Despite the reduction in the availability of blood components, the parallel decrease in demand, mainly due to a drastic reduction in

the number of surgical procedures, made it possible to satisfy all the requests without major problems.

Haematological patients were fully supported during the pandemic, both those hospitalised in the ward and those admitted in the transplant unit. There was a slight decrease in the number of transfusions in outpatients (−16%), for whom online consultations were performed for non-urgent cases; if transfusion was deemed necessary, the patients were referred to the blood centre closest to their home. Patients with hemoglobinopathies such as sickle cell disease and thalassemia were regularly followed up and transfused at our centre.

The markedly lower number of transfusions in surgical and non-Covid-19 internal medicine wards was counterbalanced by the increase in demand associated with the SARS-CoV-2 infection in the ICU and infectious diseases department. Of note, one in five inpatients with Covid-19 overall, and one in three of those admitted to the ICU, required a transfusion during their hospital stay. In these patients, transfusions can help counteract hypoxia; furthermore, the anaemic status can worsen as a consequence of frequent blood sampling, inflammation, haemorrhagic episodes, acute respiratory distress syndrome or sepsis. The role of PBM in patients positive for Covid-19 has also been recently emphasised: The correction of anaemia during the early phases of the infection could be of benefit to avoid the most severe consequences of the respiratory infection.[4,5]

In our hospital, a restrictive transfusion threshold (Haemoglobin ≤ 7 g/L) is applied in stable patients, even those admitted to the ICU. In Covid-19-transfused patients, the threshold increased to an average of 7.8 g/L due to the severe, unstable clinical conditions. The average post-transfusion increment of 0.84 g/L was satisfactory; however, the increment was significantly lower for patients admitted to the ICU compared to those admitted in the IDD.

The dysfunction of endothelial cells induced by infection results in excess thrombin generation and fibrinolysis shutdown, which indicate a hypercoagulable state in patient with infection,[6] responsible for a poor prognosis.[7] In our case series, compared to patients admitted to the IDD, those in the ICU showed higher values of D-dimer, indicative of coagulopathy, and higher CRP levels, indicative of a more severe inflammatory status; on the other hand, no major differences between the two groups emerged as for other coagulation parameters. This can be at least partially related to the adoption in our hospital of protocols for the management of coagulopathy with low-molecular-weight heparin and for blocking the inflammatory cascade with tocilizumab.

We documented a high mortality rate among Covid-19-positive, transfused patients. About 60% of patients admitted to the ICU and one in three of those admitted to the IDD died during their hospital stay. Patients who died did not differ from those who survived in terms of age and gender distribution; however, markers of inflammatory activity, particularly PCT levels, were markedly higher in patients who died than in survivors, together with a more severe lymphopenia.

Based on the available current evidence, restrictive RBC transfusions are associated with decreased morbidity and mortality,[8,9] and a transfusion trigger of 7 g/L is clinically acceptable for most non-acutely bleeding critically ill patients. In certain groups of critically ill patients, such as those with septic shock, acute respiratory failure, severe or acute ischemic heart disease and brain injury, who may be at increased risk of the adverse effects related to anaemia, a trigger of 7–9 g/L is clinically acceptable. Although patients with Covid-19 in the ICU share many characteristics with patients admitted to the ICU for other reasons, at the moment, there are no studies demonstrating that transfusion per se in patients with Covid-19 has a negative effect on the evolution of the disease.

From an organisational point of view, the need to address both donations and patient care posed additional challenges to our hospital. In fact, active strategies had to be implemented to ensure the safety of donations and satisfy the demand for transfusions from one side and reduce the non-necessary procedures from the other side, thus ensuring the possibility to meet the needs of Covid-19 and non-Covid-19 patients. On the other hand, the existence of the blood donation centre within the hospital and the coordination of the activities of collection and distribution of blood components made it possible to better plan care and adapt the provision of services to the actual availability of units to be transfused. A multidisciplinary evaluation of the appropriateness of transfusion requests was also put in place, helping to meet the shortage of blood components. Overall, the tight coordination of the different activities and expertise represented a key factor in limiting the negative impact of the epidemics on the provision of care.

4.2 | Strengths and limitations

Several reports have described the impact of Covid-19 on transfusion services[1,10-13]; however, to our knowledge, this is the first report showing the impact of the Covid-19 pandemic not only on transfusion practices but also on outcomes. As such, it offers important information, particularly regarding the measures needed to face the increasing demand for transfusion support. The major limitation of our study is the origin of the data from a single, large hospital. Additional experiences will help to better define the burden posed by Covid-19 to transfusion services and the strategies that should be implemented to meet the needs of all patients.

5 | CONCLUSIONS

The Covid-19 epidemic has had a profound impact on transfusion activities. During the emergency phase, the important blood demand for Covid-19-positive patients was satisfied, despite the reduction in donations, because of the reduction in activities in the other hospital wards. However, in recent weeks, specific structures have been gradually created for people with Covid-19, freeing hospitals from caring for these patients. This has important implications in light of the new outbreak of the epidemic. In fact, in these circumstances, the request for transfusion support for Covid-19 patients would add to the routine requests of hospitals that would resume their usual care activities. All this could create a dramatic shortage of transfusion products,

which could be at least partially remedied by increasing the stocks of frozen blood cells, optimising PBM and promoting donor awareness campaigns.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Anna Quaglietta: study design; acquisition, analysis or interpretation of data; drafting of the manuscript; approved the submitted version of the paper. **Antonio Nicolucci**: drafting of the manuscript; statistical analysis; approved the submitted version of the paper. **Raffaella Posata**: acquisition, analysis or interpretation of data; approved the submitted version of the paper. **Antonella Frattari**: acquisition, analysis or interpretation of data; approved the submitted version of the paper. **Giustino Parruti**: acquisition, analysis or interpretation of data; approved the submitted version of the paper. **Patrizia Accorsi**: acquisition, analysis or interpretation of data; approved the submitted version of the paper.

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ORIGINAL ARTICLE

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Convalescent plasma therapy for the treatment of patients with COVID-19: Assessment of methods available for antibody detection and their correlation with neutralising antibody levels

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Abstract

Introduction: The lack of approved specific therapeutic agents to treat coronavirus disease (COVID-19) associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has led to the rapid implementation of convalescent plasma therapy (CPT) trials in many countries, including the United Kingdom. Effective CPT

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is likely to require high titres of neutralising antibody (nAb) in convalescent donations. Understanding the relationship between functional neutralising antibodies and antibody levels to specific SARS-CoV-2 proteins in scalable assays will be crucial for the success of a large-scale collection. We assessed whether neutralising antibody titres correlated with reactivity in a range of enzyme-linked immunosorbent assays (ELISA) targeting the spike (S) protein, the main target for human immune response.

Methods: Blood samples were collected from 52 individuals with a previous laboratory-confirmed SARS-CoV-2 infection. These were assayed for SARS-CoV-2 nAbs by microneutralisation and pseudo-type assays and for antibodies by four different ELISAs. Receiver operating characteristic (ROC) analysis was used to further identify sensitivity and specificity of selected assays to identify samples containing high nAb levels.

Results: All samples contained SARS-CoV-2 antibodies, whereas neutralising antibody titres of greater than 1:20 were detected in 43 samples (83% of those tested) and >1:100 in 22 samples (42%). The best correlations were observed with EUROimmun immunoglobulin G (IgG) reactivity (Spearman Rho correlation coefficient 0.88; $p < 0.001$). Based on ROC analysis, EUROimmun would detect 60% of samples with titres of >1:100 with 100% specificity using a reactivity index of 9.1 (13/22).

Discussion: Robust associations between nAb titres and reactivity in several ELISA-based antibody tests demonstrate their possible utility for scaled-up production of convalescent plasma containing potentially therapeutic levels of anti-SARS-CoV-2 nAbs.

KEYWORDS

convalescent plasma, COVID-19, neutralising antibody level, SARS-CoV-2, testing of plasma

1 | INTRODUCTION

The emergence of a novel coronavirus as a cause of respiratory disease occasionally leading to severe acute respiratory syndrome (SARS) was first noted in the Hubei province, China in December 2019. From there, it rapidly spread to a number of countries, including Italy, Iran, Spain and France.[1] Subsequently, this virus was classified as SARS coronavirus 2 (SARS-CoV-2) within the genus *Betacoronavirus*[2] and its associated disease termed COVID-19. Mortality due to COVID-19 is as high as 50% for patients admitted to intensive care units.[3]

The first imported cases of SARS-CoV-2 were identified in the United Kingdom at the end of January 2020, and local transmission within the United Kingdom became evident 1 month later. As of 1st May 2020, a total of 182 260 cases and 28 131 deaths have been reported, and the numbers are predicted to continue to rise in this first pandemic wave. Currently, there are no approved specific antivirals targeting the novel virus, and convalescent plasma therapy (CPT) has been suggested as an immediately available therapy. A systematic review and retrospective meta-analysis, including 699 treated patients with SARS-CoV-1 infection or severe influenza and 568 untreated controls, demonstrated a statistically significant reduction in mortality and in the pooled odds of mortality following treatment, compared with placebo or no therapy (odds ratio 0.25; 95% CI: 0.14–0.45).[4]

Convalescent plasma may be an effective treatment for COVID-19, with success linked to levels of neutralising antibody present in plasma, which reduce viral replication and increase viral clearance.[5,6] Virus-specific neutralising antibodies play a key role in viral clearance. The spike (S) protein is responsible for the SARS-CoV-2 attachment and entry to the target cells via the ACE-2 receptor, and neutralising antibodies recognising the receptor-binding domain (RBD) on the S protein have been shown to block viral entry.[7] Antibodies against other domains of S protein or possibly even against other proteins may contribute to the functional neutralisation of the virus. Neutralising antibodies are known to be detectable in patients approximately 10–15 days after the onset of SARS-CoV-2 infection,[8] but this antibody response continues to mature for at least 3 weeks[9] and potentially longer.

The issue of the potential toxicity of convalescent plasma via antibody-dependent enhancement (ADE) also needs to be addressed carefully. It has been shown to occur when non-neutralising or heterotypic antibodies facilitate viral entry into host cells and enhance viral infectivity.[10] It is likely to occur when antibody levels or specificities do not permit neutralisation.[11] For these reasons, it is important to determine neutralising antibody titres in donated plasma, as well as a practical cut-off titre level, to evaluate not only its safety but also its effectiveness for convalescent plasma transfusion.

Neutralising antibody levels can either be determined directly using native or pseudo-type virus in cellular bioassays or be estimated by ELISA if there is an adequate correlation between neutralising antibody titre and ELISA reactivity. Neutralising antibody titre can be detected and quantified in a microneutralisation assay format in which samples are assayed for their ability to block infection of cells by SARS-CoV-2. Similarly, a pseudo-type assay can be used to measure neutralising antibody levels using a virus construct containing SARS-CoV-2 S protein in the surface of a luciferase tagged vesicular stomatitis virus or lentivirus viral vector.[12,13] Both types of assays use suitably characterised target cells. Although a limitation of microneutralisation assays using live virus is the necessity to undertake work at biosafety level (BSL)-3 laboratory, a pseudo-type assay is more suitable for high-throughput screening of convalescent plasma donors as it can be performed at a BSL-2 facility.

In the current study, we have first determined the neutralising antibody levels in our convalescent plasma donors and estimated a cut-off to be used in clinical trials. Second, we have also assessed whether there is a correlation between neutralisation antibody titres (measured either using microneutralisation or pseudo-type assay) and ELISA reactivity using a variety of assays formats including cell lysate, in-house assays and two commercial ELISAs. Identification of a suitable high-throughput assay is required urgently to support scaling up convalescent plasma production and to support the comparison of data between countries.

2 | MATERIALS AND METHODS

2.1 | Convalescent plasma donors

We initiated the collection of convalescent plasma using the established infrastructure and standard UK donor selection guidelines during March 2020; serum and EDTA blood samples were collected from individuals with a previous laboratory-confirmed SARS-CoV-2 infection at least 28 days after the resolution of their symptoms. These donor samples were submitted to Public Health England and tested initially for SARS-CoV-2 RNA by in-house reverse transcription polymerase chain reaction assay,[14] as well as for SARS-CoV-2 antibodies using a native virus antigen ELISA and microneutralisation assays, both based on the UK prototype strain (GISAID accession number EPI/ISL/407073), and the samples were subsequently subjected to testing by pseudo-type neutralisation assay and trimeric S ELISA. Basic donor information including age, gender and virology testing data were collected.

2.2 | Ethical statement

Signed consent was obtained from each donor at the time of donation. Donors consent to the NHS blood and transplant holding information about them, including their health, attendances and donations, and using their information for the purposes explained in the donor welcome booklet and data protection leaflet, which donors are asked to read at the time of donation. This includes using data for the purposes of clinical audit to assess and improve the service and for research, specifically to improve our knowledge of the donor population.

2.3 | Infected virus lysate assay

Native virus antigen ELISA was modified from a previously described MERS-CoV assay.[15] Serial dilutions of convalescent plasma samples were added to microplates containing the bound detergent-extracted lysates of SARS-CoV-2 (isolate England/02/2020)-infected Vero E6 cells and uninfected cells. The reactivity was determined using a chemiluminescent substrate labelled secondary antibody. Virus lysates contain a mixture of viral proteins expressed in Vero E6 cells, including viral nucleocapsid and S proteins, and these proteins are presented in the same structure as the native virus infecting the host. ELISA index value was defined as the difference between infected and uninfected cell reactivity expressed relative to control calibrator serum.

2.4 | Microneutralisation assay and neutralising antibody titre

SARS-CoV-2 (isolate England/02/2020)-specific neutralising antibody levels were measured using a modification of the World Health Organization (WHO) influenza microneutralisation methodology.[16] Briefly, the virus was incubated with a serial dilution of convalescent plasma obtained from recovered patients, after which a suspension of VeroE6 cells was added. After 22 h, cells were fixed, and in-cell SARS-CoV-2 nucleoprotein (NP) expression was determined by ELISA. The virus-neutralising antibody titre was determined as the serum concentration that inhibited 50% of SARS-CoV-2 NP expression. All work was undertaken in a BSL-3 laboratory.

2.5 | Enzyme-linked trimeric S immunosorbent assay (ELISA–Oxford)

Antibodies to the trimeric S (based on YP009724390.1) protein were detected by ELISA as previously described, using 2% skimmed milk in phosphate buffered saline as a blocking agent and alkaline phosphatase-conjugated anti-human IgG (A95455; Sigma) at 1:10 000 dilution.[12] Optical densities (ODs) were measured at 405 nm.

2.6 | Pseudoparticle neutralisation test

A lentivirus-based SARS-CoV-2 pseudovirus particle was constructed displaying the full S protein on the surface of pseudoparticle as previously described (accession number: YP009724390.1).[12] Neutralising antibody titres were measured by the reduction in luciferase gene

expression after 72 h incubation of HEK 293T ACE2-transfected cells at 37°C. The 50% inhibitory dilution (IC_{50}) was defined as the plasma dilution at which the relative light units (RLUs) were reduced by 50% compared with the virus control wells after subtraction of the background RLUs in the groups with cells only.

2.7 | Commercial assays, EUROimmun (IgG) and Fortress (total antibodies)

EUROimmun assay is based on the S1 protein and Fortress assay on the RBD of S protein. These assays were performed according to the manufacturer's recommendation (EUROimmun, PerkinElmer, London, UK and Fortress Diagnostics, Belfast, Northern Ireland).

2.8 | Statistics

Associations between test assays were compared using Pearson correlation coefficients and the non-parametric Spearman's rank correlation. *p*-Values were derived using Student's *t* test for correlations and Pearson correlation coefficient under the null hypothesis that the correlation was zero. The sensitivity and specificity were calculated to assess the performance of the different assays in classifying the level

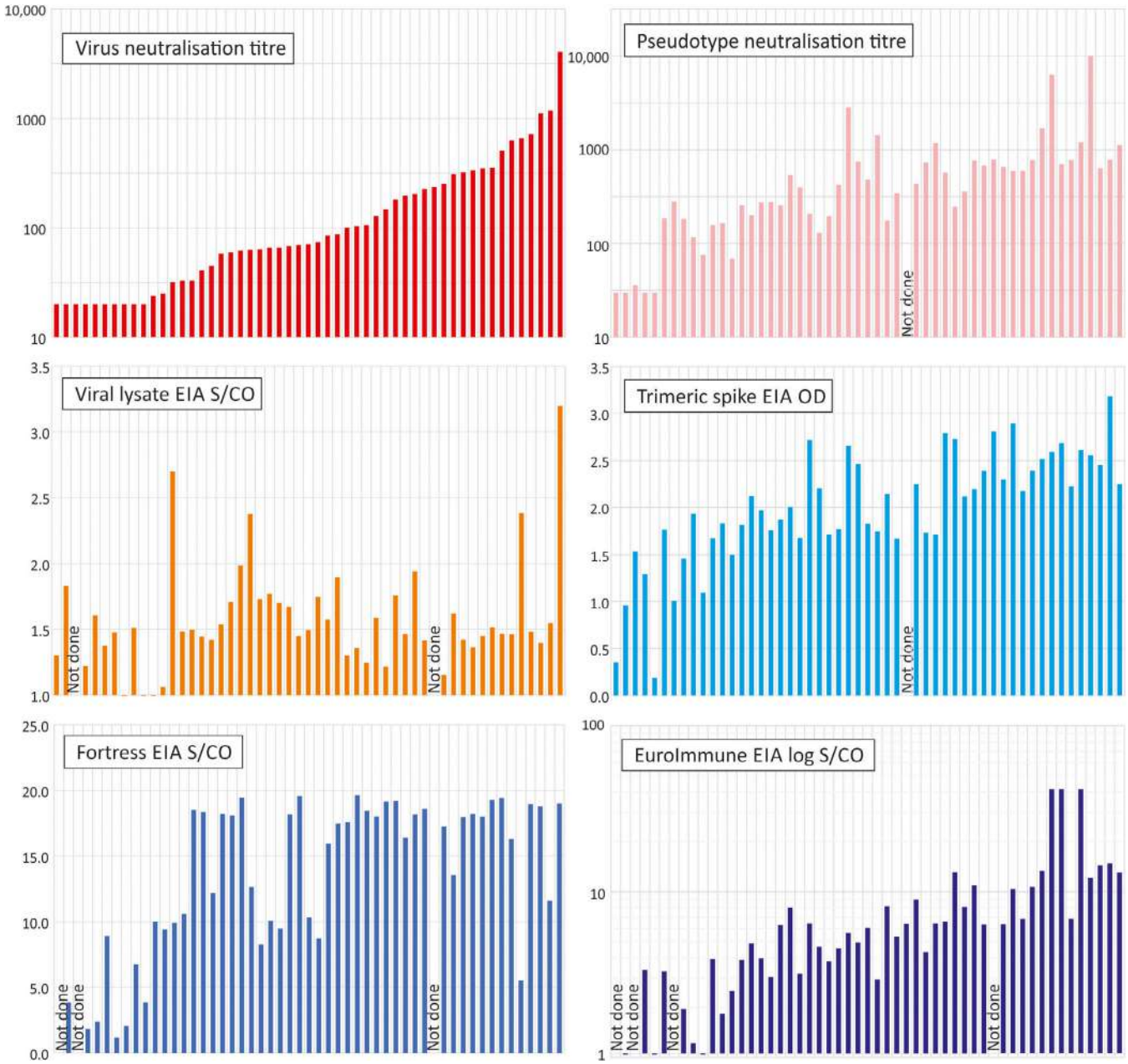

FIGURE 1 Comparison of neutralising antibody titres with reactivity in other assays. Comparison of neutralising antibody titres of the 52 test samples in the virus neutralisation assay with those of the pseudo-type assay and reactivities in enzyme immunoassay (EIAs). In all graphs, samples were ordered by virus-neutralising antibody titres. The following assay cut-off values were used: 0.049 for trimeric spike EIA, 1.0 for Fortress EIA and 1.1 for EUROimmun [Color figure can be viewed at wileyonlinelibrary.com]

of neutralising antibody titres obtained by microneutralisation assay using live SARS-CoV-2 virus. Exact binomial confidence intervals were used to derive confidence intervals.

3 | RESULTS

The initial assessment included samples from 52 recovered patients who would qualify as donors of convalescent plasma for clinical trials. They were all males (to avoid the need for additional human leukocyte antigen and human neutrophil antigen antibody testing that was not available at the required scale at the time of the study) and at least 28 days from the recovery after laboratory-confirmed SARS-CoV-2 infection. They were sampled during the first 2 weeks of April, implying that their illness began at the beginning of March. Therefore, they would all have been hospitalised as a part of the containment strategy. However, no data on the severity of their infection are currently available. EDTA and serum samples were obtained from each individual, and a whole-blood donation was collected from 10. All samples were submitted to Public Health England Colindale, and available

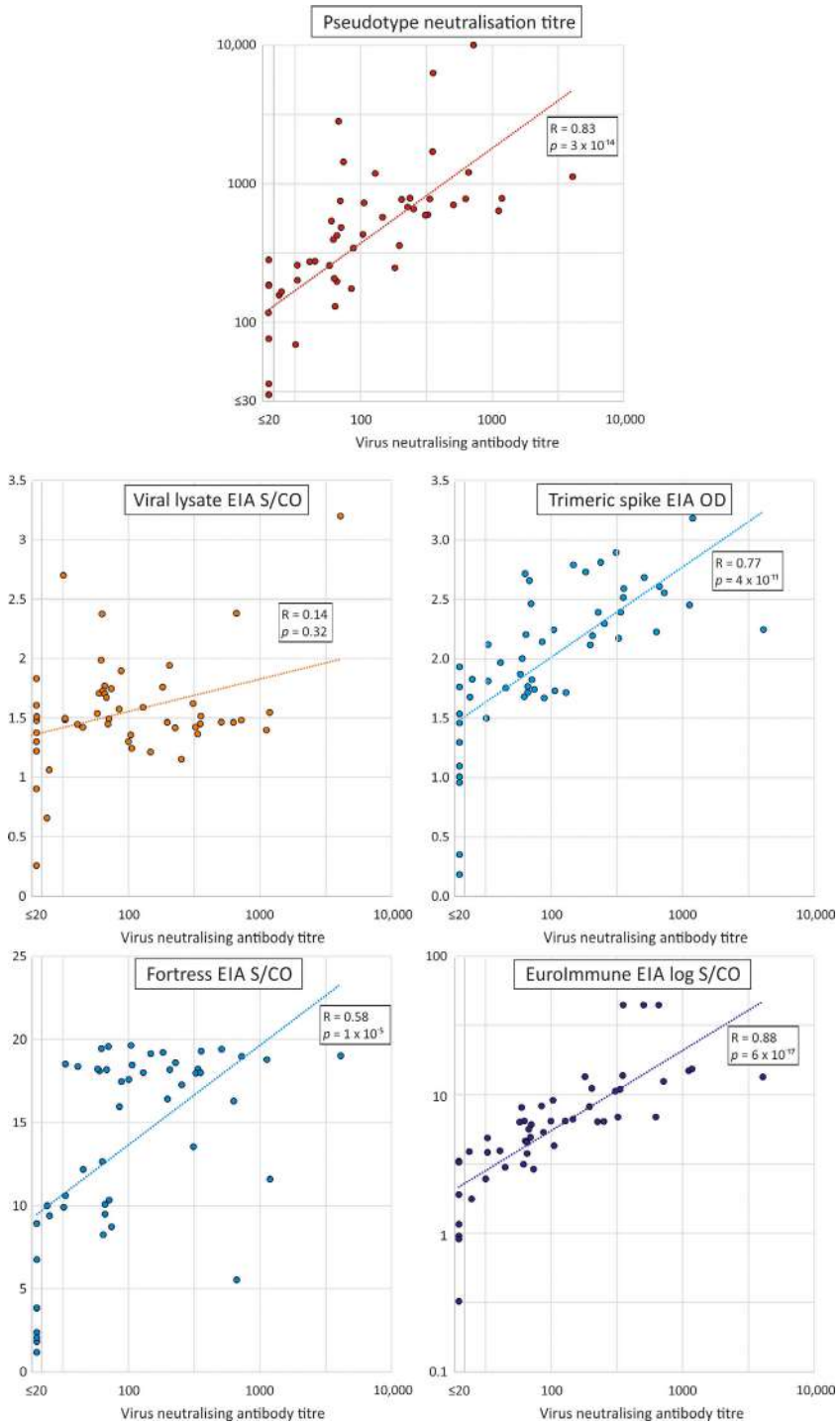

FIGURE 2 Correlations between neutralising and pseudo-type antibody titres and reactivities in EIAs. Scatter plots of neutralising antibody titres of test samples in the virus neutralisation assay with those of the pseudo-type assay and reactivities in EIAs. A line of best fit was estimated by linear regression using log-transformed values for the virus and pseudo-type neutralising antibody assays and the EUROimmun EIA. Correlation coefficients and (two-tailed) *p* values were calculated by Spearman non-parametric test [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Threshold values for optimal sensitivity and specificity of EUROimmun and pseudo-type neutralisation assays by ROC analysis

Cut-off value	Sensitivity (95% CI)	Specificity (95% CI)
EUROimmun S/CO		
6.37	0.95 (0.76, 1.00)	0.89 (0.98, 0.77)
6.64	0.76 (0.53, 0.92)	0.93 (1.00, 0.83)
8.19	0.68 (0.48, 0.83)	0.96 (0.99, 0.85)
9.1[a]	0.65 (0.45, 0.81)	1.00 (1.00, 0.92)
10	0.52 (0.30, 0.74)	1.00 (1.00, 1.00)
Pseudo-type neut. titre		
573	0.86 (0.64, 0.97)	0.90 (0.98, 0.73)
770	0.48 (0.26, 0.70)	0.93 (0.99, 0.78)

Note: These calculations are based on 48 samples, from which 22 had neutralising antibody levels of or over 1:100, and the remaining 26 were below 1:100.
[a]Optimal value selected for donation selection shown in bold.

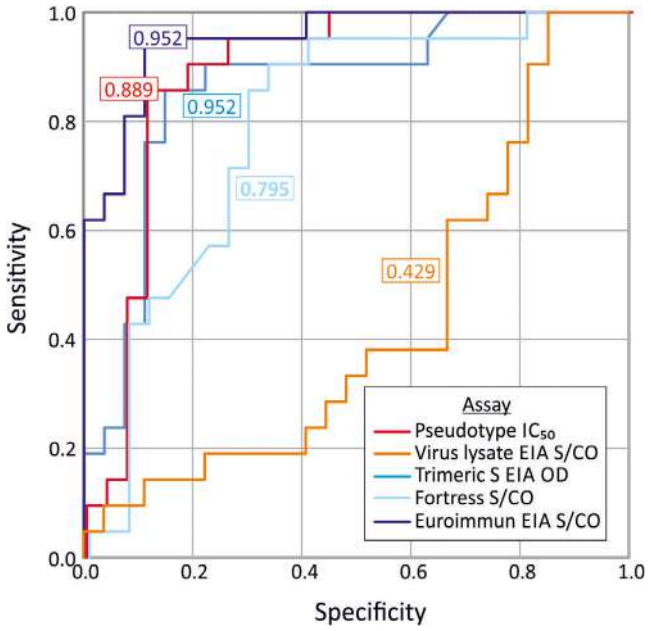

FIGURE 3 ROC analysis of serology assays predicting virus-neutralising antibody titres of ≥1/100. OC curves for the pseudo-type, virus lysate and three EIAs to correctly identify samples with neutralising antibody titres of 1:100 and over in the virus neutralisation assay. A total of 48 samples were included in these calculations (22 with neutralising antibody levels of or over 1:100 and the remaining 26 below 1:100). Areas under the curve for each assay are shown in colour-coded boxes [Color figure can be viewed at wileyonlinelibrary.com]

samples were distributed from there to the University of Oxford and Public Health England Porton Down for further testing. All samples tested negative for SARS-CoV-2 RNA. Assay specificity (particularly the rate of false reactives) has not been included in this analysis.

Neutralising antibodies were detected by microneutralisation assay in 43 of 52 tested samples using a cut-off titre 1:20; the highest detectable titre was 1:4096 (Figure 1). In other assays, SARS-CoV-2 antibodies were detected in most samples tested by pseudo-type assay (47/51), lysate ELISA (47/50) and EUROimmun (47/50) and in all samples by trimeric S ELISA (51/51) and Fortress total antibody ELISA (50/50). Based on these initial observations, all assays demonstrated good sensitivity for detecting antibodies in the study subjects 28 days after their recovery. For most assays, quantitative measures of serological reactivity (IC_{50} in the pseudo-type assay, ODs or signal to cut-off ratios (S/CO)) suggested a trend with neutralising antibody titres based on the live virus microneutralisation assay (Figure 1).

We have further assessed the correlation between neutralising antibody titre and serological reactivities in different ELISA platforms (Figure 2) where Pearson correlation coefficients and the non-parametric Spearman's Rank correlation tests were performed. The Pearson correlation tests were used for a linear association between variables (using log-transformed values for the neutralisation, pseudo-type and EUROimmun assays; R^2 values), whereas Spearman's coefficient determined correlations in ranking irrespective of magnitude. A further comprehensive pairwise comparison between all assays is provided in Figure S1.

The strongest correlation was observed between neutralising antibody titres and reactivity in the EUROimmun IgG ELISA (Spearman's rank correlation: 0.88; $p < 0.0001$, $n = 48$). Correlations were also observed between neutralising antibody titres with IC50 values in the pseudo-type assay (Spearman's rank correlation: 0.82; $p < 0.0001$, $n = 51$) and trimeric S ELISA (Spearman's rank correlation: 0.76; $p < 0.0001$, $n = 51$).

We selected a neutralising antibody titre of 1:100 as a likely therapeutic threshold for plasma donation selection (see discussion) and determined the best corresponding cut-off value in the EUROimmun ELISA by ROC analysis (Table 1; Figure 3). A total of 22 of 48 samples with a EUROimmun result had a neutralising antibody titre higher than or equal to 1:100 and hence contributed to the sensitivity calculations. Similarly, the remaining 26 samples with neutralising antibody titre below 1:100 contributed to the specificity calculations. Five potential cut-off values in the EUROimmun ELISA (S/CO values between 6.37 and 10) were investigated for sensitivity and specificity; a value of 9.1 correctly identified 65% of donations (14/22) above the 1:100 neutralising antibody threshold, whereas all donations below this neutralising antibody threshold were identified correctly using this value (26/26). In contrast, the pseudo-type assay was unable to identify 50% or more donations >1:100 without false identification.

4 | DISCUSSION

Here, we have described the first evaluation of the relationship between neutralising antibody titres and measures of antibodies to SARS-CoV-2 proteins in a variety of assays. These data can guide the selection of units of convalescent plasma for clinical use and for randomised clinical trials.

Our initial observation of convalescent plasma donors sampled at least 28 days after recovery from a laboratory-confirmed SARS-CoV-2 infection showed that all of them demonstrated serological evidence of past SARS-CoV-2 infection in one or more assays, whereas the neutralising antibody levels detected by microneutralisation assay varied from low (1:20) to high (1:4096; Figure 1). Furthermore, approximately 43% of donor samples showed neutralising antibody titres greater than 1:100. These neutralising antibody titres obtained by the microneutralisation assay correlated with values obtained by pseudovirus assay; a titre of 1:100 corresponded to 1:300 calculated based on luminescence reading. Although the pseudo-type assay can be automated and does not require working with the live virus in a biosafety level 3 laboratory, it is still time-consuming compared to the ELISA-based assay and requires the use of live cells and BSL-2 facilities that are often lacking from blood donation screening and reference laboratories.

In a previous study, most convalescent plasma donors with previous COVID-19 infection showed high neutralising antibody titres of at least 1:160 determined by the plaque reduction neutralisation test (PRNT; 39/40). For CPT, only donations with antibody titres above 1:640 were used.[5] In a separate study, donations with a neutralising antibody titre equal or higher than 1:80 based on the microneutralisation test were used successfully.[6] It is important to note that antibody titres obtained by different assays may not be comparable; based on previous data on SARS-CoV-2, neutralising antibody titres obtained by PRNT were approximately four-fold higher than those obtained by a cytopathic effect (CPE)-based microneutralisation assay.[17] CPE refers to structural changes in host cell, caused by virus invasions. Further comparative work is required to determine how the neutralising antibody level obtained by our microneutralisation assays compares with the PRNT titres and also with assays performed outside the United Kingdom. The future availability of WHO international standards will facilitate such comparisons; this is anticipated to be available in December 2020.

A minimum neutralising antibody titre in convalescent plasma needs to be determined before plasma is supplied for clinical trials. This needs to be balanced with the difficulty of collecting a required number of such components and providing a sufficient dose of antibodies to potentially be effective. For the planned trial, the use of plasma with a too-low cut-off may prevent or prolong a clear demonstration of efficacy; conversely, a too high cut-off may prevent a sufficient supply of plasma to fulfil trial needs. The chosen neutralising antibody level, 1:100, was selected as a pragmatic cut-off that enables an estimated 40% of collected plasma to be used. The actual dose of neutralising antibody given to patients also depends on the number of units given, and giving two units from different donors may substantially increase the mean dose to more than 1:300. Although considered potentially effective, how this level obtained by the microneutralisation assay compares with PRNT titres used in previous studies requires further work. This cut-off will be reviewed after a larger number of samples have been analysed to see if supply is meeting demand.

In order to support the scaling up the convalescent plasma production, it is important to identify a suitable high-throughput ELISA assay that can be used to estimate the neutralising antibody levels in convalescent plasma samples and thus could determine which donations are offered for clinical use. Serological reactivity in both the EUROimmun SARS-CoV-2 IgG ELISA and the trimeric S SARS-CoV-2 ELISA showed a strong correlation with neutralising antibodies obtained either by microneutralisation test or by pseudo-type assay. Although the EUROimmun assay has been shown to lack sensitivity for samples collected from patients with recent infection,[18] we have shown that it could be used to identify donations containing high levels of neutralising antibodies with a good level of specificity. By selecting an S/CO cut-off value of 9.1, the assay would only identify units if the neutralising antibody titre was 1:100 or higher. This is consistent with a previous finding where plasma with high titres of neutralising SARS-CoV-2 antibodies also showed higher titres of RBD, S domain 1 or 2 and specific binding antibodies.[8] Trimeric S ELISA falls within the RBD domain located in the S domain 1, whereas EUROimmun targets S domain 1. However, it is important to note that this is based on testing a preselected cohort of individuals at least 28 days after recovery from a previous laboratory-confirmed SARS-CoV-2 infection. The evaluation should be repeated if these criteria are changed or if the screening of native blood donor populations without a prior history of SARS-CoV-2 infection is considered.

As only a small number of samples from preselected convalescent plasma donors have been tested so far, which is a limitation of this study, we propose that several assay formats should be employed in a larger group of donors to validate these findings before the scaling up can be finalised. For practical and economic reasons, we decided to extend neutralising antibody testing up to 300 samples and then finalise analysis. Nevertheless, the results provide guidance for the many convalescent plasma programmes in progress around the world.

Neutralising antibody levels are partly dependent on the timing of collection relative to the recovery from infection. Seroconversion following SARS-CoV-2 infection has been observed between 8 and 21 days after the onset of symptoms,[9,19-21] and higher levels of antibodies have been determined in plasma collected at least 14 days after the symptom resolution.[5] It is likely that the antibody maturation continues for longer as demonstrated for other viruses, and hence, the collection point of 28 days after recovery has been chosen here. This maximises the chances of collecting the most clinically effective donations. However, it is still unclear how long neutralising antibody levels are maintained, and hence, repeat testing will be performed at every donation.

Higher neutralising SARS-CoV-2 antibody levels have been associated with older age and a worse clinical outcome,[8,21] although good neutralising antibody levels have also been measured in individual patients with milder infections.[22,23] The monitoring of neutralising antibody levels in different patient groups (including females not included in this study) and over time is required and will inform future screening strategies.

In conclusion, here, we have demonstrated a correlation between the neutralising antibody level and antibody reactivity measured by ELISA, which will allow scaling up of the convalescent plasma production. However, continuous monitoring of assay performance, antibody

decay and adaptation of selection strategies will be required in order to deliver the best clinical outcomes for patients receiving neutralising SARS-CoV-2 antibodies through CPT.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Heli Harvala: designed the study; coordinated the testing and collected and analysed the data, as well as wrote the first draft of manuscript; reviewed and accepted the final manuscript. **Matthew L. Robb**: drafted the statistical analysis; reviewed and accepted the final manuscript. **Nick Watkins**: critically reviewed the manuscript; supported the logistics of this study via the convalescent plasma group; reviewed and accepted the final manuscript. **Samreen Ijaz**: responsible for sample aliquoting and logistics between different sites; reviewed and accepted the final manuscript. **Steven Dicks**: responsible for sample aliquoting and logistics between different sites; reviewed and accepted the final manuscript. **Monika Patel**: performed testing with live virus neutralisation and lysate assays in PHE Colindale; reviewed and accepted the final manuscript. **Piyada Supasa**: responsible for developing spike-ELISA in Oxford and performing testing of samples with that assay; reviewed and accepted the final manuscript. **Wanwisa Dejnirattisai**: responsible for developing spike-ELISA in Oxford and performing testing of samples with that assay; reviewed and accepted the final manuscript. **Chang Liu**: responsible for developing spike-ELISA in Oxford and performing testing of samples with that assay; reviewed and accepted the final manuscript. **Juthathip Mongkolsapaya**: responsible for developing spike-ELISA in Oxford and performing testing of samples with that assay; reviewed and accepted the final manuscript. **Abbie Brown**: organised and performed EUROimmun testing at PHE Porton Down; reviewed and accepted the final manuscript. **Daniel Bailey**: organised and performed EUROimmun testing at PHE Porton Down; reviewed and accepted the final manuscript. **Richard Vipond**: organised and performed EUROimmun testing at PHE Porton Down; reviewed and accepted the final manuscript. **Nicholas Grayson**: organised and performed pseudo-type neutralisation testing of these samples; reviewed and accepted the final manuscript. **Nigel Temperton**: organised and performed pseudo-type neutralisation testing of these samples; reviewed and accepted the final manuscript. **Sunetra Gupta**: organised and performed pseudo-type neutralisation testing of these samples; reviewed and accepted the final manuscript. **Rutger J. Ploeg**: reviewed and accepted the final manuscript. **Jai Bolton**: organised and performed pseudo-type neutralisation testing of these samples; reviewed and accepted the final manuscript. **Alex Fyfe**: organised and performed pseudo-type neutralisation testing of these samples; reviewed and accepted the final manuscript. **Robin Gopal**: critically reviewed the manuscript; performed testing with live virus neutralisation and lysate assays in PHE Colindale; reviewed and accepted the final manuscript. **Peter Simmonds**: critically reviewed the manuscript; drafted the statistical analysis; reviewed and accepted the final manuscript. **Gavin Screaton**: responsible for developing spike-ELISA in Oxford and performing testing of samples with that assay; reviewed and accepted the final manuscript. **Craig Thompson**: organised and performed pseudo-type neutralisation testing of these samples; reviewed and accepted the final manuscript. **Tim Brooks**: organised and performed EUROimmun testing at PHE Porton Down; reviewed and accepted the final manuscript. **Maria Zambon**: designed the study; critically reviewed the manuscript; performed testing with live virus neutralisation and lysate assays in PHE Colindale; reviewed and accepted the final manuscript. **Gail Miflin**: critically reviewed the manuscript; supported the logistics of this study via the convalescent plasma group; reviewed and accepted the final manuscript. **David J. Roberts**: designed the study; critically reviewed the manuscript; reviewed and accepted the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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ORIGINAL ARTICLE

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Motivators of and barriers to becoming a COVID-19 convalescent plasma donor: A survey study

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Abstract

Objectives: To determine the motivators and barriers to COVID-19 convalescent plasma donation by those in the United Kingdom who have been diagnosed with or who have had symptoms of SARS-CoV-2 (COVID-19) but who have not donated.

Background: Convalescent plasma from people recovered from COVID-19 with sufficient antibody titres is a potential option for the treatment and prevention of COVID-19. However, to date, recruiting and retaining COVID-19 convalescent plasma donors has been challenging. Understanding why those eligible to donate COVID-19 convalescent plasma have not donated is critical to developing recruitment campaigns.

Methods/Materials: A total of 419 UK residents who indicated that they had been infected with COVID-19 and who lived within 50 km of sites collecting COVID-19 convalescent plasma completed an online survey between 25th June and 5th July 2020. Respondents completed items assessing their awareness of convalescent plasma, motivations and barriers to donation and intention to donate COVID-19 convalescent plasma.

Results: Awareness of COVID-19 convalescent plasma was low. Exploratory factor analysis identified six motivations and seven barriers to donating. A stronger sense of altruism through adversity and moral and civic duty were positively related to intention to donate, whereas generic donation fears was negatively related.

Conclusions: Once potential donors are aware of convalescent plasma, interventions should focus on the gratitude and reciprocity that those eligible to donate feel, along with a focus on (potentially) helping family and norms of what people ought to do. Fears associated with donation should not be neglected, and strategies that have been successfully used tor recruit whole-blood donors should be adapted and deployed to recruit COVID-19 convalescent plasma donors.

KEYWORDS
barriers, blood donors, COVID-19 convalescent plasma, motivations, pandemic

1 | INTRODUCTION

With currently limited treatment options for COVID-19, convalescent plasma from people recovered from COVID-19 with sufficient antibody titres is a potential option for treatment and prevention.[1,2] Convalescent plasma has previously been investigated as a treatment for many infectious diseases, including those caused by other coronaviruses such as SARS-CoV and MERS-CoV, and early reports of its

use in SARS-CoV-2 infection (COVID-19) showed some promise.[1] As a consequence, many trials of COVID-19 convalescent plasma are in progress, with 65 centres across 24 countries indicating that they were planning to collect and administer COVID-19 convalescent plasma to COVID-19 patients.[3] If these trials confirm the efficacy of COVID-19 convalescent plasma, such as a mortality reduction, then demand for COVID-19 convalescent plasma will grow substantially.

Although the focus of research has been on establishing the efficacy of COVID-19 convalescent plasma as a direct or manufactured treatment for COVID-19, little attention has been paid to the producers of COVID-19 convalescent plasma—the donors. Given the scale of the pandemic, Bloch and colleagues[1] noted that "finding donors is not anticipated to be a problem". In reality, the effective recruitment and retention of sufficient numbers of COVID-19 convalescent plasma donors who are eligible, have sufficient antibody titres and are willing to donate has proved challenging, with both the American Red Cross[4] and UK's National Health Service Blood and Transplant[5] (NHSBT) issuing urgent appeals for COVID-19 convalescent plasma donors in August 2020. Such a reticence in eligible convalescent plasma donors has been seen previously.[6,7] However, little is known about why this is, and indeed, nothing is known about what deters and motivates someone to become and remain a COVID-19 convalescent plasma donor.

Work on influenza A virus subtype H1N1, the pandemic influenza strain that originated in 2009 and Ebola convalescent plasma donors suggested that fear regarding the process (e.g., fear of needles), the stigma of having been infected and a sense that donating will impede recovery all deterred potential donors.[6-8] Furthermore, trust in the institutions collecting convalescent plasma, solidarity with those currently infected and belief in the efficacy of the treatment resulting from convalescent plasma enhanced willingness to be a convalescent plasma donor for Ebola.[7]

Although these studies are informative, they are limited as they are specific to H1N1 and Ebola. Early research on survivors of COVID-19 has identified similar themes in their illness narratives (e.g., guilt, fear, dichotomy of praise and stigma); however, we do not yet know if we can generalise previously identified barriers to and motivators of convalescent plasma donation.[9] H1N1, Ebola and COVID-19 are thought to differ substantially in mortality rates, and this may influence how survivors feel about their illness, their survival and donating convalescent plasma.[10-12] Furthermore, work on the preference/motivations for cooperative behaviour linked to tissue donation (blood, organs, gametes) has advanced greatly in recent years.[13] Thus, a comprehensive analysis of the motivation structure of convalescent plasma donors requires that we move beyond what we know for convalescent plasma in Ebola and H1N1. For example, constructs like reluctant altruism—whereby people are motivated to help as the majority either cannot or will not—are very pertinent here as those eligible to donate COVID-19 convalescent plasma are a minority.[13-17]

Likely also important in the decision to donate COVID-19 convalescent plasma are beliefs that result from the experience of COVID-19. Those eligible to donate COVID-19 convalescent plasma may experience gratitude at having survived a traumatic event, promoting a greater desire to help others: altruism borne from adversity.[18,19] Gratitude can engender direct reciprocity (paying back a debt to the health services) or upstream (pay-it-forward) indirect reciprocity, where the COVID-19 convalescent plasma donor feels gratitude for having been helped and a want to help others.[19] Similarly, those who have survived COVID-19 may experience aspects of post-traumatic growth, with perceptions of personal strength and the finding of meaning in survival potentially motivating donation.[20] These motivating factors may, however, be tempered by uncertainty about infectiousness, both in terms of potentially infecting others or becoming re-infected themselves.[21]

Aside from personal experience, context is also likely important. The media narrative of the COVID-19 pandemic has varied across countries, but in the United Kingdom, it has focused on the fight against an unseen enemy.[22] Consistent with appeals in times of crisis, this has very much mirrored a wartime "call to arms" to fight a national threat.[23] In the framing of COVID-19, notions of patriotism and the moral and civic duty of individuals have been common both in proclamations by governments and in the popular media.[23-25]

Accordingly, those members of the public with the (potential) ability to save others have been hailed as "heroes."[23,26,27] Civic duty motivates the donation of biological material (specifically organs) and, given the contextual salience of the link between COVID-19 and moral/civic duty in the United Kingdom, may influence how those eligible think about donating COVID-19 convalescent plasma.[28]

An understanding of the impact of the framing of the pandemic on potential donors is important in developing strategies to ensure a future supply of COVID-19 convalescent plasma. The approach of identifying the motivators first and then developing interventions to reflect these has proven to be successful in other areas of health-based cooperation around tissue donation. This is especially the case for whole-blood and plasma donation.[29,30] The objective of this study is to do the same for COVID-19 convalescent plasma, with this being the first study to report on the motivations and barriers of potential COVID-19 convalescent plasma donors. We also provide recommendations on how to most effectively recruit COVID-19 convalescent plasma donors based on the findings.

2 | MATERIALS AND METHODS

2.1 | Sampling procedure and sample size calculations

This study was approved by the University of Queensland Health and Behavioural Sciences, Low and Negligible Risk Ethics Sub-Committee (Ref: 2020001347), the Australian Red Cross Lifeblood Human Research Ethics Committee (Ref: 11062020) and the University of Nottingham Ethics Committee (Ref: F1257). Potentially eligible COVID-19 convalescent plasma donors were recruited via the online panel Prolific Academic between 25th June and 5th July 2020. At the time of data collection, confirmed cases of COVID-19 in the United Kingdom had fallen from their initial peak in early April of over 5000

TABLE 1 Motivation and barriers factors, internal reliability, factor stability coefficients, items and item factor loadings

	Factor saturation	Y	Cronbach's alpha (α) or correlation (*r*) for 2 item measures	Items	Factor loading
Barriers					
Worry that others will know of COVID-19 infection	0.838	0.014	0.90	In general, I do not want people to know that I have had coronavirus	0.712
				If people found out that I have had coronavirus, I am worried how they would react to me	0.918
				I am concerned that some people may avoid me if they know I've had coronavirus	0.884
Infection and process risk to self and others	0.635	0.038	0.83	I worry that I may inadvertently infect others with coronavirus through donating	0.598
				Donating convalescent plasma will set my recovery back	0.727
				I will become ill again if I donate convalescent plasma	0.834
				I would feel like a guinea pig if I donated convalescent plasma	0.348
				I am scared of what might be involved in donating convalescent plasma	0.502
				I don't understand what donating convalescent plasma involves	0.802
Logistics	0.724	0.028	0.83	I do not want to travel to the donor centre to donate convalescent plasma	0.739
				It is just too inconvenient to donate	0.787
				Logistically, it is just too difficult for me to donate convalescent plasma (because of childcare/transport limitations etc.)	0.647
Not well enough	0.488	0.061	0.82	I do not really feel well enough to donate convalescent plasma	0.511
				Others who are fitter than me can donate convalescent plasma	0.343
				I need more time to recover from coronavirus before I could donate	0.464
				I have spent too long in hospital settings recently	0.480
				I have been through enough recently	0.481
				I do not think I would physically be able to donate convalescent plasma	0.411
Generic donation fears	0.691	0.032	0.79	I do not like needles	0.875
				I am frightened of blood	0.826
				I do not like the idea of donating convalescent plasma	0.372
Lack of trust in institutions	0.804	0.018	$r = 0.75$	I do not trust the Blood Collection Agencies	0.804
				I do not trust doctors	0.805
Fear of re-infection	0.435	0.063	$r = 0.48$	I do not want to be around other people in the donor centre in order to donate convalescent plasma	0.549
				I am worried about getting re-infected if I donate convalescent plasma	0.321
Facilitators					
Signalling reluctant altruism	0.508	0.054	0.82	Donating convalescent plasma will make others feel more positively about me	0.674
				If I donate convalescent plasma it will be a story I can tell others about	0.578
				Through donating convalescent plasma, I can be a hero and help others	0.645
				I am in a unique position to help by donating convalescent plasma where other people cannot	0.375
				There are very few people who can help through donating convalescent plasma	0.292
				Donating convalescent plasma would make me feel proud	0.486

(Continues)

TABLE 1 (Continued)

	Factor saturation	Y	Cronbach's alpha (α) or correlation (*r*) for 2 item measures	Items	Factor loading
Altruism from adversity	0.604	0.043	0.78	I feel grateful that I survived coronavirus	0.731
				I want to feel part of the amazing effort to beat coronavirus	0.515
				I feel a debt to the medical staff and care workers who looked after me	0.597
				I like to help others, and donating convalescent plasma is just one way I can help	0.444
				I want to help others not get as ill as I was with coronavirus	0.732
Post-traumatic growth	0.726	0.028	*r* = 0.63	I survived coronavirus, and feel that this must have been for a reason	0.693
				Surviving coronavirus makes you a strong person	0.759
Moral and civic duty to help research	0.449	0.061	0.77	Donating convalescent plasma will help research into coronavirus treatments	0.498
				Donating convalescent plasma will potentially help my family and friends if they get ill	0.525
				I do not think that convalescent plasma will be an effective therapy for coronavirus®	−0.483
				My friends and family would not want me to donate convalescent plasma	−0.322
				I would feel guilty if I did not donate convalescent plasma	0.328
				Donating convalescent plasma would be the morally right thing to do	0.609
				For me, donating convalescent plasma would be - The wrong thing to do: The right thing to do	0.376
Patriotism and control	0.683	0.033	0.84	Donating convalescent plasma is a way to repay being saved	0.602
				Donating convalescent plasma would give me a sense of patriotic duty and national pride	0.726
				Donating convalescent plasma would help me get some sense of control back over my life	0.910
				I have felt a little "down" since recovery and donating convalescent plasma is something I can do to pull myself back up	0.495
Reluctant altruism	0.831	0.015	*r* = 0.65	I do not trust that others in my position would be able to donate their plasma	0.928
				I do not trust that others in my position will want to donate their plasma	0.734

Note: Cronbach's alphas/*r* calculated with whole sample. Y = factor stability estimate. All items responded to on 1–7 scales. Scale endpoints are strongly disagree to strongly agree. (*R*) indicates reversed item in composite scale.

per day to fewer than 900,[31] and the widespread lockdown restrictions imposed during the early phases of COVID-19 were beginning to ease.[32] Reflecting the eligibility criteria to donate COVID-19 convalescent plasma in the United Kingdom, respondents were eligible to participate in this cross-sectional survey if they indicated that they had previously been infected with SARS-CoV-2; were fit and healthy; weighed between 50 and 158 kg; were aged between 17 and 66 years (or 70+ if they had given a full blood donation in the last 2 years); and lived within 32 miles (50 km) of one of the listed COVID-19 convalescent plasma collection sites in England, Scotland, Wales and Northern Ireland.

The main focus of the analyses was to explore the latent structure of the motivations and barriers of potentially eligible COVID-19 convalescent plasma donors. As such, we aimed to sample enough participants to ensure we could recover a stable factor structure. Although many rules of thumb guide this decision,[33,34] a Monte Carlo simulation showed that factor saturation (the average loading on a factor) and absolute sample size are the key determinants.[35] If saturation is high (0.6 or greater), then an absolute sample size of 150 is sufficient; if it is lower (0.4), then a minimum sample size of 300 is required. We assumed low saturation and sought a minimum sample of 300.

2.2 | Materials and measures

After reading information about the study and providing informed consent, participants were initially asked to indicate the month in which they tested positive or had symptoms of COVID-19 before

being asked about their current health using a single item adapted from the Short Form Health Survey[36] ("Right now, would you say your health is?" with response options of very good, good, fair, bad, very bad). Respondents were then asked to indicate whether they had heard of convalescent plasma (yes, no). Those who answered "yes" were asked to indicate how they knew about convalescent plasma and from where they had obtained this information.

Participants were then given standard information on COVID-19 convalescent plasma adapted from the NHSBT websites[37,38] before being asked if they had attempted to donate convalescent plasma (yes, no). Those who indicated yes were asked if they had successfully donated convalescent plasma (yes, no) and whether they intended to continue donating convalescent plasma ("I intend to continue donating convalescent plasma," 1 *strongly disagree* to 7 *strongly agree).* Those who indicated that they had not attempted to donate were asked to indicate their agreement with the statement "I intend to donate convalescent plasma" on a 1, *strongly disagree* to 7, *strongly agree* scale.

Following this, participants were presented with 56 statements assessing (potential) motivators for/facilitators of donating COVID-19 convalescent plasma and deterrents and barriers to donating COVID-19 convalescent plasma (see Table 1). Barrier statements focused on participants' self-perception that they were not yet well enough to donate,[7,39] concern about poor recovery following donation,[6,7] lack of familiarity with the plasmapheresis process,[6,7] general physical and logistical barriers to donating,[6,7] stigma associated with being identified as someone who had been infected with COVID-19,[7,9] (lack of) trust in medical personnel/institutions[7] and fear of infecting others/self. Motivating statements focused on solidarity with those currently experiencing COVID-19,[7] trust in the efficacy of the treatment,[7] moral and civic duty to donate,[23-25] altruism through adversity,[17,18] post-traumatic growth,[20,40] reluctant altruism[13] and patriotism and control.[25] All items were responded to using 1 (*strongly disagree*) to 7 (*strongly agree*) scales.

2.3 | Statistical analyses

Following initial examination of the data through descriptive statistics, we conducted exploratory factor analysis (EFA) using M*Plus* 8.1.[41] An exploratory, rather than a confirmatory, analytic approach was justified as (1) we had no formal model to represent the broad theoretical domains drawn on, and (2) these analyses focused on a novel domain with a mix of constructs that had not been examined together before. Therefore, an EFA approach was the most informative. However, the interpretation was informed by the conceptual domains examined.

The EFA analysis was estimated using a weighted least squares with mean- and variance-adjusted (WLSMV) estimator and GEOMIN oblique rotation. We used oblique rotation as some degree of association is assumed and expected between factors within behavioural science research, and oblique rotation allows for factors to have varying degrees of association, including no association, whereas orthogonal rotation does not. Several different factor models were compared with respect to the following goodness-of-fit indices: chi-square, a comparative fit index (CFI), Tucker-Lewis index (TLI) and root mean square error of approximation (RMSEA). Within EFA, the chi-square statistic should be non-significant; however, as this statistic is sample size-specific, a non-significant chi-square is rarely achieved, and its use is contentious.[42] As such, it is included for completeness only. The CFI and TLI should be 0.95 or greater, and the RMSEA should be below 0.08.[43-45] The chi-square difference test was used for comparisons across the different model solutions. If the chi-square difference is significant, the model with the greater number of factors is selected. As an additional test of the adequacy of the solution, we calculated the factor stability coefficient (Y: the average distance between the sample and population loading) for each factor using the equation specified in Guadagnoli and Velicer[35] There is no calibration for this coefficient, so the smaller the number, the more stable the factor.[34]

Following identification of the optimal factor solution, composite measures of each factor were created. Correlations between these measures, self-perceptions of eligibility to donate blood and intention were examined prior to multiple regression being undertaken to determine which barriers and motivators were significantly related to intention.

3 | RESULTS

3.1 | Sample characteristics

Participants were 432 (281 female, 150 male, 1 gender non-specified) UK residents aged 18–71 years (M = 34.38, SD = 10.41). Of these participants, 306 (70.8%) believed themselves currently eligible to donate blood, 85 (19.7%) were unsure, and 41 (9.5%) believed themselves currently ineligible to donate. In addition, 56 (13.0%) had donated blood in the last 12 months, whereas a further 37 (9.9%) had attempted to donate. Participants were asked to self-identify their ethnicity. We used the UK Office of National Statistics (ONS) system to categorise these self-identifications into five higher-order codes. Of those who provided a self-identified ethnicity (some reported a religion or that they were British), 85.6% identified as White, 3% as Black/African/Caribbean/Black British, 4.2% as Asian/Asian British, 5.9% as Mixed/Multiple Ethnic Groups and 1% as other ethnic groups. These broadly correspond to the UK statistics on ethnic diversity of 87.2% White, 3% Black/African/Caribbean/Black British, 3.8% Asian/Asian British, 2% Mixed/Multiple Ethnic Groups and 7.2% other ethnic groups.[46]

Most participants (213; 49.8%) reported that they experienced COVID-19 in March 2020 and that their current health was "very good" (25.5%) or "good" (58.3%). Only 1.9% indicated that their current health was "bad."

A total of 148 respondents (34.3%) indicated that they had heard of convalescent plasma, with a further 40 respondents (9.3%) unsure as to whether they had heard of it or not. Nine stated that they had attempted to donate COVID-19 convalescent plasma, 419 stated that they definitely had not, and 4 were unsure. Among the nine respondents (2.1%) who had attempted to donate, only one successfully donated. Of those who had attempted, four had enquired about donating but had not yet heard back, two had veins that were not

suitable for plasmapheresis, and two could not secure an appointment when they were able to donate.

3.2 | Exploratory factor analysis

The EFA was conducted on the data from those who had not yet attempted to donate convalescent plasma (n = 419, there was no missing data). The chi-square difference test showed that the 13-factor model was a significantly better fit to these data than a 12-factor model, $\chi^2_{(diff)}$ = 144.390 (41), p = 0.0000. This model showed an excellent fit to these data—TLI = 0.954, CFI = 0.975, RMSEA = 0.044 (90% CI = 0.040, 0.047, p = 0.998), $\chi^2_{(40)}$ = 1307.624 (728), p = 0.0000—and was readily interpretable with respect to the initial constructs considered (see Table 1). We also examined the fit and interpretability of models with fewer potential factors (8–12). The RMSEA was significant for the 8- and 9-factor models, and the TLI was below the 0.95 cut-off for the 8–11-factor models. Although the fit was good for the 12-factor model (CFI = 0.971, TLI = 0.951, RMSEA = 0.045 (90% CI = 0.041, 0.049, p = 0.990), the 13-factor model showed incremental fit in terms of the chi-square difference test ($\chi^2_{(diff)}$ = 144.390 (41), p = 0.0000). Thus, the 13-factor model was selected.

Ten of the factors showed good factor saturation (0.6 or greater), and three showed lower saturation (0.4 or great), which with an absolute sample size of 419 suggests that the solution is stable. Indeed, all the factors had small to negligible factor stability estimates, indicating that the sample factor and loadings were close to the population values. Finally, all of the factors demonstrated good internal reliability. Therefore, the psychometric properties of these factors and this solution are excellent.

3.3 | Factor descriptives

An examination of mean scores on the composite measures showed that, on average, perceptions of barriers to donating COVID-19 convalescent plasma were low (and significantly below 4, the midpoint of the scale, ts > −6.77, ps < 0.001). Endorsement of the facilitators signalling reluctant altruism, altruism through adversity and moral and civic duty were significantly above the scale midpoint (ts > 6.15, ps < 0.001), whereas endorsement of post-traumatic growth and patriotism and control were significantly below the scale midpoint (ts > −6.30, ps < 0.001; Table 2). Participants' endorsement of reluctant altruism as a motive did not differ significantly from the scale's midpoint. Furthermore, participants' endorsement of all facilitators and barriers did not differ significantly by perceived eligibility to donate (see Table 2).

3.4 | Predicting COVID-19 convalescent plasma behavioural intentions

Variables with significant bivariate correlations with intention (Table S1) were entered into a multivariable, hierarchical ordinary least

TABLE 2 Means and standard deviations on continuous measures for whole sample and by perceived eligibility, significance of deviation from the midpoint of the scale (4) and significance of the difference in endorsement for those eligible and not eligible to donate (n = 419)

Scale	Overall (n = 419)	Significance of deviation from midpoint of the scale (4)[a]	Eligible (n = 378)	Not eligible (n = 41)	Significance of difference between those eligible and not eligible to donate[a]
Barriers					
Worry that others will know of COVID-19 infection	2.71 (1.61)	t(418) = −16.36, p < 0.001	2.73 (1.62)	2.60 (1.47)	t (417) = −0.472, p = 0.637
Infection and process risk to self and others	2.88 (1.26)	t(418) = −18.23, p < 0.001	2.89 (1.24)	2.83 (1.42)	t (417) = −0.273, p = 0.785
Logistics	3.45 (1.67)	t(418) = −6.78, p < 0.001	3.45 (1.67)	3.45 (1.72)	t (417) = 0.003, p = 0.997
Not well enough	2.78 (1.23)	t(418) = −20.29, p < 0.001	2.72 (1.22)	3.27 (1.31)	t (417) = 2.704, p = 0.007
Generic donation fears	3.18 (1.68)	t(418) = −10.08, p < 0.001	3.19 (1.68)	3.02 (1.62)	t (417) = −0.638, p = 0.524
Lack of trust in institutions	2.12 (1.36)	t(418) = −28.26, p < 0.001	2.11 (1.35)	2.18 (1.54)	t (417) = 0.320, p = 0.749
Fear of re-infection	3.15 (1.62)	t(418) = −10.75, p < 0.001	3.14 (1.60)	3.23 (1.82)	t (417) = 0.348, p = 0.728
Facilitators					
Signalling reluctant altruism	4.33 (1.09)	t(418) = 6.16, p < 0.001	4.31 (1.08)	4.48 (1.14)	t (417) = 0.962, p = 0.336
Altruism from adversity	4.90 (1.04)	t(418) = 17.77, p < 0.001	4.90 (1.04)	4.95 (1.07)	t (417) = 0.280, p = 0.780
Post-traumatic growth	3.53 (1.52)	t(418) = −6.31, p < 0.001	3.52 (1.53)	3.65 (1.38)	t (417) = 0.507, p = 0.613
Moral and civic duty to help research	5.01 (0.96)	t(418) = 21.52, p < 0.001	5.01 (0.96)	5.01 (0.96)	t (417) = 0.004, p = 0.997
Patriotism and control	3.34 (1.39)	t(418) = −9.68, p < 0.001	3.32 (1.39)	3.60 (1.38)	t (417) = 1.230, p = 0.219
Reluctant altruism	3.90 (1.34)	t(418) = −1.49, p = 0.136	3.88 (1.35)	4.13 (1.26)	t (417) = 1.165, p = 0.245

[a]Bonferroni correction ($p \leq 0.003$) applied to alpha to protect against Type 1 errors.

TABLE 3 Hierarchical multiple regression of perceptions of eligibility, barriers and facilitators onto intention to donate convalescent plasma (n = 418)

Step	Predictor	*B*	Std. Error	Beta	*t*	Significance	95% lower CI	95% higher CI
1								
	Constant	3.756	0.208		18.040	0.000	3.347	4.165
	Perceived eligibility to donate	0.458	0.219	0.102	2.090	0.037	0.027	0.889
2								
	Constant	1.611	0.562		2.867	0.004	0.507	2.716
	Perceived eligibility to donate	0.527	0.188	0.117	2.797	0.005	0.157	0.898
	Worry that others will know of COVID-19 infection	−0.011	0.042	−0.013	−0.251	0.802	−0.094	0.072
	Infection and process risk to self and others	−0.125	0.072	−0.117	−1.738	0.083	−0.266	0.016
	Logistics	−0.053	0.043	−0.066	−1.211	0.227	−0.138	0.033
	Not well enough	−0.028	0.071	−0.026	−0.394	0.694	−0.168	0.112
	Generic donation fears	−0.126	0.042	−0.158	−2.992	0.003	−0.209	−0.043
	Lack of trust in institutions	0.058	0.052	0.059	1.098	0.273	−0.045	0.160
	Fear of re-infection	0.006	0.049	0.007	0.122	0.903	−0.091	0.103
	Signalling reluctant altruism	0.045	0.076	0.036	0.588	0.557	−0.105	0.194
	Altruism from adversity	0.318	0.073	0.247	4.362	0.000	0.174	0.461
	Moral and civic duty to help research	0.187	0.092	0.134	2.043	0.042	0.007	0.367
	Patriotism and control	0.101	0.055	0.105	1.822	0.069	−0.008	0.210
	Reluctant altruism	−0.009	0.043	−0.009	−0.200	0.841	−0.094	0.077

squares regression model with perceived eligibility at step 1 and the motivations and barriers at step 2. At step 1, perceived eligibility was a positive predictor, with those who perceived themselves as eligible more likely to intend to donate COVID-19 convalescent plasma. Motivators and barriers accounted for an additional 32% of variance in intention to donate COVID-19 convalescent plasma, with altruism from adversity (β = 0.25, 95% CI [0.17, 0.46], p < 0.001, sr^2 = 0.03) and moral and civic duty (β = 0.13, 95% CI [0.01, 0.37], p = 0.042, sr^2 = 0.01) positively related to intention, while generic donation fears (β = −0.16, 95% CI [−0.20, −0.043], p = 0.003, sr^2 = 0.01) was negatively related (Table 3).

4 | DISCUSSION

To increase the number of COVID-19 convalescent plasma donors and progress trials and eventual large-scale deployment of COVID-19 convalescent plasma, we need to understand what motivates and deters donation. Creating awareness of convalescent plasma among potential donors is a necessary but not sufficient[47] first step, with understanding eligible donors' motivations and barriers also important. Although all of our sample met basic eligibility criteria to donate COVID-19 convalescent plasma, surprisingly, 55% had not heard of convalescent plasma. Thus, at the time of data collection, awareness of convalescent plasma among those potentially eligible to donate in the United Kingdom was low. Attention or awareness is the first step for effective persuasion,[47] and recruitment efforts need to focus on disseminating information about the importance of COVID-19 convalescent plasma and eligibility criteria through the optimal channels to reach those people that recruiting agencies wish to donate. These may be donors with demographic characteristics that are typically associated with higher rates of retention (e.g., older individuals[48]) or, if demonstrated, donors with demographic or infection characteristics that make them more likely to have sufficient antibody titres.[49]

Critically, however, our data suggests that even when the barrier of awareness is addressed, broader beliefs about donating and COVID-19 impact intentions to donate COVID-19 convalescent plasma. The strongest motivator of intention was "altruism from adversity"—beliefs centred around gratitude and reciprocity. The emergence of this as a main predictor is theoretically and practically significant. Theoretically, it is consistent with approaches which highlight that adversity results in people aligning with wanting to help others.[17,18] This want is motivated by gratitude and debt that reflects both upstream and downstream indirect reciprocity. In the context of COVID-19, gratitude is focussed on having survived and is generalised (e.g., grateful for the beauty of the world[19]). This type of gratitude should link to upstream indirect (pay-it-forward) reciprocity, motivating people to want to help those who have not been directly involved in helping the donor. For those without other ways to assist, donating COVID-19 convalescent plasma may be a comparatively easy way to help. The desire to repay a debt to medical services is linked to downstream (pay-it-back) reciprocity. Although this can sometimes be a "dark side" of altruism associated with coercion,[50] the association of both gratitude and debt in the same factor with the general goal of

helping others suggests that, here, debt has a positive sense of repayment.

Practically, this suggests that emphasising the gratitude felt at surviving COVID-19 and what that means for the person[51-53] may be useful to recruit and potentially retain COVID-19 convalescent plasma donors. Furthermore, the principle of Voluntary Reciprocal Altruism (VRA) that has been effectively used in organ donation[54,55] could also be useful. A VRA intervention would ask people to consider if they would have a transfusion of COVID-19 convalescent plasma in the future if they needed it and, if so, would they consider donating COVID-19 convalescent plasma. This could be effectively applied in recruiting new donors in a general advertisement and could be adapted slightly for those who have been treated with convalescent plasma and who are now eligible to donate (e.g., "as someone who had convalescent plasma, and is now recovered, would you be willing to help others in a similar position?"). This would also tap into the idea of advantageous inequality aversion that has been highlighted as a motivation for donating blood.[56]

Moral and civic duty was also a significant predictor of intention to donate COVID-19 convalescent plasma. In this predictor, the focus was more on family and friends, rather than others in general, and links to the mechanism of duty and injunctive norms (what people ought to do). There is growing evidence that norms can be used effectively to motivate cooperation and prosocial behaviour.[57] For example, "what do you personally think is the morally right thing to do in this situation?" However, caution is needed here not to trigger guilt but to activate prosocial emotions. One option is to actively encourage the potential donor to think of helping those close to them and rely on models of inclusive fitness and kin selection, which shows that people differentially help family over strangers.[58,59] Interventions here could ask people to consider donating to help a diversity of people from strangers to family, with this triggering kin mechanisms.

Both of these approach motivations, however, were countered by general fears about donating and the donation process. It is not surprising that donation fears were negatively associated with intention to donate. Fears associated with donation, particularly of needles, are well-known barriers to blood donation,[60] while concerns specific to the apheresis procedure, particularly the return of red cells, are known deterrents to donating plasma.[61] The cultural context must be taken into consideration in interpreting these findings as UK residents have not previously been able to routinely donate plasma by apheresis nor donate blood products if they have previously received a transfusion. Recruitment and retention materials could therefore pair VRA messaging with information designed to demystify the apheresis process by explaining what donors can expect when donating COVID-19 convalescent plasma and building self-efficacy to attempt donation.[62] This strategy may be particularly effective for those without prior donation experience who are contacted to donate only on the basis of their positive COVID-19 test result. Several interventions have been developed and trialled to encourage those without experience of donation to attempt donation,[28,29] and adaption of these materials may be useful in encouraging those eligible to donate COVID-19 convalescent plasma.

Although this research drew on the interdisciplinary literature and represents the first attempt to identify beliefs critical to target and encourage non-donors to donate COVID-19 convalescent plasma, this contribution needs to be considered in light of the limitations of our approach. In order to rapidly obtain data, we employed a convenience sampling method, recruiting from the four nations comprising the United Kingdom, and the disproportionate representation of women, the young and those not targeted by recruiting agencies[49] (e.g., Asian, Asian/British) in our sample potentially limits the generalisability of our results. Furthermore, as we were primarily concerned with the general motivational profile of potential donors, the variation in background information about donating COVID-19 convalescent plasma from the different recruitment strategies of the four nations comprising the United Kingdom was not detected in our data. However, it is notable that, despite the different strategies of the four nations, general awareness of the need for convalescent plasma at this time was low.

In addition, given our focus on those who had not donated COVID-19 convalescent plasma and our measurement rather than intervention focus, we assessed only intention rather than behaviour. However, this was carried out knowing that behavioural intentions are strong predictors of actual behaviour.[63] Finally, our data are limited to only reflecting the motivations and barriers for those eligible to donate in the United Kingdom. Identifying how these motives and barriers are present and influence behaviour in other countries and contexts remains critical to ensure a sufficiency of COVID-19 convalescent plasma in the global fight against COVID-19.

The implications of these results for UK policymakers is clear. First, awareness of the importance COVID-19 convalescent plasma as a potential treatment option for COVID-19 needs to be ensured using diverse channels to target (likely) optimal groups—either demographic groups with typically higher (blood donation) retention rates[48] or groups with a statistically greater chance of having sufficient antibody titres.[49] Campaigns should target the motivating power of altruism from adversity, harnessing the gratitude and desire to repay those eligible to donate COVID-19 convalescent plasma feel.[51-53] Interventions derived from VRA may be useful.[54,55] Similarly, targeting perceptions of moral and civic duty through a focus on (potentially) helping family and injunctive norms or what people ought to do may be productive in interventions.[57] Fears associated with donation should be explicitly acknowledged, and strategies that have previously been successfully deployed to recruit whole-blood donors could be adapted and deployed to recruit convalescent plasma donors.[29,30]

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the overall content of the manuscript and, as corresponding author, attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

CONFLICT OF INTEREST

The authors have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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ORIGINAL ARTICLE

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Prevalence of leucocyte antibodies in non-transfused male and female platelet apheresis donors

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Abstract

Objectives: In our study group of Thai PLT apheresis donors, we assessed the prevalence of anti-leucocyte antibodies.

Background: Antibodies against human leucocyte antigens (anti-HLA), neutrophil antigens (anti-HNA), and major histocompatibility complex class I related chain A (anti-MICA) in blood products can lead to transfusion-related acute lung injury (TRALI). To reduce the risk of TRALI, some blood centres are implementing strategies based on screening platelet (PLT) apheresis donors for the presence of anti-leucocyte antibodies.

Methods/Materials: Blood samples were collected from non-transfused individuals, 340 males and 63 females (50 nulliparous and 13 parous). Anti-HLA class I and II and anti-MICA were analysed using the Luminex assay, and anti-HNA-3 was detected using the granulocyte agglutination test.

Results: Anti-HLA was found in 14 of 403 subjects (3.5%). Ten subjects (2.5%) tested positive for HLA class I, 2 (0.5%) for HLA class II, and 2 (0.5%) for both HLA class I and HLA class II. Anti-HLA class I or II were detected in 2 of 13 (15.4%) parous females and only anti-HLA class I was found in 4 (8.0%) nulliparous females. Six of 327 subjects tested (1.8%), all males, were positive for anti-MICA. Anti-HNA-3 was not found in any of the 403 individuals.

Conclusions: Screening for anti-HLA class I and II should be implemented for Thai PLT apheresis donors. Although immunisation against HNA and MICA seems to be a rare event in Thais, further work is necessary to decide whether our PLT apheresis donors should be screened for HNA and MICA antibodies.

KEYWORDS
HLA antibodies, HNA-3, MICA antibodies, platelet apheresis donors

1 | INTRODUCTION

Leucocyte antibodies can cause several complications in blood transfusion such as febrile-non-haemolytic transfusion reaction (FNHTR) and transfusion-related acute lung injury (TRALI).[1] TRALI is a serious hazard, which contributes to morbidity and death.[2] The diagnosis of TRALI is based clinically on the symptomatic expression of hypoxemia with bilateral pulmonary oedema on imaging (e.g., chest radiograph) occurring within 6 h after transfusion in which circulatory overload is excluded.[3] There are many TRALI mediators. A majority of cases are related to alloantibodies against human leucocyte antigens (HLA) class I or class II and against human neutrophil antigens (HNA),[2,4,5] especially antibodies directed against HNA-3a, which are associated with severe and fatal TRALI.[6] Epidemiologic studies in the last two decades have found TRALI to be associated with anti-leucocyte antibodies in donated blood[7-9] and on rare occasions in recipient blood.[10] In

general, anti-HLA and anti-HNA are typically found in female donors with a previous history of pregnancies. The causative antibodies in TRALI cases are frequently associated with transfused blood components containing high plasma volumes, especially fresh-frozen plasma (FFP) and platelet (PLT) products.[11,12] The use of plasma products from males only has reduced the incidence of TRALI cases.[5] However, male donors have also been implicated in antibody-associated TRALI.[13] Around 48% of donors implicated in TRALI cases were male.[13,14] Consequently, these antibodies have been detected also in male donor plasma.[14-19] Besides HLA and HNA, major histocompatibility complex class I related chain A (MICA) antigens can also induce alloantibodies and constitute a possible risk factor related to transfusion reaction as well as TRALI.[20]

In this study, we assess the prevalence and specificities of anti-HLA, anti-HNA and anti-MICA in Thai PLT apheresis donors with focus on their clinical significance in non-transfused PLT apheresis donors.

2 | MATERIALS AND METHODS

2.1 | Subject and sample collection

Four hundred and three consecutive eligible PLT apheresis donors were enrolled via the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. These subjects were all regular donors. After informed consent, all donors completed a standardised questionnaire about transfusion and pregnancy history. Peripheral blood samples were collected during routine apheresis donation. Whole blood was collected into tubes containing ethylene diamine tetraacetic acid. Plasma was stored at −80°C until use. Genomic DNA was extracted from blood samples using the guanidine-HCL method[21] and was then stored at −20°C until used for genotyping. The research protocol was approved by the Ethics Committee of Khon Kaen University (HE621063).

2.2 | HLA antibody testing

Plasma samples were screened for anti-HLA class I and class II using LABScreen Mixed® according to the manufacturer's instructions (One Lambda Inc., Canago Park, CA, USA.). Samples positive according to LABScreen Mixed® were further analysed with LABScreen single antigen beads (SAB) class I and/or class II (One Lambda Inc.). Antibodies with mean fluorescence intensity (MFI) >2000 were assessed for specificity using the HLA-Visual™ software (One Lambda Inc.). The cut-off was used derived from data from patients awaiting solid organ transplantation.

2.3 | MICA antibody testing

Plasma samples were screened for anti-MICA antibodies using LABScreen Mixed® according to the manufacturer's instructions (One Lambda Inc.). The specificity of anti-MICA was determined using SAB (One Lambda Inc.). The MICA-SAB assay could identify anti-MICA 001, 002, 004, 005, 007, 009, 012, 017, 018, 019, 027, 028 and 046 antibodies. Specificity was assigned following the MFI >2000 rule.

2.4 | Detection of anti-HNA-3

Plasma samples were analysed for anti-HNA-3 using the granulocyte agglutination test (GAT) as previously described.[22] Briefly, 2 μl of isolated neutrophil cells (5×10^3 neutrophils/μl) from donors typed for the HNA-3 (two with HNA-3aa and two with HNA-3bb) suspension with autologous plasma was incubated with 6 μl of donor or control serum for 2 h at 37°C under oil on Terasaki plates. The GAT was performed in duplicate. Our GAT testing was run in parallel with anti-HNA-3a and anti-HNA-3b antibody-positive control sera; from the Institute for Clinical Immunology and Transfusion Medicine, Giessen, Germany. Agglutinations were evaluated by inverted microscope (Oympus® IX71, Life Science, NY, USA).

2.5 | HLA and MICA genotyping

DNA samples from PLT apheresis donors with reactive antibodies were genotyped. HLA class I and class II alleles were genotyped by polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) as previously described.[23] Samples exhibiting rare alleles or ambiguous SSP patterns were confirmed by LABType® SSO HLA (One Lambda Inc.). MICA genotype was determined by the PCR-SSP method described previously.[24]

2.6 | Statistical analysis

Fisher's exact tests were performed to assess the difference in HLA antibody frequencies among the male, nulliparous female and parous female groups using the GraphPad Prism software (GraphPad, Inc., La Jolla, CA, USA). Differences between categories were considered statistically significant if the p value was less than 0.05.

3 | RESULTS

3.1 | Frequency of HLA immunisation

In total, 403 healthy, regular PLT apheresis donors were enrolled in this study. Only 63 (15.6%) subjects were female, of whom 13 (3.2%) were parous, but data of number of pregnancies were not available (Table 1). Fourteen donors (3.5%), eight males and six females, were positive for anti-HLA class I and/or II. Anti-HLA class I and class II were detected in seven and two males, respectively, while two (15.4%) parous females showed anti-HLA class I or II. Among

TABLE 1 Prevalence of leucocyte antibodies in female and male PLT donors with and without allo-exposure

Antibodies	Males (n = 340)	Females			Total (n = 403)
		Total females (n = 63)	Nulliparous females[a] (n = 50)	Parous females [b,c] (n = 13)	
HLA class I	6 (1.8%)	4 (6.3%)	4 (8.0%)	0	10 (2.5%)
HLA class II	1 (0.3%)	1 (1.6%)	0	1 (7.7%)	2 (0.5%)
HLA class I + II	1 (0.3%)	1 (1.6%)	0	1 (7.7%)	2 (0.5%)
HNA-3	0	0	0	0	0
Total	8 (2.4%)	6 (9.5%)	4 (8.0%)	2 (15.4%)	14 (3.5%)

[a]p = 0.031 males vs. nulliparous females.
[b]p = 0.005 males vs. parous females.
[c]p = 0.419 nulliparous females vs. parous females.

nulliparous females, four (8.0%) subjects exhibited only anti-HLA class I (Table 1).

The frequency of HLA class I and/or HLA class II antibodies was low among male donors, and significantly lower than among females (male and nulliparous females, 2.4 vs 8.0%, p = 0.031; male and parous females, 2.4 vs 15.4%, p = 0.005). Although the frequencies of HLA antibodies seemed to be high in parous females compared with nulliparous females, this difference was not significant (15.4 vs 8.0%; p = 0.419) (Table 1).

3.2 | Specificity of HLA antibodies

The SAB assay was performed to identify the specificity of antibodies. Table 2 shows the frequencies of anti-HLA class I (A, B and Cw) and anti-HLA class II (DR and DQ) antibodies. For anti-HLA-A, the highest frequency was A1 (0.5%). For anti-HLA-B, the common antibodies were B13, B27, B37, B44, B52 and B77. For anti-HLA-Cw, antibodies to Cw5, Cw12 and Cw15 were found in this study. Antibodies to HLA-DR and HLA-DQ antigens were also found but not to HLA-DP. Six subjects were followed up on three different occasions over a 2-year period and the same specificity of antibodies was found.

The corresponding specificities of antibodies with MFI >3000 are shown in Figure 1. Of these, 23 of 37 (62.2%) anti-HLA class I and 7 of 12 (58.3%) anti-HLA class II had an MFI >5000.

TABLE 2 Specificity of anti-HLA (MFI > 2000) in PLT apheresis donors

Anti-HLA class I						Anti-HLA class II		
HLA	n	%	HLA	n	%	HLA	n	%
A1	2	0.5	B51	2	0.5	DR1	1	0.2
A2	1	0.2	B52	3	0.7	DR103	1	0.2
A24	1	0.2	B53	1	0.2	DR4	1	0.2
A30	1	0.2	B54	1	0.2	DR7	1	0.2
A31	1	0.2	B55	2	0.5	DR9	1	0.2
A32	1	0.2	B57	1	0.2	DR10	1	0.2
A68	1	0.2	B58	1	0.2	DR12	1	0.2
			B60	2	0.5	DR51	1	0.2
B7	2	0.5	B61	2	0.5	DR52	1	0.2
B8	2	0.5	B62	2	0.5			
B13	5	1.2	B63	2	0.5	DQ4	1	0.2
B18	1	0.2	B67	2	0.5	DQ7	2	0.5
B27	3	0.7	B71	2	0.5	DQ8	1	0.2
B35	1	0.2	B72	2	0.5	DQ9	2	0.5
B37	3	0.7	B75	2	0.5			
B38	2	0.5	B76	2	0.5			
B39	2	0.5	B77	3	0.7			
B41	2	0.5	B78	1	0.2			
B44	3	0.7						
B45	2	0.5	Cw5	1	0.2			
B48	1	0.2	Cw12	1	0.2			
B50	2	0.5	Cw15	1	0.2			

3.3 | Frequency of MICA immunisation in the study

We analysed data from 327 PLT apheresis donors from whom plasma samples were available for detection of MICA antibodies. Six of these 327 sera (1.8%) were positive for anti-MICA antibodies (Table 3). All MICA antibody-positive samples were from male donors. Only one of the six donors also demonstrated anti-HLA-A24. Figure 2 shows the specificities of MICA antibodies with an MFI >2000 according to the SAB assay. The most frequently found antibody was anti-MICA 002 (n = 5:1.5%), followed by antibodies to MICA 009 and MICA 019, which occurred in 4 (1.2%) sera.

3.4 | HNA-3 antibody testing

Among 403 donors, as expected, we identified none with anti-HNA-3. Neutrophil-specific antibodies are rare in blood donors without allo-exposure.

4 | DISCUSSION

To reduce the risk of TRALI, most blood centres only use plasma products from males or nulliparous females. For PLT apheresis collection,

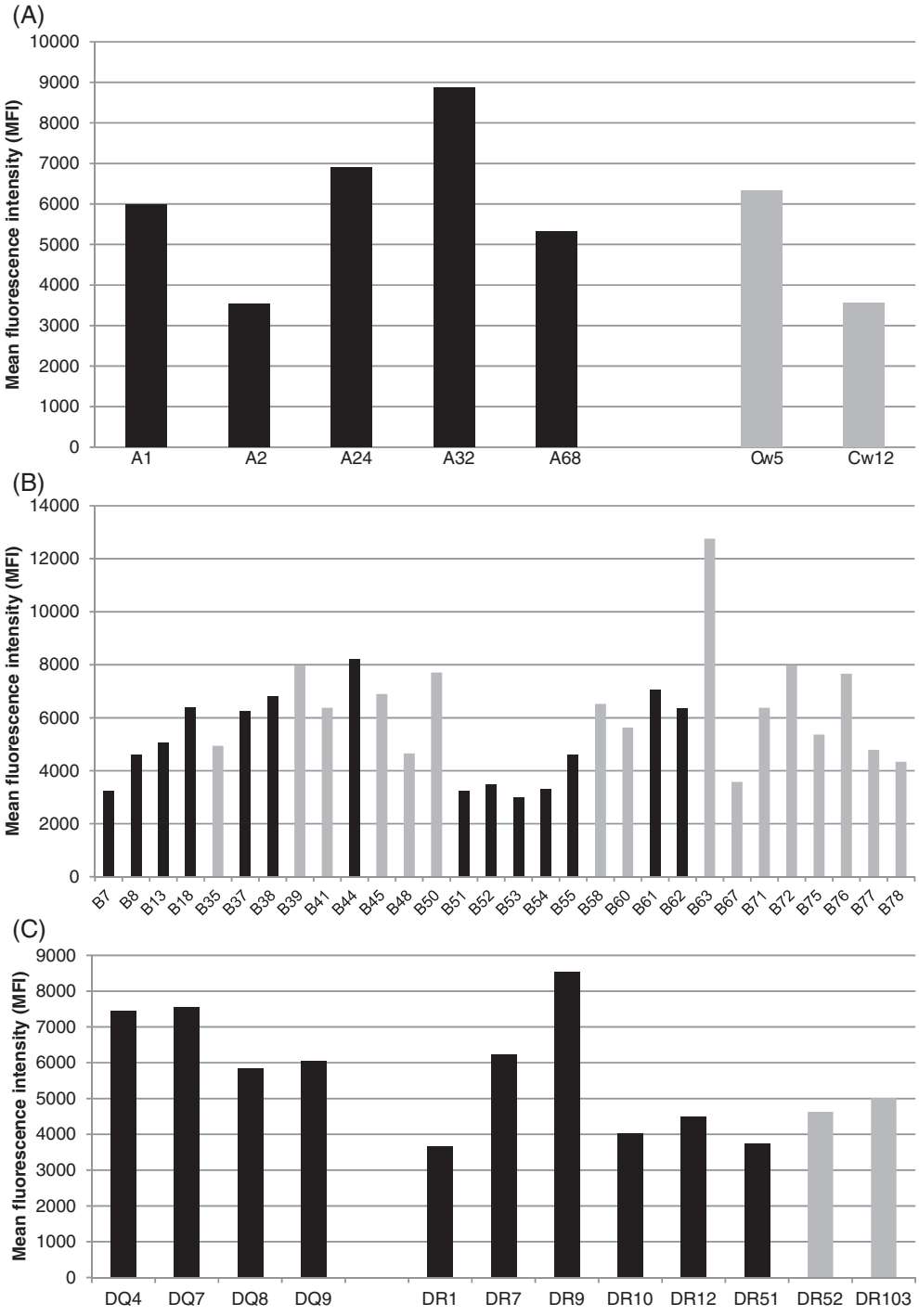

FIGURE 1 Specificities of antibodies in PLT pheresis donors with MFI > 3000 in the study. (A) anti-HLA-A and anti-HLA-Cw (B) anti-HLA-B and (C) anti-HLA class II. The black columns indicate specificities detected in donors associated with TRALI cases in the literature.[9,29,34,35] The corresponding specificities are given only the highest MFI

Donor	Sex	MICA antibody reactivity	HLA antibody reactivity
1	Male	MICA 002, 004, 009, 019	Negative
2	Male	MICA 009, 019	Negative
3	Male	MICA 002, 004, 009, 017, 019	Negative
4	Male	MICA 002	Negative
5	Male	MICA 002	HLA-A24
6	Male	MICA 002, 004, 009, 012, 017, 018, 019	Negative

TABLE 3 MICA antibody specificities (assayed in 327 subjects among whom 6 were positive)

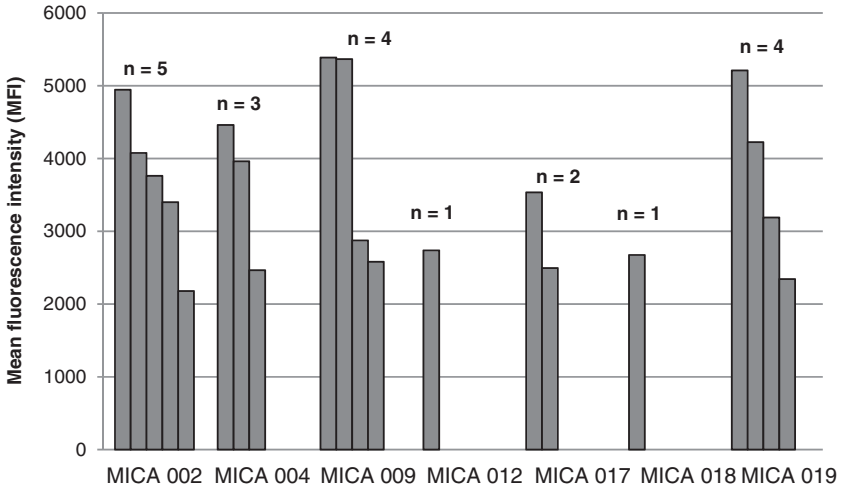

FIGURE 2 Specificities of MICA antibodies in six male PLT donors (See also Table 3). Anti-MICA specificities of MFI > 2000 are shown. Antibodies against only seven alleles of MICA were detected in this study

many centres screen for anti-leucocyte antibodies and exclude donors with these antibodies.[25-27] In our blood centre, PLT products originate mostly from male donors without a history of transfusion (84.4%): only 3.2% are produced from parous females.

In our sample of donors, HLA class I antibodies alone were found in 2.5% of subjects, HLA class II in 0.5%, and HLA class I together with HLA class II antibodies were in 0.5% of subjects. This is in accordance with the result of Reil and colleagues,[28] who reported that HLA class I antibodies were the most frequently detected leucocyte antibodies among blood donors. The most common antibody in this study was B13 (1.2%), which has been implicated with TRALI cases.[29] Of note, this antibody recognises HLA antigens that are common in ethnic Northeast Thais.[23] This observation may indicate that patients with HLA B13 are at a higher risk of developing TRALI.

Among 340 non-transfused male PLT apheresis donors, we detected anti-HLA in only eight individuals (2.4%). This was a significantly lower proportion than in parous females (15.4%). Our result is in accordance with the studies of Xia and colleagues[30] and others.[31,32] Clippel and colleagues[33] also showed that a former pregnancy constituted a major risk factor for the development of HLA antibodies. In our study, nulliparous females also had a higher prevalence of detectable antibodies than males. A similar finding was reported by Nguyen and colleagues[14] but Middelburg and colleagues[17] and Xia and colleagues[30] did not find such a difference. The discrepancies between studies might be due to the presence of multi-specific antibodies in nulliparous females. We found that the prevalence of HLA antibodies in parous females was higher than among nulliparous females (15.4% vs. 8.0%); however, the difference was not statistically significant. This may be due to the relatively small parous female study group and the lack of information concerning number and outcomes of pregnancies in this group. These factors may stimulate production of HLA antibodies.[17]

Hashimoto and colleagues[29] noted that an MFI >3000 was the lowest fluorescence intensity of donor antibodies implicated in TRALI. In our study, 51.4% of HLA class I antibodies and 83.3% of HLA class II antibodies exhibited MFI values >3000, levels comparable with the TRALI cases in the literature.[9,29,34,35] Reil and colleagues[28] also demonstrated that HLA class II antibodies more frequently triggered TRALI than did HLA class I antibodies. It is important to note that antibodies against A2, A24 and DR12 found in this study recognise high-frequency HLA antigens in ethnic Northeast Thais.[23] Antibodies to HLA-A2 have been shown to be involved in severe TRALI cases.[1] In our study, anti-HLA-A2 was only found in one individual out of 403 tested (0.2%). It is of interest that anti-HLA-A2 was the most common antibody identified in kidney transplant patients with antibody-mediated rejection (unpublished observations). The importance of this antibody in the development of TRALI and the significance of our finding needs to be further investigated. Furthermore, about 48.6% of HLA class I and 16.7% of HLA class II antibody specificities (MFI >3000) detected in this study have not been reported in donors implicated in TRALI cases so far. Of these, 66.7% of anti-HLA class I and 50.0% of anti-HLA class II specificities had an MFI >5000 and most antibodies recognised HLA antigens of low frequency in ethnic Northeast Thais.[23] Thus, the role of these antibodies in pathogenesis of TRALI remains to be explored.

We were surprised to identify anti-MICA antibodies in 6 of 327 sera tested. The following specificities were confirmed: MICA 002, 004, 009, 012, 017, 018 and 019. MICA 002 and MICA 019 recognised MICA antigens present at high frequencies in ethnic Northeast Thais.[24] Anti-MICA antibodies are implicated in allograft rejection in solid organ transplantation.[36] However, there has been little investigation of any association between anti-MICA antibodies and transfusion reactions as well as TRALI.

None of our 403 PLT apheresis donors were positive for neutrophil antibodies (HNA-3). Similarly, Reil and colleagues[28] did not find anti-neutrophil-specific antibodies in male blood donors. This antibody is rarely found, even in pregnant women (in 1/1000 or less).[37,38] However, Nguyen and colleagues[14] recently reported that neutrophil antibodies in male blood donors could trigger TRALI.

The screening and identification of HNA alloantibodies have been not quite "trivial"; requires different methodologies that need certain expertise. However, GAT remains the best technique for detection of anti-HNA-3a and anti-HNA-3b [39] and only this method was used in this study. Thus, we only tested for HNA-3 antibodies. However, other HNA antibodies could trigger neutrophil aggregation and can be easily identified by the GAT assay.

Our data demonstrate that 3.5% and 1.8% of non-transfused PLT apheresis donors had anti-HLA and anti-MICA, respectively, with MFI >2000. Our retrospective study did not find that these antibody-positive donors were associated with TRALI. Indeed, the question why leucocyte antibodies trigger TRALI in some patients but not in others remains to be elucidated. Several factors are possible, such as cognate antigen density, antibody titre, affinity/avidity and the presence of non-immunological factors in the patients.[37] Thus, the finding of these antibodies in our study group raises the question whether they are clinically significant or just noise?

The reasons for the presence of specific leucocyte antibodies of the IgG class in males and females with no allo-exposure are unknown. A possible explanation is that these could be naturally occurring alloantibodies as a consequence of immunisation to microbial antigens or vaccination.[14,19] In addition, these donors were genotyped for their HLA and MICA antigens and antigens corresponding with their antibodies were not detected. Thus, we could exclude autoantibodies in our study.

5 | CONCLUSIONS

PLT apheresis donors with no previous allo-exposure had anti-leucocyte antibodies that could potentially cause TRALI. HLA antibodies occurred at low prevalence in males and in females without a history of pregnancy. Although immunisation against HNA and MICA seems to be a rare event in Thailand, further investigation is necessary to decide whether we should screen for HNA and MICA antibodies in our blood donors.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

P.S. designed the study, performed the experiments, analysed the data and wrote this manuscript; Y.S. and S.C. performed the experiments; C.L. edited the manuscript; and A.V.R. designed the study, analysed the data and edited the manuscript.

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ORIGINAL ARTICLE

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Risk of hepatitis-E virus infections among blood donors in a regional blood transfusion centre in western India

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Abstract

Background: Hepatitis-E virus (HEV) is an emerging infectious threat to blood safety. The enormity of the transmission of HEV and its clinical consequence are issues currently under debate. This study aimed to evaluate the prevalence of HEV-RNA in blood donors in western India.

Materials and Methods: We screened 13 050 blood donors for HEV using HEV-RNA screening of 10 mini-pools using RealStar HEV RT-PCR Kit (95% limit of detection (LOD): 4.7 IU/ml). Furthermore, all HEV-RNA-positive donors were investigated for the presence of IgM/IgG antibody along with liver function tests.

Results: Of the 13 050 blood donations, 7 (0.53%) were found to be HEV-RNA positive, and the prevalence of HEV nucleic acid testing yield cases among blood donors was 1 in 1864. All seven HEV-RNA-positive samples were tested with anti-HEV IgM and anti-HEV IgG antibodies; this resulted in two (28.5%) positive anti-HEV IgM and two (28.5%) positive anti-HEV IgG antibodies. Hepatic activity was measured, with two of seven HEV-RNA-positive donors demonstrating abnormal serum glutamic oxaloacetic transaminase (SGOT) andserum glutamic pyruvic transaminase (SGPT). Two HEV-RNA-positive blood donors who had abnormal SGOT and SGPT were found to have a high HEV viral load. Furthermore, we were able to follow up two HEV-RNA donors, and both were HEV-RNA positive and had anti-HEV IgM and anti-HEV IgG antibodies; moreover, their liver function tests were also abnormal. One of the HEV-RNA donors with high viral load did show hepatitis-E-like virus on electron microscopy.

Conclusion: Our studies indicate that there is a significant risk of blood-borne transmission of HEV. This finding may help to provide a direction towards the safety of blood transfusions in clinical settings in countries like India, which fall under the endemic category for HEV infection.

KEYWORDS
RT-PCR, blood donors, hepatitis-E virus, HEV-RNA

1 | INTRODUCTION

The global scenario of hepatitis-E virus (HEV) is very scary; based on the Global Burden of Disease study, it was found that as many as 20.1 million people were infected with HEV in 2005 in nine regions.[1] This accounted for 71% of the world's population, with 3.4 million symptomatic cases, 70 000 deaths and 3000 stillbirths.[2] The global burden of HEV infection is greater due to sporadically transmitted HEV cases

than because of cases due to epidemic HEV. The safety of blood and blood products remains an important public health concern due to the continual emergence of infective viruses. In this context, HEV is an emerging infectious threat to blood safety; since 2004, HEV has gained importance in public health as a transfusion-transmitted infectious (TT-HEV) agent. In the recent decade, there have been several reports of the high incidence of TT-HEV infection.[3,4] HEV-infected immunocompromised patients develop chronic hepatitis-E in approximately 60% of cases.[5]

In India, HEV was first identified during an epidemic of hepatitis, which occurred in Kashmir Valley in 1978. There are documented cases from India, Japan, the United Kingdom, Saudi Arabia and France on the transmissibility of HEV via blood transfusions in patients.[6-12] HEV has also been detected in human blood products.[13-17] Blood donors can be silently infected with HEV, as indicated by plasma pools testing positive for HEV-RNA[13,16] and by a high prevalence of antibodies to HEV among blood donors. HEV has an effect on adolescent to middle-aged adults and causes elevated mortality in pregnant women, 20%–30%, in contrast with 0.2%–1% in general population.[18] HEV was detected in 1 of 815 blood donations in Germany[19] and is 100 times more prevalent compared with human immunodeficiency virus (HIV), hepatitis-B virus (HCV) and hepatitis-C virus (HBV) combined.

Recent studies from India reporting the seroprevalence for HEV, which ranges from 17% to 23% among healthy young adults (18--40 years), indicate the inclination of the virus towards this population; hence, HEV could be a subject of worry in the transfusion medicine community.[20,21] Furthermore, current overall worldwide data indicate that asymptomatic HEV infection is very common among adults who are potential blood donors.[22] Moreover, the occurrence of blood-borne transmission is supported by the demonstration of HEV viraemia among healthy blood donors in several countries.[23] Furthermore, knowing that there is no definitive management for HEV-induced hepatitis, selective screening should be performed in blood products for the high-risk patients in endemic areas.

Therefore, to investigate the infection pressure of HEV in western India, we assessed the presence of HEV-RNA in a large number of recent blood donations, collected throughout the city of Surat. The detection of HEV-RNA also has the advantage of a lower likelihood of being affected by genomic variations in the virus.[24] Data obtained were used to estimate the detection rate of HEV infections in blood donors and the risk of HEV transmission by blood transfusion.

2 | MATERIALS AND METHODS

The study was designed to evaluate the incidence and prevalence of HEV-RNA in the blood donors of Surat Raktadan Kendra and Research Centre, Surat, Gujarat. Blood samples were collected from 13 050 healthy volunteers who participated in the blood donation camps organised by us at various locations in Surat during January 2017 and August 2017. Informed consent was obtained prior to collection. This project has been approved by the Institutional Ethics Committee (IEC) of Surat Raktadan Kendra & Research Centre.

2.1 | Plasma samples

Plasma was separated by centrifugation at room temperature and was frozen and stored at −40°C and thawed once before use.

2.2 | Serological assay

Samples from the donated blood units have to undergo routine HIV, HBV and HCV screening using a third-generation assay: for HCV, Abs with SD HCV ELISA 3.0 test system (SD Bio standard diagnosis Pvt. Ltd, Gurgaon, India); for HIV, HIV-1/2 Abs with Microlisa (J. Mitra & Co. Pvt. Ltd, New Delhi, India); and for HBV, Ags with SD HIV ELISA 3.0 test system (SD Boi standard diagnosis Pvt. Ltd, Gurgaon, India).

2.3 | Pooling of plasma samples for HEV viral nucleic acid test

Pooling of samples was performed using separate aerosol tips (certified free from RNase, DNase and pyrogen) under the laminar hood using aseptic precautions. In the pool containing 10 samples (10-MP), each donation represented 100 μl for a total of 1000 μl. If any 10-MP was positive, further pools were subdivided into two pools, that is, 5-MP-A and 5-MP-B, and in the pool of five samples, each donation represented 200 μl. Furthermore, if any 5-MP was positive, the run was repeated individually (ID) for the plasma sample.

2.4 | Isolation of viral nucleic acid

Viral nucleic acid of HEV-RNA was extracted from individual (ID) or pooled (10-MP) plasma samples using the Chemagic Prepito-D automated extractor (Perkin Elmer), in combination with reagents/buffers of the Prepito Viral DNA/RNA Kit as described in the manufacturer's protocol, and was stored at −80°C until further analysis.

2.5 | Real-time PCR amplification of HEV viral nucleic acid

HEV-RNA was amplified by kits of RealStar HEV RT-polymerase chain reaction (PCR) Kit 1.0 (95% LOD: 4.7 IU/ml) (Altona Diagnostics, Hamburg, Germany) as described in the manufacturer's protocol. The PCR was performed on ABI Prism 7500 Real Time PCR System (Life Technologies).

2.6 | Hepatitis-E virus quantisation

HEV cDNA quantisation was performed using the RealStar HEV RT-PCR Kit 2.0 (Altona Diagnostics), which is an in vitro diagnostic test based on real-time PCR technology for the detection and quantification of HEV-

specific RNA. HEV quantification assay was carried out as per the manufacturer's protocol. Results were measured in IU/ml.

2.7 | Detection of the amplified products

Amplified products of RealStar HEV RT-PCR Kit 1.0 (Altona Diagnostics) were detected by fluorescence, and data were analysed using the 7500 SDS software, version 2.3. Fluorescence from the specific amplification of viral target HEV-RNA was captured in the FAM channel, whereas a signal from IC amplification was read in the JOE channel. During data analysis, ROX was switched on only for the HEV test. An analysis was performed using Auto-Threshold and Auto-Baseline by default.

2.8 | Serological testing (HEV IgM/IgG)

All HEV-RNA-positive samples were investigated for the presence of HEV-specific anti-HEV IgM by anti-HEV IgM and anti-HEV IgG antibodies using an anti-HEV IgG enzyme immunoassay of DIA. PRO, Diagnostic Bioprobes Srl, Milano, Italy, as described in the manufacturer's protocol.

2.9 | Liver function tests: Measurements of liver enzyme activities

The entire samples that were positive for HEV-RNA were further processed for the measurement of serum alanine aminotransferase (ALT/SGPT) levels and aspartate aminotransferase (AST/SGOT) levels. ALT and AST assays were performed using the respective test kits ALTP2: ACN 20140 and ASTP2: ACN 20230, cobas, Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, at an NABL-accredited clinical laboratory, Metropolis Healthcare Ltd., Surat, as described in the manufacturer's protocol. An in-vitro test was used for the quantitative determination of ALT and AST in human serum and plasma on Roche cobas c systems.

2.10 | Electron microscopy of HEV-RNA positive samples

For further confirmation of the presence of virus particles, we sent all seven HEV-RNA-positive samples to Dr Atanu Basu, National Institute of Virology, Pune, India, for electron microscopy.

3 | RESULTS

3.1 | Prevalence rate of HEV-RNA

Of the 13 050 blood donations (seronegative of HIV/HBV/HCV), 7 (0.53%) were found to be HEV-RNA NAT-positive; the prevalence of HEV NAT yield cases among our routine donors was 1 in 1864 donations tested (Table 1). The HEV RT-PCR cycle threshold (Ct) value range was 13–35. Among the seven HEV-RNA-positive blood donors, all were males in the age group of 29–44 years; furthermore, two (28.5%) donors were AB positive, two (28.5%) were O positive, and two (28.5%) were B positive, whereas one (14.2%) donor was AB negative. Details of the HEV-RNA-positive sample are given in Table 1.

3.2 | Characteristics and relationship between HEV-RNA-positive donors and viral load levels and liver function of blood donors and anti-HEV IgM/IgG antibody

Because the HEV infection can perturb hepatic function, hepatic activity was also measured (Table 2), and two blood donors of the seven HEV-RNA-positive blood donors demonstrated abnormal SGOT and SGPT, whereas the remaining five HEV-RNA-positive tested donors showed normal serum ALT and AST levels (Table 2). Furthermore, these two HEV-RNA-positive blood donors who had abnormal SGOT and SGPT demonstrated high HEV viral load (Table 3).

In this study, 13 050 blood donors were screened for the presence of HEV-RNA, and all 7 HEV-RNA-positive samples were further tested with anti-HEV IgM antibody and anti-HEV IgG antibody as anti-HEV IgM is the serologic marker of choice for the diagnosis of acute HEV infection. This resulted in two cases were positive for IgM anti-HEV and two (28.5%) were positive for IgG anti-HEV antibody (Table 3).

3.3 | Electron microscopy of HEV-RNA-positive samples

Of 13 050 blood units tested, 7 blood samples were positive for HEV-RNA. Six of the seven samples that had a very high Ct value (low viral

TABLE 1 HEV NAT yield rate among blood donors and HEV-RNA positivity details

HEV NAT yield rate				
Total unit tested	**Total HEV-RNA positives**			**HEV NAT yield rate**
13 050	07			1 in 1864
HEV-RNA positivity details				
Sr. no.	**Age/ gender**	**Blood group**	**HEV-RT-PCR Ct value**	**IC Ct value**
HEV-1	44/M	AB^{+ve}	32.78	31.14
HEV-2	35/M	AB^{+ve}	25.98	39.65
HEV-3	30/M	B^{+ve}	19.13	0.00
HEV-4	29/M	O^{+ve}	35.96	31.95
HEV-5	36/M	AB^{-ve}	29.04	30.97
HEV-6	30/M	O^{+ve}	27.01	32.43
HEV-7	31/M	B^{+ve}	13.37	0.00

Abbreviations: *Ct*., cycle threshold; IC, internal control.

TABLE 2 Liver function tests of HEV-RNA-positive blood donors

Sr no.	SGOT (AST) (BRI: 19–48 U/L)	SGPT (ALT) (BRI: 16–63 U/L)	Bilirubin total (BRI: 0.3–1.2 U/L)
HEV-1	21	11	0.43
HEV-2	25	16	0.38
HEV-3	71	96	1.35
HEV-4	29	34	0.72
HEV-5	27	46	0.41
HEV-6	27	20	0.21
HEV-7	136	417	1.38

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

TABLE 3 The relationship between HEV-RNA-positive donors and anti-HEV IgM/IgG antibody and viral load levels and liver function of blood donors

Sr. no.	Age/gender	HEV Ct	HEV viral load (IU/ul)	Anti HEV-IgM (negative: <1.0; equivocal: 1.0–1.2; positive: >1.2)	Anti HEV-IgG (negative: <0.9; equivocal: 0.9–1.1; positive: >1.1)	SGOT	SGPT
HEV-1	44/M	32.78	4.8×10^4	0.32 (–ve)	0.08 (–ve)	Normal	Normal
HEV-2	35/M	25.98	4.9×10^4	0.36 (–ve)	0.17 (–ve)	Normal	Normal
HEV-3	30/M	19.13	2.5×10^6	9.62 (+ve)	1.69 (+ve)	Abnormal	Abnormal
HEV-4	29/M	35.96	2.1×10^4	0.21 (–ve)	0.32 (–ve)	Normal	Normal
HEV-5	36/M	29.04	4.6×10^3	0.19 (–ve)	0.11 (–ve)	Normal	Normal
HEV-6	30/M	27.01	1.5×10^4	0.23 (–ve)	0.19 (–ve)	Normal	Normal
HEV-7	31/M	13.37	2.2×10^4	9.6 (+ve)	7.62 (+ve)	Abnormal	Abnormal

load) did not show the virus particle on transmission electron microscopy (EM). However, one of the samples with a Ct value of 13.37 (high viral load) (Table 3) did show hepatitis-E-like virus on EM (Figure 1).

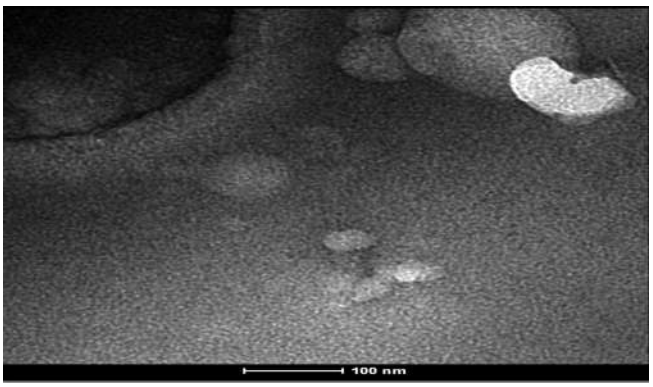

FIGURE 1 Electron microscopy (EM) of HEV-RNA-positive samples (image of a morphologically consistent HEV). Courtesy by: Dr Atanu Basu, National Institute of Virology, Pune, India

3.4 | Follow-up of HEV-RNA-positive blood donors

For the blood donors who were considered HEV-RNA positive, follow-up samples were tested for HEV-RNA and HEV IgG and IgM antibodies and serum ALT (ALT/SGPT) and AST (AST/SGOT) levels to find if anti-HEV seroconversion was detected in a follow-up sample. Follow-up blood samples were collected at 3 months, and of the seven HEV-RNA-positive blood donors, we were able to follow up two donors (HEV-4 and HEV-7). Both were HEV-RNA positive and had anti-HEV IgM and anti-HEV IgG antibodies. Furthermore, their liver function tests were also abnormal (Table 4). Follow-up blood donors demonstrated evidence of infection.

4 | DISCUSSION

There are published reports of HEV infections by contaminated blood products[25] and the detection of HEV in plasma fractionation pools;[26] moreover, samples from blood donors[27-30] suggest that the transfusion transmission of HEV is probably not uncommon. The threat of HEV is currently being experienced by blood banks; moreover, the occurrence of asymptomatic infection in blood donors raised many questions of blood safety regarding the transmission of HEV.[31] Although the disease is usually self-limiting, acute HEV may progress to a fulminant fatal form.

Despite the elevated prevalence and high rate of transmission by HEV-infected blood or blood products; the limited range of HEV-

TABLE 4 Follow-up study of HEV-RNA-positive blood donors

Sr. no.	HEV-RNA	IgM	IgG	SGOT	SGPT
HEV-4	Positive	Positive	Positive	Abnormal	Normal
HEV-7	Positive	Positive	Positive	Abnormal	Abnormal

specific, efficient antiviral therapy; and no vaccines to prevent HEV diseases in those who are still healthy, routine screening for HEV is presently not performed in most blood banks. HEV screening is implemented only in developed countries like the United Kingdom, Ireland, Netherlands and Japan.[32] Furthermore, studies performed worldwide indicate a high HEV seroprevalence (1%–52%) in blood donors.[33] Therefore, the risk and importance of TT-HEV infections by contaminated blood products is currently a debatable discussed topic in transfusion medicine.

The subclinical nature of HEV infection among blood donors has huge implications for blood banks as a donor may provide no history of jaundice despite being anti-HEV antibody positive. Anti-HEV IgM antibody indicates acute infection, and the anti-HEV IgG titre may remain detectable for up to 15 years.[34] Anti-HEV antibodies serve as a surrogate marker for HEV viraemia. However, in the existing studies, viraemia was equally common in the seropositive and seronegative HEV units;[35] besides that, only a minority of HEV viraemic blood units were seropositive. Therefore, tests for these HEV antibodies are unlikely to be useful for screening donated blood. Moreover, it has previously been reported that HEV-PCR might be a better indicator than ELISA of acute HEV infection,[36] and the identification of HEV-RNA also has the advantage of a lower likelihood of being affected by genomic variations of the virus.[24,35]

Furthermore, in the course of a viral infection, viral proteins are released in the host body fluids, and a test for the detection of the HEV antigen in body fluids has been developed and provides another best option for the detection of HEV viraemia. The test appears to be in good strong harmony with the detection of HEV-RNA using nucleic acid testing (NAT). Therefore, molecular NAT for HEV-RNA is critically needed. NAT is currently used in conjunction with serological test, and it has reduced the risk of HIV-1, HBV and HCV where it has been implemented.[37-42] In addition, NAT is also useful for determining the incidence of active infection by these viruses in blood donor populations.[37]

In India, only a few studies have analysed the performance of RNA assays with HEV; in the current study, HEV-RNA was detected in 7 of 13 050 blood donors (0.53%). Furthermore, the prevalence of HEV NAT yield cases among our routine blood donors was 1 in 1864 donations tested. Countrywide estimations of positive HEV-RNA in blood donations were 1 in 1430 in China,[15] 1 in 3090 in the Netherlands,[17] 1 in 3179 in Germany[43] and 1 in 7040 in the United Kingdom.[16] Furthermore, Arankalle from Pune, India, has shown that 1.5% (3/200) of blood donors were positive for HEV-RNA and has suggested the possibility of transmission by transfusion.[44] All the above studies suggested that HEV-RNA is present in the healthy blood donors, and there is always potential risk for the transmission of HEV through the blood and blood products. Boxall et al from the United Kingdom have recently shown the transmission of HEV from a donor to recipient, which was proven by serology and molecular methods.[10]

In the present study and previously published studies, the overall prevalence of HEV-RNA viraemia among blood donors was relatively low.[16,17,43] However, such HEV-RNA screening may still be important and necessary for blood units destined for administration to recipients in whom HEV infection may carry serious consequences. This includes individuals with inherited or acquired immunodeficiency disorders, including those with organ transplantation; those who are at risk of developing chronic HEV infection and consequently chronic liver disease; and those with an underlying chronic liver disease who are at risk of acute-on-chronic liver failure.[34] Furthermore, there are currently no specific antiviral therapies available for acute or chronic HEV infections, and there is no approved vaccine for the protection of HEV. Thus, preventive measures are needed for the HEV RNA testing of blood donations.

Furthermore, all HEV-RNA positive samples were tested with anti-HEV IgM antibody and anti-HEV IgG antibody. This resulted only in two (28.5%) positive anti-HEV IgM and two (28.5%) positive anti-HEV IgG cases (Table 3). It may be possibility is, rest of five HEV-RNA donors were in window period, therefore detecting the virus in the pre-seroconversion, window period thereby providing higher sensitivity as compared to serological tests.[45] Another possibility is that the amounts of antibodies in those samples were lower than the detection level of the kit. Viral RNA can be detected for 6 months before seroconversion;[46] because of this, a viral RNA method to detect HEV is recommended.[47]

In the present study, as anti-HEV IgM/IgG was tested only in HEV-RNA-positive donors, of seven HEV-RNA-positive donors, only two donors were positive for anti-HEV IgM/IgG; this is might be due to the early stage of infection (window period), where plasma HEV-RNA viremia peaks before IgM and IgG antibodies have reached a detectable level. Thus, blood donors can be HEV-RNA positive even though they are anti-HEV IgM/IgG negative, and the probability of missing HEV-RNA-positive samples cannot be ruled out if we only depend on antibody detection methods.

Since the innovation of the ABO blood group, there has been an ongoing interest in the potential role of blood groups in infectious disease.[48] However, there was no major variation in HEV-RNA prevalence in blood group screening, with the highest and equal prevalence in the AB, O and B blood groups (28.5%) and the lowest in the AB^{-ve} blood group (14.2%), and none of the HEV-RNA positive cases were found in the A and B blood groups. However, this a small study, and this variation in HEV-RNA positivity in relation to the blood groups may suggest that the conclusion cannot be decided as only a small number of samples is tested; testing of a huge number of blood donations is probably required.

HEV infection could be the most important trigger of liver decompensation, and the evaluation of hepatic activity was also a measure of all HEV-RNA positive donors. Of seven HEV-RNA-positive blood donors, two demonstrated abnormal SGOT and SGPT and had high viral load, whereas the remaining five HEV-RNA-positive donors had normalised SGOT and SGPT. In a study of the Chinese population, among four HEV-RNA-positive donors, only two had elevated ALT levels.[49] Similar results have also been reported from Japan.[30,50] In the five HEV-RNA-positive donors with normalised SGOT and SGPT, HEV viraemia was observed to last beyond normalisation of transaminases in a number of patients, and this suggest that liver injury is independent of viral loads.[51]

Of these seven HEV-RNA-positive blood donors, we were able to follow up two donors (HEV-4 and HEV-7), and both were HEV-RNA positive. Furthermore, during follow-up, one of the donors (HEV-4) became anti-HEV IgM and anti-HEV IgG antibody positive, and his SGOT tests were also abnormal. It might be due to the window period; during the window period, a donor can have HEV and will be positive for HEV-RNA and be infectious, but still, the tests for HEV IgM and HEV IgG antibodies will be negative. The HEV window period is usually 4–10 weeks from the time of exposure; after 6 months, most people will have developed enough antibodies. In rare cases, however, antibodies can take up to 9 months to develop.[52] Therefore antibody tests are unlikely to be useful for HEV screening in donated blood, but HEV-RNA screening might be a better indicator than ELISA of HEV infection.

Furthermore, none of the HEV-RNA-positive blood donors had any signs and symptoms at the time of blood donation, as well as during follow-up. The HEV RT-PCR Ct value range was 13–35. Of seven HEV-RNA-positive blood donors, only two donors had a Ct value ≤20, and five donors' Ct values were ≥27. Present data suggested the presence of silent HEV infection at the time of donation because of the confirmation of viremia in 7 of 13 050 blood donors by RT-PCR.[53]

EM is considered an old technique; however, it is still at the forefront of both clinical viral diagnoses and viral ultrastructure and pathogenesis studies. In the diagnostic setting, it is particularly valuable in the surveillance of emerging diseases and potential bioterrorism viruses.[54] In the present study, six of seven HEV-RNA-positive samples that had a very high Ct value (low viral load) did not show the virus particle on transmission EM. However, one of the samples with a Ct value of 13.37 (high viral load) did show hepatitis-E-like virus on EM. One of the biggest advantages of using EM for viral detection is that it does not require organism-specific reagents for recognising the pathogenic agent.[55] Other tests involving molecular and serological methods require that a specific probe be available for virus identification.[51]

India falls under the endemic category for HEV infection, and routine screening for this virus is currently not performed in Indian blood banks. These seven of 13 050 (0.53%) blood donors demonstrate a meaningful HEV-RNA positive rate, furthermore, the present study suggest a cost-effective mini-pool HEV-RNA screening approach to reduce transmission risk for the benefit of blood safety and public health, and it will be worthwhile in HEV-endemic regions in India. However, further studies are required to assess the amount of transmission and the clinical relevance of transfusion-associated hepatitis-E infection.

5 | CONCLUSION

Hepatitis-E is being considered a re-emerging infectious disease across the world. HEV transmission by blood transfusion may have detrimental outcomes for the recipients, considering their immunosuppressive status, underlying disease, pregnant women or other circumstances requiring blood transfusion. In the present study, we find the relative risk (1 in 1864) of transmission of HEV through blood products. Furthermore, based on our finding and earlier reports, it appears that screening blood donors for HEV-RNA will be worthwhile because of the absence of definitive treatment and vaccination and because a country like India falls under the endemic category for HEV infection.

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AUTHOR CONTRIBUTIONS

Dr Atanu Basu carried out the electron microscopy experiment for further confirmation of the presence of virus particles in HEV-RNA positive samples.

CONFLICT OF INTEREST

The authors have no competing interests.

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ORIGINAL ARTICLE

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Prevalence of leucocyte antibodies in non-transfused male and female platelet apheresis donors

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Abstract

Objectives: In our study group of Thai PLT apheresis donors, we assessed the prevalence of anti-leucocyte antibodies.

Background: Antibodies against human leucocyte antigens (anti-HLA), neutrophil antigens (anti-HNA), and major histocompatibility complex class I related chain A (anti-MICA) in blood products can lead to transfusion-related acute lung injury (TRALI). To reduce the risk of TRALI, some blood centres are implementing strategies based on screening platelet (PLT) apheresis donors for the presence of anti-leucocyte antibodies.

Methods/Materials: Blood samples were collected from non-transfused individuals, 340 males and 63 females (50 nulliparous and 13 parous). Anti-HLA class I and II and anti-MICA were analysed using the Luminex assay, and anti-HNA-3 was detected using the granulocyte agglutination test.

Results: Anti-HLA was found in 14 of 403 subjects (3.5%). Ten subjects (2.5%) tested positive for HLA class I, 2 (0.5%) for HLA class II, and 2 (0.5%) for both HLA class I and HLA class II. Anti-HLA class I or II were detected in 2 of 13 (15.4%) parous females and only anti-HLA class I was found in 4 (8.0%) nulliparous females. Six of 327 subjects tested (1.8%), all males, were positive for anti-MICA. Anti-HNA-3 was not found in any of the 403 individuals.

Conclusions: Screening for anti-HLA class I and II should be implemented for Thai PLT apheresis donors. Although immunisation against HNA and MICA seems to be a rare event in Thais, further work is necessary to decide whether our PLT apheresis donors should be screened for HNA and MICA antibodies.

KEYWORDS
HLA antibodies, HNA-3, MICA antibodies, platelet apheresis donors

1 | INTRODUCTION

Leucocyte antibodies can cause several complications in blood transfusion such as febrile-non-haemolytic transfusion reaction (FNHTR) and transfusion-related acute lung injury (TRALI).[1] TRALI is a serious hazard, which contributes to morbidity and death.[2] The diagnosis of TRALI is based clinically on the symptomatic expression of hypoxemia with bilateral pulmonary oedema on imaging (e.g., chest radiograph) occurring within 6 h after transfusion in which circulatory overload is excluded.[3] There are many TRALI mediators. A majority of cases are related to alloantibodies against human leucocyte antigens (HLA) class I or class II and against human neutrophil antigens (HNA),[2,4,5] especially antibodies directed against HNA-3a, which are associated with severe and fatal TRALI.[6] Epidemiologic studies in the last two decades have found TRALI to be associated with anti-leucocyte antibodies in donated blood[7-9] and on rare occasions in recipient blood.[10] In

general, anti-HLA and anti-HNA are typically found in female donors with a previous history of pregnancies. The causative antibodies in TRALI cases are frequently associated with transfused blood components containing high plasma volumes, especially fresh-frozen plasma (FFP) and platelet (PLT) products.[11,12] The use of plasma products from males only has reduced the incidence of TRALI cases.[5] However, male donors have also been implicated in antibody-associated TRALI.[13] Around 48% of donors implicated in TRALI cases were male.[13,14] Consequently, these antibodies have been detected also in male donor plasma.[14-19] Besides HLA and HNA, major histocompatibility complex class I related chain A (MICA) antigens can also induce alloantibodies and constitute a possible risk factor related to transfusion reaction as well as TRALI.[20]

In this study, we assess the prevalence and specificities of anti-HLA, anti-HNA and anti-MICA in Thai PLT apheresis donors with focus on their clinical significance in non-transfused PLT apheresis donors.

2 | MATERIALS AND METHODS

2.1 | Subject and sample collection

Four hundred and three consecutive eligible PLT apheresis donors were enrolled via the Blood Transfusion Center, Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand. These subjects were all regular donors. After informed consent, all donors completed a standardised questionnaire about transfusion and pregnancy history. Peripheral blood samples were collected during routine apheresis donation. Whole blood was collected into tubes containing ethylene diamine tetraacetic acid. Plasma was stored at −80°C until use. Genomic DNA was extracted from blood samples using the guanidine-HCL method[21] and was then stored at −20°C until used for genotyping. The research protocol was approved by the Ethics Committee of Khon Kaen University (HE621063).

2.2 | HLA antibody testing

Plasma samples were screened for anti-HLA class I and class II using LABScreen Mixed® according to the manufacturer's instructions (One Lambda Inc., Canago Park, CA, USA.). Samples positive according to LABScreen Mixed® were further analysed with LABScreen single antigen beads (SAB) class I and/or class II (One Lambda Inc.). Antibodies with mean fluorescence intensity (MFI) >2000 were assessed for specificity using the HLA-Visual™ software (One Lambda Inc.). The cut-off was used derived from data from patients awaiting solid organ transplantation.

2.3 | MICA antibody testing

Plasma samples were screened for anti-MICA antibodies using LABScreen Mixed® according to the manufacturer's instructions (One Lambda Inc.). The specificity of anti-MICA was determined using SAB (One Lambda Inc.). The MICA-SAB assay could identify anti-MICA 001, 002, 004, 005, 007, 009, 012, 017, 018, 019, 027, 028 and 046 antibodies. Specificity was assigned following the MFI >2000 rule.

2.4 | Detection of anti-HNA-3

Plasma samples were analysed for anti-HNA-3 using the granulocyte agglutination test (GAT) as previously described.[22] Briefly, 2 μl of isolated neutrophil cells (5×10^3 neutrophils/μl) from donors typed for the HNA-3 (two with HNA-3aa and two with HNA-3bb) suspension with autologous plasma was incubated with 6 μl of donor or control serum for 2 h at 37°C under oil on Terasaki plates. The GAT was performed in duplicate. Our GAT testing was run in parallel with anti-HNA-3a and anti-HNA-3b antibody-positive control sera; from the Institute for Clinical Immunology and Transfusion Medicine, Giessen, Germany. Agglutinations were evaluated by inverted microscope (Oympus® IX71, Life Science, NY, USA).

2.5 | HLA and MICA genotyping

DNA samples from PLT apheresis donors with reactive antibodies were genotyped. HLA class I and class II alleles were genotyped by polymerase chain reaction amplification with sequence-specific primers (PCR-SSP) as previously described.[23] Samples exhibiting rare alleles or ambiguous SSP patterns were confirmed by LABType® SSO HLA (One Lambda Inc.). MICA genotype was determined by the PCR-SSP method described previously.[24]

2.6 | Statistical analysis

Fisher's exact tests were performed to assess the difference in HLA antibody frequencies among the male, nulliparous female and parous female groups using the GraphPad Prism software (GraphPad, Inc., La Jolla, CA, USA). Differences between categories were considered statistically significant if the p value was less than 0.05.

3 | RESULTS

3.1 | Frequency of HLA immunisation

In total, 403 healthy, regular PLT apheresis donors were enrolled in this study. Only 63 (15.6%) subjects were female, of whom 13 (3.2%) were parous, but data of number of pregnancies were not available (Table 1). Fourteen donors (3.5%), eight males and six females, were positive for anti-HLA class I and/or II. Anti-HLA class I and class II were detected in seven and two males, respectively, while two (15.4%) parous females showed anti-HLA class I or II. Among

TABLE 1 Prevalence of leucocyte antibodies in female and male PLT donors with and without allo-exposure

Antibodies	Males (n = 340)	Females			Total (n = 403)
		Total females (n = 63)	Nulliparous females[a] (n = 50)	Parous females [b,c] (n = 13)	
HLA class I	6 (1.8%)	4 (6.3%)	4 (8.0%)	0	10 (2.5%)
HLA class II	1 (0.3%)	1 (1.6%)	0	1 (7.7%)	2 (0.5%)
HLA class I + II	1 (0.3%)	1 (1.6%)	0	1 (7.7%)	2 (0.5%)
HNA-3	0	0	0	0	0
Total	8 (2.4%)	6 (9.5%)	4 (8.0%)	2 (15.4%)	14 (3.5%)

[a]p = 0.031 males vs. nulliparous females.
[b]p = 0.005 males vs. parous females.
[c]p = 0.419 nulliparous females vs. parous females.

nulliparous females, four (8.0%) subjects exhibited only anti-HLA class I (Table 1).

The frequency of HLA class I and/or HLA class II antibodies was low among male donors, and significantly lower than among females (male and nulliparous females, 2.4 vs 8.0%, p = 0.031; male and parous females, 2.4 vs 15.4%, p = 0.005). Although the frequencies of HLA antibodies seemed to be high in parous females compared with nulliparous females, this difference was not significant (15.4 vs 8.0%; p = 0.419) (Table 1).

3.2 | Specificity of HLA antibodies

The SAB assay was performed to identify the specificity of antibodies. Table 2 shows the frequencies of anti-HLA class I (A, B and Cw) and anti-HLA class II (DR and DQ) antibodies. For anti-HLA-A, the highest frequency was A1 (0.5%). For anti-HLA-B, the common antibodies were B13, B27, B37, B44, B52 and B77. For anti-HLA-Cw, antibodies to Cw5, Cw12 and Cw15 were found in this study. Antibodies to HLA-DR and HLA-DQ antigens were also found but not to HLA-DP. Six subjects were followed up on three different occasions over a 2-year period and the same specificity of antibodies was found.

The corresponding specificities of antibodies with MFI >3000 are shown in Figure 1. Of these, 23 of 37 (62.2%) anti-HLA class I and 7 of 12 (58.3%) anti-HLA class II had an MFI >5000.

TABLE 2 Specificity of anti-HLA (MFI > 2000) in PLT apheresis donors

Anti-HLA class I						Anti-HLA class II		
HLA	n	%	HLA	n	%	HLA	n	%
A1	2	0.5	B51	2	0.5	DR1	1	0.2
A2	1	0.2	B52	3	0.7	DR103	1	0.2
A24	1	0.2	B53	1	0.2	DR4	1	0.2
A30	1	0.2	B54	1	0.2	DR7	1	0.2
A31	1	0.2	B55	2	0.5	DR9	1	0.2
A32	1	0.2	B57	1	0.2	DR10	1	0.2
A68	1	0.2	B58	1	0.2	DR12	1	0.2
			B60	2	0.5	DR51	1	0.2
B7	2	0.5	B61	2	0.5	DR52	1	0.2
B8	2	0.5	B62	2	0.5			
B13	5	1.2	B63	2	0.5	DQ4	1	0.2
B18	1	0.2	B67	2	0.5	DQ7	2	0.5
B27	3	0.7	B71	2	0.5	DQ8	1	0.2
B35	1	0.2	B72	2	0.5	DQ9	2	0.5
B37	3	0.7	B75	2	0.5			
B38	2	0.5	B76	2	0.5			
B39	2	0.5	B77	3	0.7			
B41	2	0.5	B78	1	0.2			
B44	3	0.7						
B45	2	0.5	Cw5	1	0.2			
B48	1	0.2	Cw12	1	0.2			
B50	2	0.5	Cw15	1	0.2			

3.3 | Frequency of MICA immunisation in the study

We analysed data from 327 PLT apheresis donors from whom plasma samples were available for detection of MICA antibodies. Six of these 327 sera (1.8%) were positive for anti-MICA antibodies (Table 3). All MICA antibody-positive samples were from male donors. Only one of the six donors also demonstrated anti-HLA-A24. Figure 2 shows the specificities of MICA antibodies with an MFI >2000 according to the SAB assay. The most frequently found antibody was anti-MICA 002 (n = 5:1.5%), followed by antibodies to MICA 009 and MICA 019, which occurred in 4 (1.2%) sera.

3.4 | HNA-3 antibody testing

Among 403 donors, as expected, we identified none with anti-HNA-3. Neutrophil-specific antibodies are rare in blood donors without allo-exposure.

4 | DISCUSSION

To reduce the risk of TRALI, most blood centres only use plasma products from males or nulliparous females. For PLT apheresis collection,

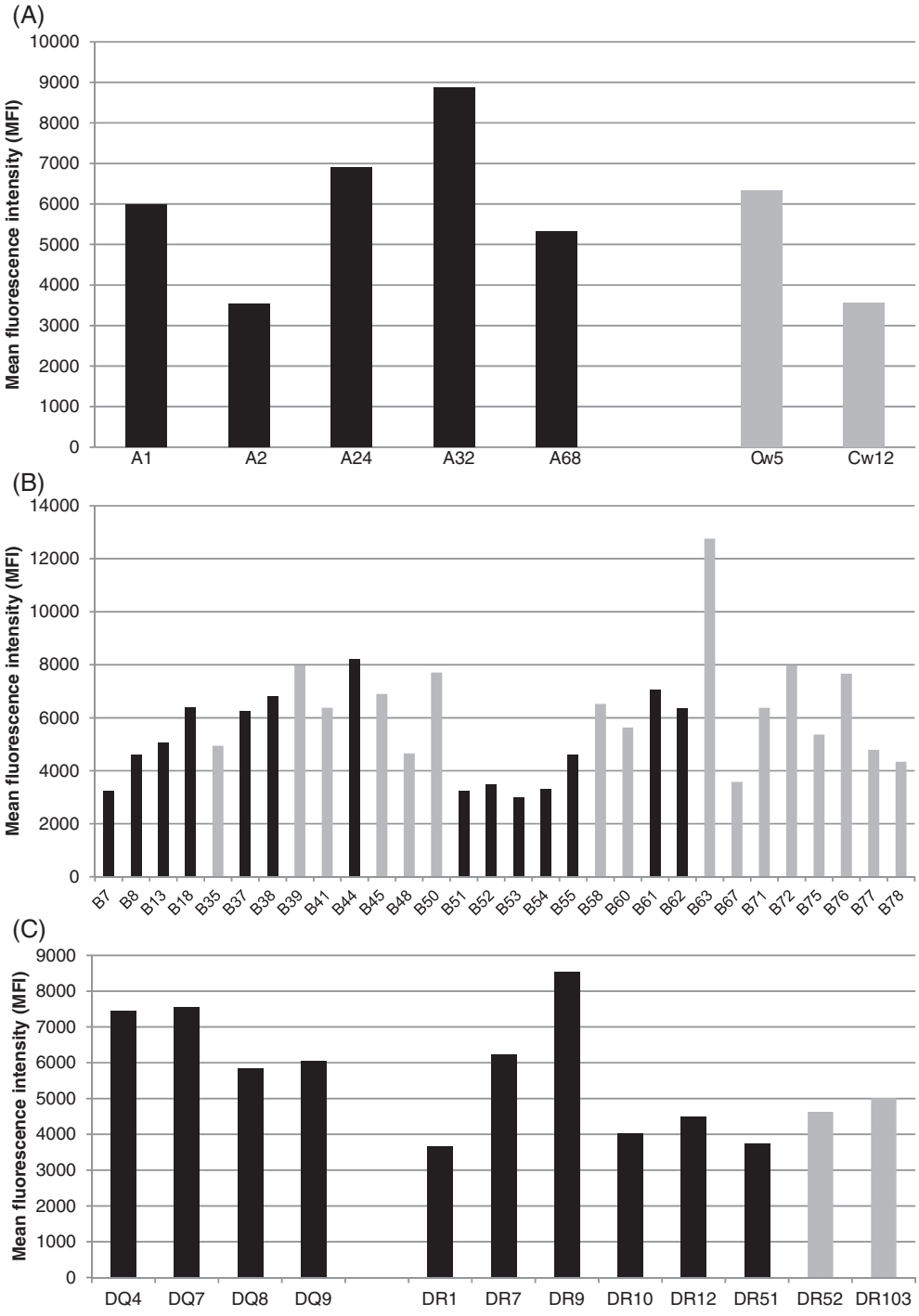

FIGURE 1 Specificities of antibodies in PLT pheresis donors with MFI > 3000 in the study. (A) anti-HLA-A and anti-HLA-Cw (B) anti-HLA-B and (C) anti-HLA class II. The black columns indicate specificities detected in donors associated with TRALI cases in the literature.[9,29,34,35] The corresponding specificities are given only the highest MFI

Donor	Sex	MICA antibody reactivity	HLA antibody reactivity
1	Male	MICA 002, 004, 009, 019	Negative
2	Male	MICA 009, 019	Negative
3	Male	MICA 002, 004, 009, 017, 019	Negative
4	Male	MICA 002	Negative
5	Male	MICA 002	HLA-A24
6	Male	MICA 002, 004, 009, 012, 017, 018, 019	Negative

TABLE 3 MICA antibody specificities (assayed in 327 subjects among whom 6 were positive)

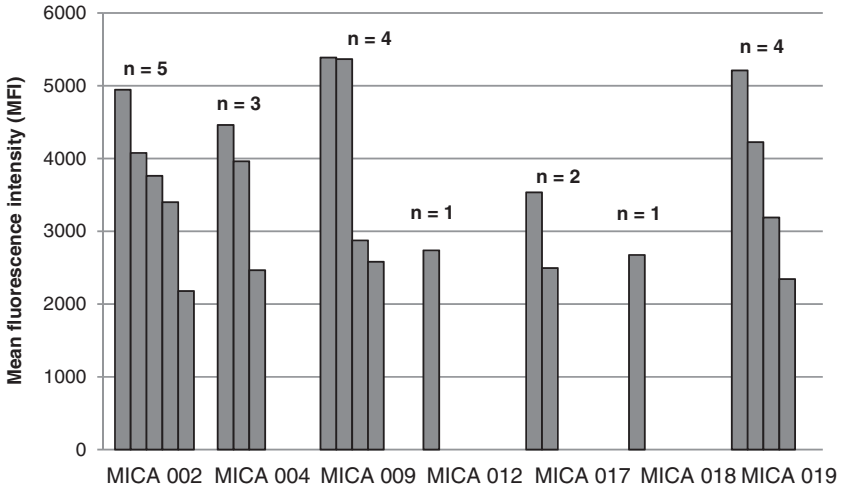

FIGURE 2 Specificities of MICA antibodies in six male PLT donors (See also Table 3). Anti-MICA specificities of MFI > 2000 are shown. Antibodies against only seven alleles of MICA were detected in this study

many centres screen for anti-leucocyte antibodies and exclude donors with these antibodies.[25-27] In our blood centre, PLT products originate mostly from male donors without a history of transfusion (84.4%): only 3.2% are produced from parous females.

In our sample of donors, HLA class I antibodies alone were found in 2.5% of subjects, HLA class II in 0.5%, and HLA class I together with HLA class II antibodies were in 0.5% of subjects. This is in accordance with the result of Reil and colleagues,[28] who reported that HLA class I antibodies were the most frequently detected leucocyte antibodies among blood donors. The most common antibody in this study was B13 (1.2%), which has been implicated with TRALI cases.[29] Of note, this antibody recognises HLA antigens that are common in ethnic Northeast Thais.[23] This observation may indicate that patients with HLA B13 are at a higher risk of developing TRALI.

Among 340 non-transfused male PLT apheresis donors, we detected anti-HLA in only eight individuals (2.4%). This was a significantly lower proportion than in parous females (15.4%). Our result is in accordance with the studies of Xia and colleagues[30] and others.[31,32] Clippel and colleagues[33] also showed that a former pregnancy constituted a major risk factor for the development of HLA antibodies. In our study, nulliparous females also had a higher prevalence of detectable antibodies than males. A similar finding was reported by Nguyen and colleagues[14] but Middelburg and colleagues[17] and Xia and colleagues[30] did not find such a difference. The discrepancies between studies might be due to the presence of multi-specific antibodies in nulliparous females. We found that the prevalence of HLA antibodies in parous females was higher than among nulliparous females (15.4% vs. 8.0%); however, the difference was not statistically significant. This may be due to the relatively small parous female study group and the lack of information concerning number and outcomes of pregnancies in this group. These factors may stimulate production of HLA antibodies.[17]

Hashimoto and colleagues[29] noted that an MFI >3000 was the lowest fluorescence intensity of donor antibodies implicated in TRALI. In our study, 51.4% of HLA class I antibodies and 83.3% of HLA class II antibodies exhibited MFI values >3000, levels comparable with the TRALI cases in the literature.[9,29,34,35] Reil and colleagues[28] also demonstrated that HLA class II antibodies more frequently triggered TRALI than did HLA class I antibodies. It is important to note that antibodies against A2, A24 and DR12 found in this study recognise high-frequency HLA antigens in ethnic Northeast Thais.[23] Antibodies to HLA-A2 have been shown to be involved in severe TRALI cases.[1] In our study, anti-HLA-A2 was only found in one individual out of 403 tested (0.2%). It is of interest that anti-HLA-A2 was the most common antibody identified in kidney transplant patients with antibody-mediated rejection (unpublished observations). The importance of this antibody in the development of TRALI and the significance of our finding needs to be further investigated. Furthermore, about 48.6% of HLA class I and 16.7% of HLA class II antibody specificities (MFI >3000) detected in this study have not been reported in donors implicated in TRALI cases so far. Of these, 66.7% of anti-HLA class I and 50.0% of anti-HLA class II specificities had an MFI >5000 and most antibodies recognised HLA antigens of low frequency in ethnic Northeast Thais.[23] Thus, the role of these antibodies in pathogenesis of TRALI remains to be explored.

We were surprised to identify anti-MICA antibodies in 6 of 327 sera tested. The following specificities were confirmed: MICA 002, 004, 009, 012, 017, 018 and 019. MICA 002 and MICA 019 recognised MICA antigens present at high frequencies in ethnic Northeast Thais.[24] Anti-MICA antibodies are implicated in allograft rejection in solid organ transplantation.[36] However, there has been little investigation of any association between anti-MICA antibodies and transfusion reactions as well as TRALI.

None of our 403 PLT apheresis donors were positive for neutrophil antibodies (HNA-3). Similarly, Reil and colleagues[28] did not find anti-neutrophil-specific antibodies in male blood donors. This antibody is rarely found, even in pregnant women (in 1/1000 or less).[37,38] However, Nguyen and colleagues[14] recently reported that neutrophil antibodies in male blood donors could trigger TRALI.

The screening and identification of HNA alloantibodies have been not quite "trivial"; requires different methodologies that need certain expertise. However, GAT remains the best technique for detection of anti-HNA-3a and anti-HNA-3b [39] and only this method was used in this study. Thus, we only tested for HNA-3 antibodies. However, other HNA antibodies could trigger neutrophil aggregation and can be easily identified by the GAT assay.

Our data demonstrate that 3.5% and 1.8% of non-transfused PLT apheresis donors had anti-HLA and anti-MICA, respectively, with MFI >2000. Our retrospective study did not find that these antibody-positive donors were associated with TRALI. Indeed, the question why leucocyte antibodies trigger TRALI in some patients but not in others remains to be elucidated. Several factors are possible, such as cognate antigen density, antibody titre, affinity/avidity and the presence of non-immunological factors in the patients.[37] Thus, the finding of these antibodies in our study group raises the question whether they are clinically significant or just noise?

The reasons for the presence of specific leucocyte antibodies of the IgG class in males and females with no allo-exposure are unknown. A possible explanation is that these could be naturally occurring alloantibodies as a consequence of immunisation to microbial antigens or vaccination.[14,19] In addition, these donors were genotyped for their HLA and MICA antigens and antigens corresponding with their antibodies were not detected. Thus, we could exclude autoantibodies in our study.

5 | CONCLUSIONS

PLT apheresis donors with no previous allo-exposure had anti-leucocyte antibodies that could potentially cause TRALI. HLA antibodies occurred at low prevalence in males and in females without a history of pregnancy. Although immunisation against HNA and MICA seems to be a rare event in Thailand, further investigation is necessary to decide whether we should screen for HNA and MICA antibodies in our blood donors.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

P.S. designed the study, performed the experiments, analysed the data and wrote this manuscript; Y.S. and S.C. performed the experiments; C.L. edited the manuscript; and A.V.R. designed the study, analysed the data and edited the manuscript.

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ORIGINAL ARTICLE

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Risk of hepatitis-E virus infections among blood donors in a regional blood transfusion centre in western India

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Abstract

Background: Hepatitis-E virus (HEV) is an emerging infectious threat to blood safety. The enormity of the transmission of HEV and its clinical consequence are issues currently under debate. This study aimed to evaluate the prevalence of HEV-RNA in blood donors in western India.

Materials and Methods: We screened 13 050 blood donors for HEV using HEV-RNA screening of 10 mini-pools using RealStar HEV RT-PCR Kit (95% limit of detection (LOD): 4.7 IU/ml). Furthermore, all HEV-RNA-positive donors were investigated for the presence of IgM/IgG antibody along with liver function tests.

Results: Of the 13 050 blood donations, 7 (0.53%) were found to be HEV-RNA positive, and the prevalence of HEV nucleic acid testing yield cases among blood donors was 1 in 1864. All seven HEV-RNA-positive samples were tested with anti-HEV IgM and anti-HEV IgG antibodies; this resulted in two (28.5%) positive anti-HEV IgM and two (28.5%) positive anti-HEV IgG antibodies. Hepatic activity was measured, with two of seven HEV-RNA-positive donors demonstrating abnormal serum glutamic oxaloacetic transaminase (SGOT) andserum glutamic pyruvic transaminase (SGPT). Two HEV-RNA-positive blood donors who had abnormal SGOT and SGPT were found to have a high HEV viral load. Furthermore, we were able to follow up two HEV-RNA donors, and both were HEV-RNA positive and had anti-HEV IgM and anti-HEV IgG antibodies; moreover, their liver function tests were also abnormal. One of the HEV-RNA donors with high viral load did show hepatitis-E-like virus on electron microscopy.

Conclusion: Our studies indicate that there is a significant risk of blood-borne transmission of HEV. This finding may help to provide a direction towards the safety of blood transfusions in clinical settings in countries like India, which fall under the endemic category for HEV infection.

KEYWORDS
RT-PCR, blood donors, hepatitis-E virus, HEV-RNA

1 | INTRODUCTION

The global scenario of hepatitis-E virus (HEV) is very scary; based on the Global Burden of Disease study, it was found that as many as 20.1 million people were infected with HEV in 2005 in nine regions.[1] This accounted for 71% of the world's population, with 3.4 million symptomatic cases, 70 000 deaths and 3000 stillbirths.[2] The global burden of HEV infection is greater due to sporadically transmitted HEV cases

than because of cases due to epidemic HEV. The safety of blood and blood products remains an important public health concern due to the continual emergence of infective viruses. In this context, HEV is an emerging infectious threat to blood safety; since 2004, HEV has gained importance in public health as a transfusion-transmitted infectious (TT-HEV) agent. In the recent decade, there have been several reports of the high incidence of TT-HEV infection.[3,4] HEV-infected immunocompromised patients develop chronic hepatitis-E in approximately 60% of cases.[5]

In India, HEV was first identified during an epidemic of hepatitis, which occurred in Kashmir Valley in 1978. There are documented cases from India, Japan, the United Kingdom, Saudi Arabia and France on the transmissibility of HEV via blood transfusions in patients.[6-12] HEV has also been detected in human blood products.[13-17] Blood donors can be silently infected with HEV, as indicated by plasma pools testing positive for HEV-RNA[13,16] and by a high prevalence of antibodies to HEV among blood donors. HEV has an effect on adolescent to middle-aged adults and causes elevated mortality in pregnant women, 20%–30%, in contrast with 0.2%–1% in general population.[18] HEV was detected in 1 of 815 blood donations in Germany[19] and is 100 times more prevalent compared with human immunodeficiency virus (HIV), hepatitis-B virus (HCV) and hepatitis-C virus (HBV) combined.

Recent studies from India reporting the seroprevalence for HEV, which ranges from 17% to 23% among healthy young adults (18--40 years), indicate the inclination of the virus towards this population; hence, HEV could be a subject of worry in the transfusion medicine community.[20,21] Furthermore, current overall worldwide data indicate that asymptomatic HEV infection is very common among adults who are potential blood donors.[22] Moreover, the occurrence of blood-borne transmission is supported by the demonstration of HEV viraemia among healthy blood donors in several countries.[23] Furthermore, knowing that there is no definitive management for HEV-induced hepatitis, selective screening should be performed in blood products for the high-risk patients in endemic areas.

Therefore, to investigate the infection pressure of HEV in western India, we assessed the presence of HEV-RNA in a large number of recent blood donations, collected throughout the city of Surat. The detection of HEV-RNA also has the advantage of a lower likelihood of being affected by genomic variations in the virus.[24] Data obtained were used to estimate the detection rate of HEV infections in blood donors and the risk of HEV transmission by blood transfusion.

2 | MATERIALS AND METHODS

The study was designed to evaluate the incidence and prevalence of HEV-RNA in the blood donors of Surat Raktadan Kendra and Research Centre, Surat, Gujarat. Blood samples were collected from 13 050 healthy volunteers who participated in the blood donation camps organised by us at various locations in Surat during January 2017 and August 2017. Informed consent was obtained prior to collection. This project has been approved by the Institutional Ethics Committee (IEC) of Surat Raktadan Kendra & Research Centre.

2.1 | Plasma samples

Plasma was separated by centrifugation at room temperature and was frozen and stored at −40°C and thawed once before use.

2.2 | Serological assay

Samples from the donated blood units have to undergo routine HIV, HBV and HCV screening using a third-generation assay: for HCV, Abs with SD HCV ELISA 3.0 test system (SD Bio standard diagnosis Pvt. Ltd, Gurgaon, India); for HIV, HIV-1/2 Abs with Microlisa (J. Mitra & Co. Pvt. Ltd, New Delhi, India); and for HBV, Ags with SD HIV ELISA 3.0 test system (SD Boi standard diagnosis Pvt. Ltd, Gurgaon, India).

2.3 | Pooling of plasma samples for HEV viral nucleic acid test

Pooling of samples was performed using separate aerosol tips (certified free from RNase, DNase and pyrogen) under the laminar hood using aseptic precautions. In the pool containing 10 samples (10-MP), each donation represented 100 μl for a total of 1000 μl. If any 10-MP was positive, further pools were subdivided into two pools, that is, 5-MP-A and 5-MP-B, and in the pool of five samples, each donation represented 200 μl. Furthermore, if any 5-MP was positive, the run was repeated individually (ID) for the plasma sample.

2.4 | Isolation of viral nucleic acid

Viral nucleic acid of HEV-RNA was extracted from individual (ID) or pooled (10-MP) plasma samples using the Chemagic Prepito-D automated extractor (Perkin Elmer), in combination with reagents/buffers of the Prepito Viral DNA/RNA Kit as described in the manufacturer's protocol, and was stored at −80°C until further analysis.

2.5 | Real-time PCR amplification of HEV viral nucleic acid

HEV-RNA was amplified by kits of RealStar HEV RT-polymerase chain reaction (PCR) Kit 1.0 (95% LOD: 4.7 IU/ml) (Altona Diagnostics, Hamburg, Germany) as described in the manufacturer's protocol. The PCR was performed on ABI Prism 7500 Real Time PCR System (Life Technologies).

2.6 | Hepatitis-E virus quantisation

HEV cDNA quantisation was performed using the RealStar HEV RT-PCR Kit 2.0 (Altona Diagnostics), which is an in vitro diagnostic test based on real-time PCR technology for the detection and quantification of HEV-

specific RNA. HEV quantification assay was carried out as per the manufacturer's protocol. Results were measured in IU/ml.

2.7 | Detection of the amplified products

Amplified products of RealStar HEV RT-PCR Kit 1.0 (Altona Diagnostics) were detected by fluorescence, and data were analysed using the 7500 SDS software, version 2.3. Fluorescence from the specific amplification of viral target HEV-RNA was captured in the FAM channel, whereas a signal from IC amplification was read in the JOE channel. During data analysis, ROX was switched on only for the HEV test. An analysis was performed using Auto-Threshold and Auto-Baseline by default.

2.8 | Serological testing (HEV IgM/IgG)

All HEV-RNA-positive samples were investigated for the presence of HEV-specific anti-HEV IgM by anti-HEV IgM and anti-HEV IgG antibodies using an anti-HEV IgG enzyme immunoassay of DIA. PRO, Diagnostic Bioprobes Srl, Milano, Italy, as described in the manufacturer's protocol.

2.9 | Liver function tests: Measurements of liver enzyme activities

The entire samples that were positive for HEV-RNA were further processed for the measurement of serum alanine aminotransferase (ALT/SGPT) levels and aspartate aminotransferase (AST/SGOT) levels. ALT and AST assays were performed using the respective test kits ALTP2: ACN 20140 and ASTP2: ACN 20230, cobas, Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim, at an NABL-accredited clinical laboratory, Metropolis Healthcare Ltd., Surat, as described in the manufacturer's protocol. An in-vitro test was used for the quantitative determination of ALT and AST in human serum and plasma on Roche cobas c systems.

2.10 | Electron microscopy of HEV-RNA positive samples

For further confirmation of the presence of virus particles, we sent all seven HEV-RNA-positive samples to Dr Atanu Basu, National Institute of Virology, Pune, India, for electron microscopy.

3 | RESULTS

3.1 | Prevalence rate of HEV-RNA

Of the 13 050 blood donations (seronegative of HIV/HBV/HCV), 7 (0.53%) were found to be HEV-RNA NAT-positive; the prevalence of HEV NAT yield cases among our routine donors was 1 in 1864 donations tested (Table 1). The HEV RT-PCR cycle threshold (Ct) value range was 13–35. Among the seven HEV-RNA-positive blood donors, all were males in the age group of 29–44 years; furthermore, two (28.5%) donors were AB positive, two (28.5%) were O positive, and two (28.5%) were B positive, whereas one (14.2%) donor was AB negative. Details of the HEV-RNA-positive sample are given in Table 1.

3.2 | Characteristics and relationship between HEV-RNA-positive donors and viral load levels and liver function of blood donors and anti-HEV IgM/IgG antibody

Because the HEV infection can perturb hepatic function, hepatic activity was also measured (Table 2), and two blood donors of the seven HEV-RNA-positive blood donors demonstrated abnormal SGOT and SGPT, whereas the remaining five HEV-RNA-positive tested donors showed normal serum ALT and AST levels (Table 2). Furthermore, these two HEV-RNA-positive blood donors who had abnormal SGOT and SGPT demonstrated high HEV viral load (Table 3).

In this study, 13 050 blood donors were screened for the presence of HEV-RNA, and all 7 HEV-RNA-positive samples were further tested with anti-HEV IgM antibody and anti-HEV IgG antibody as anti-HEV IgM is the serologic marker of choice for the diagnosis of acute HEV infection. This resulted in two cases were positive for IgM anti-HEV and two (28.5%) were positive for IgG anti-HEV antibody (Table 3).

3.3 | Electron microscopy of HEV-RNA-positive samples

Of 13 050 blood units tested, 7 blood samples were positive for HEV-RNA. Six of the seven samples that had a very high Ct value (low viral

TABLE 1 HEV NAT yield rate among blood donors and HEV-RNA positivity details

HEV NAT yield rate				
Total unit tested	**Total HEV-RNA positives**			**HEV NAT yield rate**
13 050	07			1 in 1864
HEV-RNA positivity details				
Sr. no.	**Age/ gender**	**Blood group**	**HEV-RT-PCR Ct value**	**IC Ct value**
HEV-1	44/M	AB^{+ve}	32.78	31.14
HEV-2	35/M	AB^{+ve}	25.98	39.65
HEV-3	30/M	B^{+ve}	19.13	0.00
HEV-4	29/M	O^{+ve}	35.96	31.95
HEV-5	36/M	AB^{-ve}	29.04	30.97
HEV-6	30/M	O^{+ve}	27.01	32.43
HEV-7	31/M	B^{+ve}	13.37	0.00

Abbreviations: *Ct*., cycle threshold; IC, internal control.

TABLE 2 Liver function tests of HEV-RNA-positive blood donors

Sr no.	SGOT (AST) (BRI: 19–48 U/L)	SGPT (ALT) (BRI: 16–63 U/L)	Bilirubin total (BRI: 0.3–1.2 U/L)
HEV-1	21	11	0.43
HEV-2	25	16	0.38
HEV-3	71	96	1.35
HEV-4	29	34	0.72
HEV-5	27	46	0.41
HEV-6	27	20	0.21
HEV-7	136	417	1.38

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

TABLE 3 The relationship between HEV-RNA-positive donors and anti-HEV IgM/IgG antibody and viral load levels and liver function of blood donors

Sr. no.	Age/gender	HEV Ct	HEV viral load (IU/ul)	Anti HEV-IgM (negative: <1.0; equivocal: 1.0–1.2; positive: >1.2)	Anti HEV-IgG (negative: <0.9; equivocal: 0.9–1.1; positive: >1.1)	SGOT	SGPT
HEV-1	44/M	32.78	4.8×10^4	0.32 (–ve)	0.08 (–ve)	Normal	Normal
HEV-2	35/M	25.98	4.9×10^4	0.36 (–ve)	0.17 (–ve)	Normal	Normal
HEV-3	30/M	19.13	2.5×10^6	9.62 (+ve)	1.69 (+ve)	Abnormal	Abnormal
HEV-4	29/M	35.96	2.1×10^4	0.21 (–ve)	0.32 (–ve)	Normal	Normal
HEV-5	36/M	29.04	4.6×10^3	0.19 (–ve)	0.11 (–ve)	Normal	Normal
HEV-6	30/M	27.01	1.5×10^4	0.23 (–ve)	0.19 (–ve)	Normal	Normal
HEV-7	31/M	13.37	2.2×10^4	9.6 (+ve)	7.62 (+ve)	Abnormal	Abnormal

load) did not show the virus particle on transmission electron microscopy (EM). However, one of the samples with a Ct value of 13.37 (high viral load) (Table 3) did show hepatitis-E-like virus on EM (Figure 1).

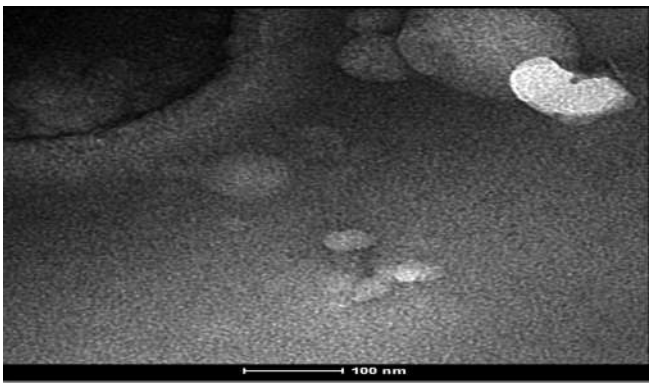

FIGURE 1 Electron microscopy (EM) of HEV-RNA-positive samples (image of a morphologically consistent HEV). Courtesy by: Dr Atanu Basu, National Institute of Virology, Pune, India

3.4 | Follow-up of HEV-RNA-positive blood donors

For the blood donors who were considered HEV-RNA positive, follow-up samples were tested for HEV-RNA and HEV IgG and IgM antibodies and serum ALT (ALT/SGPT) and AST (AST/SGOT) levels to find if anti-HEV seroconversion was detected in a follow-up sample. Follow-up blood samples were collected at 3 months, and of the seven HEV-RNA-positive blood donors, we were able to follow up two donors (HEV-4 and HEV-7). Both were HEV-RNA positive and had anti-HEV IgM and anti-HEV IgG antibodies. Furthermore, their liver function tests were also abnormal (Table 4). Follow-up blood donors demonstrated evidence of infection.

4 | DISCUSSION

There are published reports of HEV infections by contaminated blood products[25] and the detection of HEV in plasma fractionation pools;[26] moreover, samples from blood donors[27-30] suggest that the transfusion transmission of HEV is probably not uncommon. The threat of HEV is currently being experienced by blood banks; moreover, the occurrence of asymptomatic infection in blood donors raised many questions of blood safety regarding the transmission of HEV.[31] Although the disease is usually self-limiting, acute HEV may progress to a fulminant fatal form.

Despite the elevated prevalence and high rate of transmission by HEV-infected blood or blood products; the limited range of HEV-

TABLE 4 Follow-up study of HEV-RNA-positive blood donors

Sr. no.	HEV-RNA	IgM	IgG	SGOT	SGPT
HEV-4	Positive	Positive	Positive	Abnormal	Normal
HEV-7	Positive	Positive	Positive	Abnormal	Abnormal

specific, efficient antiviral therapy; and no vaccines to prevent HEV diseases in those who are still healthy, routine screening for HEV is presently not performed in most blood banks. HEV screening is implemented only in developed countries like the United Kingdom, Ireland, Netherlands and Japan.[32] Furthermore, studies performed worldwide indicate a high HEV seroprevalence (1%–52%) in blood donors.[33] Therefore, the risk and importance of TT-HEV infections by contaminated blood products is currently a debatable discussed topic in transfusion medicine.

The subclinical nature of HEV infection among blood donors has huge implications for blood banks as a donor may provide no history of jaundice despite being anti-HEV antibody positive. Anti-HEV IgM antibody indicates acute infection, and the anti-HEV IgG titre may remain detectable for up to 15 years.[34] Anti-HEV antibodies serve as a surrogate marker for HEV viraemia. However, in the existing studies, viraemia was equally common in the seropositive and seronegative HEV units;[35] besides that, only a minority of HEV viraemic blood units were seropositive. Therefore, tests for these HEV antibodies are unlikely to be useful for screening donated blood. Moreover, it has previously been reported that HEV-PCR might be a better indicator than ELISA of acute HEV infection,[36] and the identification of HEV-RNA also has the advantage of a lower likelihood of being affected by genomic variations of the virus.[24,35]

Furthermore, in the course of a viral infection, viral proteins are released in the host body fluids, and a test for the detection of the HEV antigen in body fluids has been developed and provides another best option for the detection of HEV viraemia. The test appears to be in good strong harmony with the detection of HEV-RNA using nucleic acid testing (NAT). Therefore, molecular NAT for HEV-RNA is critically needed. NAT is currently used in conjunction with serological test, and it has reduced the risk of HIV-1, HBV and HCV where it has been implemented.[37-42] In addition, NAT is also useful for determining the incidence of active infection by these viruses in blood donor populations.[37]

In India, only a few studies have analysed the performance of RNA assays with HEV; in the current study, HEV-RNA was detected in 7 of 13 050 blood donors (0.53%). Furthermore, the prevalence of HEV NAT yield cases among our routine blood donors was 1 in 1864 donations tested. Countrywide estimations of positive HEV-RNA in blood donations were 1 in 1430 in China,[15] 1 in 3090 in the Netherlands,[17] 1 in 3179 in Germany[43] and 1 in 7040 in the United Kingdom.[16] Furthermore, Arankalle from Pune, India, has shown that 1.5% (3/200) of blood donors were positive for HEV-RNA and has suggested the possibility of transmission by transfusion.[44] All the above studies suggested that HEV-RNA is present in the healthy blood donors, and there is always potential risk for the transmission of HEV through the blood and blood products. Boxall et al from the United Kingdom have recently shown the transmission of HEV from a donor to recipient, which was proven by serology and molecular methods.[10]

In the present study and previously published studies, the overall prevalence of HEV-RNA viraemia among blood donors was relatively low.[16,17,43] However, such HEV-RNA screening may still be important and necessary for blood units destined for administration to recipients in whom HEV infection may carry serious consequences. This includes individuals with inherited or acquired immunodeficiency disorders, including those with organ transplantation; those who are at risk of developing chronic HEV infection and consequently chronic liver disease; and those with an underlying chronic liver disease who are at risk of acute-on-chronic liver failure.[34] Furthermore, there are currently no specific antiviral therapies available for acute or chronic HEV infections, and there is no approved vaccine for the protection of HEV. Thus, preventive measures are needed for the HEV RNA testing of blood donations.

Furthermore, all HEV-RNA positive samples were tested with anti-HEV IgM antibody and anti-HEV IgG antibody. This resulted only in two (28.5%) positive anti-HEV IgM and two (28.5%) positive anti-HEV IgG cases (Table 3). It may be possibility is, rest of five HEV-RNA donors were in window period, therefore detecting the virus in the pre-seroconversion, window period thereby providing higher sensitivity as compared to serological tests.[45] Another possibility is that the amounts of antibodies in those samples were lower than the detection level of the kit. Viral RNA can be detected for 6 months before seroconversion;[46] because of this, a viral RNA method to detect HEV is recommended.[47]

In the present study, as anti-HEV IgM/IgG was tested only in HEV-RNA-positive donors, of seven HEV-RNA-positive donors, only two donors were positive for anti-HEV IgM/IgG; this is might be due to the early stage of infection (window period), where plasma HEV-RNA viremia peaks before IgM and IgG antibodies have reached a detectable level. Thus, blood donors can be HEV-RNA positive even though they are anti-HEV IgM/IgG negative, and the probability of missing HEV-RNA-positive samples cannot be ruled out if we only depend on antibody detection methods.

Since the innovation of the ABO blood group, there has been an ongoing interest in the potential role of blood groups in infectious disease.[48] However, there was no major variation in HEV-RNA prevalence in blood group screening, with the highest and equal prevalence in the AB, O and B blood groups (28.5%) and the lowest in the AB^{-ve} blood group (14.2%), and none of the HEV-RNA positive cases were found in the A and B blood groups. However, this a small study, and this variation in HEV-RNA positivity in relation to the blood groups may suggest that the conclusion cannot be decided as only a small number of samples is tested; testing of a huge number of blood donations is probably required.

HEV infection could be the most important trigger of liver decompensation, and the evaluation of hepatic activity was also a measure of all HEV-RNA positive donors. Of seven HEV-RNA-positive blood donors, two demonstrated abnormal SGOT and SGPT and had high viral load, whereas the remaining five HEV-RNA-positive donors had normalised SGOT and SGPT. In a study of the Chinese population, among four HEV-RNA-positive donors, only two had elevated ALT levels.[49] Similar results have also been reported from Japan.[30,50] In the five HEV-RNA-positive donors with normalised SGOT and SGPT, HEV viraemia was observed to last beyond normalisation of transaminases in a number of patients, and this suggest that liver injury is independent of viral loads.[51]

Of these seven HEV-RNA-positive blood donors, we were able to follow up two donors (HEV-4 and HEV-7), and both were HEV-RNA positive. Furthermore, during follow-up, one of the donors (HEV-4) became anti-HEV IgM and anti-HEV IgG antibody positive, and his SGOT tests were also abnormal. It might be due to the window period; during the window period, a donor can have HEV and will be positive for HEV-RNA and be infectious, but still, the tests for HEV IgM and HEV IgG antibodies will be negative. The HEV window period is usually 4–10 weeks from the time of exposure; after 6 months, most people will have developed enough antibodies. In rare cases, however, antibodies can take up to 9 months to develop.[52] Therefore antibody tests are unlikely to be useful for HEV screening in donated blood, but HEV-RNA screening might be a better indicator than ELISA of HEV infection.

Furthermore, none of the HEV-RNA-positive blood donors had any signs and symptoms at the time of blood donation, as well as during follow-up. The HEV RT-PCR Ct value range was 13–35. Of seven HEV-RNA-positive blood donors, only two donors had a Ct value ≤20, and five donors' Ct values were ≥27. Present data suggested the presence of silent HEV infection at the time of donation because of the confirmation of viremia in 7 of 13 050 blood donors by RT-PCR.[53]

EM is considered an old technique; however, it is still at the forefront of both clinical viral diagnoses and viral ultrastructure and pathogenesis studies. In the diagnostic setting, it is particularly valuable in the surveillance of emerging diseases and potential bioterrorism viruses.[54] In the present study, six of seven HEV-RNA-positive samples that had a very high Ct value (low viral load) did not show the virus particle on transmission EM. However, one of the samples with a Ct value of 13.37 (high viral load) did show hepatitis-E-like virus on EM. One of the biggest advantages of using EM for viral detection is that it does not require organism-specific reagents for recognising the pathogenic agent.[55] Other tests involving molecular and serological methods require that a specific probe be available for virus identification.[51]

India falls under the endemic category for HEV infection, and routine screening for this virus is currently not performed in Indian blood banks. These seven of 13 050 (0.53%) blood donors demonstrate a meaningful HEV-RNA positive rate, furthermore, the present study suggest a cost-effective mini-pool HEV-RNA screening approach to reduce transmission risk for the benefit of blood safety and public health, and it will be worthwhile in HEV-endemic regions in India. However, further studies are required to assess the amount of transmission and the clinical relevance of transfusion-associated hepatitis-E infection.

5 | CONCLUSION

Hepatitis-E is being considered a re-emerging infectious disease across the world. HEV transmission by blood transfusion may have detrimental outcomes for the recipients, considering their immunosuppressive status, underlying disease, pregnant women or other circumstances requiring blood transfusion. In the present study, we find the relative risk (1 in 1864) of transmission of HEV through blood products. Furthermore, based on our finding and earlier reports, it appears that screening blood donors for HEV-RNA will be worthwhile because of the absence of definitive treatment and vaccination and because a country like India falls under the endemic category for HEV infection.

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AUTHOR CONTRIBUTIONS

Dr Atanu Basu carried out the electron microscopy experiment for further confirmation of the presence of virus particles in HEV-RNA positive samples.

CONFLICT OF INTEREST

The authors have no competing interests.

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SHORT COMMUNICATION

TRANSFUSION MEDICINE WILEY

A forecasting model to estimate the drop in blood supplies during the SARS-CoV-2 pandemic in Italy

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Abstract

Objectives: To estimate the number of actually Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) infected blood donors applying a statistical forecasting model.

Background: Following the outbreak of the SARS-CoV-2 epidemic, a drop in blood donation has been observed. It is crucial to determine the actual number of potential SARS-CoV-2-positive donors to define the measures and ensure adequate blood supply.

Methods: The cumulative incidence of SARS-CoV-2 positivity, calculated on the general population, was applied to the donor population by estimating the number of positive subjects. The calculation model was validated by the linear interpolation method. The number of blood units actually discarded based on post-donation information was also taken into account.

Results: Three months after the outbreak, 5322 donors were estimated to be positive for SARS-CoV-2 and were therefore potentially excluded from donation. A total of units of blood components were discarded following post donation information. The estimated number of donors deceased (180) and the number of clinically recovered individuals in the same period was also considered.

Conclusion: This forecasting model can be used to obtain information on blood donors' involvement during future SARS-CoV-2 outbreaks, especially in case of changes concerning epidemiology, incidence by age bracket and geographical distribution and also for new outbreaks of emerging viruses.

KEYWORDS
blood donations, safety, SARS-CoV-2, self-sufficiency

1 | INTRODUCTION

After the first warnings of Severe acute respiratory syndrome Coronavirus-2 (SARS-CoV-2) infection cases in Wuhan, Hubei Province in China, the epidemic spread very rapidly worldwide, with a steep rise in new cases and deaths from Coronavirus disease 2019 (COVID-19). On 30 January 2020, the World Health Organization (WHO) declared the epidemic a Public Health Emergency of International Concern, and on the 11th March the WHO made the assessment that COVID-19 could be characterised as a pandemic.[1,2]

The spread of the new disease had an initial impact on blood and blood component donations, causing a significant reduction in donations in China and in the other countries affected by the epidemic. The reason for this reduction was probably related to logistics

(transfer becoming more difficult as a consequence of lockdown measures adopted) and, above all, to blood donors' fear of contracting the infection in blood donation centres.[3-6]

In Italy, after an initial drop in donations in the first 10 days of March 2020, the pace stepped up because of among other things, the introductions of new national measures aimed at reassuring blood donors and guaranteeing adequate organisational conditions.[7]

Based on the unlikely transmission of SARS-CoV-2 through labile blood components,[8] the Italian National Blood Centre (Centro Nazionale Sangue, CNS), acting as national competent authority issued clear guidelines for blood collection centres (social distancing, temperature control etc.) and put in place a telephone triage service for recruiting donors, including specific questions about any risk behaviours or the appearance of symptoms compatible with SARS-CoV-2 infection, and suggested recruitment only by appointment.

Therefore, during the first phase, the pandemic had a very strong impact on the national health system's ability to respond, particularly with regard to intensive care and resuscitation units. However, overall, the level of self-sufficiency in blood and blood components was not overly affected because, at the same time, hospitals greatly reduced their non-urgent medical and surgical activities.[7]

With the gradual exit from the state of emergency and the lifting of lockdown restrictions, hospitals will restart their routine activities, and the demand for blood components will return to pre-pandemic levels.

In order to evaluate the level of pressure the Italian transfusion system is experiencing during the pandemic and considering the possibility of even more severe future outbreaks with possible different epidemiological pictures, the CNS has adopted a calculation model to estimate the number of donors potentially excluded from donations because they are positive to SARS-CoV-2 and the consequent drop in the number of available blood units.

The forecast function of the calculation model was based on the data relating to the trend of the SARS-CoV-2 pandemic in the general population provided by the national health institutions in the period under examination.

To assess the overall number of units that have failed in transfusion stocks, the number of blood component units actually discarded as a precaution in the same period, following clinical information provided by donors after the donation (post-donation information, PDI), was added to this estimate.

Finally, to evaluate the impact of the epidemic event on the donor population, the number of positive donors who, after resolution of symptoms or discontinuation of therapies, are theoretically readmitted to donation and the number of donors definitively excluded due to death from complications from COVID-19 were also estimated.

2 | MATERIALS AND METHODS

The duration considered for the study ranges from 18 February 2020 (epidemic start date in Italy) to 28 May 2020 (3 months). The estimates presented in this paper are based on national epidemiological data, more specifically:

1. the COVID-19 integrated surveillance data relating to the number of positive subjects and the number of deceased subjects in the general population, published online by the Italian National Institute of Health (Istituto Superiore di Sanità, ISS). Here are reported all the cases of SARS-CoV-2-positive subjects divided by 10-year age brackets (between 0 and over 90) and by gender.[9,10]
2. The number of clinically recovered patients available on the Ministry of Health website.[11]
3. The number of PDIs reported by the competent regional authorities to the CNS.

The estimated number of donors who were potentially infected with SARS-CoV-2 was obtained first by calculating the probability that an infected subject in the general population belonging to the 18–69 age bracket, that is, those that include the blood donor population, may have tested positive for SARS-CoV-2.

The cumulative incidence of SARS-CoV-2 positivity, calculated on the general population for each 10-year age bracket, was then applied to the donor population age bracket (18–69). Therefore, the probability values per year of age, taken from the incidence classes of the general population, were recomposed to constitute the age brackets used for donors.

Finally, to estimate the number of positive cases expected in the donor population per each age bracket, the estimated probability for each donor age bracket was multiplied by the number of donors detected for the year 2019 on the information system of the Italian transfusion services.

Considering the 3 month period of observation, the estimated number of positive donors was considered equivalent to the number of donations.

To validate the calculation made, an additional statistical method based on linear interpolation was applied using the R Stats Package programme (version 4.0.0).

The number of blood component units effectively not transfused due to PDI was calculated on the basis of the real number of post-donation reports notified by the Regional Blood Centers to the CNS in the first 3 months of the epidemiological emergency. Donors were made aware of the importance of promptly informing the Transfusion service of reference if they noticed any symptoms compatible with the SARS-CoV-2 infection, if they had been diagnosed with SARS-CoV-2 infection in the 14 days following donation or if they had been in close contact with a person prior to the donation who was only subsequently diagnosed with SARS-CoV-2 infection.

In the first two cases, the Transfusion services discarded the donated blood components as an extreme precautionary measure.

The number of clinically recovered donors was calculated using general population data provided by the Ministry of Health.[11] Distribution by age bracket is not available for these individuals, so the total percentage figure on the number of infected individuals was therefore considered. The number of potentially deceased donors was also

estimated by applying the same calculation method used for estimating positive donors and on the basis of weekly reports published by the ISS and available online.[10]

3 | RESULTS

As shown in Table 1, the ISS reports, on which this study was conducted, showed 230 778 positive cases in the general population, 27% of them asymptomatic, and 31 851 deaths distributed by gender and by 10-year age brackets. The number of clinically recovered patients on 28 May 2020, was 147 101 (64% of the total).

3.1 | Estimate of SARS-CoV-2-positive donors

The data, obtained from the analysis on the general population, made it possible to estimate 5322 (95% CI: 5187–5460) cases of SARS-CoV-2 expected in the donor population, 3 months after outbreak, against 650 000 active donors in the same quarter of 2019.[7]

The calculation of the cumulative incidence of SARS-CoV-2 on the general population, on 26 May 2020, highlighted a greater distribution of positivity in over 69 years of age, that is, those not eligible to donate blood (Figure 1A).

The age brackets that include the blood donor population (18–69), highlighted with the pattern filling in Figure 1A, show positivity values that gradually decrease in the younger age brackets.

The cumulative incidence value, calculated for each 10-year age bracket of the general population, was then redistributed to the age brackets of the donor population (Figure 1B).

Against the 230 778 positives detected overall in the Italian population, in the 3 months following the outbreak of the epidemic, 134 058, or slightly more than half, belonged to the age brackets of the population that include blood donors and were equally distributed between males and females (64 404 males, 69 654 females).

The results show a higher probability of positivity in the male population, especially for those in the older age brackets. On the contrary, females belonging to the older age brackets have a probability level of positivity very close to that of subjects aged between 26 and 45 years. For both genders, however, more generally, the estimated probability is lower in the younger age brackets. The linear interpolation method applied to the same data provided overlapping results.

Overall, of these 5322 estimated positive donors, the estimated number of males was 3545 compared to 1777 females. On the whole, the distribution of expected cases shows a greater concentration of positivity in the northern regions (Lombardy, Emilia-Romagna, Piedmont and Veneto) and a decreasing gradient towards central-southern Italy (Figure 2A).

The distribution of expected cases by gender and age brackets highlights very similar values between males and females for the 18–25 age bracket and with an increase in the male/female ratio in subsequent age brackets (Figure 2B).

3.2 | Post donation information

In the 3-month period considered, a total of 982 donors informed the Transfusion Service of the appearance of symptoms compatible with the SARS-CoV-2 infection in the 14 days following donation.

In particular, 96 donors reported a confirmed diagnosis of SARS-CoV-2 infection, 669 donors reported the sudden onset of at least one of the symptoms compatible with SARS-CoV-2 infection (fever, cough or respiratory distress), and 217 donors reported close contact with a person in the 2 days preceding donation who was subsequently diagnosed with SARS-CoV-2 infection.

TABLE 1 General data and results of the study

	General population (*N*)			Blood donor population (*N*)					
				Real data			Estimated cases		
	Male	Female	Total	Male	Female	Total	Male	Female	Total
SARS-CoV-2-positive subjects	-	-	230 778[a]	-	-	-	-	-	-
SARS-CoV-2-positive subjects belonging to age brackets of blood donors	64 404	69 654	134 058	-	-	-	3545	1777	5322
SARS-CoV-2-positive dead subjects	-	-	31 851[a]	-	-	-	-	-	180
Recovered subjects	-	-	147 101[b]	-	-	-	-	-	3652
Blood units discarded on PDI data 2020						765[c]			
Blood donations in the quarter						650 000[c]			
Total blood unit missed									6087[d]

Note: General data obtained from the reports of Italian National Institute of Health, Ministry of Health and Italian National Blood Centre from February to May 2020 and results generated from application of the forecasting model.
Abbreviations: *N*, number; PDI, post-donation information.
[a]Data from Italian National Institute.
[b]Data from Ministry of Health.
[c]Data from Italian National Blood Centre.
[d]Obtained from 5322 + 765.

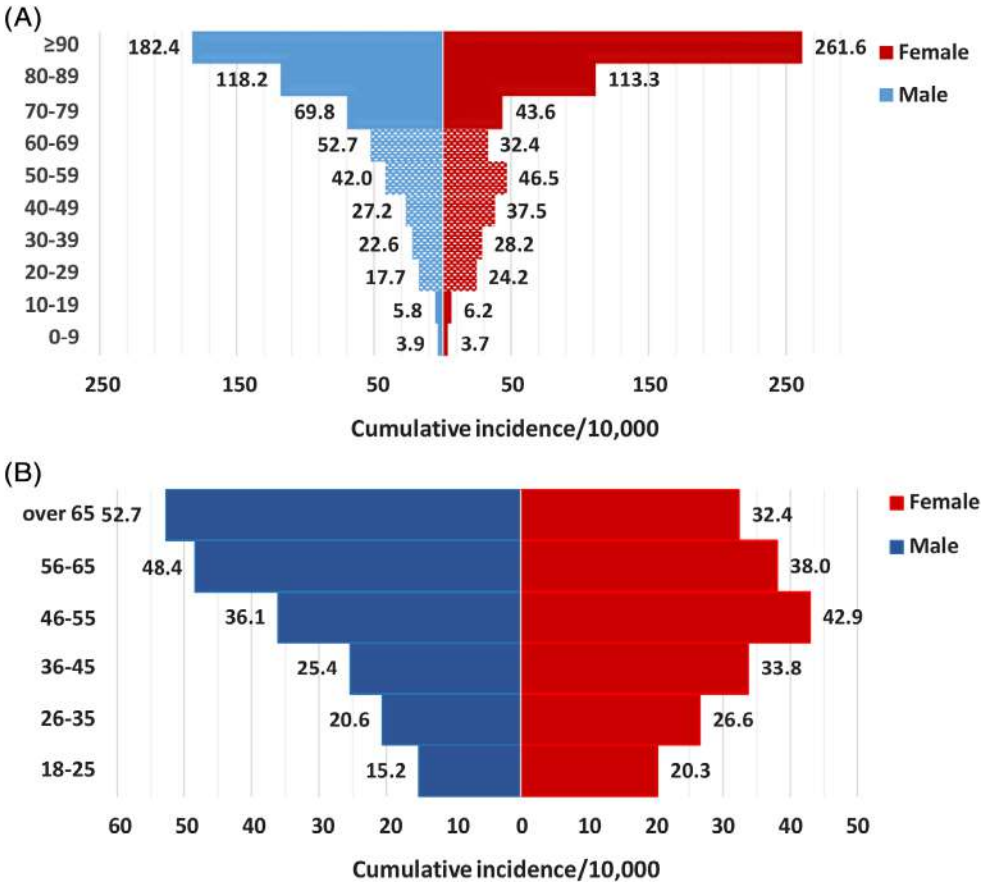

FIGURE 1 SARS-CoV-2 cumulative incidence in the general and blood donor population in Italy. (A) Distribution of the SARS-CoV-2 cumulative incidence in the general population in Italy. The age brackets that include the blood donor population (18–69) are highlighted with the pattern filling. (B) Redistribution in the blood donor population of the cumulative incidence values observed in the general population, divided by age brackets [Color figure can be viewed at wileyonlinelibrary.com]

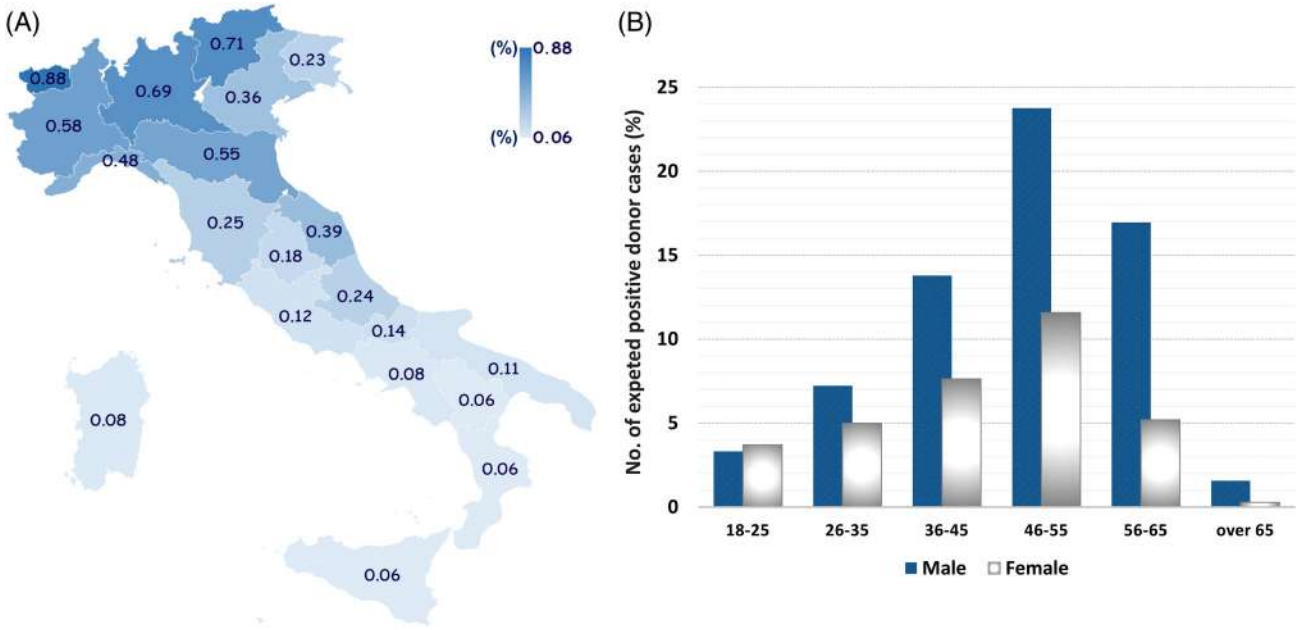

FIGURE 2 Distribution of blood donor cases expected in Italy by north–south gradient and by gender and age brackets. (A) Regional distribution, with north–south colour gradient, of expected SARS-CoV-2 cases in Italian blood donors from February to May 2020. The data are reported as a percentage of the total donor population for each region. (B) Distribution of expected cases of SARS-CoV-2 positivity in the Italian blood donor population divided by gender and age brackets from February to May 2020. The data are reported as a percentage of the total donor population [Color figure can be viewed at wileyonlinelibrary.com]

As the CNS' indication to the Transfusion Services was to eliminate the blood components donated by donors that fall within the first two conditions as a precautionary measure, 765 (96 + 669) units of blood components were discarded.

3.3 | Estimate of SARS-CoV-2 positive donors deaths

The number of donors potentially deceased following SARS-CoV-2 infection was 180. This estimate was arrived at using the same method utilised for the evaluation of infected cases expected in the donor population 3 months after outbreak.

3.4 | Estimate of donors readmitted to donation after recovery

The number of clinically recovered patients in the same period, provided by the Ministry of Health and equal to 147 101, corresponds to 64% of the total infected subjects. Therefore, it is conceivable, that out of 6087 deferred donors, 3652 are theoretically readmitted for donation.

4 | DISCUSSION

The rapid spread of the SARS-CoV-2 pandemic caused a serious overload for healthcare facilities in numerous countries, with the need to increase the reception capacity in hospitals or even to set up new ones in a very short time.

In the first weeks of the spread of the epidemic, the transfusion system also detected a reduction in blood donations, creating the concern of not being able to satisfy all transfusion requests.

This was followed by numerous initiatives aimed at reducing the consumption of blood components, ensuring the safety of donors and premises and preventing access to subjects at risk of SARS-CoV-2 infection.

In Italy, the response of donors to the appeal launched by the authorities was immediate, and the restoration of blood stocks was achieved in a short time.[7]

At the moment, the number of new cases of infection in the national population is on a downward trend, but the proportion of the population susceptible to infection remains very high.[12] There is therefore concern that an epidemic resurgence could also lead to new epidemic peaks in the near future.

By using the institutional surveillance data of the general population and for a defined period of time, the adopted calculation model makes it possible to make a quick estimate of the impact of SARS-CoV-2 infection on blood availability in the country on the basis of the probable distribution of the infection among donors.

The number of blood units discarded following PDI was added to this estimate.

The first parameter used was that of the total number of SARS-CoV-2 positive cases observed in the general population in the 3 months following the outbreak of the epidemic: compared to the 230 778 positives detected, 134 058, slightly more than half, belonged to the age brackets of eligible blood donors. Among the 134 058 eligible blood donors, our forecasting model allowed us to estimate 5322 potentially infected donors, corresponding to an equal number of lost donations.

The second figure considered, equal to 765 units, was that of the blood component units discarded after donation following a PDI reporting symptoms compatible with SARS-CoV-2 infection or a confirmed diagnosis of SARS-CoV-2 infection.

In fact, if on the one hand we can estimate the number of potentially infected donors, and therefore of the units lost, on the other hand, not knowing the recovery times and the clinical evolution of each case of infection, we are unable to define the period necessary for healing. Even assuming, on the basis of data from the Italian National Institute of Health, that 27% of donors (asymptomatic donors) are readmitted to donate in a short time, this is insufficient to estimate the effects on the donor population in quantitative terms. Therefore, to understand the impact of the epidemic on the donor population in quantitative terms, the number of subjects healed in Italy in the same period (equal to 64% of the total number of infected) was considered.

This figure could correspond to the share of clinically recovered patients (tested negative for SARS-CoV-2 RNA in at least two consecutive nasopharyngeal swab specimens collected $\geq$24 h apart) and, therefore, also to the total number of unsuitable donors who currently can be theoretically readmitted for donation 14 days from the resolution of the symptoms or from the suspension of the therapy.

The estimated number of donors who died following SARS-CoV-2 infection is included in the number of estimated positives and corresponds to 3.4% of them. Therefore, for a correct evaluation of the quota of patients who, once cured, could be readmitted for donation, the estimate of deaths should be subtracted from the total number of estimated positives. However, considering that the number of deaths compared to the total number of donors is negligible, this figure is not relevant for the purposes of this study.

The forecast function of the calculation model used is based on individuals in the general population diagnosed with SARS-CoV-2. The projection made, therefore, refers to donors who would not be able to donate because they tested positive for SARS-CoV-2 infection or because they were considered unsuitable on the basis of anamnestic screening or because they were self-excluded.

The positivity data, collected by the ISS and processed in this study, also include a quota of asymptomatic individuals (~27%); however, this is not an exact figure as it does not include any asymptomatic individual not tested for SARS-CoV-2.[13] This category also includes the quota of asymptomatic donors not tested for SARS-CoV-2 who, at least in part, could still be intercepted by the triage or medical selection process that has been put in place in the Italian transfusion system, for example, in the event of close contact with infected individuals.

However, the actual magnitude of asymptomatic subject is currently unknown, and in absence of symptoms or other historical events related to SARS-CoV-2, they will not be deferred from blood donation and could not be included in this forecasting model.

Therefore, in the period under examination, overall, there was an estimated loss of 6087 blood units due to the SARS-CoV-2 infection (5322 unsuitable donors estimated to be positive for SARS-CoV-2 + 765 units discarded following PDI).

It should be noted that, although the number of units discarded following PDI is real and refers to the 3-month period examined, the estimate of donors potentially infected with SARS-CoV-2 was made with respect to the entire blood donor population in 1 year in Italy.

However, even if we want to consider the worst case scenario, in which it can be assumed that the maximum number of blood units (total 6087) could be lost during the quarter considered, without readmitting clinically recovered individuals for donation, the drop in the number of available blood units would correspond to 0.9% of the total donations usually collected in the same period of the year in Italy, which is an average of 650 000 units.

In conclusion, the estimated number of infected donors in the 3-month period examined and considering the worst-case scenario is much lower than the actual reduction in donations in the first week of the epidemic (10%)[7] and was largely compensated in the following weeks after the appeals to donate blood launched by the competent authorities. The readmission to donate of the previously infected donors could be also considered one of the reasons of this response.

This confirms that, to date, the number of donors infected with SARS-CoV-2 is relatively low and the reduction or increase in donations detected during this outbreak, therefore, is not attributable to the SARS-CoV-2 infection in blood donors but rather to motivations related to donors' fear of contracting the virus or organisational aspects of the transfusion network.

This forecasting model can be a useful tool to obtain information on blood donors involvement both during future SARS-CoV-2 outbreaks, especially in case of changes concerning epidemiology, incidence by age bracket and geographical distribution, and also for new outbreaks of emerging viruses.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Giancarlo M. Liumbruno, **Claudio Velati** and **Ilaria Pati:** Conceptualised and designed the study; **Claudio Velati**, **Ilaria Pati** and **Carlo Mengoli:** Performed the research work; all authors contributed to drafting of the manuscript.

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SHORT COMMUNICATION

TRANSFUSION MEDICINE WILEY

Impact of COVID-19 in the attendance of blood donors and production on a Brazilian Blood Centres

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Summary

Background: One of the effects of the coronavirus disease 2019 (COVID-19) pandemic is the risk of shortages in Blood Centres.

Objectives: To verify the impact of the COVID-19 pandemic on the blood donor's attendance and production of blood components in Fundação Hemominas, a Brazilian public institution was formed by several Blood Centres.

Methods: A cross-sectional study was carried out from January to June 2020. Data collected were compared to a historical series from 2016 to 2019.

Results: The study showed a reduction in the attendance of blood donors, whole blood collections and blood component production from March 2020, when the first case of COVID-19 was notified in Minas Gerais, Brazil. The results evidenced that Hemominas Blood Centres were affected in a very distinct way by the pandemic with a general mean reduction around 17% in attendance of blood donors and in production of blood components in the period of March to June. On the other hand, the return of blood donors rate increased.

Conclusion: The reduction in blood donation during the pandemic period was significant, despite the measures adopted. Still, the recruitment of return donors appears to be an important measure to be considered to decrease the pandemic's effect on blood stocks.

KEYWORDS

blood donors, coronavirus, COVID-19, hemotherapy, SARS-CoV-2

1 | INTRODUCTION

Severe acute respiratory syndrome caused by coronavirus 2 (SARS-CoV-2), also known as COVID-19, has spread to more than 213 countries with almost 18 million cases reported until 1 July 2020[1-4] The first case of COVID-19 in Brazil was reported on 26 February 2020. At 22 May, Brazil became the second country with the highest number of COVID-19 cases worldwide. In the state of Minas Gerais, Southeast Brazil, the first case of COVID-19 was reported on 8 March in a patient with a history of travelling to Italy. On 17 March, the state of Minas Gerais was considered a community transmission area.

One of the effects of the COVID-19 pandemic is the risk of shortages in Blood Centres due to decreased attendance of candidates for blood donation. In the Chinese province of Zhejiang, total blood collection fell from 15 609 in 2019 to 5253 in 2020 in the same period analysed.[5] In Italy, the Italian National Blood Centre (Centro Nazionale Sangue [CNS]) reported a 10% reduction in the number of blood donations in the first week of March.[6] In India, despite the actions of the National Blood Transfusion Council (NBTC) encouraging donation

during the lockdown period, a reduction of 64% in blood collections was identified in the Dehradun district (North India) when compared to the pre-pandemic period.[7] Studies on the impact of the COVID-19 pandemic are important to understand the consequences for the hemotherapy and to assist decision-making to mitigate its impacts on hemotherapeutic systems.

Fundação Hemominas is a Brazilian public institution formed by several Blood Centres responsible for more than 95% of hemotherapeutic coverage of the state of Minas Gerais. With 22 centres for blood collection, Hemominas operates in all macroregions of the state. In 2019, it received 348 158 blood donor candidates and produced approximately 825 000 blood components.

Hemominas has reported a reduction of attendance of blood donors since the increase of COVID-19 cases in Brazil, despite the reinforcement of the campaigns, online scheduling and the disclosure of the need for blood donors. This study aimed to verify the impact of COVID-19 pandemic on the attendance of blood donors, whole blood collection, production of blood components, besides deferral and return rates of blood donation candidates in the first semester of 2020 in Hemominas when compared to an institutional historical series from 2016 to 2019.

2 | MATERIALS AND METHODS

Fundação Hemominas operates as a network that consists of 22 regional units and six external collection points that collect blood in Minas Gerais. Minas Gerais is a state located in southeastern Brazil with approximately 21 million inhabitants distributed in 853 counties and 588.384 km^2 (Figure 1). Blood Centres located in the counties of Belo Horizonte, Governador Valadares, Juiz de Fora, Montes Claros, Pouso Alegre, Uberlândia and Uberaba are the larger and more complex regional Blood Centres, accounting for approximately 60% of the blood collections performed by Hemominas. Monthly data from the first semester of these Blood Centres as well as the global data from Hemominas in the period of 2016 to 2020 were collected. The following variables were included for analysis: number of attendance of blood donors, production of blood components, collection of whole blood, deferral rates based on physical examination and health and behavioural interview, and return rate of blood donors. Averages and SDs of the monthly data for the period 2016 to 2019 were calculated for comparison with the 2020 data. The comparison with the mean of the four previous years were performed to avoid the influence of both seasonal variation and annual fluctuation.

Data of confirmed COVID-19 cases and deaths were extracted from epidemiological bulletins published by the Department of Health of Minas Gerais (SES/MG).[8] Data from the last day of each month in the counties where Blood Centres are located and the global data from Minas Gerais were considered. Demographic data were obtained from the SES/MG website and were used to calculate cases and deaths per 100 000 inhabitants.[9] Data analysis was carried out using Graphpad Prisma 5 software (San Diego, California). This study was approved by the institutional ethics committee (CAAE: 31087720.2.0000.5118).

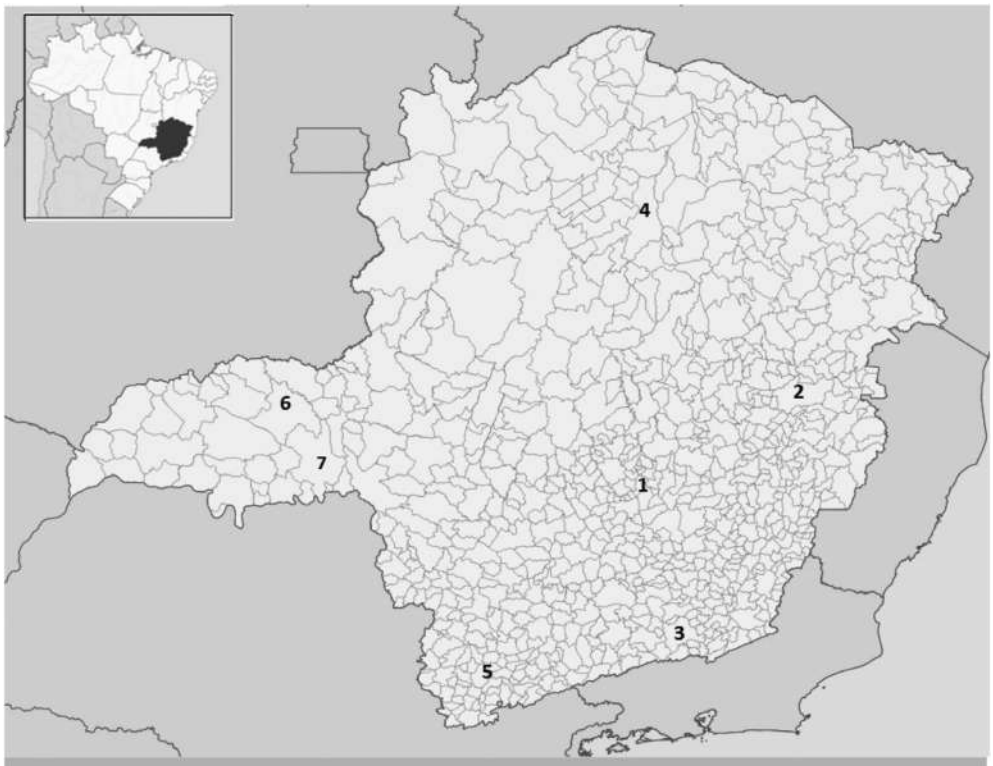
3 | RESULTS

We collected the data of attendance of blood donors, whole blood collections, blood component production, deferral and return rates of blood donors at Fundação Hemominas (global data) from 2016 to 2020 in the first semester (Table S1). The Hemominas global data showed a reduction in the quantity of attendance of blood donor, whole blood collections and blood component production since March 2020, the month in which the first case of COVID-19 was reported in Minas Gerais (Figure 2A-C). April was the month with the greatest reduction in the quantity of attendance of candidates for blood donation and collections of whole blood, when these parameters dropped 19.11% and 19.22%, respectively, in comparison to the same period of 2016-2019 (Table 1). In May and June a slight recovery in these parameters was observed, which reflected in improvement in the production of blood components. The fall in blood components production was higher in March (−16.39%), but reached the lower decrease in May (−9.43%), which remained in June (Table 1).

It is interesting to note that the return rate of blood donors was increased for all months analysed in 2020 when compared to the means of previous years (Figure 2D). In its turn, the deferral rates seem to have been little affected over the evaluated period.

The data were also analysed for the biggest Hemominas Blood Centres (Table 1). The results showed that although the Blood Centres have been affected differently by the pandemic, a reduction in the number of candidates for blood donation and collections of whole blood was observed in all of them, reaching the maximal level of reduction in April for the majority of them. The rate of return donors increased in the first half of 2020 compared to previous years for these Blood Centres, except for Juiz de Fora. This Blood Centre also shows a divergent result about production of blood component, that increased from April to June, different that was observed for the other ones.

According to data released by SES/MG of cases of COVID-19 infection and death in counties from Minas Gerais, and considering the population of the analysed cities, we calculated the number of confirmed COVID-19 cases and death per 100 000 inhabitants (Figure 1). The data showed a heterogeneous distribution of the number of COVID-19 cases and deaths in the geographic space of the state of Minas Gerais, which have increased since March, reaching an extraordinary elevation from April to June. Despite this growing increase, the fall in the parameters evaluated did not follow this trend, showing a certain recovery of Blood centres in the attendance of blood donors, and consequently, blood collection and production, except Juiz de Fora. Among the counties evaluated, the highest cumulative number of COVID-19 cases per 100 000 inhabitants was observed in Uberlândia (920), while the lowest number of cases was observed in Montes Claros (62) (Figure 1), but they were not the Blood centres with, respectively, greater or lesser decrease in the evaluated parameters. Thus, although all analysed Blood Centres showed a decrease in the number of blood donation candidates and collection of whole blood, the magnitude of this reduction varied



Local	Population	COVID-19	March	April	May	June
Belo Horizonte (1)	2501576	Cases	6.52	23.03	72.07	214.82
		Deaths	0.08	0.68	1.96	5.44
Governador Valadares (2)	278685	Cases	0.72	7.18	60.28	368.52
		Deaths	0.00	1.08	2.51	12.92
Juiz de Fora (3)	564310	Cases	4.08	25.87	103.49	273.79
		Deaths	0.00	0.89	5.32	8.86
Montes Claros (4)	404804	Cases	0.00	2.96	16,30	61.51
		Deaths	0.00	0.25	0.49	0.99
Pouso Alegre (5)	148862	Cases	0.00	14.78	75.24	213.62
		Deaths	0.00	1,34	2.02	2.69
Uberlândia (6)	683247	Cases	1.46	15.51	128.80	920.17
		Deaths	0.00	1.17	2.49	11.42
Uberaba (7)	330361	Cases	0.91	14.83	58.72	210.98
		Deaths	0.00	0.91	1.82	7.87
Minas Gerais	21040662	Cases	1.31	8.68	49.73	213.88
		Deaths	0.01	0.39	1,29	4.59

FIGURE 1 Geographic location, estimated population, cumulative number of confirmed cases and deaths by COVID-19 per 100 000 inhabitants in the first half of 2020 in the Minas Gerais counties where the Fundação Hemominas Blood Centres are located. *Source*: Fundação Hemominas, Secretaria de Saúde do Estado de Minas Gerais, 2020

considerably and was not directly correlated with the quantity of local cases of COVID-19.

4 | DISCUSSION

Maintenance of blood components production during the COVID-19 pandemic has been a challenge for Blood Centres in different parts of the world. The fast spread of the disease has led to the need for social isolation to prevent contagion, which is an important factor to decrease blood donation in several countries worldwide, such as Italy, Iran, China, United States and Brazil.[10-15] In the first half of 2020, Brazil became the second country in the world with the most reported cases of COVID-19 and this study aimed to verify the impact of this pandemic on the production indicators of Blood Centres from Fundação Hemominas, which is a public institution responsible for more than 95% of hemotherapeutic coverage of the state of Minas Gerais, located in the southeast of Brazil.

Comparing to an institutional historical series from the first semester of 2016-2019, we observed in all of the correspondent months of

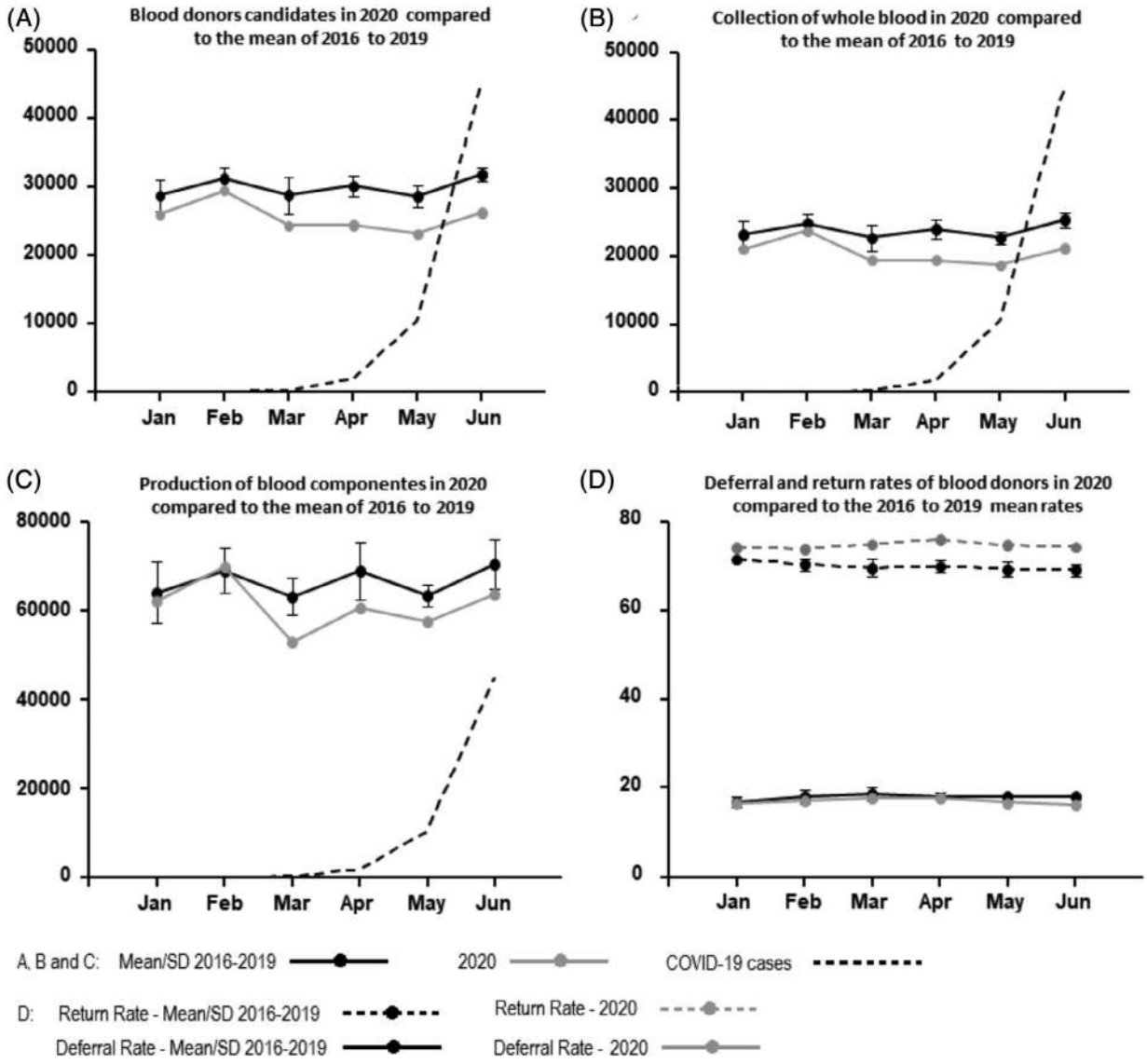


FIGURE 2 Variation of indicators from Fundação Hemominas in the first half of 2020 compared to the means of the same month in the period of 2016-2019. A, Attendance of blood donor candidates; B, whole blood collections; C, production of blood components; D, rate of deferral and return donors

2020 a reduction in the quantity of attendance of blood donors, collections of whole blood and production of blood components (Table 1 and Figure 2). This fall became more intense from March, the month in which the first case of COVID-19 was reported in Minas Gerais. However, it is important to note that March has always been a critical month for Brazilian Blood Centers, because of the carnival. Thus, the impact of COVID-19 appears to have been felt most strongly since April, when occurred the greatest drop in these indicators in most of the Blood centres evaluated and in Hemominas in general (that means, all its Blood centres). In the subsequent months, a slight recovery was noted. In addition, we can observe that despite the reduction in the present year in the parameters evaluated, their seasonal variation over the months has been maintained (Figure 2).

It is interesting to note that the only indicator that showed increase for all months of 2020 compared to the mean of previous years was the return rate of blood donors, whereas the deferral rate was not affected over the pandemic period (Figure 2D). This increase was possibly caused by the adoption of differentiated recruitment measures, including active recruitment of repeat donors. Another result that draws attention is the increase in blood production in Juiz de Fora Blood Centre, from April, despite the drop in blood collection. This can be explained by the sending to Juiz de Fora of the blood collected by other units from Hemominas, which shows the advantage of working cooperatively in a network, especially to face critical situations.

From March, Hemominas adopted several instructions and recommendations to face the impacts of the pandemic. The conducts adopted by Hemominas that could decrease the attendance of blood donors were related to: (a) deferral for candidates from areas with community transmission of SARS-CoV-2, adopted only in March,

TABLE 1 Percentage change in data of Hemominas blood centres in 2020 in comparison to the means of 2016-2019 period

	Regional Blood Centres							
Months	HBH	GOV	JFO	MOC	PAL	UDI	URA	Hemominas (global data)
Blood donor candidates								
January	−14.29	−10.18	−14.65	−1.89	−9.38	3.47	−16.05	−9.78
February	−14.66	19.40	−13.78	0.04	14.12	−1.69	−18.74	−5.71
March	−25.73	3.42	−17.09	−0.25	−7.20	−23.71	−8.47	−15.30
April	−20.26	−19.70	−24.03	−29.76	−25.23	−25.23	−31.80	−19.11
May	−17.65	−33.14	−24.38	−18.45	−20.81	−14.34	−24.56	−19.11
June	−16.10	−28.56	−32.26	−22.78	−14.68	−21.05	−23.58	−17.73
Whole blood collections								
January	−14.14	−9.71	−14.06	−1.69	−5.92	4.28	−9.51	−9.11
February	−16.33	24.95	−11.33	2.65	18.55	−1.11	−11.69	−4.59
March	−25.93	−2.91	−14.59	0.79	−6.29	−20.20	0.41	−14.38
April	−21.64	−21.12	−21.43	−28.34	−31.08	−26.36	−27.68	−19.22
May	−17.48	−29.20	−21.48	−11.75	−24.13	−10.89	−17.09	−17.86
June	−17.03	−25.01	−29.64	−20.68	−11.25	−18.87	−17.11	−16.58
Production of blood components								
January	2.42	−9.11	−5.74	−2.08	−7.26	22.47	−10.56	−2.78
February	−0.35	25.84	−10.96	3.63	12.62	21.79	−5.72	1.20
March	−42.76	−3.35	−4.80	3.80	−5.09	−0.90	1.12	−16.39
April	−7.61	−19.54	5.51	−29.01	−29.55	−15.14	−22.93	−12.01
May	0.02	−25.66	6.89	−12.77	−23.38	3.17	−12.39	−9.43
June	−7.48	−22.22	2.92	−20.11	−8.43	−2.01	−13.11	−9.56
% clinical deferral rate								
January	−0.40	0.77	0.29	0.41	−2.53	−0.71	−6.16	−0.44
February	0.23	−3.43	−2.70	−0.78	−1.94	−1.18	−5.71	−1.09
March	−0.88	5.57	−2.43	−0.43	0.30	−4.24	−7.01	−0.89
April	−0.40	2.43	−5.11	−0.68	6.37	0.89	−5.81	−0.27
May	−1.16	−3.02	−4.22	−5.58	3.86	−3.69	−7.89	−1.45
June	−1.72	−2.68	−4.90	−2.57	−2.85	−2.65	−6.69	−1.89
% return blood donors rate								
January	4.69	0.73	−3.53	1.02	2.02	5.44	−0.58	2.59
February	7.02	1.39	−1.11	0.91	3.69	4.21	4.70	3.46
March	9.21	−0.48	2.24	3.39	4.22	13.43	4.20	5.34
April	5.31	6.08	2.48	10.00	3.88	7.88	8.58	5.99
May	3.76	10.81	−1.28	6.97	5.04	7.22	8.98	5.36
June	5.88	10.97	−0.60	6.30	5.76	6.36	4.48	5.12

Abbreviation: GOV, Governador Valadares Blood Centre; HBH, Belo Horizonte Blood Centre; JFO, Juiz de Fora Blood Centre; MOC, Montes Claros Blood Centre; PAL, Pouso Alegre Blood Centre; UDI, Uberlândia Blood Centre; URA, Uberaba Blood Centre.

when the state of Minas Gerais had no evidence of local transmission; (b) deferral for 30 days (initially 90 days) of individuals who related COVID-19; (c) guidance to individuals who belong to risk groups to wait until the end of social isolation to apply for the donation; (d) temperature checks at entrance and prevention of entry of people with flu-like symptoms in Blood Centres; (e) prior scheduling and limiting the number of individuals in external blood collections and groups of people travelling together to donate blood; and (f) determining greater distance between donors, which can limit the number of donors at certain times. On the other hands, other measures sought to promote blood donation, such as: (a) active blood donor recruitment, including those with known blood groups (eg, police, army); (b) operation of Blood Centres at alternative schedules; (c) contact with blood donors via cell phone and sending messages for recruitment and confirmation of attendance; (d) increase of in-hospital recruitment of blood donors. Concomitantly, media campaigns were

widely publicised, including social media, warning about the need for blood and reinforcing that Blood Centres are safe places with very low probability of SARS-CoV-2 transmission. These measures appear to have stabilised the decline since April, with a better performance in June.

The adoption of measures to face the pandemic was also reported by Blood Centres in different countries. In general, Blood Centres have adopted actions not only to alert the population to the need for blood donation, but also to gain donor confidence that the donation can be made safely.[12,13] In comparison to Blood Centres from different countries, that related a more dramatic decrease in blood collections (33% in Zhejiang [China] and 64% in Dehradun [India]),[5,7] Hemominas showed a relatively better performance during the first four months of the pandemic in the state, with a drop below 20%. Measures similar to those adopted by Hemominas were also followed by other Brazilian Blood Centre (Campinas, São Paulo), resulting in an average increase of 14% in the number of weekly donations compared to the pre-pandemic period.[15]

The management of the stocks of blood components during the pandemic is a complex task. The drop in blood donations does not imply a shortage of blood components, since a decrease in the number of transfusions has also been observed in different countries due, among other factors, to the postponement of elective surgeries.[16,17] Thus, blood centres should be alert to inventory management to avoid both shortages and the disposal of expired units due to decreased demand. At Hemominas actions are carried out to optimise the management of blood components, such as daily monitoring of stocks, including in hospitals, discussion with hospitals about the suspension of elective surgeries, and priority for the use of blood components with shorter expiration dates. In this context, the number of transfusions recorded by Hemominas decreased in 2020 in relation to the historical series in all months evaluated, with the largest decrease occurring in May (23.8%, Table S1). In addition, the number of red blood cell units discarded due to the expiration date has fallen in every month since March compared to the previous year (average reduction of 32.2%), while the discard of platelet units showed a huge variation among the months, indicating a greater challenge in the management of blood components with shorter expiration dates (data not shown).

We also analysed whether the confirmed cases of COVID-19 influenced directly the attendance of blood donors, blood collection and blood production. The confirmed cases of COVID-19 were distributed in a very heterogeneous manner in the territory of Minas Gerais. The most affected regions were those in which Belo Horizonte and Uberlândia are located (known as the Central and Northern Triangle, respectively) (Figure 1). In addition, over the period studied, the cases of COVID-19 presented a pattern of dissemination from large urban regions to smaller cities. This pattern seems to have reflected in the Blood Centres evaluated, since the Blood Centre in Belo Horizonte (capital of Minas Gerais) showed earlier falls in blood collections (even in March), while this drop was only observed later in the other Blood Centres. It is important to note that in Minas Gerais, all regions did not adhere to a standard protocol to contain the spread of infection, as manner as the number of COVID-19 cases have evolved at very heterogeneous rates between regions and counties. In addition, it is important to note that the results presented in this study refer to the number of confirmed cases of COVID-19 per 100 000 inhabitants. Is it important to emphasise there is no population testing program in Minas Gerais and only patients who fit the protocols are tested,[18] which result in considerable underreporting of asymptomatic or mild SARS-CoV-2 infection in the analysed regional population.

The measures above described to face pandemic were adopted uniformly in all units of the Hemominas, regardless of the regional progression of COVID-19 cases. This action may have contributed to mitigate the reduction in the number of blood donations even in regions that experienced a sharp increase in the number of cases of COVID-19. Thus, the drop in blood collections was not proportional to the evolution of COVID-19 cases observed in the regions. For example, in Uberlândia, where the number of COVID-19 cases increased more than seven times in June, the drop in whole blood collection (−18.87%) was not greater than that observed in other regions where the number of cases remained much lower, such as Montes Claros (−20.68%) (Figure 1 and Table 1).

These results suggest that the measures adopted by Hemominas, that are similar to those adopted in Blood Centres worldwide, have been useful to control the decrease in blood donations, even in regions where the spread of COVID-19 is markedly increased. The appearance of new emerging diseases and pandemics is a challenge for which Blood Centres have to be attentive and ready to quickly adopt measures to mitigate the impact on donor attendance and blood component production, and consequently in meeting demand transfusion. Acting as a network of cooperative units can be a strategy for Blood Centres to overcome these challenges.

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CONFLICT OF INTEREST

The authors have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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LETTER TO THE EDITOR

TRANSFUSION MEDICINE WILEY

Role of donor self-reporting in securing blood safety during COVID-19 pandemic

Dear Editor,

Blood safety is of paramount importance, and many advances in transfusion medicine lower current transfusion risk. Emerging infectious diseases always put pressure on blood service with regard to the protection of blood safety. Although the transfusion transmissibility of SARS-CoV-2 in the COVID-19 pandemic has not been reported and remains theoretical,[1-3] several precautious measures have been in place since early 2020 to ensure blood safety and ease public concerns. These include travel and contact history deferral, eligibility screening, use of face mask, hand sanitizer and temperature checking before and during blood donation.[4] In addition, all donors should be reminded to report any symptom or sign of medical illnesses after blood donation so that mitigating measures can be taken as soon as possible. Even with this approach, there are still case reports of donors reporting to the blood centre with confirmed COVID-19 a few days after donation. Although their blood samples at donation were negative for viraemia, it continues to raise concerns regarding transmissibility and blood safety.[3,5]

Reminding donors to report or about post-donation self-exclusion (PDSE) could be a simple but important tool to enhance blood safety. It not only allows opportunities for donors to provide additional health- or blood safety-related information, as in the case of confidential unit exclusions in preventing potential transfusion-transmitted viral diseases such as HIV and hepatitis, but also to report on any medical illness that is developed after blood donation.[6,7] Locally, the blood service has routinely implemented this voluntary PDSE reminder for more than 20 years as part of its comprehensive blood safety measures. Given the similarities between symptoms and signs of COVID-19 and upper respiratory tract infection (URTI), surveillance of URTI-related PDSE can play

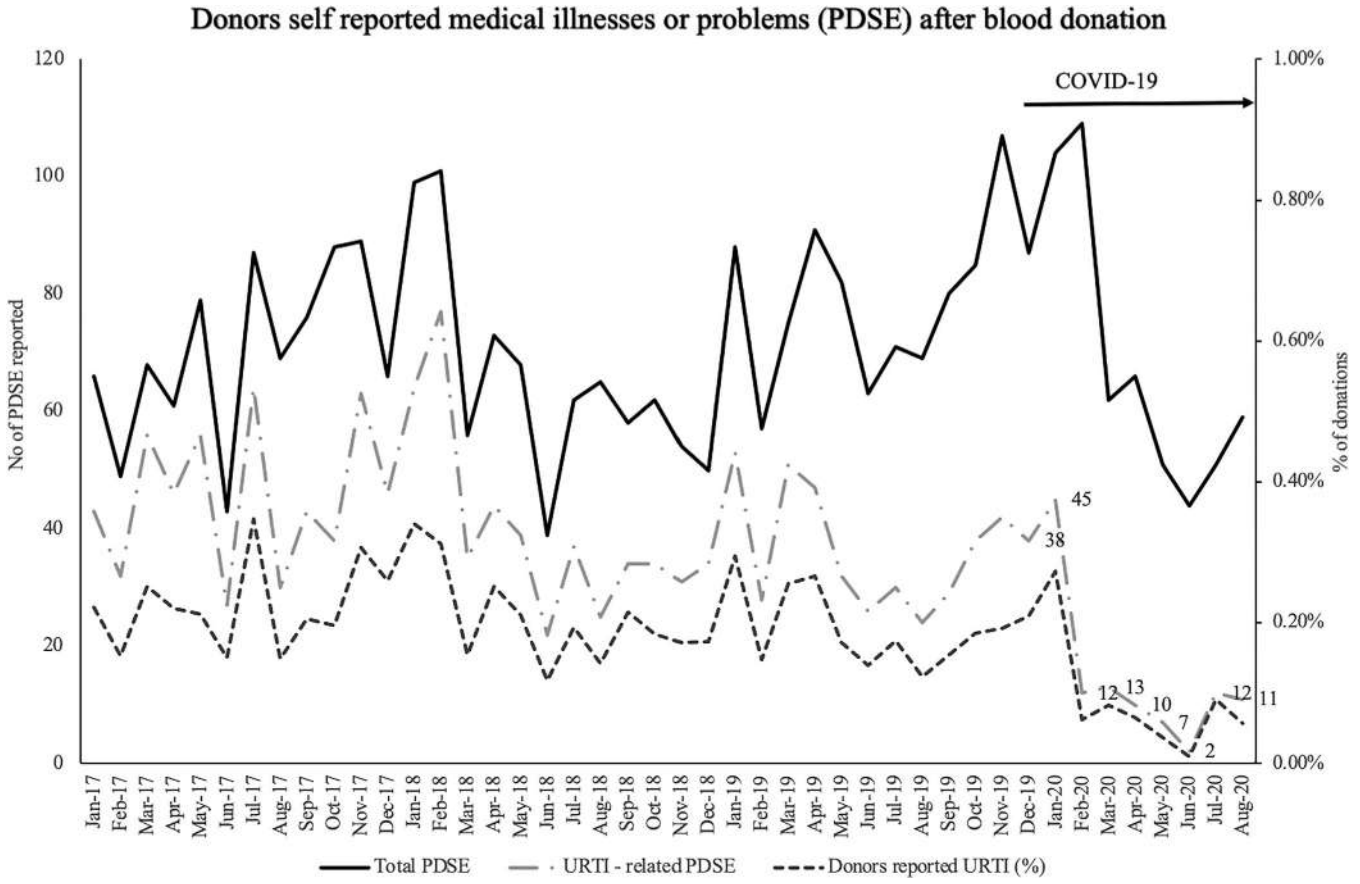

FIGURE 1 Trend of donors' self-reported medical illnesses or problems (post-donation self-exclusion; PDSE) after blood donation

a surrogate role for the early detection of COVID-19 in blood donors, such that precautionary measures could be taken promptly to prevent the issue or transfusion of the blood components.

For the period from January 2017 to December 2019, PDSE was reported in 0.37% of blood donations, with an average of 57% related to symptoms and signs of URTI. Some seasonal trends of increasing reports were seen when the influenza season prevailed (Figure 1). Interestingly, the number of URTI-related PDSE was observed to decrease significantly from 12 (0.06% of donation) in February 2020 to the lowest number of 2 in June 2020 (0.01% of donation). The findings are indeed consistent with local reports of fewer influenza cases during the same period.[8,9] It could be postulated that, during the COVID-19 epidemic, the public are concerned with stringent infection control measures such as social distancing and the use of face mask and hand sanitizer, which render them less likely to get or transmit the respiratory virus infection. As a result, this led to fewer reported URTI-related PDSE.

On the other hand, the blood service continues to receive callbacks from donors for safety precautions as a result of either exposure or contact history with family members or colleagues who were diagnosed to have COVID-19. Over the same period, 24 donors reported to the blood service post-donation as they had recalled recent travel history (n = 3) or were informed of exposure to and/or contact with persons who were COVID-19 positive (n = 21). The donors' own follow-up actions were variable, from none and self-quarantine to COVID-19 testing. Nevertheless, upon contact by the blood service at 14 and 28 days, none of them were found to have COVID-19.

In summary, the COVID-19 pandemic has put much pressure on the healthcare system and the society. Blood services are no exception, with continuous challenges in managing blood supply, the need to implement proactive measures in securing blood safety and efforts to follow up donors post-donation. Nevertheless, it not only creates an opportunity to enhance the existing contingency response plan to ensure business continuity but also engages donors further in securing blood safety. In this short review, it is recommended that reminding donors to report any symptoms developed after blood donation should be adopted as routine practice as an additional blood safety measure.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

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LETTER TO THE EDITOR

TRANSFUSION MEDICINE WILEY

ABO-incompatible convalescent plasma transfusion: Yes, you can

Dear Sir,

On the basis of interventional studies published to date, transfusion of convalescent plasma (CP) is hypothesized as an effective treatment in nonmechanically ventilated severe COVID-19 patients.[1] This modality continues to be pursued worldwide, and randomised clinical trials are underway to test the hypothesis.

Because the vast majority of COVID-19 cases are asymptomatic or paucisymptomatic, generating neutralising antibody (nAb) titres which are too low, the number of suitable donors of CP is limited. Repeat plasmapheresis is currently the standard of collection in westernised countries. Several studies indicate that group AB patients have greater disease severity,[2] and are hence less likely to fully recover and become CP donors before the nAb titre declines to levels which are therapeutically useless.[1] In small-scale CP programs, this and other biases have led to a patient-donor imbalance, often leaving group AB and B (and more rarely group A) patients devoid of ABO-matched CP units.

Three approaches can theoretically be implemented to address this deficiency:

1. Repeat plasma donations from the suitable donors. This is of limited scaling up, as the nAb titre can drop rapidly over the timeframe needed to harvest a useful number of donations in a large part of donors, and that is the reason why the nAb titre has to be reassessed at every donation.
2. Sourcing units from different geographic areas. This will not obviate the aforementioned imbalance as these will occur within as well as across borders.
3. ABO-incompatible (ABOi) CP transfusion. ABOi plasma transfusion has long been used under emergency setting, and no major immediate intravascular haemolytic transfusion reactions (IHTR) occurs when isoagglutinin titres are below 1:64.[3] According to the AABB Technical Manual, ABOi plasma transfusion in group AB patients should be attempted with group A before group B in order to minimise haemolysis. Group A units are generally more abundant and less likely to introduce additional unbalance within the pool of available donors. Anti-B isoagglutinin titration can be performed using high-throughput automated platforms, and, when discordance occurs between platforms, the highest signal should be prudentially used as output reported in the validation label.[4] Blood group O remains the last choice for ABOi plasma transfusion in recipients of group A, B and AB.

ABOi CP transfusion has been successfully implemented in at least one case in South Korea.[5] In the COVID-19 setting, the typical therapeutic dose under investigation is 200–400 ml, which is considerably lower than in the massive plasma transfusion setting where a larger volume of transfused ABOi plasma may pose a risk, but we reasonably expect a high degree of hesitancy from non-transfusion specialists who finally are legally responsible for patient treatment.

The issue of incompatible recipients has been accommodated by the US FDA, whose emergence investigational new drug (eIND) approach has never mandated ABO-compatibility for COVID-19 CP. Similarly, the expanded access program (EAP) led by Mayo Clinic has amended its initial protocol to state that *"ABO compatible convalescent plasma units will be transfused preferentially. If ABO compatible convalescent plasma is not available, investigators may follow their institution's guidelines for administration of incompatible plasma with respect to ABO mismatch, titer, and volume limits"* (https://www.uscovidplasma.org/pdf/COVID-19%20Plasma%20EAP.pdf).

The use of ABOi CP transfusion should be discussed in COVID-19 guidelines and included in medical education programs.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Focosi Daniele: designed the manuscript and wrote the first draft. Farrugia Albert: critically revised the manuscript.

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LETTER TO THE EDITOR

TRANSFUSION MEDICINE WILEY

Safety and efficacy of COVID-19 convalescent plasma in severe pulmonary disease: A report of 17 patients

Dear Sir,

To date, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 30 million people, resulting in over 900 000 deaths globally and counting.[1] Investigational therapies, including hydroxychloroquine/azithromycin and lopinavir/ritonavir, have been disappointing. Remdesivir shortened median recovery time by 4 days but led to a non-significant decrease in mortality.[2] Nevertheless, remdesivir is unlikely to be available on a large scale in the near future. Thus, immediate interventions to improve COVID-19 mortality represent a public health emergency. COVID-19 convalescent plasma (CCP) is a promising approach, whereby plasma carrying antibodies against SARS-CoV-2 from recently recovered donors is transfused into patients, conferring passive immunity in patients susceptible to having poor outcomes.

Passive immunity was first applied in a pandemic during the 1918 influenza outbreak, where recovered patients' sera successfully treated acutely ill patients, reducing fatality from 37% to 16%. The benefit was even more pronounced when convalescent plasma was infused within 4 days of decompensation, reducing the fatality rate from 59% to 19%.[3] In the SARS-CoV-1 epidemic, patients' overall survival improved from 12.5% to 17% when treated with convalescent plasma, with the highest benefit seen when plasma was infused within 14 days of symptom onset.[4]

Studies regarding the clinical use of CCP have been inconsistent due to the pressing need for immediate effective treatment during this pandemic. A Cochrane Review by Valk et al highlighted eight studies regarding the use of CCP.[5] Based on the wide spectrum of patients, small total number of treated patients and lack of an ubiquitous endpoint, the authors were unable to draw any concrete conclusions about overall mortality or clinical improvement with regard to CCP use. However, despite various differences, many studies demonstrated some improvement. An early study by Ye et al from Wuhan showed an improvement in chest computed tomography findings post-transfusion of CCP in six patients. It is worth noting that, in this study, only four of the six patients required supplemental oxygen via nasal cannula prior to transfusion (highest need was 5 L/min), and none were intubated. Original data from China showed that only 41% of symptomatic people required supplemental oxygen, of which 6% required mechanical ventilation.[6] Therefore, it is unclear whether those six patients treated with CCP would have progressed to more severe disease or recovered independently. Nonetheless, the Shen et al case series of five critically ill patients from the Shenzhen province highlighted successful extubation of all five patients following CCP infusion.[7] A clinical trial by Li et al noted that patients with severe COVID-19 infection who had CCP added to standard treatment, compared with standard treatment alone, did not demonstrate a statistically significant improvement in both time to clinical improvement and overall mortality.[8] However, this study had a significantly older patient population, as well as a delayed time from symptom onset to administration of CCP. In contrast, a recent clinical trial by Joyner et al demonstrated a mortality benefit between early transfusion of CCP, as well as higher antibody titres.[9] Recently, a propensity score-matched case-control study of 39 patients by Liu et al demonstrated a benefit in both clinical symptoms and overall survival.[10] Despite the uncertainty surrounding CCP, the food and drug administration (FDA) recently announced emergency authorisation use (EUA) for CCP.[11]

For COVID-19, the optimal timing and frequency of CCP infusion remains largely unknown. Similarly, the role of CCP in cancer patients, particularly those with haematological malignancies, remains unknown. Here, we describe the outcomes of 17 critically ill patients with COVID-19, including six with haematological malignancies, displaying varying ranges of severe illness and length of infection, who were treated with CCP with marked clinical improvement.

Thirteen donors with blood types O, A and B donated two to four CCP units each (200 mL per unit) 18 to 56 days following full recovery from COVID-19. Ten men and seven women between the ages of 24 and 81 years (mean 56) received CCP following informed consent (Data S1). All patients were diagnosed by a reverse transcription polymerase chain reaction (RT-PCR)-based technique with the exception of patients 2, 4, 11 and 13, who were diagnosed using the highly sensitive *clustered regularly interspaced short palindromic repeats* (CRISPR)-based qualitative COVID-19 assay, as detailed in Table S1.[12] Interestingly, these four patients had haematological malignancies and had multiple false negative RT-PCR results prior to the CRISPR diagnosis. Patients 1 and 12 also had haematological malignancies. Most patients had multiple medical comorbidities, and 14 of the 17 patients were treated in the intensive care unit (Table S1). The average time from illness to treatment with convalescent plasma was 12 days (range 4-41) (Table S1). Further patients' characteristics are summarised in Table S1.

At the time of CCP infusion, all patients were either mechanically ventilated (six patients), on non-invasive support with high-flow nasal cannula (four patients), on bilevel ventilation (one patient) or on nasal cannula (six patients). Using enzyme-linked immunosorbent assay (ELISA), we were able to determine the Spike protein IgG titres on

CCP units used to treat patients 5, 6, 9, 11, 12, 13, 14, 15 and 17 to be 1:1600, 1:3200, 1:3200, 1:800, 1:3200, 1:400, 1:1600, 1:6400 and 1:3200, respectively. The recent FDA EUA recommended an IgG titre of at least 1:250 in transfused CCP,[11] whereas a study by Salazar et al noted a reduction in mortality with CCP IgG titres greater than 1:1350.[13] Joyner et al noted a mortality gradient with better outcomes associated with higher titre and early administration.[9] The nine CCP IgG titres obtained in our study were all above the FDA recommendation of 1:250, whereas seven of the nine were above the 1:1350 mortality benefit seen by Salazar et al. Treatment was with a single unit of 200 mL of CCP given over 1 to 2 hours, with the exception of patients 4, 10 and 11, who received two units roughly 8 days apart (Table S1) due to severe immunosuppression, continual hypoxia with exertion and goal to discharge without oxygen. Patient 4 was on rituximab and steroids for chronic graft vs host disease following haploidentical stem cell transplantation, whereas patient 11 had T-cell acute lymphoblastic leukaemia, receiving lymphodepleting induction chemotherapy. Patient 10 was critically ill and had not responded to any other treatment (Table S1). Nevertheless, steady improvement in oxygenation levels was observed following each CCP infusion. No adverse events were reported in patients with the exception of a fever during CCP transfusion in patient 7, resulting in infusion of only 100 mL. Details of the treatment can be found in Data S1.

Patient disease progression and outcomes are summarised in Figure 1 and Table S2. Overall, of the six intubated patients, three were extubated between 1 and 13 days post-CCP infusion. The other 11 patients showed a dramatic decline in oxygen needs and did not require ventilatory support. Of the 17 patients included here, 2 patients, patients 3 and 10, died in the hospital (patient 3 died 2 days following extubation secondary to progression of medical comorbidities and the family's decision to transition to comfort care, whereas patient 10 died after developing an intraparenchymal haemorrhage resulting in complete brain herniation, after which the family transitioned to comfort care). Of the 15 survivors, 14 were discharged from the hospital, whereas 1 was extubated to tracheostomy. Two patients (patients 1 and 11) with advanced haematological malignancies died at home after being discharged off oxygen with home hospice.

Interpretation of the data could potentially be affected by the concomitant clinical trial enrolment of some patients. Patients 12 and 15 were enrolled in ACTT-1, a randomised, double-blind, placebo-controlled trial to evaluate the safety and efficacy of remdesivir in hospitalised adults diagnosed with COVID-19 (ClinicalTrials.gov Identifier: NCT04280705); patients 3, 15 and 17 were enrolled in REGN88, a randomised, double-blind, placebo-controlled trial to evaluate the safety and efficacy of sarilumab in hospitalised adults diagnosed with COVID-19 (ClinicalTrials.gov Identifier: NCT04327388), whereas patients 1, 2, 5, 6 and 10 received remdesivir outside of a clinical trial context, and patients 5, 6, 8, 9, 10, 15 and 16 received dexamethasone. The blinded nature of the ACTT-1 and REGN88 trials make it impossible to determine whether patients 12 and 15 received remdesivir or placebo and whether patients 3, 15 and 17 received sarilumab or placebo. Thus, it is difficult to determine whether the evaluated medication played any role in the observed clinical improvement of these patients. Patients 4, 7, 11, 13 and 14 received no additional COVID-19-directed therapies of CCP infusion.

The mechanism driving improvement in patients receiving passive antibody therapy is currently unclear. Antibodies are known to work by destroying viral particles via complement activation; opsonisation; or through neutralising the virus by blocking attachment, cell entry or uncoating inside the cell cytoplasm. These mechanisms imply that the optimal timing for CCP infusion is early in the disease process when the virus is still actively replicating. Later phases of COVID-19 are characterised by widespread tissue damage secondary to uncontrolled inflammation, with minimal to no viral detection, which challenges any role of CCP. A report from the Henan province challenged the benefit of CCP if administered late in the disease course of patients with

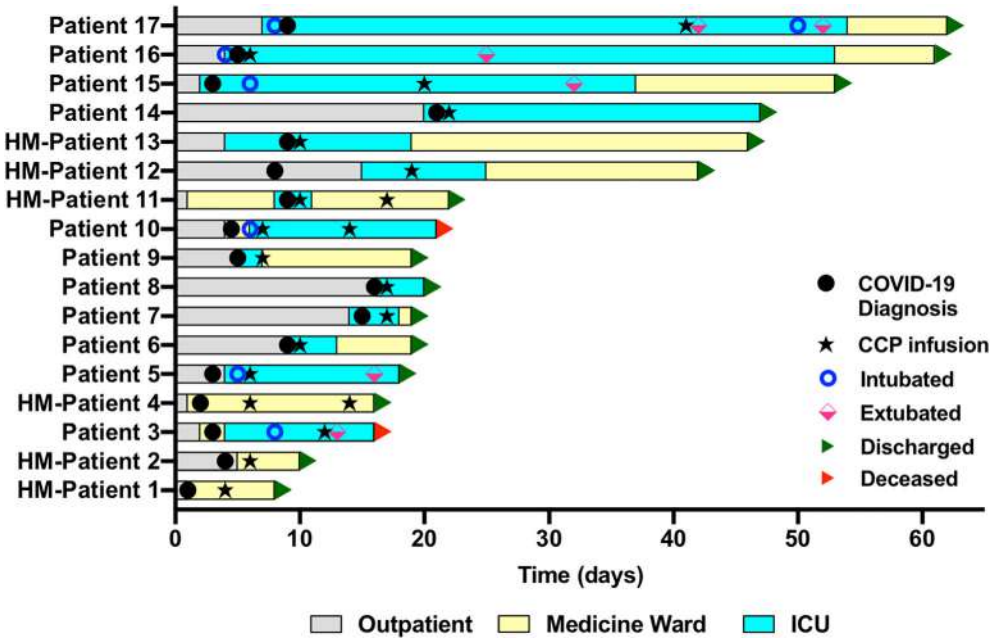

FIGURE 1 Overall outcomes of COVID-19 convalescent plasma therapy. Swimmer plot detailing the clinical course for each patient. Outpatient time denotes time of symptoms prior to presentation to the hospital. ICU, intensive care unit [Color figure can be viewed at wileyonlinelibrary.com]

severe COVID-19.[14] Although all of our patients showed improvement following CCP, the most impressive effects were seen in patients 2, 4, 5, and 7 and 13 when CCP was administered early in their disease course (days 5, 5, 5, 17 and 5 of disease, respectively). Two CCP infusions were used in three patients, and in each case, we saw an incremental improvement in patient oxygenation. Two patients were considered lymphodepleted secondary to ongoing therapy related to their underlying haematological malignancy. Therefore, those patients were less likely to mount an appropriate humoral response to SARS-CoV-2 due to B-cell depletion. Thus, a second CCP infusion was likely beneficial. Patient 10 did not show improvement following the first unit; thus, a second unit was given, with rapid improvement in oxygenation following the second unit.

Although a randomised controlled clinical trial is needed to determine with certainty the role of CCP in treating severe COVID-19, our limited data represent a sign that CCP is safe and may be efficacious in COVID-19; this underscores the potential role for passive immunity in this disease.

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CONFLICT OF INTEREST

The authors have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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LETTER TO THE EDITOR

TRANSFUSION MEDICINE WILEY

Kinetics of anti-SARS-COV2 spike protein IgG and IgA antibodies at 4°C: Implications for convalescent plasma stability

Dear Editor,

Both diagnostic laboratory samples and convalescent plasma (CP) are usually preserved frozen at temperatures below −25°C for long-term storage. Nevertheless, investigating the stability of anti-SARS-CoV-2 antibodies at refrigerator temperature (+4°C) is of paramount importance in logistical settings where freezers are not available, or when usage cannot be accomplished within time limits after thawing imposed by law (usually 1–5 days, to preserve stability of labile clotting factors, as currently recommended by the European Commission) and plasma refreezing is not allowed by law.

We repeated anti-SARS-CoV-2 Spike (S) protein S1 subunit IgG and IgA testing with Euroimmun ELISA anti-SARS-CoV-2 kits (Euroimmun Medizinische Labordiagnostika AG, Lubeck, Germany) on 24 residual diagnostic serum samples stored at +4°C for variable amount of time after the initial determination, without any freeze/thaw cycle. The study was approved by the internal review board (protocol 17437/2020). Statistical analyses were run using Spearman's Rho test on SPSS software v.23. The result plotted in Figure 1, lower panel, shows that both IgG and IgA levels (expressed as ratio between sample and calibrator) linearly declined by up to 30% at day 95. There was no correlation between intensity of reduction and baseline antibody levels (Figure 1, upper panel), and, as per manufacturer's instructions for user, the intra-laboratory coefficient of variation for the assay run in the same lab at different timepoints is lower than 8% (data not shown). Preliminary reports by Stadlbauer et al. [1] showed stable IgG levels in 15 plasma samples for up to 42 days using an in-house ELISA targeting the Spike protein: no details were disclosed regarding the kinetics of different immunoglobulin isotypes or the exact domain targeted by the assay. Our findings were instead achieved with a commercially available assay targeting the S1 subunit of the Spike protein: we extend the observation to 100 days, and for the first time report the kinetics of IgA isotype.

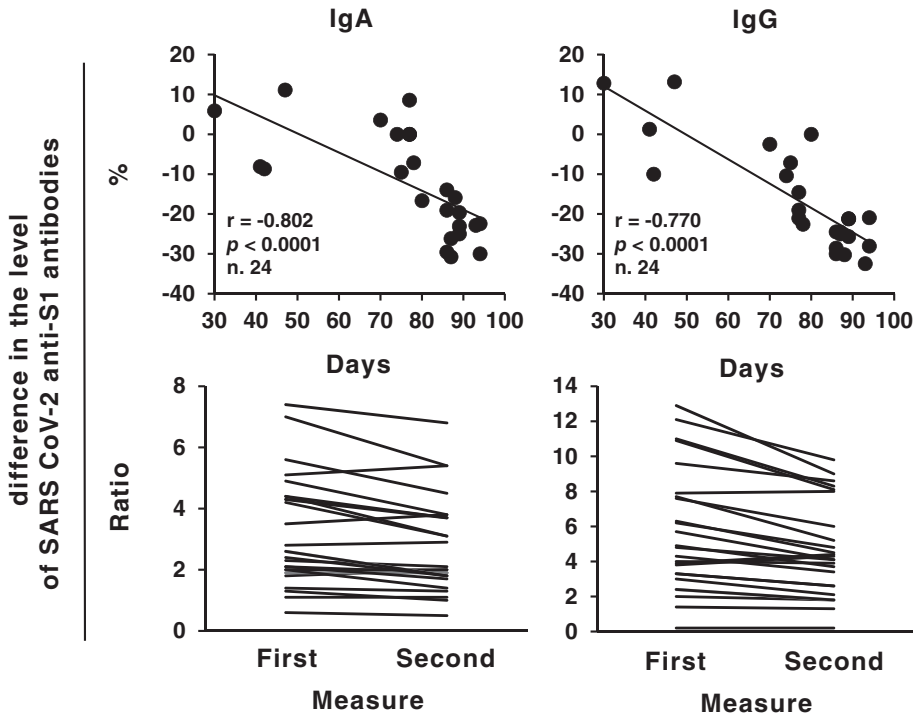

FIGURE 1 Kinetics of IgG and IgA antibody levels against SARS-CoV-2S1 subunit expressed either as % difference (lower panel) or absolute values of ratio (upper panel)

Previous studies on antibody stability in serum or plasma at 4°C are very scarce and old and mostly related to whole blood cells units, where anti-cytomegalovirus IgG, IgA and IgM mean decrease for the fluorescence signals at week 8 was 1.2% both in serum and plasma.[2] Hodgkinsons et al. reported that IgG, IgA and IgM to epitopes from various viruses can be measured reliably from serum and plasma 4°C for up to 6 days before processing.[3] Similarly, anti-CMV IgG were stable at day 14 in packed red blood cell units stored at 4°C.[4]

The main limitations of our study are usage of serum rather than plasma samples (but no variations in antibody levels are seen between the two matrices in our experience) and reliance over paired testing of different sera rather than on sequential multiple testing of the same serum. Additionally, the implications for CP therapy, whose efficacy is largely based on neutralising antibody (nAb) levels, should be better assessed using a virus neutralisation test: nevertheless, receiver operating characteristic curve analysis showed Euroimmun ELISA area under the curve outperformed six different in-house ELISAs and pseudotyped microneutralization test at predicting nAb titres >1:100 against the native isolate. A cut-off value of 9.1 S/CO in the Euroimmun ELISA identified 65% of donations above the 1:100 nAb threshold, with no false identification of donations below this nAb threshold.[5]

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

Daniele Focosi and Fabrizio Maggi designed the study and performed statistical analyses. Giovanna Moscato performed the serological assays. Mauro Pistello approved the final version.

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LETTER TO THE EDITOR

TRANSFUSION MEDICINE WILEY

Absence of SARS-CoV-2 viraemia in a blood donor with COVID-19 post-donation

The recent COVID-19 pandemic caused by SARS-CoV-2 has posed significant challenges to the healthcare system and the safety and sustainability of blood supply. Most blood centres face a shortage of blood supply because of much lesser donation due to lockdown or stay-home requirements, although the reduction of non-emergency clinical services contributes to a lower blood transfusion demand.[1] Although previous experience with similar coronaviruses suggested that the transfusion transmission risk is theoretical, SARS-CoV-2 viraemia was found in symptomatic and asymptomatic patients[2] and archived samples from blood donation.[3] On the other hand, Kwon et al reported the absence of viraemia in archived samples from seven persons who donated blood 6 to 16 days prior to COVID-19 confirmation.[4] Recently, Chang et al updated, using a large-scale blood donation screening study in Hubei, that no SARS-CoV-2 viraemia was found in 98 342 donations between 9 February and April 30, 2020[5].

To further the understanding on whether viraemia is present in asymptomatic individuals, here, we report a blood donor who donated blood 7 days prior to COVID-19 confirmation. The individual gave a unit of whole blood on 5 July after passing the latest donation requirement[1] and was instructed to report any symptoms developed post-donation. The collected blood was processed into red cells, platelets and plasma. Platelet was issued to a patient with haematological disease on 9 July, whereas red cells and plasma were stored in the blood storage fridge. On 13 July, the donor presented to the hospital with upper respiratory tract symptoms that began on 12 July and was confirmed to have COVID-19. He had fever, cough and headache since 9 July, that is, 4 days after blood donation. Clinician notification, product recall and quarantine of unused blood components were then performed immediately. Archived samples from the index blood donation were sent to three laboratories to test for SARS-CoV-2 RNA, which were negative. At the same time, the recipient was followed up but remained asymptomatic and negative for SARS-CoV-2 RNA. Table 1 summarised the SARS-CoV-2 RNA results of different donor and recipient samples. Finally, as advised by Department of Health, a limited tracing was conducted for the four collecting staff members who served the donor on 5 July, and they were all negative.

In conclusion, we did not detect SARS-CoV-2 RNA in the blood donor's sample (by three testing platforms) 7 days prior to confirmation or 4 days before onset of symptoms. This suggests that transfusion transmissibility of SARS-CoV-2 remains theoretical. As a routine blood donation screening test for SARS-CoV-2 RNA is not available nor recommended by the World Health Organization, the blood safety measures against COVID-19 continue to be secured via a number of pre- and post-donation means.[1]

TABLE 1 Summarized the SARS-CoV-2 RNA results on donor's and recipient's samples

Sample	Donor	Recipient
Respiratory specimen	Nasopharyngeal and throat swab (12/7/2020)	Nasopharyngeal swab (13/7/2020)
	Lab1: Positive	Lab1: Not detected
	Lab2: Positive	
EDTA whole blood	From blood donation archived sample	Taken on 13/7/2020
	Lab1: not detected	Lab1: not detected
	Lab2: not detected	Lab2: not detected
	Lab3: not detected	Lab3: not detected
EDTA plasma	From blood donation archived sample	
	Lab1: not detected	
	Lab2: not detected	
	Lab3: not detected	

SARS-CoV-2 RNA Testing platform:
Lab1-Xpert® Xpress SARS-CoV-2 (Cepheid, Califonia, USA).
Lab2-in house RT-PCR.
Lab3-LightMix® Modular SARS and Wuhan CoV E-gene kit (TIB Molbiol, Berlin, Germany).

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CKL decided upon and wrote the manuscript. JNSL collected donor and donation information and reviewed the manuscript. PC, DCL and KKWT performed the molecular test and reviewed the manuscript. DNCT reviewed the manuscript.

CONFLICT OF INTEREST

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