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REVIEW

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Physician autonomy and patient rights: lessons from an enforced blood transfusion and the role of patient blood management

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This article provides an ethical and medico-legal analysis of ruling no. 465 of 30 May 2018 issued by the Court of Termini Imerese (Palermo) and confirmed on appeal on 11 November 2020, which, in the absence of similar historical precedents in Europe, convicted a medical doctor of a crime of violent assault for having ordered the administration of a blood transfusion to a patient specifically declining blood transfusion on religious grounds. We analyse the Court's decision regarding the identification of assault in performing the blood transfusion and its decision not to accept exculpatory urgent 'necessity' as a defence. In addition, we present an updated revision of the current standard of care in transfusion medicine as well as the ethical principles governing the patient's declining of transfusion. In doing so, we highlight that respect for the patient's self-determination in declining transfusions and respect for the professional autonomy of the doctor protecting the safety and life of the patient could be equally satisfied by applying the current peer-reviewed evidence.

Key words: ethics, Jehovah's Witness, patient blood management, transfusion medicine.

Introduction

A frequently discussed topic in clinical legal medicine is the right of patients who have mental capacity or have an advanced healthcare directive. Choice of specific medical interventions can be either accepted or declined by patients. The choice of declining blood transfusions by Jehovah's Witness patients is a context for such discussion. In recent decades, in many parts of the world, declining transfusion has been the focus of important reflections on the rights of patients and a frustrating event for legal and medical professionals. For the latter, it seemed impossible to reconcile these patients' rights for self-determination and the ethical purpose of medical practice to provide the best care to safeguard the patient's life and safety. Numerous published examples of such cases are in the literature [1,2]. However, there are no cases in Europe that have been detailed in the scientific literature in which the administration of a blood transfusion has been considered as an assault on a patient perpetrated by health professionals. In this article, we discuss a Court's decision in Italy to convict a medical practitioner of criminal assault for administering a blood transfusion to a patient who specifically declined transfusion under any circumstance. The court did not accept exculpatory urgent 'necessity' as a defence. The case is analysed, and an updated review into the current standards of care in transfusion medicine and patient blood management is presented. This landmark ruling received significant media coverage in Italy in 2018 and 2020 and has broader relevance for clinical medicine.

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The case report

On 6 November 2010, a 24-year-old woman was admitted to the emergency department of the 'Cimino' Hospital in Termini Imerese (Palermo, Sicily). The patient was in her 14th week of gestation and from the beginning of the third month of pregnancy experienced vomiting episodes that were difficult to manage resulting in 3–4 kg of weight loss. Her medical history comprises a previous term pregnancy requiring Caesarean section as well as a history of episodes of tachycardia. The young woman was hospitalized for further management, during which she declared herself as a Jehovah's Witness. She expressed her intention to decline blood transfusion of any blood components (red cells, platelets and plasma), while accepting all patient blood management strategies aimed at the management of her own blood including the administration of blood derivatives such as factor concentrates. On 13 November 2010, the woman was discharged with the recommendation to take folate and supplements containing magnesium and potassium; the state of health of the fetus was satisfactory. On 21 November 2010, the patient was once again admitted to the emergency department of the 'Cimino' Hospital for epigastric pain and vomiting, followed by admission to the Gynecology and Obstetrics department. She again confirmed to be a Jehovah's Witness and reiterated her refusal of any transfusion of blood components. Investigations during hospitalization included an ultrasound examination showing the gallbladder contained abundant biliary sand and micro-gallstones. The patient was initially treated conservatively, but in the following days, symptoms of pain and vomiting reappeared and on 28 November 2010 the onset of hyperbilirubinaemia were observed. The clinicians determined the need to perform a cholecystectomy. Blood tests were performed, showing a haemoglobin level of 12.8 g/dl. On the morning of 1 December 2010, laparoscopic surgery was performed. The medical record does not report the time of start and end of surgery; however, the findings included in the medical record show that shortly after the end of surgery, a significant blood loss occurs both from the drains and from the surgical access points. Despite this, laboratory checks and gynaecological examination were not performed until several hours later. A gynaecological consultation was carried out at 3:00 p.m. and revealed fetal bradycardia (83 bpm). Atropine was prescribed and administered; a blood count and a reassessment after one hour were scheduled. Around 4:00 p.m., the patient was hypotensive and reoperation revealed active bleeding at the original surgical incision site, which was controlled via affixing parietal stitches and there was no further blood loss. The following morning, erythropoietin 40 000 U and intravenous iron 1 g/day were administered. The gynaecological consultation around 6:00 p.m. confirmed fetal death. On the morning of 3 December 2010, the haemoglobin level was 5.3 g/dl. A subsequent haemoglobin three hours later was 5.8 g/dl. However, 30 min earlier at 11:00 a.m. the medical record documented that, in light of the haemoglobin of 5.3 g/dl and in view of the patient's religious commitment to decline blood transfusion, the physician considered informing the magistrate on duty at the Court of Termini Imerese, in order to proceed with an emergency blood transfusion. At 12:00 p.m., two nurses, following a direct order from the medical director of the clinical unit, and after making the relatives leave the hospital room, removed the ongoing infusion from the venous access on the upper right limb and attached a unit of packed red blood cells. The patient immediately demanded an explanation from the nurses involved, and in response, she was told that the blood transfusion had been authorized by the magistrate and she must not object and resist. The patient strongly expressed her dissent and attempted to thwart the actions of the nurses by moving her limbs; intervening staff forcibly immobilized her, and after some time, the patient desisted from trying to free herself. The staff made it clear not to think about removing the infusion apparatus in that 'there would have been serious consequences'. The nurse coordinator, present during the transfusion that was administered by another nurse, later stated during the trial that 'the woman was very sad and cried'. Subsequently, the patient was yet again transfused with two further units of packed red blood cells. (Fig. 1 presents a summary of key hospitalization events.)

It should be noted that the medical director reported to the patient that he would transfuse her because he had received authorization from the magistrate by telephone. The physician had requested the authorization from the magistrate, emphasizing that the life of the woman and of the fetus were in danger. However in actual fact, at the time of the call, the fetus had already died. The magistrate subsequently reported that he had been contacted by telephone about the matter but denied granting any authorization. On 7 December 2010, a labour induction abortion was performed, and on 9 December 2010, the patient was discharged home.

The patient subsequently filed a complaint, and the Public Prosecutor investigated the clinicians who took part in the various events already described. The investigation included the potential crime of culpable abortion relating to incorrect surgical procedures that seemingly caused the death of the fetus and the crime of assault pursuant to Art. 610 of the Italian criminal code for having voluntarily forced the patient to undergo the blood transfusion that she had repeatedly and 'stubbornly' (as cited in the medical record) refused.

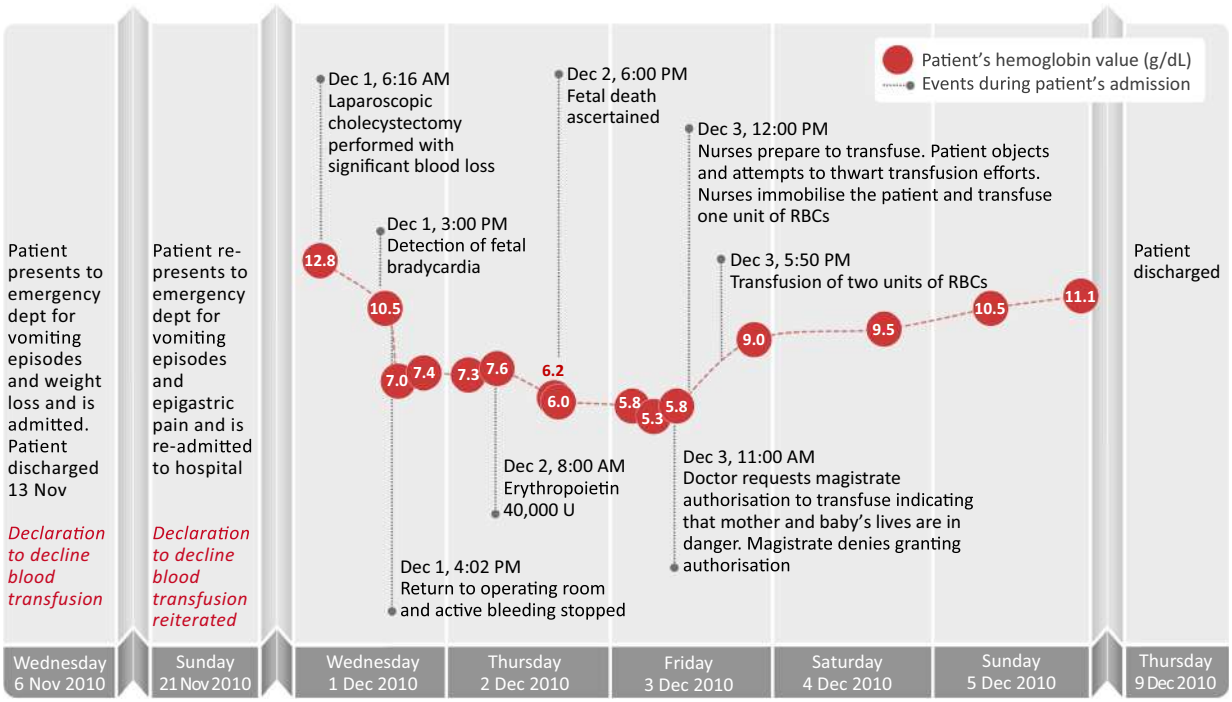

Fig. 1 Summary of key hospitalization events. [Colour figure can be viewed at wileyonlinelibrary.com]

The legal ruling

At the end of a long trial, ruling no. 465 of 30 May 2018 was delivered, absolving the defendants of the offence of culpable abortion but convicting the medical director of the clinical unit for criminal assault by having ordered the blood transfusion that was declined by the patient. This ruling was later confirmed on appeal on 11 November 2020. In this publication, the focus is on this latter crime and not issues relating to the fetal death. With reference to the crime of assault, the ruling is of specific interest as it represents a legal precedent in European jurisdictions, in which coercion to undergo a blood transfusion in spite of the dissent expressed by a competent adult patient satisfies the legal definition of a crime of assault in criminal terms.

The ruling provides a comprehensive summary for the legal basis and legitimization of the medical and surgical activity in the Italian legal system, as well as of the issue of informed consent, recalling the most significant Court rulings on the subject, issued in Europe.

Informed consent as the legal basis for medical and surgical activity

The current ruling cites and acknowledges the conclusions of a previous case of the Court of Cassation, Italy's highest judicial body. They reported that consent given by a patient is an actual assumption of lawfulness of the activity performed by the doctor who administers the treatment, to whom a general right to treat irrespective of the will of the patient cannot be ascribed. In this regard, it maintains the principle of self-determination; the will of the patient is the ultimate boundary (inalienable and enduring) of the exercise of medical practice. Indeed, the criterion defining and dictating the doctor–patient relationship is that of the free availability of the benefit of health for the patient in possession of his/her intellectual and decisional capabilities, according to a full autonomy of choices. This can also entail the loss of life that must always be respected by healthcare professionals. Therefore, the court concluded every individual has the right to choose between the 'salvation of the body and the salvation of the soul'.

The Ruling of the Court of Termini Imerese also refers to various international sources such as the Convention on the Rights of the Child, signed in New York on 20 November 1989; the Convention on Human Rights and Biomedicine, signed in Oviedo on 4 April 1997; and the Charter of Fundamental Rights of the European Union, proclaimed in Nice on 7 December 2000.

Blood transfusion in the absence of consent and necessity

The ruling of the Court of Termini Imerese convicted the doctor for the crime of assault and determined not

to accept the argument of the defendant who, in his defence, invoked having acted out of necessity. In Italian law, the defendant can claim exemption from conviction if the medical intervention was performed in order to protect others from the imminent risk of serious personal injury. In this case, neither the presence of risk factors nor inadequate physiological compensation for anaemia or abnormalities in the patient's vital signs was documented in the medical record. It is indicated in the ruling that, although the laboratory findings revealed a low haemoglobin value (5.8 g/dl at the time of transfusion, after a *nadir* of 5.3 g/dl), the patient was not in a life-threatening condition. The court ruling includes clinical and laboratory data from which it may be inferred that physiological compensatory mechanisms for anaemia were present and were responding adequately for the reduced haemoglobin levels. To this end, the expert witnesses for the plaintiff argued that haemoglobin results 'cannot constitute the sole parameter to be considered in the decision to carry out a transfusion, nor can it become an irrational element of psychological terror in clinical decision-making'. In addition, case reports of patients, from the international scientific literature, with particularly low haemoglobin values but displaying adequate compensation were presented [3,4].

The Court determined that the patient was not in a life-threatening emergency state; however, even if the patient's anaemia had deteriorated to engender the presumption of imminent death only avertable with transfusion, it would still not have been possible to deem exculpatory necessity applicable in pursuance to Art. 54 of the Criminal Code. The patient, fully aware of the possible repercussions of her decision, had expressly and repeatedly denied her consent to the blood transfusion.

The ruling indicates that 'indeed, no so-called compulsory emergency aid is provided for in our legal system, able to extend beyond the contrary intention of the subject concerned, given that the limit of exculpatory necessity, in the light of the above-mentioned constitutional principles, is strictly limited to the premise whereby the patient is unable - due to his/her condition – to lend his/her dissent or consent… the doctor cannot, therefore, impose the health treatment that s/he deems life-saving upon any patient who knowingly and lucidly refuses it'.

This stresses that the only case in which it is possible to consider exculpatory necessity as a valid defence is that in which the patient is in a situation of incapacity of manifesting his/her will and has not previously expressed any preferences regarding the clinical picture entailing imminent and present risk of serious personal injury. The same indication is also acknowledged by the new law that in Italy regulates the consent to the medical act and the anticipated treatment provisions of 22 December 2017 no. 219, in article 1, paragraph 7, stating that 'in an emergency or in emergency situations, the doctor and the members of the health team ensure the necessary treatment, in compliance with the wishes of the patient should the latter's clinical conditions and circumstances allow for their implementation'.

Unwanted blood transfusion and grounds of the crime of assault

For the crime of assault to exist, the ruling indicates two key elements must be clearly identified: (1) violent conduct; and (2) the event, namely what the person is forced to suffer against his/her will. In this case, the violent conduct first materialized in all the manoeuvres to introduce the peripheral venous catheter into the vein and thus inside the patient's body. The event was implemented via the introduction of blood inside the patient's body and via the haemotransfusion. The doctor was not faced with an unexpected emergency; on the contrary, he planned the transfusion well in advance despite the patient's repeated denial.

The ruling identifies the requisite of violence as any suitable means to quash the freedom of determination and action of the injured party and that the interest protected that describes the crime is moral freedom, to be understood as the freedom of spontaneous self-determination. The law in question protects the psychological freedom of the individual and represses coercion, explicable in myriad forms used to exert pressure on the will of others, preventing their free choice. In the crime in question, the transfusion, combined with all associated preparatory activities, is envisaged as an act of violence against the patient refusing it.

Aspects of a bioethical nature

The Court of Termini Imerese reiterates and determines the legal principle based on the personalist conception of humans: the will of the patient as the ultimate limit of the exercise of medical activity, and more broadly health care, in which the criterion governing the doctor–patient relationship, as well as the healthcare professional–patient relationship, coincides with the free availability of the benefit of health for the patient in possession of his/her intellectual and cognitive capabilities, according to a freedom of choice that can imply the sacrifice of life itself and that must always be respected by health professionals. The Court also underlines that 'necessity', if present, cannot be used as a 'strategy for undermining the rights of every person' [5].

The clinical case presented contains important teachings and reflections useful to the entire scientific and legal community. From a moral and ethical point of view, the doctor has acted on the basis of the principle of his own autonomy in making decisions in terms of appropriateness and usefulness of the health treatments to be performed on the patient. However, this principle did not take into account the limits imposed by the respect, no less important, of the patient's self-determination. The doctor is necessarily autonomous as regards the strictly technical-scientific field of patient care and management but cannot ignore considerations relating to the patient's lifestyle, values, needs and aspirations, who exercises his own autonomy in health choices.

In the specific case, it must be considered that the doctor acted on the basis of an inappropriate technical-scientific autonomy, as the blood transfusions he imposed on the patient were not based on scientific evidence and therefore were in no way justifiable by failing to comply with the rules that define autonomy. This word is made up of two ancient Greek terms, αυτός and νόμος (which mean 'own' and 'rule'), and expresses the competence to operate according to the 'proper rules' of the profession. According to a certain culture, still quite widespread, a paternalistic attitude towards the patient persists, according to which the doctor is the good father who knows the good of the patient-children and acts accordingly.

The shift from a paternalistic doctor–patient relationship to one of shared decision-making has been a slow and problematic one dating as far back to Hippocrates. The traditional view of this relationship became well imbedded for two millennia on the basis that a patient should be 'protected' from knowing the truth about their disease and its likely outcome. Hippocrates stated: 'Reveal nothing of the patient's present or future condition'. The rationale was that the fully informed patient may not be able to absorb, understand and psychologically cope with the information. Indeed, the meaning and origin of the word 'patient' exemplified the doctor/patient communication and interface. The word patient is from the Latin verb pati, to suffer. The words passive and passion have the same origin, and over time, the term patient has come to mean somebody who suffers their disease with calmness and composure and having patience. It was implied that appropriately informing the patient might cause stress or worse, harm the patient.

This paternalistic relationship between the doctor and patient was a modus operandi that excluded truth telling and informed patient consent. This almost implied the doctors' service was a commodity that is assumed to be fit for purpose and 'buyer beware' (caveat emptor). The patient was expected to tacitly and unquestionably trust their doctor.

This type of approach is no longer tolerable in any way from an ethical point of view, in an evolved society where the person is at the centre of care and informed decisions about his life and his future, even up to the extreme consequences.

The paternalistic doctor distorts and mystifies the principle of charity [6], under the illusion that the patient's good may come from the doctor's wisdom and not from the evaluation of the interested party, who is free to avail himself of the support of others, without, however, others being able to arrogantly intrude on his decision-making process or even, as in the case under discussion, to completely replace him. In this case, paternalism even led the doctor who carried out the transfusions not to apply the principle of non-maleficence. In fact, through the transfusion he violated the intimacy (by creating a constraint) and the dignity (by disregarding religious beliefs) of the person; it then determined, as a consequence, a psychological insult and damage. To further clarify, it is worth considering the religious aspects of the case.

The religious component of identity also assumes importance, whenever the transfusion is offered to a person who practices a religion that prescribes rules of conduct that prohibit it. These are binding norms that do not lend themselves to personal re-elaboration and that make the transfusion, if practiced, a permanent treatment that damages the religious identity of those who suffer it. In these cases, the irresolvable compromise of religious identity affects personal identity as a whole, since it is unthinkable that, in these irreversible circumstances, the person can be able to process the bodily aspects of his own identity compromised by the extraneous biological mass.

The compromise of religious identity, in addition to preventing the elaboration of bodily identity per se, intrinsically compromised [7], also has an impact on social and family identity: the first with reference to the social group of those who practice the same religion and the second with particular (but not exclusive) reference to family relationships when one or more members of the family belong to the same creed. In these cases, therefore, the transfusion carried out against consent leads to such a compromise of the identity, that is, involving different aspects of the same: religious, corporeal, family, social.

The doctor who practices transfusion, indifferent to these consequences, expresses lack of respect for the person as the bearer and expression of an intrinsic value and therefore also damages their dignity. If this lack of respect is public, because it is made known, with concrete acts, both to relatives and to those who accompany the person and follow the human story, the damage to dignity is also perceived and suffered by the family and social context.

Patient blood management: a solution to manage the patient's blood with better outcomes

Both the patient's and the doctor's best interests could have been satisfied by applying current peer-reviewed evidence. In fact, the issue of minimising and avoiding blood in medicine and surgery is a topic of great interest, not only for patients who decline transfusions but also for the population at large [8–10].

For example, since 2002 the World Health Organization (WHO) has recommended 'transfusion alternatives' where possible to avoid exposing patients to the risks associated with blood transfusions [11]. More recently, in 2010, the World Health Assembly endorsed patient blood management (PBM) as the standard of care. PBM being defined as 'an evidence-based bundle of care to optimize medical and surgical patient outcomes by clinically managing and preserving a patient's blood' [12].

The principles and practical application of PBM initiatives first took place when assisting Jehovah's Witness patients; however, the methods and techniques applied would later benefit all patients [13]. For example, in 2008 the Government of Western Australia successfully implemented a state-wide PBM programme which led to significant reductions in transfusions and concurrent improvements in patient outcomes. As a result of the reduction in transfusions, tens of millions of dollars were saved [14]. In March 2017, the European Commission introduced a Guide intended to implement PBM as a standard of care throughout the European Union [15–16].

Patients treated according to the principles of PBM have their own blood optimized prior to surgery and their blood loss minimized during surgery. With the application of these proactive approaches, the patient may not reach a restrictive transfusion threshold, minimising or eliminating the administration of blood products. Even when it is not possible to act prior to surgery, strategies for the management of postoperative anaemia after major surgery remain applicable, [17] and the literature has many examples of complex interventions performed without using blood transfusion, with results that are similar, if not better, than those of transfused patients [18–31].

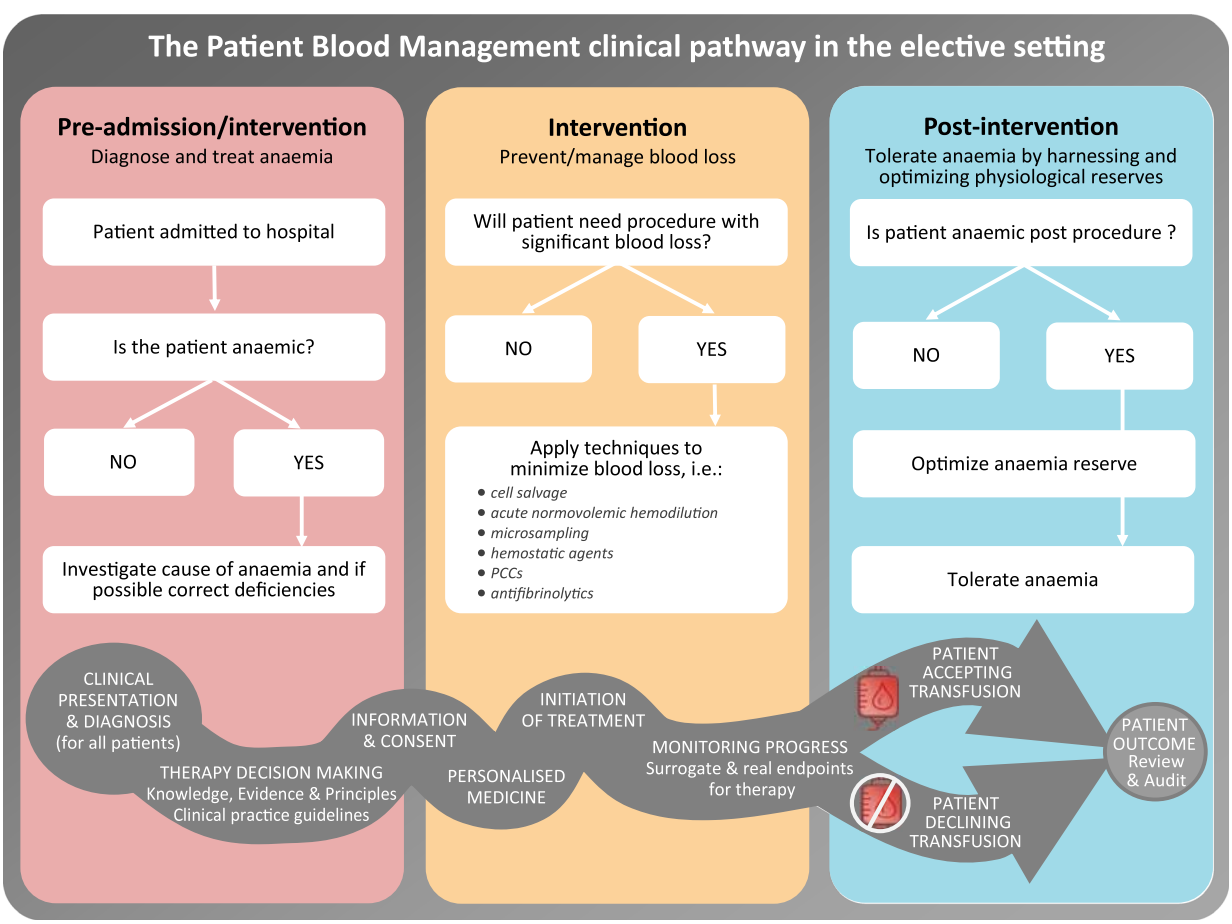

Fig. 2 The patient blood management clinical pathway in the elective setting. [Colour figure can be viewed at wileyonlinelibrary.com]

Patient blood management is not a specific medical intervention or an alternative to allogeneic blood transfusion; it is sound evidence-based clinical practice. Minimising and avoiding blood transfusion is a corollary stemming from successful PBM. The principles of PBM are based on a robust understanding of core physiological and pathophysiological aspects of haemopoiesis, haemostasis and oxygen transport. The haematological and immune systems are the fundamental physiological infrastructure to maintain the body's homeostasis, responses to injury and tissue repair. These systems have considerable adaptive reserve responding to deficiencies or increased demands. It is the responsibility of all clinicians who have primary accountability for the quality and safety of a patient's clinical management to ensure the patient's blood is managed appropriately [32–34]. It is no longer acceptable or ethical to continue adopting a *laissez-faire* approach to the assumed benefits and known risks of allogeneic blood transfusion. Blood transfusion can no longer be regarded as default therapy in the context of clinical uncertainty. Managing a patient's own blood appropriately is now the clinical decision-making focus, based on the three pillars of PBM [35–36]. If after following the principles of PBM, evidence-based medicine suggests an allogeneic blood transfusion is appropriate, the consent process will still require a doctor to discuss the risk and benefits as well as any possible alternatives [37]. Where alternatives are not available, or the risk/benefit equation is not clear, and the patient does not decline transfusion, evidence is needed that the patient's ultimate clinical outcome is likely to be improved. Surrogate endpoints are necessary for many clinical interventions, but it is necessary that these immediately measurable surrogate endpoints causally correlate with better long-term patient outcomes.

In circumstances in which a patient declines allogeneic blood transfusion, it is imperative that a pre-emptive strategy is in place. This management strategy should be initiated and documented for such patients at the point in their clinical course that blood transfusion would normally be regarded as standard of care. An argument can be made that all patients should be managed on this basis up until blood transfusion is considered appropriate management (Fig. 2).

In the case reported, if PBM and the legal empowerment of the patient had been the *modus operandi* from the initial admission, the outcome for the patient and the doctor would have been quite different. To use current *lingua franca,* a lose–lose outcome could have been a win–win outcome.

Conclusions

The ruling concerning the Termini Imerese case has roused much media interest in Italy. It is based on the fundamental principles of freedom and self-determination universally acknowledged in the Western world for adults with mental capacity. The same situation, related to therapeutic choices in the case of potentially life-saving therapies, can manifest itself in other situations, which are now regulated in Italy by law No. 219 of 2017 also referred to as the 'Living will' providing for the respect of the patient's current will, as well as any anticipated treatment provisions, thus guaranteeing the right of self-determination of the adult subject, possibly even expressing the declining of life-saving treatments, in any clinical situation. In the case of declining transfusion, modern practice including PBM, when correctly applied, has greatly minimized the clinical problems and opened up a new perspective for the application of the principles of legal medicine in the field of medical professional liability.

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A. Shander has received consulting, research and lecture fees, as well as expenses, from Masimo, CSL Behring, IL Werfen, Merck, Vifor Pharma and HbO2 Therapeutics. Other authors declare that they have no conflicts of interest.

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ORIGINAL PAPER

Blood donation in times of crisis: Early insight into the impact of COVID-19 on blood donors and their motivation to donate across European countries

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Vox Sanguinis

Background In this survey, we aimed to provide early insight into the impact of COVID-19 on blood donors and their motivation to donate during the crisis.

Study design and methods We asked representative samples in 7 European countries (Denmark, France, Germany, Italy, Portugal, the Netherlands and the UK) about their blood donation activity and motivation to donate using an online survey. We analysed donor turnout during the COVID-19 period descriptively and using logistic regression.

Results Of the 7122 people that responded to the survey, 1205 (16·9%) blood donors were identified, with 33·8% donating during the first 4–5 months of the COVID-19 period. We observed that around half of donors donated less than normal. The vast majority of donors that did donate made a special effort to do so in response to COVID-19. The majority of donors were also not aware of their blood being tested for COVID-19 antibodies. Although the perceived risk of infection among all respondents whilst donating blood was relatively low, those who anticipated a high risk of infection were much less likely to donate (OR = 0·540; *P*-value = 0·006). Furthermore, those that were adherent to COVID guidelines were also less likely to donate (OR = 0·583; *P*-value = 0·000).

Discussion We suggest that blood collection services consider specialist campaigns that focus on the altruistic motivation of donors during the crisis and that they continue to communicate the additional safety measures in place with the aim of reducing the fear of infection whilst donating blood.

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Introduction

The COVID-19 pandemic has caused an unprecedented impact on blood transfusion and collection with large-scale disruption to both the supply and demand for blood. Early evidence suggests European countries and across the world have experienced initial drops in whole blood donations, despite centres implementing extra safety measures and public appeals across Europe to encourage continued donation [1–7]. Research from Hong Kong and China has suggested that anxiety and fear of contracting COVID-19 were deterrents to donating blood [7, 8], which is consistent with findings from studies on the SARS and avian flu outbreaks [9, 10]. Falling donations have been partly mitigated in the short term by the postponement of elective surgeries, but future demand remains

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unpredictable and is dependent on how the pandemic evolves [1, 11]. Therefore, maintaining blood supplies remains an important public health concern during the pandemic. In the past, donors have responded well to public appeals to donate in situations of perceived exceptional need, such as the September 11 attacks, mass shootings in the United States and bush fires in Australia where large influxes of donors in short periods of time were documented [11, 12]. Such reactionary spikes have already been observed in some settings across Europe and in Brazil in response to public calls to donate [3, 13–15]. However, motivations that drive such responses may wane over time and especially so if driven by first-time donors [12].

Large interruptions to donation activity may have stark consequences for healthcare systems and should be avoided by careful tracking of the supply and demand of blood during these uncertain times. It is therefore essential to gain an initial perspective on the impact of COVID-19 on blood donors and an understanding of the key aspects of their motivation to donate (or not donate) during this crisis. The aim of this paper was to provide early insight into blood donation activity across seven European countries and the motivation of blood donors to donate or not to donate during the first phase of the COVID-19 crisis. We do so to understand what is driving changes in donation behaviour and which policies might help to restore donation levels.

Methods

We asked approximately 7000 people about their blood donation activity and motivation to donate or not to donate within the second wave of the European Covid Survey (ECOS) in June 2020. ECOS is an online survey across seven European countries. Around 1000 people in Denmark, France, Germany, Italy, Portugal, the Netherlands and the UK, representative of their country, participated in the study. The survey was organized in a way to avoid self-selection, as respondents were not aware of the survey questions beforehand. Participants completed the survey during the period 9–22 June 2020 using online panels provided by the market research company Dynata. Diverse recruiting campaigns reaching out to around 120 000 people were administered online (open recruitment, loyalty programmes, affiliate networks and mobile apps). Survey respondents received payment, which varied depending on the platform they were recruited through. The survey was programmed in the Qualtrics research suite where quotas were implemented to ensure that the country samples matched national census shares, which were representative in terms of age, sex, region and education level. A declaration of compliance for the project was reviewed and approved before the start of the project by the Vice Dean for Research according to the terms of use and ethical standards of the Faculty of Business, Economics and Social Sciences at the University of Hamburg and the European Commission's RESPECT Code of Practice [16].

Survey questions and scales

Firstly, we asked all participants whether they had donated blood during the previous 10 years before February 2020 (COVID-19 period), and for those that answered 'yes', we asked how many times they had donated in the 2 years prior to COVID-19. A timeframe of 10 years was used to capture donors that may have not been active for some time, but could have responded to the crisis by donating blood. Furthermore, we asked whether the entire sample had donated blood since the beginning of February 2020, as this marks the beginning of the COVID-19 pandemic in Europe. Overall, we were able to identify (a) blood donors, (b) those that donated during COVID-19 and (c) active donors with at least one donation in the 2 years prior to COVID-19.

To understand the impact of COVID-19, we asked donors directly whether they donated less than, more than or the same as they normally would (in the absence of COVID-19). To gain a better understanding of the motivations underlying the decision to donate, we asked those that stated that they did donate during COVID-19, about the extent to which they made a special effort to do so in order to help their healthcare system. Possible responses included 'yes, somewhat', 'yes, definitely', 'no' and 'I don't know'. The phrasing of the question aimed to identify whether donors saw COVID-19 as a 'call to arms' and responded by helping their healthcare system during the crisis by relieving blood shortages.

As a potential motivator to donate during the pandemic, and following suit from the World Health Organization, we asked all donors how worried they were about their healthcare system being overloaded [17]. Possible responses were on a 5-point scale from (1) do not worry at all, (2) slightly worry, (3) moderately worry, (4) worry quite a bit and (5) worry a lot. Responses 4 and 5 were combined to create a binary variable for analysis. We included the question as an indicator of how concerned donors were with how their healthcare system was handling the crisis and as a signal for the need for additional support. Despite blood donations not normally being needed directly to treat COVID-19 patients, blood donation supply concerns during the period were heavily reported in the media [11, 18–20].

Furthermore, all survey respondents were asked to assess their likelihood of being infected with the novel coronavirus whilst donating blood during the COVID-19 period (1 = no risk; 5 = very high risk), measuring infection risk as a potential barrier to donation, for example risk of travelling to the donation site, contact with staff and other donors. Lastly, we asked those who donated during the COVID-19 period if they were aware of being offered and receiving an antibody test, which we considered a potential incentive to donate. Responses included 'yes, my blood was tested for COVID-19 antibodies', 'no, my blood was not tested for COVID-19 antibodies' and 'I don't know if my blood was tested for COVID-19 antibodies'.

Statistical analysis

The analysis was conducted in two parts (1) a descriptive analysis of blood donors during COVID-19 and (2) a logistic regression of donation turnout during COVID-19 among all respondents and active donors.

To analyse the likelihood of donating during the COVID-19 period, we performed logistic regression analysis across the entire sample of individuals in order to understand which factors were driving the decision to donate during the pandemic. Our dependent variable was binary, indicating whether the sample donated during the COVID-19 period or not (0 or 1). The focal independent variables of interest were (a) having a perceived high or very high (4 or 5) risk of infection risk whilst donating blood (0 or 1), (b) being worried quite a bit or a lot about the healthcare system being overloaded (0 or 1), (c) whether individuals had a vulnerable person living in their household or not (elderly, disabled or with chronic conditions) (0 or 1) and (d) whether they quite strongly or fully adhered to COVID-19 guidelines, for example hand washing and social distancing (0 or 1). We expected that individuals with a vulnerable person at home might be dissuaded from donating to avoid exposing them to additional risk, for example through contact with blood donation staff, other donors and those encountered when travelling to the blood donation site. We therefore anticipated that people who were adherent to COVID guidelines would also be less likely to donate blood.

Additionally, we included variables used previously in the literature in our analysis of donation behaviour, which included a measure of donation experience (no. of self-reported donations in the 2 years prior to the COVID period), age, education level, gender and field of work. We included country fixed effects to account for differences between countries, for example donation eligibility requirements and the structure of country-specific blood donation systems.

Results

Of the 7122 people that responded to the survey, 1205 (16·9%) blood donors were identified across the seven European countries. Table 1 presents the characteristics of all survey respondents, and Table 2 presents the sample of blood donors and their background characteristics. Germany, closely followed by France, had the highest number of self-reported blood donors with 226 (21·5%) and 209 (20·8%) donors, respectively. In contrast, the Netherlands had the lowest number of donors with only 10·2% of the sample.

Regarding the impact of COVID-19 on self-reported blood donation activity, the results suggest a high number of donors across countries donated less than they normally would compare to their individual reference point, with 61·2% of donors in Portugal selecting this option and around half of donors in France (49·3%) compared with 40-45% in the remaining countries (Fig. 1). Only a small proportion of donors donated more than they normally would during the COVID-19 period. Some differences between countries were observed, with only 4·8% of donors in Portugal stating that they donated more than normal, compared with 19·4% in the UK and 18·1% in

Table 1 Background characteristics of all survey respondents

Country	Overall sample	Number of donors (%)	Age (SE)	Male %	Years in full-time education (SE)
Germany	1050	226 (21·5%)	48·7 (0·5)	48·6%	12·9 (0·1)
United Kingdom	1041	186 (17·9%)	47·5 (0·5)	46·9%	14·2 (0·1)
Denmark	1005	151 (15·0%)	49·4 (0·5)	48·0%	14·0 (0·1)
The Netherlands	1000	102 (10·2%)	48·1 (0·5)	49·0%	13·0 (0·2)
France	1003	209 (20·8%)	47·7 (0·5)	47·2%	13·8 (0·2)
Portugal	1015	165 (16·3%)	44·6 (0·5)	47·6%	14·2 (0·1)
Italy	1008	166 (16·5%)	48·6 (0·5)	48·0%	14·3 (0·1)
All countries	7122	1205 (16·9%)	47·8 (0·2)	47·9%	13·8 (0·1)

Table 2 Background characteristics of blood donors in sample

Country	Number of donors (% of country sample)	% donating during COVID-19 period	Mean age (SE)	% Male	Years in full-time education. Mean (SE)	No. of donations 2 years pre-COVID-19. Mean (SE)	Perceived COVID-19 infection risk whilst donating blood (SE)*
Germany	226 (21·5%)	35·8%	41·4 (1·0)	54·0%	13·0 (0·4)	4·3 (0·2)	2·28 (0·1)
United Kingdom	186 (17·9%)	40·9%	40·0 (1·1)	45·7%	15·1 (0·4)	4·1 (0·2)	2·44 (0·1)
Denmark	151 (15·0%)	29·8%	43·8 (1·3)	55·0%	14·7 (0·3)	3·8 (0·3)	1·95 (0·1)
The Netherlands	102 (10·2%)	37·3%	42·6 (1·6)	58·8%	12·9 (0·6)	4·5 (0·4)	2·41 (0·1)
France	209 (20·8%)	31·1%	41·3 (1·0)	51·7%	14·3 (0·4)	3·8 (0·2)	2·16 (0·1)
Portugal	165 (16·3%)	21·2%	40·5 (1·1)	52·7%	14·7 (0·3)	3·1 (0·2)	2·03 (0·1)
Italy	166 (16·5%)	40·4%	43·5 (1·2)	57·2%	15·4 (0·4)	4·3 (0·3)	2·26 (0·1)
All countries	1205 (16·9%)	33·8%	41·7 (0·4)	53·1%	14·3 (0·1)	4·0 (0·1)	2·21 (0·0)

*(1 = no risk; 5 = very high risk).

Italy. In total, however, 407 (33·8%) of the identified donors stated that they donated during the COVID-19 period of 4–5 months, with some variation between countries, which can be seen in Table 2.

For donors who donated during COVID-19 (n = 407), we saw that the majority of COVID-19 donors answered that they 'yes, somewhat' or 'yes, definitely' made extra effort to donate during the epidemic (Fig. 2). Portugal and the UK reported a high majority, making up 73·7% and 75·9% of donating donors. Furthermore, the Netherlands and France reported a number of donors who responded more explicitly with 'yes, definitely', with 37·8% and 34·7% of COVID-19 donors selecting this option, respectively. Denmark reported a large number (42·6%) of donors who answered 'no' to this question.

In Figure 3, we report how concerned donors were with their healthcare system being overloaded. Those who worried 'quite a bit' and 'a lot' were compared against the three remaining categories: worrying only moderately, slightly or not at all. We found that although the majority of donors (58·8%) were not worried or only moderately/slightly worried during the period, a substantial group were worried 'quite a bit' or 'a lot' (41·2%) including the majority of donors in Portugal (62·4%) and Italy (57·2%).

Overall, the mean risk of infection whilst donating blood was perceived to be quite low among donors (M = 2·21 SE = 0·33). We found pairwise differences (one-way ANOVA with Bonferroni correction) between the UK (M = 2·44, SE = 0·08) and Denmark (M = 1·95, SE = 0·09), P-value = 0·002, and Portugal (M = 2·03, SE = 0·09), P-value = 0·020. Furthermore, we found differences between the Netherlands (M = 2·41, SE = 0·11) and Denmark (M = 1·95, SE = 0·09), P-value = 0·032.

The results suggest that the minority of donors reported to be aware that their blood was tested for COVID-19 antibodies by their blood collection service (Fig. 4). The exception being the Danish sample where more than half of donors (57.5%) reported being tested. Of those that reported that their blood was tested for COVID-19 antibodies, 81·3% answered that they had received the results.

The results of the logistic regression analysis of donor turnout during the COVID period are presented in Table 3. Overall, we found that although the perceived risk of infection when donating blood was relatively low, those with a high perceived risk of infection were much less likely to donate (OR = 0·540; P-value = 0·006). Moreover, we identified that those who were quite strongly or fully adherent to COVID guidelines (handwashing, social distancing, etc.) were also less likely to donate (OR = 0·583; P-value = 0·000). We did

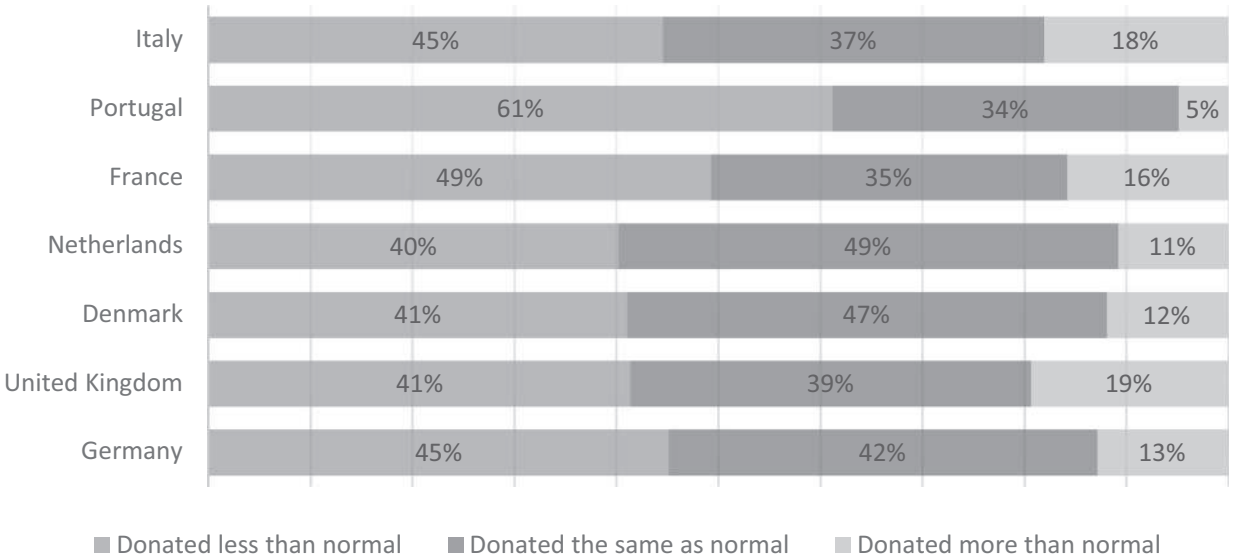

Fig. 1 How has COVID-19 affected your blood donation activity? Self-reported impact on blood donation activity from 1205 blood donors identified in the sample.

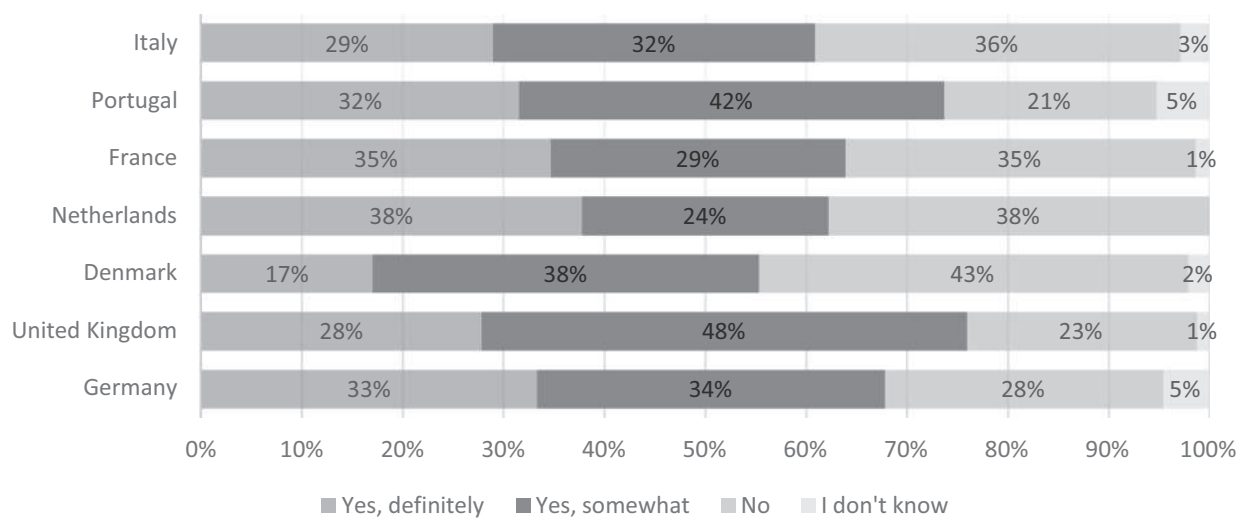

Fig. 2 Did you make a special effort to donate in order to help your healthcare system during the COVID-19 epidemic? Responses from 407 blood donors who donated during COVID-19.

not find evidence that those who worried about their healthcare system being overloaded were more likely to donate and that having a vulnerable person at home factored in the donation decision. Previous blood donation activity in the 2 years prior to COVID-19 was a strong predictor of donating during the crisis period, with each additional donation associated with a 87·7% higher donation likelihood (*P*-value < 0·000). We found that age was important, whereby individuals were incrementally less likely to donate by each age category (compared with 18-24 years olds). We did not find significant differences between countries in the analysis. Subsequently, we found that those working in the health or education sectors were over twice as likely to donate during COVID-19 compared with those working in other areas (not including food retail or research).

Lastly, for the active donor sample (n = 992) presented in the Appendix (Tables A1 and A2), we found comparable results whereby those with more donations in the past two years (OR = 1·370; *P*-value = 0·000) and highly adherent donors were more likely to donate during COVID-19 (OR = 0·569; *P*-value = 0·000). However, we

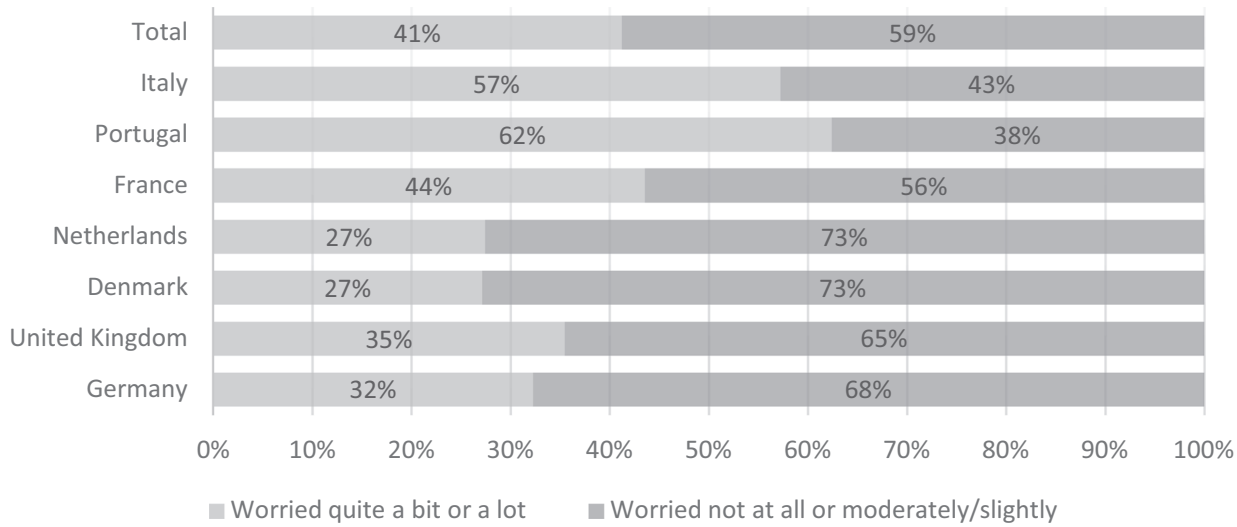

Fig. 3 Worries among blood donors about the healthcare system being overloaded. Responses from 1205 blood donors identified in the sample.

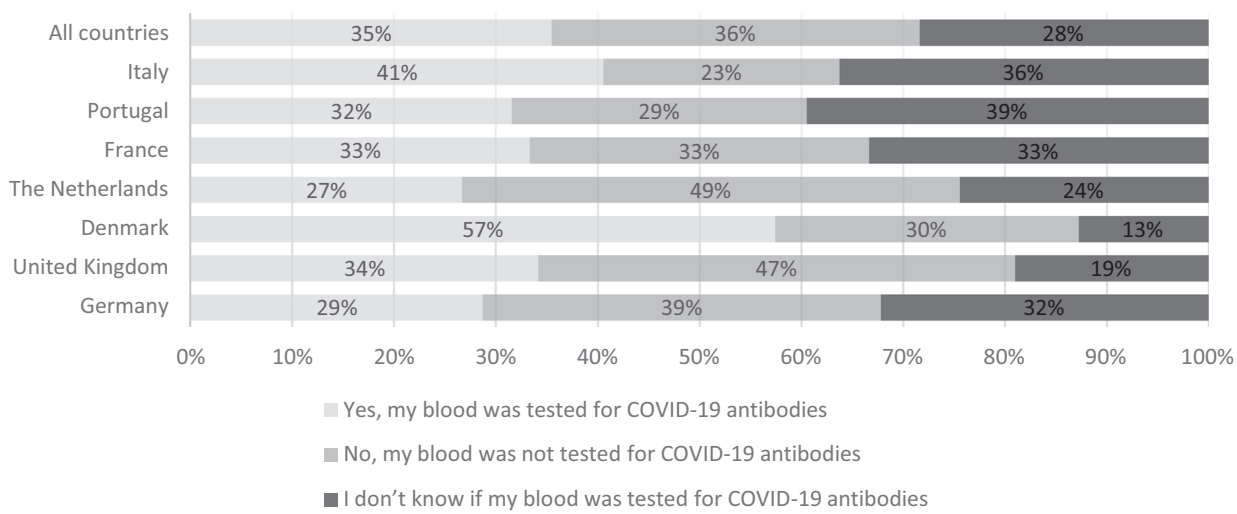

Fig. 4 Are you aware whether your blood sample was tested for COVID-19 antibodies? Responses from 407 blood donors who donated during COVID-19.

did not find evidence that having a high perceived risk of infection whilst donating blood factored into the donation decision (OR = 0·853; *P*-value = 0·485).

Discussion

In the study, we found that around half of all donors reported that they donated less than they normally would, which suggests a concerning drop in blood donations throughout the COVID-19 period across Europe. However, we also identified a small number of donors who donated more than they normally would, perhaps identifying an opportunity to help in a time of need by responding to public appeals.

In terms of donor motivation, we observed that the majority of donors that donated made a special effort to help their healthcare system during the COVID-19 period. Many of them were also concerned with overcrowding of their healthcare system, indicating that altruistic motives (at least retrospectively) played an important role in donors' decision to donate and were weighed up against potential risks [5, 21]. Blood donation centres may thus take advantage of this result by appealing to the altruistic nature of donors in times of crisis. As an example, specialist campaigns that focus on the continued need for blood donors during the pandemic could be employed. Such public appeals have been successful in past crises, where large influxes of donors were documented over

Table 3 Logistic regression analysis of donor turnout during COVID-19

	Mean (SE)/%	Odds Ratio	SE	z	*P*-value	95% Confidence Interval	
No. of blood donations two years prior to COVID-19 period	0·67 (0·02)	1·872	0·043	27·260	0·000	1·790	1·958
Perceived high risk of COVID infection whilst donating blood (4 or 5) (Yes/no)	12·29%	0·540	0·121	−2·750	0·006	0·349	0·838
Worried quite a bit/a lot about healthcare system being overloaded (Yes/no)	41·85%	1·033	0·153	0·220	0·827	0·772	1·382
Vulnerable person in household (Yes/no)	29·30%	0·975	0·161	−0·150	0·877	0·705	1·347
Quite strongly/fully adhered to COVID guidelines (Yes/no)	74·40%	0·583	0·085	−3·690	0·000	0·438	0·777
Age category							
18–24	9·66%	Reference					
25–34	16·22%	0·545	0·109	−3·030	0·002	0·368	0·807
35–44	18·23%	0·352	0·076	−4·830	0·000	0·230	0·538
45–54	17·99%	0·270	0·065	−5·480	0·000	0·169	0·432
55–64	16·29%	0·167	0·048	−6·280	0·000	0·096	0·292
65+	21·60%	0·104	0·033	−7·070	0·000	0·055	0·195
Education (years)							
<10	16·15%	Reference					
10–12	20·31%	0·689	0·164	−1·560	0·118	0·432	1·099
13–15	26·11%	0·692	0·150	−1·700	0·089	0·453	1·057
16–18	22·37%	0·499	0·113	−3·070	0·002	0·320	0·778
18+	15·06%	0·874	0·199	−0·590	0·552	0·559	1·364
Field of work							
Health-related sector (medical staff, pharmacist, medical student)	8·57%	2·362	0·479	4·240	0·000	1·587	3·514
Education (e.g. schools, nurseries)	7·82%	2·182	0·461	3·690	0·000	1·443	3·301
Food retail (Supermarkets)	3·97%	1·359	0·406	1·030	0·304	0·757	2·441
Research	2·37%	0·913	0·354	−0·230	0·815	0·427	1·954
Other	77·26%	Reference					
Female	52·12%	0·835	0·119	−1·260	0·207	0·632	1·104
Country							
Germany	14·75%	Reference					
United Kingdom	14·62%	1·052	0·249	0·210	0·831	0·662	1·672
Denmark	14·09%	0·647	0·169	−1·670	0·096	0·388	1·079
The Netherlands	14·04%	0·609	0·166	−1·820	0·068	0·357	1·038
France	14·09%	0·845	0·201	−0·710	0·480	0·530	1·348
Portugal	14·26%	0·725	0·192	−1·220	0·224	0·432	1·217
Italy	14·16%	1·083	0·264	0·330	0·745	0·671	1·745
Intercept		0·106	0·031	−7·770	0·000	0·060	0·187

CI, confidence interval; N, 7120; SE, standard error.

short periods, for example mass shootings in the United States and bush fires in Australia [11, 12].

Although the perceived risk of infection whilst donating blood was relatively low for many respondents, those who anticipated a high risk of infection were much less likely to donate. Our results coincide with previous research from Hong Kong and China that found anxiety and fear of contracting COVID-19 were deterrents to donating blood [7, 8], and with studies on the SARS and avian flu outbreaks [9, 10]. Therefore, we suggest that blood donation centres emphasize the steps that have been taken to reduce risk of infection in order to maintain confidence in their services, for example handwashing, face coverings and social distancing. Key messages could assure the public that they can still safely donate blood whilst adhering to COVID-19 measures. We found that the result did not hold for the active donor sample, which may be explained by a high level of intrinsic donor motivation, even in those that anticipated a high infection risk whilst donating.

Despite all European countries being eventually affected by COVID-19, there were notable differences in the severity of the crisis and the government response. Italy was hit particularly hard early in the pandemic and was the first European country to apply interventional measures from the beginning of March 2020 in response

to the outbreak of the virus [22]. Other EU countries followed soon after by introducing measures from around mid-March 2020 [22]. Despite this variation, we did not find evidence of country differences in our analysis of donor turnout, which suggests that the results are generalizable across Europe and states may face similar challenges in managing blood supply during the period.

With some countries including Finland, Germany and the United Kingdom discussing the use of 'immunity passports' [23–25], we conjecture that having evidence of COVID-19 antibodies could be valuable to many individuals and provide reassurance that they are not putting loved ones and those in their community at risk. However, according to our results, the low number of donors who were aware of COVID-19 antibody tests alongside their donation suggests that the tests were not a relevant incentive for donors. However, by framing such tests as incentives more explicitly, in line with free health checks, which have been used previously to motivate blood donation, donors may be more encouraged to donate [26, 27].

As has been observed in a German setting, many donors prefer to visit at lunchtime and after work in normal times [28]. We speculate that, in addition to perceived infection risk, other factors contributed to a drop in donations that warrant further research. For instance, lockdown and quarantine periods preventing normal movement, changing working conditions disrupting established routines, uncertainty around whether blood collection services were open and the cancellation of blood drives. Further research would allow blood donation centres to accommodate new donation patterns as they begin to observe them. For example, some donors may benefit from extended opening hours and flexibility as previous childcare arrangements (e.g. nurseries and schools) have been disrupted, whilst others may benefit from more blood drive locations in city suburbs to accommodate working from home.

The study findings should be considered in the light of some limitations: firstly, we did not know the blood type of the survey respondents and could therefore not incorporate it into our analysis. However, as our sample is representative across major characteristics, we do not expect large deviations in the ABO or Rhesus factor blood type from the respondents. Secondly, our data are self-reported, which may explain our donation rate of 5·7%, which was slightly higher than has been reported previously across Europe with figures ranging between 2·5% and 5·4% [29–33]. In line with other studies, we found our donors to be mostly male (53·1%) and between 30 and 44 (35·3%). We identified a higher proportion of younger donors than was reported in a large European survey, which is likely due to differences in donor definitions, for example donated in lifetime versus in the last 10 years [34].

In conclusion, we observed that despite half of donors donating less than they normally would during the pandemic, most that did donate made a special effort to do so. Furthermore, survey respondents who anticipated a high risk of infection were much less likely to donate. This change in donation behaviour and the associated motivations of donors are relevant to policymakers who are concerned with maintaining adequate blood supply during this crisis. We suggest that blood collection services consider specialist campaigns that focus on the altruistic motivation of donors during the crisis, and they continue to reassure donors of the safety measures in place in their centres. Lastly, the majority of donors appear to have not been incentivized by COVID-19 antibody tests, which should be considered if framed alongside free health checks as an incentive to elicit blood donations.

Conflict of interests

The authors declare no conflict of interests.

Author contributions

TC analysed the data and wrote the original manuscript. SNB, IS, PPB, WB, JVE, JS, AT and TS collaborated in developing the survey questions and provided substantial comments that were incorporated into the manuscript.

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Appendix 1

Table A1 Background characteristics of active donors*

Country	Number of active donors (% of country sample)	% donating during COVID-19 period	Mean age (SE)	% Male	Years in full-time education. Mean (SE)	No. of donations 2 years pre-COVID-19. Mean (SE)	Perceived COVID-19 infection risk whilst donating blood (SE)**
Germany	192 (18·3%)	41·7%	39·4 (1·1)	54·2%	12·8 (0·4)	5·0 (0·2)	2·4 (0·1)
United Kingdom	154 (14·8%)	48·1%	38·6 (1·2)	48·1%	14·9 (0·4)	4·9 (0·2)	2·5 (0·1)
Denmark	114 (11·3%)	38·6%	40·2 (1·4)	53·5%	15·0 (0·4)	5·0 (0·3)	2·1 (0·1)
The Netherlands	79 (7·9%)	48·1%	39·0 (1·7)	63·3%	13·0 (0·7)	5·8 (0·4)	2·6 (0·1)
France	178 (17·7%)	36·0%	39·9 (1·1)	51·1%	14·2 (0·4)	4·5 (0·2)	2·2 (0·1)
Portugal	131 (12·9%)	24·4%	38·8 (1·2)	50·4%	15·0 (0·3)	3·9 (0·2)	2·0 (0·1)
Italy	144 (14·3%)	46·5%	41·3 (1·2)	56·3%	15·8 (0·4)	4·9 (0·3)	2·0 (0·1)
All countries	992 (13·9%)	40·2%	39·6 (0·5)	53·1%	14·4 (0·2)	4·8 (0·1)	2·3 (0·0)

*At least one donation in two years pre-COVID-19 period.

**1 = no risk; 5 = very high risk).

Table A2 Logistic regression analysis of active donor turnout during COVID-19

	Mean (SE)/%	Odds Ratio	SE	z	*P*-value	95% Confidence Interval	
No. of blood donations two years prior to COVID-19 period	4·80 (0·096)	1·370	0·040	10·730	0·000	1·294	1·451
Perceived high risk of COVID infection whilst donating blood (4 or 5) (Yes/no)	16·13%	0·853	0·194	−0·700	0·485	0·546	1·333
Worried quite a bit/a lot about healthcare system being overloaded (Yes/No)	42·54%	1·100	0·177	0·590	0·555	0·802	1·507
Vulnerable person in household (Yes/no)	23·49%	0·964	0·177	−0·200	0·842	0·673	1·381
Quite strongly/fully adhered to COVID guidelines (Yes/no)	63·81%	0·569	0·092	−3·500	0·000	0·415	0·781
Age category							
18–24	15·32%	Reference					
25–34	28·83%	0·511	0·119	−2·880	0·004	0·323	0·807
35–44	21·77%	0·416	0·103	−3·540	0·000	0·256	0·676
45–54	16·33%	0·366	0·100	−3·670	0·000	0·214	0·626
55–64	10·18%	0·284	0·090	−3·990	0·000	0·153	0·527
65+	7·56%	0·202	0·074	−4·390	0·000	0·099	0·412
Education (years)							
<10	14·82%	Reference					
10–12	16·43%	0·795	0·218	−0·840	0·403	0·465	1·360
13–15	24·19%	0·945	0·236	−0·230	0·821	0·579	1·543
16–18	24·09%	0·616	0·158	−1·900	0·058	0·373	1·017
18+	20·46%	1·033	0·269	0·120	0·902	0·620	1·719
Field of work							
Health-related sector (medical staff, pharmacist, medical student)	13·41%	1·866	0·433	2·690	0·007	1·184	2·941
Education (e.g. schools, nurseries)	10·79%	1·751	0·428	2·290	0·022	1·085	2·826
Food retail (Supermarkets)	4·94%	0·997	0·343	−0·010	0·992	0·508	1·956
Research	4·33%	0·772	0·296	−0·670	0·500	0·364	1·637
Other	66·53%	Reference					
Female	46·88%	1·077	0·168	0·470	0·636	0·793	1·462
Country							

Table A2 (Continued)

	Mean (SE)/%	Odds Ratio	SE	z	*P*-value	95% Confidence Interval	
Germany	19·35%	Reference					
United Kingdom	15·52%	1·154	0·296	0·560	0·575	0·698	1·909
Denmark	11·49%	0·770	0·217	−0·930	0·354	0·443	1·338
The Netherlands	7·96%	0·879	0·276	−0·410	0·680	0·475	1·624
France	17·94%	0·806	0·201	−0·870	0·386	0·495	1·313
Portugal	13·21%	0·663	0·190	−1·430	0·152	0·378	1·163
Italy	14·52%	1·382	0·362	1·230	0·217	0·827	2·309
Intercept		0·458	0·160	−2·240	0·025	0·231	0·908

CI, confidence interval; N, 992; SE, standard error.

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Low ferritin levels appear to be associated with worsened health in male repeat blood donors

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Vox Sanguinis

Background and objectives Frequent blood donation depletes iron stores of blood donors. Iron depletion may lead to anaemia, but the health effects of iron depletion without anaemia in healthy blood donors are not well understood. We studied in the FinDonor cohort whether worsening of self-rated health of blood donors during the study period was associated with biomarkers for iron levels or other self-reported changes in lifestyle.

Materials and methods We included 1416 participants from the cohort who answered an 89-item questionnaire on their health and lifestyle during their enrolment visit and again at the end of the study. We performed multivariate logistic regression to test if blood donation-related factors affected the probability of reporting worsened health. To set these findings into a more holistic context of health, we subsequently analysed all other questionnaire items with a data-driven exploratory analysis.

Results We found that donation frequency in men and post-menopausal women and ferritin level only in men was associated negatively with worsened health between questionnaires. In the exploratory analysis, stable physical condition was the only questionnaire item that was associated negatively with worsened health in both women and men.

Conclusion Our results suggest that low ferritin level is associated with worsened health even in non-anaemic repeat donors, although we find that when health is analysed more holistically, ferritin and other factors primarily related to blood donation lose their importance.

Key words: blood donation, donor health, self-rated health, iron deficiency, iron deficiency without anaemia, ferritin.

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Introduction

Blood establishments are responsible of securing a safe blood donation. Donor selection criteria strive to guarantee the quality and safety of the blood components and donor safety. The long history of blood donation as such demonstrates that there are no clear acute or long-term harmful effects related to blood donation. If not properly managed, blood donation leads to iron deficiency anaemia, but this risk is typically well cared for. Lately, concern has been raised regarding the possibility of depletion of iron stores without anaemia, in particular among younger women or frequent donors [1].

Due to the healthy donor effect, blood donors are considered to be healthier than the general population [2]. Healthier individuals get selected by the blood establishment and the donors themselves as donors and are able to maintain the habit from years to decades. For example, among young individuals, better self-perceived mental health, and with the increase in age, better self-perceived physical health are associated with blood donation [3].

Despite these positive associations, blood donors are also considered to be at higher risk of iron deficiency [1, 4, 5], especially as a result of frequent blood donation [1,

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6–8]. However, iron deficiency without anaemia has not been found to be associated with reduced self-perceived health-related quality of life in blood donors [8, 9]. Recently, iron supplementation was found to reduce iron deficiency-related symptoms in non-anaemic Swiss blood donors [10]. Iron levels of Finnish blood donor population are mostly affected by blood donation activity [11]. Importantly, iron supplementation for at-risk groups has been in place since the 1980s in Finland. In addition to donation activity, other factors like age [12], BMI [12, 13] and donation frequency [6, 14] are associated with both blood donors iron status and self-rated health [15].

The factors which influence health are multiple and interactive. According to the World Health Organization constitution: 'Health is defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity' [16]. Subjective and objective determinants of health should be used together as they might have different influences on health [17]. In industrialized countries, self-rated health (alternatively self-reported or self-perceived health) is the most relevant measure of one's general health and robust predictor of objective outcomes, such as mortality [18–20].

The Finnish Red Cross Blood Service (FRCBS) set up the FinDonor 10 000 study in 2015 [21] to investigate the health effects of blood donation with a specific focus on iron status [11]. In the present study, we test if worsening of self-rated health of blood donors during the study period was associated with donation activity, biomarkers for iron levels or self-reported changes in lifestyle.

Material and methods

FinDonor 10 000 was a prospective longitudinal cohort study and ran from the 18 May 2015 to 8 December 2017. An ethical approval (approval number 282/13/03/00/14) for the study was acquired from the Hospital District of Helsinki and Uusimaa Review Board. Details of the FinDonor cohort, sample collection, laboratory analyses and quality issues are described elsewhere [21].

During their enrolment visit, participants were asked to fill out an electronic questionnaire about their health and lifestyle at the donation site after donating blood. This questionnaire included 89 questions about general health, current and past medical conditions, dietary habits, smoking, alcohol use, physical activity and sleep. During the 2018 summer, participants received a letter from FRCBS inviting them to answer the questionnaire again from home using a web page. We added questions about educational level, working status and quality of life to this second follow-up questionnaire. We used combined data from participant's donation history (FRCBS donation database, e-Progesa, MAK-SYSTEM), both questionnaires and laboratory results from the enrolment visit blood samples.

Of the 2584 donors who consented to the study, we excluded 1103 participants because they did not answer both questionnaires. We divided the participants into three different groups: men, post-menopausal and pre-menopausal women. The post-menopausal group was formed by women who were over 45 years of age and reported amenorrhoea. Women under 45 years of age reporting amenorrhoea were excluded from the analyses (N = 38). Women of all ages who reported menstruation formed the pre-menopausal group. Subsequently, we excluded donors who did not answer the question 5: 'How would you rate your health in general?' (N = 5), question 27: 'How much do you weigh?' (N = 15) in both questionnaires, who had high ferritin (≥400 (μg/l), N = 3) or who had high CRP (≥30 mg/l, N = 4) from the analyses.

To test if blood donation-related factors could explain worsening of self-rated health between the two questionnaires, we performed multivariate logistic regression. As a measure of self-rated health we used the commonly used and validated single question: 'How would you rate your health in general?' (excellent, very good, good, moderate or poor) [20]. The outcome variable (health_outcome) was defined as reporting worsened health (health_outcome = 1) rather than same or better health in the second questionnaire (health_outcome = 0). As explanatory factors, we used donor characteristics age (years), initial ferritin, initial haemoglobin, initial weight, BMI difference, during study average annual donation frequency and if initial self-rated health was very good (was not very good = 0, was very good = 1) or excellent (was not excellent = 0, was excellent = 1). Full analysis code can be found from [22].

The data were analysed separately for men, post-menopausal women and pre-menopausal women. After estimating p-values and odds ratios (OR) by fitting a model once to the original data, 95% percentile confidence intervals were estimated for ORs, that is effect sizes, with the adjusted bootstrap (BCa) method [23]. These were calculated by sampling with replacement from the original study group a sample of equal size as the original study group 10 000 times. A logistic regression model was fitted each time to the data to calculate 10 000 alternative effect sizes for each donor characteristic. The confidence interval was then calculated and adjusted for non-normality from the distribution of these 10 000 effect sizes.

In the exploratory analysis, we excluded 371 additional participants more who did not answer some of the questions selected for the analysis (see [24] for code). In brief, we first decided to exclude 26 of the 89 questions from

the analysis, because they could not be formulated as change in quantity between the questionnaires or were related to female reproductive health. For all the remaining 63 questions, 817 donors had provided complete data. Many questions in the questionnaire probe similar topics. We screened if answers of the questions had a correlation of at least 0·5 with answers of some other question and selected among the group of questions correlating with themselves a single question to represent that group (Fig. S2). We selected the question that allowed us to keep as much data as possible and had a balanced distribution to enable modelling. This enabled us to increase the number of donors with complete data to 1045 and augment the models of primary analysis with further donor characteristics (Table S5). The answers to these selected questions were then recoded as change between the two questionnaires. Questions 1–3 have the format 'Have you ever..?' and represent single health events (e.g. diagnosis of anaemia). We coded them as '0' for no new event during the study and '1' for a new diagnosis during the study. The rest of the questions asked in both questionnaires can be interpreted as ordered factors. We coded them as change of factor levels between questionnaires, that is 'decreased', 'stable' or 'increased'. The additional questions of the follow-up questionnaire were not used. The exploratory analysis was carried out by adding the selected questions to the primary analysis multivariate logistic regression models and refitting the models (see [25] for code).

We carried out all analysis in R 'a free software environment for statistical computing and graphics' [26] using tidyverse [27]. In particular, plots were created with library ggplot2 [28], ggbeeswarm [29] and GGally [30], tables with tableone [31] separation in logistic models was identified with safeBinaryRegression [32], collinearity was analysed with function vif() in car [33], and BCa confidence intervals were calculated with boot [34].

Results

After the exclusions detailed in Materials and Methods, we were able to include 1416 donors (589 men, 353 post-menopausal and 474 pre-menopausal women) of the FinDonor cohort. The time interval between answering the two questionnaires varied from 12 months to 39 months (Fig. S1). We found a slight cohort level trend towards the donors reporting worse health in the second compared to the first questionnaire, in particular in pre-menopausal women (Table 1).

To evaluate whether exclusion of donors from the data sets used for the hypothesis testing and explorative analysis could bias results from them, completion rates by initial health status (Table S1) and evolution of health status (Fig. S3) were analysed. Overall, self-reported health of 15% of women had improved and 54% reported same and 31% worsened health (14%, 57% and 29%, respectively, in men) with similar percentages in the explorative

Table 1 Characteristics of the study population

	Men	Post-menopausal women	Pre-menopausal women
n	589	353	474
Age (years) (mean (SD))	47·65 (13·20)	58·58 (5·57)	35·51 (10·04)
Initial weight (kg) (mean (SD))	85·23 (14·38)	71·09 (12·86)	70·74 (14·06)
BMI difference (second - first) (mean (SD))	0·23 (1·39)	0·28 (1·53)	0·90 (1·91)
Initial haemoglobin (g/l) (mean (SD))	150·38 (9·64)	138·07 (8·03)	135·28 (7·80)
Initial ferritin (μg/l) (median [IQR])	41·00 [25·00, 68·00]	33·00 [21·00, 51·00]	25·00 [16·00, 41·00]
During study donation frequency (yearly) (mean (SD))	3·00 (1·40)	2·41 (0·94)	1·92 (0·96)
Initial health rating (%)			
Poor	0 (0·0)	0 (0·0)	0 (0·0)
Satisfactory	15 (2·5)	6 (1·7)	9 (1·9)
Good	175 (29·7)	142 (40·2)	156 (32·9)
Very good	294 (49·9)	146 (41·4)	236 (49·8)
Excellent	105 (17·8)	59 (16·7)	73 (15·4)
Final health rating (%)			
Poor	2 (0·3)	1 (0·3)	2 (0·4)
Satisfactory	36 (6·1)	30 (8·5)	28 (5·9)
Good	213 (36·2)	136 (38·5)	195 (41·1)
Very good	256 (43·5)	149 (42·2)	192 (40·5)
Excellent	82 (13·9)	37 (10·5)	57 (12·0)

sub-cohort. In women, the completion rate tends to increase with improved initial health, unlike in men.

Our primary goal was to test whether blood donation-related factors could explain worsened self-rated health between the two questionnaires. To this end, we selected from the literature plausible donation-related factors [11, 35] and constructed logistic multivariate regression models to test the specific hypothesis of whether these factors could be associated with worsened donor health.

Results of the multivariate logistic regression models are presented in Fig. 1 and their numeric values in Table S2. The model included separate factors for worsening of higher health ratings ('Initial health rating Very good' and 'Initial health rating Excellent', Fig. 1 and Table 1). These factors were found to be the most influential, with odds ratios of worsened health between the two questionnaires from 4 to 19 in different study groups. Initial weight was found to be positively associated with worsened health in all groups. Initial ferritin and during study donation frequency were found to associate negatively with worsened health in men. Among post-menopausal women, during study donation frequency was also found to associate negatively with worsened health. The BMI difference (BMI in second – BMI in first questionnaire) in post-menopausal women was found to associate positively with worsened health. The most visible mean difference was seen in initial weight of men, where donors that reported worsened health had a mean initial weight of 88·8 kg and for stable and improved health 84·2 and 82 kg, respectively (Fig. 2).

To further verify that the above results are not confounded by initial health rating of donors, we repeated the above analysis stratified by initial health (Tables S3 and S4 and Fig. S4). The negative association of ferritin with worsened health was detected in men with 'Very good' or 'Excellent' initial self-reported health.

Given the multiple determinants of health, we wanted to find out how do the effects of the studied blood donation-related factors compare to the effects of the rich FinDonor health questionnaire data. For this exploratory analysis, we included 1045 donors (Table S5). Results of the analyses are presented in Fig. 3 (numeric values in

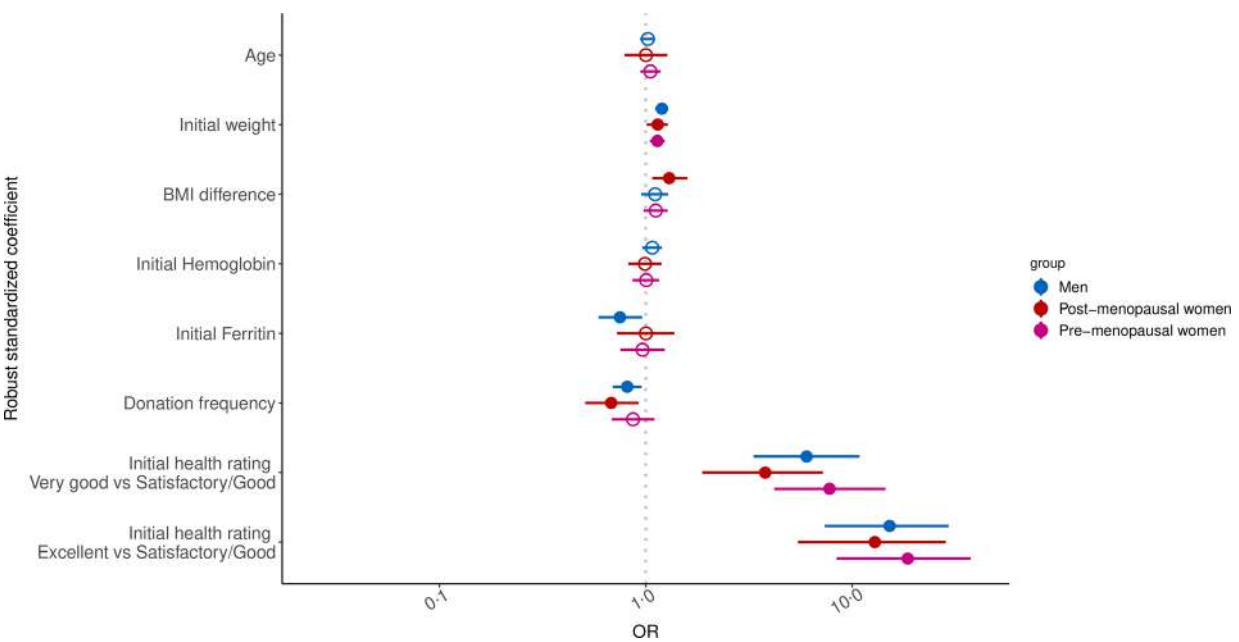
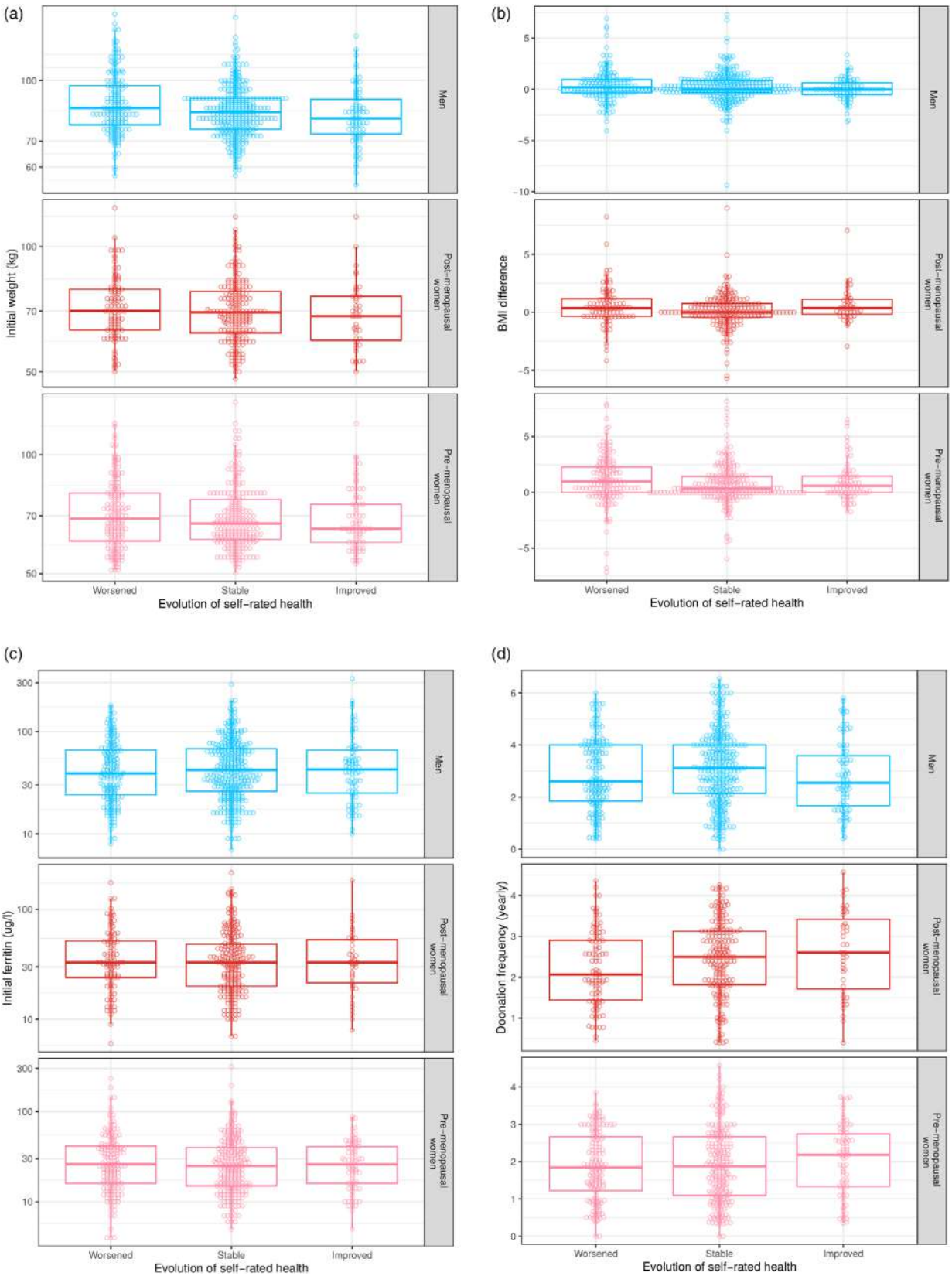


Fig. 1 Associations of donor characteristics to worsened self-reported health from hypothesis-based multivariate regression. The standardized coefficients (circle), their bootstrapped 95% BCa confidence intervals (thick line) and whether the factor was significant (Bonferroni-corrected $P < 0{\cdot}05$ in the initial regression models, filled circle) or not (non-filled circle). Ferritin is presented as log(ferritin)/log(2), that is a unit increase corresponds to doubling of ferritin. Weight, age and haemoglobin were divided with 5, that is a unit increase corresponds to 5 kg, 5 years or 5 g/l, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]

Fig. 2 Distributions of selected donor characteristics divided by change in self-reported health. Each individual shown as an open circle in a dot plot and overlaid with a boxplot where the central line shows the median and upper and lower edges of the box 25th and 75th percentiles, respectively. [Colour figure can be viewed at wileyonlinelibrary.com]



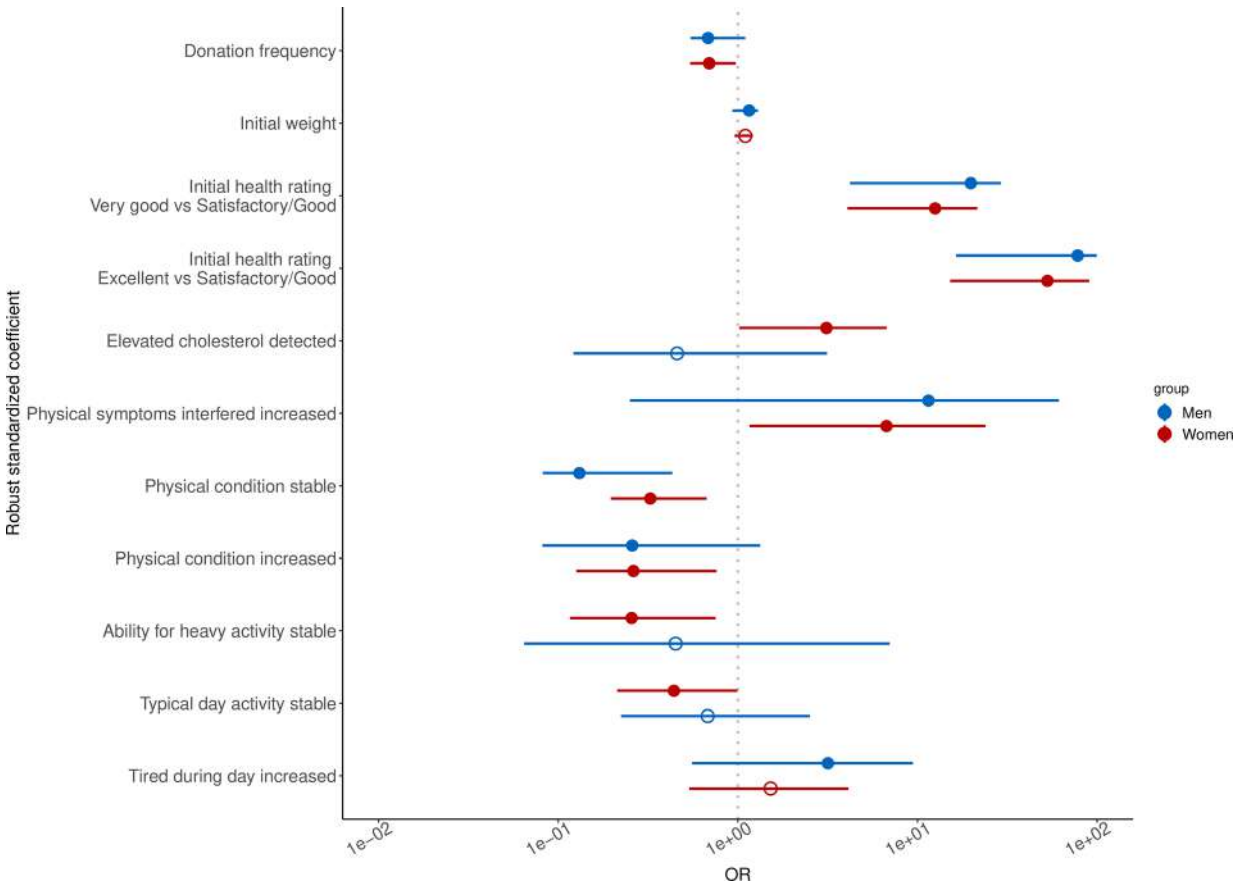


Fig. 3 Associations of selected donor characteristics to worsened self-reported health from the exploratory multivariate regression. See Fig. 1 for details. [Colour figure can be viewed at wileyonlinelibrary.com]

Table S6). Size of the data set was found to be limiting for analysis of post-menopausal women and pre- and post-menopausal women were combined into one group. After data-driven exclusion of donor characteristics, the two models included 31 donor characteristics, which covered 26 individual questions (initial self-rated health used twice), two laboratory values (ferritin and haemoglobin) and two values from donation registry (age and during study donation frequency). Of these donor characteristics, 11 had a Bonferroni-corrected p-value below 0·05 in the model fitted to non-bootstrapped data in at least one study group. These 11 are included in Fig. 3. As in the hypothesis testing models, the 'Initial health rating Very good' and 'Initial health rating Excellent' variables had the largest coefficients. Of other factors included also in the hypothesis testing model, 'during study donation frequency' was negatively associated with worsened health in men and women and 'initial weight' positively associated for men. The directions of associations between donor characteristics and worsened health were consistent between the hypothesis testing and exploratory models. However, the confidence intervals of all these coefficients, except donation frequency in women, crossed zero indicating that their direction of effect could not be estimated with 95 % certainty by the models. In men and women, an equal answer to question, '21. How would you rate your current physical condition?' in both questionnaires, in comparison to having answered an inferior level in the second questionnaire than in the first, was negatively associated with worsened health (OR of 0·13 [CI: 0·08–0·043] in men; OR of 0·32 [CI: 0·19–0·67] in women, Fig. 3: row 'Physical condition stable'). In addition, significant p-values were detected for questions related to elevated cholesterol, tiredness, physical symptoms, condition and activity, but in most cases the direction of the effects could not be estimated with 95% certainty.

Discussion

The FinDonor 10 000 cohort represents the Finnish blood donors well despite an over-representation of active

donors [21]. In the present publication, we studied only the FinDonor participants that answered both the enrolment and follow-up questionnaire. Highly educated and permanently employed people are over-represented in this population [21]. These factors are known to have a mostly positive effect on health [17, 36]. The health of non-anaemic iron-deficient blood donors is a concern for the blood donation community. According to enrolment ferritin 5% of men, 10% of post-menopausal and 21% of pre-menopausal women of the 1416 participants were iron-deficient (ferritin < 15 μg/l). Hence, our study might not have sufficient numbers of non-anaemic iron-deficient blood donors to be applicable to this subpopulation. Furthermore, we found that among women the initial health status correlated with completion rate of the second questionnaire (Table S1). Among women and men, only 3–4% of donors had a two level drop in health (Fig. S3). Hence, our data might be biased to detect only associations with moderate worsening in health.

In our sample of 1416 blood donors, we found that men (n = 589) rated their health slightly better or the same as women (Table 1), supporting results of studies in general populations [17, 18]. We also saw a slight overall trend towards worsened health in the second questionnaire in comparison to the first questionnaire (Table 1). However, we have shown that there was no consistent individual-level trend for worsened health in the FinDonor cohort, rather random variation or regression to the mean [21]. To capture the effect that the highest health rating cannot improve and the possible regression to the mean effects, the model included separate factors for initial high health ratings ('Initial health rating Very good' and 'Initial health rating Excellent', Fig. 1 and Table 1). We found that these donor characteristics had the strongest associations with the worsened self-rated health between questionnaires. Capturing variation related to the baseline health ratings in the enrolment questionnaire is important to correctly estimate the effect sizes of other factors, although it reveals little of the actual causes of worsened health. Donors replied to the first questionnaire at the donation site after donating blood. Consequently, healthy registration effect [15] or warm glow [37] was likely to have been present. Hence, the initial high health rating factors in the model were likely to capture also these effects.

We detected that initial weight was positively associated with worsened health in all groups. The OR for women was 1·4 meaning that a 5 kg increase in initial weight increases the odds of reporting worsened health by 40%. The difference in BMI (second - first) was positively associated with worsened health in post-menopausal women (Fig. 1, Table S2). Results are in accordance with previous studies about weight stability and obesity avoidance to stay healthy [38].

We found that in men and post-menopausal women, during study donation frequency was negatively associated with worsened self-rated health (Fig. 1, Table S2). The result is similar to that of Donor InSight blood donor study [15] and is likely to be related to the healthy donor effect.

We detected that in men initial ferritin level was negatively associated with worsened health. (Fig. 1, Table S2). To our knowledge, such association between ferritin and evolution of self-rated health has not been reported before. Hence, higher iron stores, reported by higher ferritin levels, might help to maintain health even in non-anaemic male repeat blood donors. In women, the completion rate was found to be higher if the initial health rating was higher. In the stratified analysis, the ferritin association is only detected in men with 'very good' or 'excellent' initial self-reported health. Hence, it seems possible that we are unable to detect the ferritin association in women due to selection bias.

To allow interpretation of the primary hypothesis-based analysis of donor characteristics associated with worsened health in a more comprehensive context of health, we carried out a secondary exploratory analysis which included as many donor characteristics from our donor health and lifestyle questionnaire as possible given limitations of data availability (Fig. 3, Table S6). In this analysis, we found that stable physical condition was the only donor characteristic for which direction of effect could be reliable estimated for men and women. In addition for women, 'increase of interference of physical symptoms' was positively and 'increase of physical condition' and 'stable ability of heavy activity' were negatively associated with worsened health. These results are in accordance with previous results about healthy lifestyle, for example physical activity is positively associated with good self-rated health and unhealthy lifestyle with ill-health [39] and that active lifestyle is associated with better self-rated health [40, 41].

Blood donors are considered to be healthy but the definition and perception of health is complex and involves several factors such vitality and physical and mental health [36, 42]. Also, health-damaging behaviours may serve to promote pleasure, relieve stress or even enhance mental health [43]. Some particular health components may be more important to an individual when they assess their health, while others are less consequential [42], for example, possible mild symptoms caused by low iron stores in otherwise healthy non-anaemic donors might be compensated by the positive feelings from blood donation [44]. Also, impact of iron metabolism to health at large is actively debated. Low iron stores may even be beneficial in some cases [45, 46].

In conclusion, our results suggest that low iron stores, reported to be low ferritin levels, could be associated with

worsened health even in non-anaemic repeat donors. This raises concern over iron store management of active donors, but the results also imply that when health is analysed more holistically, blood donation-related factors lose their importance.

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Conflict of interest

There are no conflicts identified.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Distribution of time between questionnaires in the study groups.
Figure S2 Correlations between answers of questionnaire items.
Figure S3 Health status evolution between questionnaires.
Figure S4 Forest plot of hypothesis testing stratified by initial health.
Table S1 Completion rates by level of self-rated health at the first visit.
Table S2 ORs from the hypothesis testing model.
Table S3 Population characteristics of hypothesis testing stratified by initial health.
Table S4 ORs from hypothesis testing stratified by initial health.
Table S5 Population characteristics of explorative analysis.
Table S6 ORs from the explorative model.

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Determinants of the intention to participate in a programme of plasma donation for fractionation among men who have sex with men

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Vox Sanguinis

Background and objectives Several approaches are currently under study to contribute to efforts to allow men who have sex with men (MSM) to donate blood. One of these approaches involves implementing a programme of plasma donation for fractionation, with a quarantine period. The goal of this article is to identify the determinants of intention to participate in the plasma donation programme among MSM in Montreal, Canada.

Materials and methods Based on the theory of planned behaviour, a questionnaire was developed to measure MSM's intention to donate plasma and identify influencing factors. A multiple linear regression analysis was conducted to identify the determinants of intention to donate plasma.

Results Respondents' (N = 933) intention to donate plasma in the next six months was moderate. The multiple linear regression model explained 55% ($P < 0{\cdot}001$) of the variation of intention. Intention was predicted by attitudes ($\beta = 0{\cdot}34$, $P < 0{\cdot}001$), perceived behavioural control ($\beta = 0{\cdot}28$, $P < 0{\cdot}001$), aged under 35 years ($\beta = 0{\cdot}26$, $P < 0{\cdot}001$), history of blood donation ($\beta = 0{\cdot}24$, $P < 0{\cdot}001$), subjective norm ($\beta = 0{\cdot}21$, $P < 0{\cdot}001$), income above $40,000 ($\beta = 0{\cdot}20$, $P < 0{\cdot}001$), moral norm ($\beta = 0{\cdot}18$, $P < 0{\cdot}001$) and higher level of involvement in various issues LGBTQ+ communities are fighting for ($\beta = 0{\cdot}09$, $P < 0{\cdot}001$).

Conclusion Our analyses show that intention to donate plasma within the proposed programme is associated with personal, social and structural factors, but more strongly predicted by factors related to the theory of planned behaviour. Our results also highlight the importance of involving MSM; community acceptability of the plasma donation programme would probably be higher if MSM felt respected and party to the decisions.

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Key words: plasma fractionation, apheresis donation, donors, donor motivation.

Introduction

In response to the human immunodeficiency virus (HIV) epidemic, Canada introduced in 1986 an eligibility criteria that established a lifetime ban on blood donation for men who had at least one sexual relation with another man

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since 1977. This criterion has always been controversial because of its discriminatory nature, as perceived by LGBTQ+ communities (Lesbian, Gay, Bisexual, Trans, Queer or Questioning, and other identities) [1]. In 2013, the deferral period was changed to 5 years from last men who have sex with men (MSM) contact, then to 12 months in 2016 and finally to 3 months in 2019. Currently in Canada, several studies are underway to produce evidence-based data to support reviewing this criterion or to develop other approaches to facilitate MSM's access to donation of whole blood or other blood products. One option considered is plasma donation for fractionation, a process that greatly reduces the risks of donations being contaminated with HIV or other infections. Having the plasma donation put under quarantine could be an additional measure to ensure the safety of the donation. The window period for HIV can be up to three months (maximum period between the time of infection and when it can be detected by a screening test). Therefore, this approach proposes to quarantine the donation during that period and then test the donor and release the donation when the result is negative.

Drawing inspiration from a model of plasma donation with quarantine recently implemented in France for MSM [2], Héma-Québec (agency that manages the blood donation supply in Québec, Canada) is seeking to explore this avenue. The programme being considered by Héma-Québec would be available exclusively to HIV-negative MSM who have never had hepatitis B or C. Other eligibility criteria could apply, like PrEP use. Each plasmapheresis donation would be quarantined for a period of two to four months, after which the donor would return to get tested for blood-borne infections (e.g. HIV, hepatitis B or C, syphilis) and ideally make another donation. If the test results are negative, the initial donation would be sent for fractionation.

A number of studies have explored motivations and barriers to blood donation in the general population, with the goal of better targeting and recruiting donors [3–8]. However, studies on plasma donation are rarer. Asked about their motivations or the perceived benefits, plasma donors reported that donating is useful [9] as they recognize there is a strong need for this type of blood product [9–11]. A sense of pride [9, 10] and personal satisfaction at the idea of doing a good deed [11], as well as the feeling of being able to save lives [10], are also reported. Some plasma donors had chosen to donate plasma rather than whole blood following an explicit request from staff at a donation centre [9–11]. Others did so because they had more time, since the plasma donation procedure is longer [9, 11]. Although the longer time required also enables donors to socialize with other people [9], it is sometimes considered as a deterrent to plasma donation compared with blood donation [9, 11, 12]. Other concerns mentioned include fear of contamination and unease with the idea that the blood returns to the donor [12]. Disadvantages to plasma donation were also reported, such as discomfort in the arm and fatigue due to the time required for the donation [9]. Some donors were also discouraged by the many forms to fill out [12] and having to be very knowledgeable of their state of health (e.g. medications) to answer questions on eligibility [9]. Lack of knowledge about plasma donation and the process used was additional reported barriers [12]. Charbonneau [10] compared the sociodemographic characteristics of blood donors ($n = 795$) to those of plasma donors ($n = 473$) in their sample from the province of Québec (Canada). Proportionately more plasma donors were men, were married and had a university degree.

Theories such as Ajzen's theory of planned behaviour [13] have often been used to study determinants of donation intention and behaviour. In a Canadian study of blood donors asked to make a first plasma donation, intention to donate plasma and being aged 50 years or over were identified as determinants of plasma donation [14]. Other studies using planned behaviour theories to identify determinants of intention in a population of donors have shown that the main predictors of intention – attitudes [14, 15], moral norm [16] and perceived behavioural control [14] – were associated with a higher intention to donate plasma. Other variables from these theoretical models, such as self-efficacy [15, 16], anticipated regrets [16], behavioural beliefs, normative beliefs and control beliefs [17], were also associated with a higher intention to donate plasma. Studies that used multiple linear regression models demonstrate that the variables from those theories explain 28%–77% of the variation in intention to donate plasma [14, 15, 17].

In the first qualitative phase of the present study, seven focus groups were conducted in 2018 in Montréal; 47 MSM participated, and their average age was 33 years (standard deviation [SD] = 8·8) [18]. The goal was to understand the attitudes, norms and control beliefs related to their intention to participate in Héma-Québec's proposed plasma donation programme. Participants' attitudes towards the programme were mixed. Some participants perceived the programme as discriminatory and stigmatizing towards MSM. By opting for a programme of plasma donation for fractionation, the perception was that MSM's blood is impure, and the quarantine period reinforced the prejudice that MSM take more risks. Despite these inconveniences, the programme was considered as a step forward and that it could enhance social recognition of MSM's contribution to other people's well-being. The programme could also be an opportunity for MSM to help people in need and save lives. As for norms,

most participants asserted that plasma donation is in keeping with their values of altruism, but for some, the programme is at odds with their values of equality and social justice. A majority of participants stated that most people they know would be in favour of their participating in the programme. However, some said that members of and activists in LGBTQ+ communities could be against the programme and even boycott it. As for control beliefs, obstacles to participation included fear of being judged, treated differently or outed without their consent and having to go through a long, restrictive process. Factors that would facilitate their participation in the programme include accessibility of the donation site, possibility of accessing other medical services and being treated with respect and the same as other non-MSM donors.

To our knowledge, the only study that looks at the determinants of intention to donate plasma among MSM is by Levy [19]. In this study, conducted in Israel, the authors explored the feasibility and acceptability of plasma donation for transfusion, with quarantine of donations. Two variables were associated with intention to donate plasma: younger age and low income.

Since demand for products derived from plasma has increased over the past 10 years [20] and the plasma donation process is different than for whole blood, it is important to produce evidence-based data for this type of donation. In addition, almost all of the studies on this topic have involved the general population. It is essential to have a good understanding of MSM's intention to participate in such programmes. Data specific to MSM are needed if Canada is to move forward with changing its donation policy, including for plasma donation programmes accessible to this population. Therefore, the goal of this article is to identify determinants of MSM's intention to participate in Héma-Québec's proposed plasma donation programme.

Methods

Theoretical model

Determinants of intention were studied using a model proposed by Godin [21], which integrates Ajzen's theory of planned behaviour [13] as well as other constructs emanating from theories of prediction of behaviour applied to the field of health, such as Triandis's theory of interpersonal behaviour [22]. This integrative model was used in a study on intention to donate plasma among whole blood donors [14]. According to this model, the main predictors of intention are attitudes, subjective norm and perceived behavioural control. Attitudes refer to an individual's overall cognitive and affective evaluation, whether favourable or unfavourable, when thinking about adopting a behaviour, in this case participating in a plasma donation programme. Subjective norm indicates an individual's perception of whether or not close people in his social circle approve or disapprove of their adopting this behaviour. It is also measured using a moral norm, which corresponds to the individual's perception of the behaviour as being in line with his personal values and principles. Perceived behavioural control is the individual's perception of his capacity to adopt a behaviour. External factors such as individual characteristics (gender, age, education) and environmental characteristics (social or physical) can also influence the relationship between intention and behaviour, as do past behaviours such as experiences or habits linked to the behaviour.

Study population and data collection

A questionnaire was accessible online from June 2018 to February 2019. To participate in the study, respondents had to meet the following criteria: be a man (cisgender or trans), have had a sexual relation with men, be 18 years old or over, be HIV-negative or of unknown HIV status, and be able to read French or English. Participants were recruited through various means: posters in LGBTQ+ premises, sponsored Facebook posts, email invitations, ads on dating app for men, etc. The questionnaire took about 20–30 min to complete and was available in French and English (Appendix 1). A video explaining Héma-Québec's proposed plasma donation programme was shown at the start of the questionnaire. Programme characteristics were presented to participants (apheresis donation, donation quarantine, fractionation process, etc.). All questions on intention were explicitly linked to participation in the programme. Five $100 gift certificates were drawn among participants, as financial compensation. The study was approved by the institutional ethics committee on research involving human beings at Université du Québec à Montréal.

Dependent variable

Intention to donate plasma in the next six months was measured using three items (I would intend to give plasma/I would attempt to donate plasma/I would donate plasma), using a bipolar scale ranging from −2 (strongly disagree) to +2 (strongly agree). A composite score of intention to donate plasma was created with the average of the three items ($\alpha = 0{\cdot}90$ [Cronbach's coefficients of reliability]). A higher score corresponds to a high level of intention to donate plasma in the next 6 months.

Measures of determinants

The measures chosen to identify the determinants of intention to donate plasma in the next six months were

based on the integrative theoretical model [21] and inspired by accounts collected during focus groups in the first phase of the study [18]. Variables were selected based on their applicability to plasma donation behaviour, and direct measures of intention were favoured. Most scales were bipolar, varying from −2 to +2, except connectedness to LGBTQ+ communities, which was a Likert scale varying from 1 to 5.

Attitudes

Six items were used to measure attitudes, where respondents had to rate eventual plasma donation on a semantic differential scale (e.g. donating plasma in the next 6 months would be… 'a useless experience – a useful experience'/'an experience of exclusion – an experience of inclusion'/'a frustrating experience – a gratifying experience'/'a shameful experience – an experience of pride'/'a disgusting experience – an appealing experience'/'a worrisome experience – a reassuring experience'; $\alpha = 0{\cdot}92$).

Subjective norm

A single item was used to measure subjective norm ('people important to me'… would agree or disagree with my plasma donation).

Moral norm

Moral norm was measured using three items (e.g. if I gave plasma during the next 6 months it would… 'be acting according to my values of equality and social justice'/'be acting according to my values of altruism'/'be in accordance with my principles'; $\alpha = 0{\cdot}80$).

Perceived behavioural control

Perceived behavioural control was also measured using three items (donating plasma in the next six months would be… 'difficult – easy'/'complicated – simple'/'not feasible – feasible'; $\alpha = 0{\cdot}89$).

Level of involvement in various issues LGBTQ+ communities are fighting for (as environmental characteristics [external factor])

This variable was measured using one item, that is asking respondents to rate their level of involvement in various issues LGBTQ+ communities are fighting for, varying from 'very low' to 'very high'.

Connectedness to LGBTQ+ communities (as environmental characteristics [external factor])

This Likert-type scale enabled measurement of a respondent's sense of connectedness to LGBTQ+ communities using four items, varying from 'I am not at all like this' to 'I am exactly like this' (e.g. 'I consider myself to be part of the LGBTQ+ community'/'Participating in LGBTQ+ community activities is a positive thing for me'/'I feel a sense of connection with the LGBTQ+ community'/'Being part of the LGBTQ+ community is an important aspect of who I am'; $\alpha = 0{\cdot}92$).

Proportion of friends who are gay, bisexual or queer (as environmental characteristics [external factor])

This variable was measured using one item, where respondents were asked to quantify the proportion of their friends who are gay, bisexual or queer. The variable was dichotomized as a minority or less vs. a majority or all.

Lifetime blood donation and having ever received blood products (as past behaviour)

History of blood donation and having received blood products in the past were both measured using a dichotomous answer choice (No/Yes).

Data analysis

Missing data for our variables of interest (intention and determinants) were processed using a multiple imputation model. Models were based on scale and item level for cross-sectional questionnaire data using an expectation-maximization algorithm and the Markov Chain Monte Carlo method. The proportion of missing data varied between 25% and 37% depending on the variable, mostly due to the questionnaire not being fully completed. To identify determinants of intention, a stepwise multivariate linear regression was conducted, reassigning from one block to another significant variables with *P*-values $< 0{\cdot}001$ in the preceding block. While considering multiple factors that influence the variability of intention towards plasma donation, this method produces a final parsimonious model which allow to greatly reduce measurement error, overfit and model noise and to provide a greater generalization performance across various conditions and population [23]. Attitudes, subjective norm and perceived behavioural control were the main predictors (step 1); moral norm was added in step 2; level of involvement in LGBTQ+ issues, connectedness to LGBTQ+ communities and proportion of friends who are gay, bisexual or queer were added in step 3; lifetime blood donation and having received blood products were added in step 4; and age, education, income, place of birth, language spoken at home and marital status were added in step 5. Step 5 variables correspond to individual characteristics in the theoretical model; here, the forced entry method was used since these variables also acted as control variables. The Statistical Package for the Social Sciences software (SPSS, IBM SPSS Statistics, Release 26, SPSS Inc.) was used for the regression analysis.

Results

Sample description

A total of 933 respondents participated in the online survey (Table 1), 63% of whom fully completed the questionnaire. Comparative analyses (Pearson chi-square and independent samples *t*-test) of the main sociodemographic variables showed a single statistically significant difference between respondents who had completed the questionnaire and those who had not. A significantly higher number of the latter spoke a language other than French at home. The average age of respondents was 34·5 years (SD = 11·9), ranging from 18 to 76 years. About half had a university degree (56%) and a personal income of $40 000 or over (49%). Most were born in Canada (81%), and French was their main language spoken at home (83%). A majority identified as men (95%) and homosexual (87%). Almost half were in a relationship (47%).

Intention to donate plasma and its determinants

On a descriptive level (Table 2), regarding intention to donate plasma in the next 6 months as part of Héma-Québec's proposed programme for MSM, respondents' intention to do so was moderate (mean [M] = 0·6, SD = 1·1). Their attitudes towards plasma donation were rather positive (M = 0·8, SD = 1·0), and they perceived themselves as being relatively able to adopt this behaviour (perceived behavioural control: M = 0·4, SD = 1·0). Respondents' perception that people important to them would approve their donating plasma was relatively high, with an average score of 1·2 for subjective norm (SD = 0·8). They also considered that donating plasma was rather in line with their personal values (moral norm: M = 0·7, SD = 0·9). Respondents' level of involvement in various LGBTQ+ issues was not very high, with an average of –0·3 (SD = 1·1). Their sense of connectedness to LGBTQ+ communities was moderately high (M = 3·3, SD = 1·1). A third (34%) had given blood, and 7% had received a blood product in the past.

The parsimonious multivariate linear regression model explained 55·4% ($P < 0·001$) of the variation of intention (Table 3). Intention to donate plasma as part of Héma-Québec's programme for MSM was predicted, in order of importance, by attitudes ($\beta = 0·34$, $P < 0·001$), perceived behavioural control ($\beta = 0·28$, $P < 0·001$), aged under 35 years ($\beta = 0·26$, $P < 0·001$), history of blood donation ($\beta = 0·24$, $P < 0·001$), subjective norm ($\beta = 0·21$, $P < 0·001$), income above $40,000 ($\beta = 0·20$, $P < 0·001$), moral norm ($\beta = 0·18$, $P < 0·001$) and, to a lesser degree, higher level of involvement in LGBTQ+ issues ($\beta = 0·09$, $P < 0·001$). The other variables included in the model were not statistically significant ($P > 0·001$) (connectedness to LGBTQ+ communities: $\beta = 0·07$, $P = 0·016$; proportion of friends who are gay, bisexual or queer: $\beta = 0·03$, $P = 0·209$; having ever received blood products: $\beta = –0·02$, $P = 0·365$).

Discussion

A number of studies have explored motivations and barriers to blood donation, with a goal of better targeting and recruiting donors. Some studies have looked at determinants of intention to donate blood or determinants of donation, but few have documented the factors associated with intention to donate plasma by apheresis, let alone in the MSM population. To our knowledge, our study is among the first to explore MSM's intention regarding donation of plasma by apheresis. In a context where Canada and several other countries are considering opening blood and plasma donation to MSM, our study provides evidence-based data on this population's intention to participate.

Results show that MSM in our sample have a moderate intention to participate in the plasma donation programme proposed by Héma-Québec. It should be noted that in Canada, there is currently no plasma donation programme for MSM, and that the goal here is to understand participants' intention to adopt this innovation. Although the proposed programme is explained in the questionnaire, it could be difficult for participants to project themselves into such a situation, unfamiliar to them. Should a plasma donation programme for MSM be implemented, it would be important to first target those men for whom intention is strong (early adopters) and to take note of the determinants of intention identified in the current study to increase the probability that these early adopters embrace this innovation [24].

Our analyses indicate that intention to donate plasma as part of Héma-Québec's proposed programme is associated with personal, social and structural factors. Variables in the regression model explain the 55% variation in intention, a fairly good position when compared with the studies reviewed (between 28% and 77%) [14, 15, 17]. Our results are similar to other studies carried out in the general population [14–17], where intention is mostly predicted by determinants of theory of planned behaviour. In our analyses, attitudes towards the plasma donation programme are the preponderant factor ($\beta = 0·34$). Therefore, it is important, when implementing such a programme to better understand the behavioural beliefs underlying those attitudes and highlight the many advantages of participating in the programme, while also minimizing the disadvantages. Perceived behavioural control, the second factor associated with intention to donate

Table 1 Sample description (N = 933).

Variables	Number (*n*)	Proportion (%)
Education		
None, High school diploma or Vocational diploma	182	19·5%
College or Technical	218	23·4%
University	523	56·2%
Other (mixed)	8	0·9%
Annual income, before taxes		
No income	18	1·9%
Under $39 999	420	45·0%
$40 000 or more	459	49·2%
Rather not answer	36	3·9%
Place of birth		
Canada	746	81·1%
Other (mixed)	174	18·9%
Language spoken at home		
French	767	82·5%
English	134	14·4%
Spanish	12	1·3%
Other (mixed)	17	1·8%
Gender identity		
(Not mutually exclusive)		
Man	887	95·4%
Trans	28	3·0%
Non-binary	29	3·1%
Other (queer, genderfluid, two-spirit, agender)	13	1·4%
Sexual orientation		
Homosexual or gay	812	87·0%
Bisexual	60	6·4%
Heterosexual or straight	7	0·8%
Queer, pansexual, fluctuating	40	4·3%
Other (mixed)	11	1·2%
Rather not answer	3	0·3%
Marital status		
Single	490	52·6%
Dating/in a relationship	442	47·4%
HIV status		
Unknown	79	8·5%
Negative	854	91·5%
PrEP use in the past 3 months		
No	500	83·9%
Yes	96	16·1%

plasma (β = 0·28), emphasizes the importance of expanding this perception by addressing the personal and environmental barriers (control beliefs) that could affect it. Just as important is putting facilitating conditions in place, such as flexible hours and easy access to the donation centre. The third factor associated with intention is age under 35 years (β = 0·26). This finding suggests that younger MSM should be targeted for participation in the plasma donation programme. Only Levy's study [19], conducted with MSM in Israel, also identifies younger age as being associated with intention to donate plasma. However, this link still needs to be validated in other studies of this population. Intention to donate plasma is also predicted by history of blood donation (β = 0·24). In our sample, 34% of MSM had donated blood in the past. Although we did not ask participants about this issue, we can assume that they were able to donate blood because they were eligible (in accordance with the deferral period

Table 2 Descriptive data on intention to donate plasma in the next 6 months and its determinants.

Variables	Mean ± standard deviation/*n* (%)
Intention	
Varies from −2 to +2	0·6 ± 1·1
Attitudes	
Varies from −2 to +2	0·8 ± 1·0
Subjective norm	
Varies from −2 to +2	1·2 ± 0·8
Perceived behavioural control	
Varies from −2 to +2	0·4 ± 1·0
Moral norm	
Varies from −2 to +2	0·7 ± 0·9
Involvement in LGBTQ+ issues	
Varies from −2 to +2	−0·3 ± 1·1
Connectedness to LGBTQ+ communities	
Varies from 1 to 5	3·3 ± 1·1
Proportion of friends who are gay, bisexual or queer (majority or more)	227 (24·3%)
Blood donation (lifetime)	315 (33·8%)
Having received blood products (lifetime)	69 (7·4%)

Table 3 Multiple linear regression on determinants of plasma donation.

Variables	β	Standard error	95% CI
Step 1			
Attitudes	0·34	0·03	0·27 to 0·40[a]
Subjective norm	0·21	0·04	0·13 to 0·29[a]
Perceived behavioural control	0·28	0·03	0·22 to 0·33[a]
Step 2			
Moral norm	0·18	0·04	0·10 to 0·26[a]
Step 3			
Involvement in LGBTQ+ issues	0·09	0·02	0·05 to 0·14[a]
Step 4			
Blood donation (lifetime)	0·24	0·05	0·14 to 0·34[a]
Step 5 (control variables)			
Age (34 years or less)	0·26	0·05	0·16 to 0·36[a]
Education (university degree)	−0·04	0·05	−0·13 to 0·06
Income ($40 000 or more)	0·20	0·05	0·09 to 0·30[a]
Place of birth (other than Canada)	0·10	0·06	−0·03 to 0·22
Language spoken at home (other than French)	0·06	0·06	−0·06 to 0·19
Marital status (in a relationship)	−0·03	0·05	−0·12 to 0·06

R^2: 0·554.

$P < 0·001$.

[a]Significant association.

based on time of donation), or because they omitted information, for instance, feeling that they did not present behavioural risks (e.g. monogamous/sexually exclusive). For a population who has long been banned from donating, this proportion is high and suggests a pool of potential donors. Theoretically, past behaviour is a predictor of intention; a study of the general population determined that having given blood in the past is a predictor of a second donation [25]. Subjective norm, the fifth factor associated with intention (β = 0·21), is less often reported as a predictor of intention. Given the issues surrounding MSM and blood donation, MSM

donors must feel they have the approval of significant individuals, including members of LGBTQ+ communities. As stated by focus group participants during the first stage of this study [18], some community activists could boycott the plasma donation programme if it is seen to be discriminatory, for example, if donations are quarantined. The programme to be implemented must be considered acceptable to LGBTQ+ communities. A second sociodemographic variable is also associated with intention: income above $40 000 ($\beta = 0{\cdot}20$). This variable was not identified in the studies reviewed, except by Lévy [19], who indicates the reverse, that is lower income. As is the case for age, other studies of MSM are needed to confirm a link between income and intention to donate plasma. Moral norm ($\beta = 0{\cdot}18$) is associated with intention to donate plasma, but to a lesser degree. Agreement between behaviour and personal values affects MSM's motivation to participate in a plasma donation programme. Because the association between moral norm and intention is weaker, it does not appear to be central to such decision making. To a lesser degree, intention to donate plasma is also predicted by higher level of involvement in various issues LGBTQ+ communities are fighting for ($\beta = 0{\cdot}09$). Although our sample's level of involvement in those issues is not very high ($m = -0{\cdot}3$; $SD = 1{\cdot}1$), higher level is associated with stronger intention to participate in the plasma donation programme. As focus group participants noted [18], the plasma donation programme marks a step in the right direction. Consequently, it seems essential to involve MSM in the process to change practices and review eligibility criteria so they can feel respected and be party to the decisions. Community acceptability would probably be higher if the communities were first consulted about the characteristics of the plasma donation programme targeting them.

Study limits and impacts of the findings

It is possible that the measurement of intention to donate plasma is underestimated in our study, given that it is measured in the context of the programme for MSM proposed by Héma-Québec, which included constraints such as apheresis donation, donation quarantine and plasma fractionation. Therefore, results for intention cannot be generalized to plasma donation in different contexts. The issue that resulted in the most resistance in focus groups during phase one of the study [18] is that donations would be quarantined; this characteristic of the programme is perceived as discriminatory since it does not apply to the general population of donors. Another limitation is related to participant recruitment, which was mostly done through LGBTQ+ communities. The sample may have been biased due to an overrepresentation of MSM with a strong sense of belonging to their communities. However, while analyses were controlled for this variable, there was no statistically significant association with intention to donate plasma. The presence of missing data in our sample may have caused a bias in our analyses of prediction of intention. Comparative analyses of participants who had completed the questionnaire and those who had not showed a difference only for language spoken at home. However, this variable is not associated with our variable of interest – intention to donate plasma. Moreover, missing data imputation performed for the linear regression model allowed to reduce this bias and ensure better statistical power.

In conclusion, this study provides a novel understanding of the issue of plasma donation in MSM. It can also be used to support subsequent decisions and steps towards changing policies concerning plasma donation among MSM. Future studies should explore intention to donate plasma in the absence of the characteristics of the plasma donation programme considered in the current study. Finally, if pilot projects are implemented, an evaluation of programme implementation would generate evidence-based data on planned behaviour (donating plasma) and not only on intention.

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Conflict of interest

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Appendix Online survey.

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A discrete-event simulation model for analysing and improving operations in a blood donation centre

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Vox Sanguinis

Background and objectives Healthcare systems require effective and efficient blood donation supply chains to provide an adequate amount of whole blood and blood components to hospitals and transfusion centres. However, some crucial steps of the chain, for example blood collection, are not adequately studied in the literature. This work analyses the operations in a blood collection centre with the twofold aim of analysing different configurations and evaluating the effectiveness and feasibility of schedules defined at higher planning levels.

Materials and methods The analyses are performed through a discrete event simulation (DES) model that describes a customizable collection centre. Moreover, a feedback loop from the DES to the higher planning level allows to adjust scheduling decisions if they determine criticalities or infeasibilities at the operational level.

Results Numerical tests have been conducted considering a real Italian provider. An experimental plan has been designed to compare different configurations for the blood collection centre and evaluate the best ones in terms of cost and service quality for the three main actors involved (donors, workers and managers). The best configurations have been also used to test the feedback loop.

Conclusions Results confirm the appropriateness of the proposed DES model, which can be considered a useful decision support tool for dimensioning and managing a blood collection centre, either as a standalone tool or in conjunction with a scheduler.

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Introduction

Transfusion medicine is arguably one of the most industrial-like specialities in modern medical science. Although some studies are ongoing, the state of the art for artificial substitutes of human blood is far from satisfactory, meaning that healthy donors are the only suppliers [1, 2]. Moreover, blood components have a limited shelf life (up to 42 days for red cells, 5 or 7 days for platelets depending on the regulatory environment, and 2 years for plasma), which prevents strategic long-term storage [3, 4].

Blood is provided to healthcare systems through the so-called Blood Donation Supply Chain (BDSC), whose management includes both strategic and operational decisions. Following the descriptions provided by Sundaram and Santhanam [5] and Osorio et al. [6], the BDSC can be divided into four stages: (i) collection (including donor registration, donation and blood screening); (ii)

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transportation; (iii) storage; (iv) utilization (including demand prediction, supply management and distribution).

In particular, collection is one of the most important stages in making the BDSC work properly. In fact, collecting an adequate amount of blood is a key requirement, as an unbalanced supply of units could trigger alternating periods of blood shortage and wastage. Therefore, its proper management is essential to improve all downstream steps, including patient care. Moreover, its management is particularly critical because blood collection merges the characteristics of a production system, where both demand and production are stochastic, and those of a service provider regarding the collection from donors [7, 8]. However, some stages of the BDSC are not adequately addressed in the literature yet. For example, while storage is extensively studied, collection is only marginally considered despite its relevance [9].

Given the increasing global demand for blood and the need to comply with budgetary and legal requirements, there is a growing need to reengineer the BDSC and in particular the management of collection centres by implementing efficient evidence-based policies. Moreover, as these centres are typically managed by non-technicians, these policies should be implemented using highly intuitive tools [10].

In this context, our paper focuses on the operational management of blood collection centres with a double motivation. On the one hand, we analyse different centre configurations to identify the best ones in terms of cost and service quality, considering the perspectives of the three main actors involved (donors, workers and managers). On the other hand, we assess the interactions of the operational level with higher planning levels and, in particular, we evaluate the operational effectiveness and feasibility of the schedules deriving from donor appointments defined at the tactical level.

Quantitative analyses are performed through a discrete event simulation (DES) model that describes a general and customizable blood collection centre. The DES architecture was chosen over other methodologies because of its inherent flexibility, the reduced number of assumptions required to mimic reality, and its ability to determine the best configuration among a series of alternatives under uncertainty, by exploiting what-if analysis. Even if this may lead only to nearly-optimal policies, those are sufficient in most cases where the optimal solution is indeterminable or impractical: for instance, other methods such as Integer Linear Programming require high computation power and more restrictive assumptions to provide a solution. Moreover, DES gives an intuitive representation of the flows, which can be readily understood by non-technical personnel without a strong operations research background, as often is the staff in blood collection centres. Finally, it has a short learning curve and allows users to easily verify the effectiveness and feasibility of decisions made at higher levels, enabling optimization-simulation frameworks in case these decisions need to be revisited.

The structure of the DES model is formalized with Business Process Model and Notation (BPMN) standards [11], while the inputs are provided by a donor appointment schedule. To validate the DES model and demonstrate its practical applicability, numerical experiments have been conducted considering the real case of the Milan branch of the main Italian blood provider, the *Associazione Volontari Italiani Sangue* (AVIS), hereinafter referred to as AVIS Milan. For such a case, the input schedule has been provided by the Blood Donor Appointment Scheduling (BDAS) tool presented in Baş Güre et al. [12] and Yalçındağ et al. [13].

In the literature, one of the earliest works dealing with BDSC from a non-clinical point of view was proposed by Millard [14], who suggested applying industrial inventory models to blood collection. However, BDSC only began to receive broader attention a decade later, as reported in several literature reviews [9, 15, 16]. Although BDSC has been extensively studied in the operations research literature, the different steps of the BDSC have not received the same attention. For example, as mentioned above, storage is the most addressed step, and collection the least one [9].

Several techniques can be applied to study blood collection, for example evaluation of best practices, linear and integer programming, queuing models and Markov decision processes [17–24]; however, simulation-based works outnumbered those with any other solution method over the years. Indeed, simulation is a rather effective approach to address BDSC management problems, according to Beliën and Forcé [15], because of the complexity of the system. It usually leads to the identification of near-optimal policies, which may be sufficient in cases where the optimal solution is indeterminable or impractical. In particular, DES has been highlighted as the most effective and practical approach for many aspects of BDSC management.

Finally, it is worth mentioning that DES has been successfully applied to the optimization of operations in other healthcare services, for example in operating theatres [25], in specific home care services [26] and in resource allocation for screening [27].

In the following, we focus on simulation techniques applied to the management of a blood collection centre to highlight the differences and underline the contributions of our work.

Jennings [28] used simulation to evaluate the impact of different policies on stock levels, shortages and the expiration of blood units. Pratt and Grindon [29]

developed a computer simulation model to study flow and queuing problems arising in blood collection, considering various scheduling strategies. Sirelson and Brodheim [30] built a simulation model to evaluate the performance of a platelet inventory system in terms of out-of-stock and expired units. Brennan et al. [31] studied service and productivity problems for American Red Cross blood collection using a general purpose simulation software, with the goal of understanding which donor arrival patterns make the system more efficient under different configurations, staff allocations and work rules. Michaels et al. [32] studied the impact of several planning strategies to schedule the arrival of donors at a corporate blood collection. More recently, De Angelis et al. [33] integrated a simulation model with a neural network to find an optimal resource configuration for a transfusion centre in Rome, modelling the operations as a set of consequential servers with a given nominal capacity. Rytilä and Spens [34] simulated the Finnish transfusion system, while Katsaliaki and Brailsford [35] analysed a British hospital supplied by a regional blood centre. Alfonso et al. [36] applied Petri net models and quantitative simulation to the case of a blood collection centre in France, defining performance indicators to evaluate human resource planning and donor arrival patterns both for booked apheresis donors and walk-in whole blood donors, in mobile and in fixed collection settings alike. Blake and Shimla [37] used a flow shop model to create a linear model that determines the most efficient staffing configuration in a Canadian blood centre. Finally, Moons et al. [38] exploited a software designed for industrial simulation to determine optimal staffing and resource planning.

According to Pirabán et al. [16], no work dealing with the simulation of a blood collection centre implemented an optimized appointment schedule so far. In fact, the available models are intended to identify the best input management policies given a set of organizational constraints, while in our work we focus on merging simulation with the output of an optimized scheduler. Some of the listed works also included a visual representation of the simulator, to make its functioning more intuitive for the managers of blood donation centres, who usually are medical (non-technical) personnel [10]. Some also considered multiple stakeholders' perspectives to evaluate their results. Therefore, to make its functioning more intuitive, our tool also includes a visual representation of the simulated environment.

Table 1 summarizes the main characteristics of the studies dealing with simulation for blood collection and compares them with our work. None of the other published papers included all the characteristics that are considered together in our work, and in particular the integration with an optimized schedule. Among the available works, the one proposed by Alfonso et al. [36] is the most similar to ours, but this also neglects the just-mentioned feature.

The remainder of this paper is structured as follows. Section 2 describes the proposed DES model, its implementation, validation and application to the AVIS Milan case. Section 3 presents the results for both the analysis of alternative layouts and the feedback to the appointment scheduler. Finally, Section 4 and 5 discuss the results and provides the conclusion to the work.

Materials and methods

The operations at the blood centre are based on the observations made in AVIS Milan and integrated with those reported in the literature, mainly in Alfonso et al. [36]. The resulting description is general and customizable enough to describe several blood collection centres.

Process description

The whole blood donation process for a donor generally consists of four main steps:

(1) All donors who arrive at the donation centre show up at the reception, where an employee records their information. Donors are also given a pre-donation anamnestic questionnaire to fill out.
(2) Donors enter a consultation room with a physician. Relevant vital signs are measured, including blood pressure, heart rate and haemoglobin concentration. After all data have been collected, the physician determines whether each donor can donate that day or whether they must be deferred temporarily or permanently. If not deferred, donors can proceed with the donation.
(3) Donors are assigned to a donation bed, where all necessary devices and consumables have been prepared.

Table 1 Characteristics considered in the available works dealing with a simulator for blood collection and in this work: booked donors (BD); unbooked donors (UD); fixed setting (FS); mobile setting (MS); visual representation (V); assessment of three stakeholders' perspectives (P); interaction with optimized schedule (OS)

	BD	UD	FS	MS	V	P	OS
Pratt and Grindon [29]		X		X			
Brennan et al. [31]		X		X			
Michaels et al. [32]	X	X		X			
De Angelis et al. [33]		X	X		X		
Alfonso et al. [36]	X	X	X	X	X	X	
Blake and Shimla [37]	X	X	X	X		X	
Moons et al. [38]		X	X		X		
This work	X	X	X		X	X	X

The skin of the donor's inner elbow is disinfected, a vein of the arm is punctured and the phlebotomy begins. The first millilitres of blood are collected in test tubes, which will be sent for screening. Then, the withdrawn blood is directed to the donation bag. After the target extraction level (~450 ml) is reached, the needle is removed and donors are left on the bed for a few additional minutes. This prevents vagal fainting that may occur when the donor suddenly switches from lying to standing [39].

(4) Finally, donors are directed to a canteen area to get refreshed, and above all for post-donation supervision, to detect possible negative outcomes before they leave the centre.

All works report that the blood collection process begins with donor registration and ends with the donor being offered a light meal after phlebotomy in a canteen or snack room. However, other activities such as questionnaire filling, haemoglobin testing, vital signs check and clinical counselling are taken into account differently in each work. For example, haemoglobin can also be measured before filling out the questionnaire or in the donation room just before the donation.

The arrival of donors can occur in different ways and according to different rules. In our DES model, input arrivals are given by an appointment schedule and in particular by the BDAS scheduler of Baş Güre et al. [12], whose aim is to balance the production of the different blood types (combination of group and Rhesus factor) between days while penalizing overtime and periodic accumulation of donors (queues). This scheduler divides each day into k periods (e.g. 'early morning', 'late morning' and 'early afternoon') considering already booked appointments and the expected number of walk-in (unbooked) donors for each period to pre-allocate group-specific donation slots with the aim of balancing blood type production. Then, when donors make a reservation, they fill one of these slots. The system is dimensioned on the basis of the overall physician capacity for each period and standard time for a medical consultation and is capable of penalizing donor accumulation using period-specific weights. The choice of physician time as the scarce resource derives from the characteristic of the system under analysis. Indeed, different from other health facilities where beds represent the bottleneck of the system, the cost for adding a physical bed, if there is room, is not so relevant for the considered system. Here the highest costs are associated with disposables, tests performed on donated units, and staff; while the former two items are fixed, given the number of units, staff represents the most relevant cost item on which a proper management of the blood collection centre may have an impact. Finally, if the number of booked donors for a given blood type is high, the scheduler dynamically reacts by increasing the number of new slots to allocate to that given blood group rather than simply allowing overbooking. The interested reader is referred to Baş Güre et al. [12] for more details.

The following assumptions have been made to formalize the design of the DES model. Only donations at a fixed-site blood collection centre are included, while mobile settings are excluded (e.g. blood collection vehicles sent by the centre for collection in schools, companies and at events). Only whole blood donations are considered, and they are all assumed to be successful, because the actual usability of a donation does not have an impact on the system in terms of resource occupation. A given percentage of booked donors is expected to not show up at the assigned appointment. Moreover, a given percentage of donors is estimated to be deferred from the donation after being visited; these donors leave the centre without going through the next steps and will return from a date set by the physician. Apart from this, donors do not voluntarily leave the process once they enter, contrary to what was assumed by Alfonso et al. [36]; instead, we model queues without a maximum waiting time and use the time spent in the queue as a performance metric.

The DES model refers to voluntary centres in Western countries; however, international standards contribute to unify the key steps, and the World Health Organization is actively promoting homogeneity among all transfusion systems [2, 40].

The DES includes the following configurations to cope with the alternative layouts for the activities in steps 1 and 2, to guarantee generality and flexibility to the description:

- Questionnaire filling: it is combined with waiting and physician's consultation times.
- Haemoglobin measurement: it can be performed either in conjunction with the consultation or separately, before or after it.
- Vital signs measurement: it is included in the consultation.
- Consultation: it is the last step before phlebotomy, performed in a physician's office or equivalent setting. It must be remarked that, in some contexts, consultation can be performed by non-physician personnel. However, this is not a decision that can be taken individually by a blood centre, as it intertwines with local legislations, and, moreover, it does not impact on queuing or staff deployment.

Conceptual modelling with BPMN notation

A BPMN scheme of the system has been constructed to serve as the basis for the DES model. The BPMN

notation allows to present the actions taken by the different actors involved in the process separately and to easily show how they interact and at which stage of the process [11].

The BPMN diagram is shown in Fig. 1. Each horizontal section is devoted to the actions taken by an actor: (i) donors, (ii) receptionists and administrative staff, (iii) physicians and (iv) nurses. The two blocks within coloured lines refer to the activities carried out at a higher level by the BDAS [12], included here as they characterize the system input. In particular, the green dashed line and the red dotted line identify the offline pre-allocation and the online allocation phases, respectively. All logical blocks, included in the DES model, represent the actual blood donation process according to the 4 steps presented in Section 2. Summing up, during the reception phase the donors arrive at the centre and interact with an administrative figure (receptionist or employee), who registers their arrival. The medical consultations are done by physicians, who decide whether each donor can donate that day or whether they should be deferred. During the phlebotomy, donors are assisted by a nurse, who takes care of the pre-setup and post-setup of the collection equipment. Finally, donors get a refreshment before leaving the facility.

Model implementation

The DES model has been implemented in FlexSim (FlexSim Software Products Inc., Orem, UT, USA), in a parametric form so that the parameters of the distributions can be easily adjusted or tailored. This implementation also provides a real-time 3D visual counterpart of the simulated environment, shown in Fig. 2.

The arrivals of both booked and unbooked donors at the centre are taken for each period from the BDAS pre-allocation solution. As for booked donors, their arrival times are generated using a uniform probability distribution within their respective period. Since the scheduler assigns donors to periods, rather than giving them a specific appointment, the uniform distribution provides a satisfactory and unbiased proxy for the behaviour of booked donors, reflecting the situation of AVIS Milan in which no particular donor arrival pattern has been observed. By directly considering the BDAS pre-allocation as input rather than the actual reservations of donors who call to make a reservation (see Baş Güre et al. [12]), not all pre-allocated slots are expected to be filled and converted into booked donors' slots. Therefore, each pre-allocated slot is considered to become booked following a Bernoulli distribution with probability p_{fill}. In addition, some booked donors may not show up for the donation with respect to the nominal arrivals. To model this, each booked donor is considered not to show up following a Bernoulli distribution with probability p_{ns}. Finally, unbooked donors are generated in quantities equal to the expected value considered in the BDAS pre-allocation solution, within their respective period, using a uniform probability distribution for the arrival time. This also reflects the situation of AVIS Milan where these donors tend to show up randomly.

The activities are organized and modelled as detailed below.

Each donor goes immediately to the registration desk upon arrival. Registration is set up to run differently depending on whether the donor is booked or not. In particular, the registration time is longer for unbooked donors, due to the fact that their data must be entered into the database by the clerk who serves them, and this process takes additional time.

After being successfully registered, donors must see a physician for consultation. Each physician is located in a separate office. If there is a free physician, donors can access the office directly; otherwise, they must stay in a waiting area until their turn comes. Different logics for queue management have been considered in the DES model; in the baseline scenario, the queue is served using a *First In First Out* (FIFO) logic. During the waiting time, donors fill out a questionnaire they received during registration; therefore, the time for compilation is not modelled separately.

When a physician is available, donors go to his/her office and undergo a consultation. One aspect of the assessment is haemoglobin measurement, which as mentioned can be carried out together with the consultation or not. It is considered jointly in the baseline scenario, and separately in the variants. The two process times (consultation and haemoglobin measurement) are modelled separately; in the baseline scenario, we consider their sum.

The outcome of the clinical assessment for donation eligibility depends on whether donors are booked or not. In fact, unbooked donors are assumed to have a rejection rate at least five times higher than the booked ones, who receive initial feedback during the reservation and are more familiar with the reasons why one should self-abstain from donating. Separately for the two types of donors, rejections are modelled using Bernoulli distributions with different parameter values.

Afterwards, accepted donors enter the donation room. The first available nurse accompanies them to the first available bed according to a FIFO policy in all scenarios. Then, the pre-setup of the machine is performed by the nurse, including the puncture of the donor's vein.

During phlebotomy, the nurse is able to attend other activities that need to be performed, including taking care

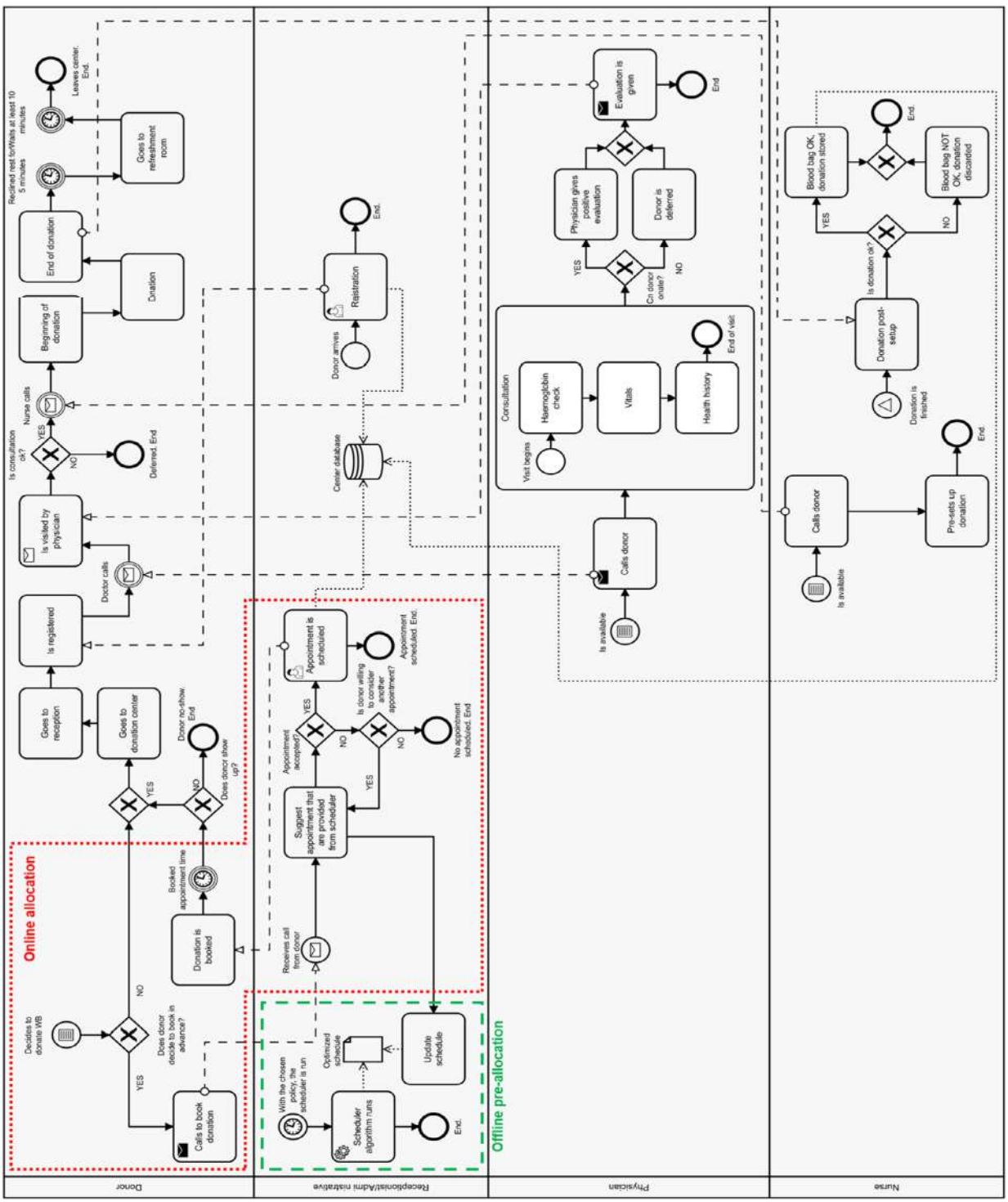

Fig. 1 BPMN model of the operations carried out at the blood collection centre. [Colour figure can be viewed at wileyonlinelibrary.com]

of other donors. After the donation, when the planned amount of blood has been withdrawn, a nurse is again engaged to disconnect the donor from the bag and for the post-setup activities.

After successfully completing the donation, donors are asked to rest on the bed to avoid vasovagal fainting related to the orthostatic reflex. Then, they can leave the donation room and move to a canteen area, where they

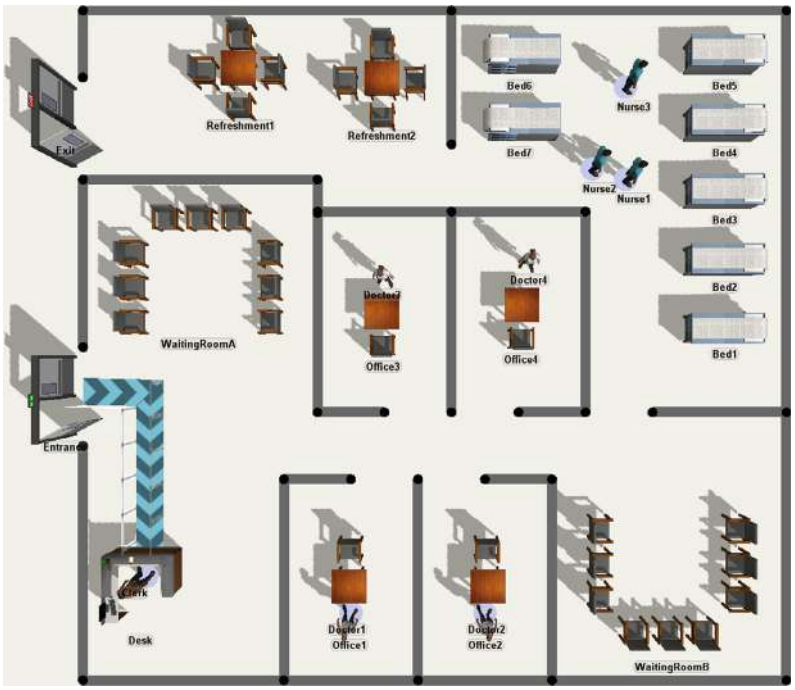

Fig. 2 Bird's eye view of the 3D environment of the simulator. [Colour figure can be viewed at wileyonlinelibrary.com]

have a refreshment before leaving the centre. This last step is not fundamental to the workflow of the donation centre, especially because it is managed separately and usually organized as a self-service area. However, the time spent in the canteen is an integral part of the psychological time that donors spend inside the centre. In this light, the refreshment phase is included in the calculation of the total donors' cycle time.

Experimental setting

A first set of experiments was conducted to validate the DES model. Then, an experimental plan was designed to compare different configurations and evaluate the best ones in terms of cost and service quality for the three main stakeholders involved (donors, workers and managers). Finally, a feedback loop from the DES to the higher scheduling level was implemented and evaluated, to adjust scheduling decisions if they determined criticalities or infeasibilities at the operational level. More specifically, the feedback consisted in the re-calibration of some parameters used by scheduler, based on the outcomes obtained from the DES model.

All experiments have been tailored to the AVIS Milan case, in terms donor flow and number of resources involved.

In the following, after reporting the adopted parameters and the Key Performance Indicators (KPIs) designed to evaluate the performance of the collection centre in Sections 2.4.1 and 2.4.2, respectively, we present the validation of the DES model in Section 2.4.3 and the tested alternative layouts in Section 2.4.4.

Simulation parameters

Already booked donors, pre-reserved slots and expected walk-in donors were taken from the first day solution of the BDAS scheduler was taken in Baş Güre et al. [12]. In particular, we randomly extracted a solution generated after the ramp-up period from the scheduling experiments reported in that work. Moreover, as similar amounts of donors were observed in the BDAS between days, the solution is representative of the considered collection centre.

The distributions of the different process times used are from the available literature. Since AVIS Milan could not provide us all the data required to build the whole simulation model, we referred to the literature as a proxy. The adopted distributions were evaluated against qualitative observations reported by AVIS Milan, and it was observed that the two sets were comparable. On this basis, process times were defined as follows:

- Registration for booked donors: normal distribution with mean value $\mu_{ru} = 1 \cdot 77$ and standard deviation $\sigma_{rb} = 0 \cdot 612$ (Alfonso et al. [36]).
- Registration for unbooked donors: log-normal distribution with mean value $\mu_{rb} = 2 \cdot 4$ and standard deviation $\sigma_{ru} = 0 \cdot 82$ (Brennan et al. [31]).
- Consultation: triangular distribution with minimum value $a_c = 16 \cdot 5$, maximum value $b_c = 20$ and modal value $c_c = 18 \cdot 33$ (shape from Alfonso et al. [36], with parameters modified to fit the AVIS Milan case).
- Haemoglobin testing: uniform distribution between minimum value $m_h = 1 \cdot 34$ and maximum value $M_h = 2$ (Alfonso et al. [36]).
- Pre-setup of collection equipment: normal distribution with mean value $\mu_{s1} = 1 \cdot 6$ and standard deviation $\sigma_{s1} = 0 \cdot 284$ (Alfonso et al. [36]).
- Phlebotomy: Weibull distribution with scale $\lambda_p = 4 \cdot 23$, shape $k_p = 1 \cdot 82$ and location $\gamma_p = 9 \cdot 5$ (Alfonso et al. [36]).
- Post-setup of collection equipment: uniform distribution between minimum value $m_{s2} = 1$ and maximum value $M_{s2} = 2$ (Alfonso et al. [36]).
- Resting: triangular distribution with minimum value $a_l = 4$, maximum value $b_l = 10$ and modal value $c_l = 5$ (Tomasulo et al. [39]).
- Post-donation refreshment: log-normal distribution with mean value $\mu_r = 14 \cdot 22$ and standard deviation $\sigma_r = 7 \cdot 11$ (Alfonso et al. [36]).

Note that parameters a_c, $b_c c_c$, m_h and M_h related to the consultation and haemoglobin distributions are calculated such that the expected value of the sum of these two times is equal to the standard consultation time used in the BDAS pre-allocation.

The $k = 3$ periods used in the BDAS solution were translated into 3 blocks of two hours: 7:30 a.m. to 9:30 a.m.; 9:30 a.m. to 11:30 a.m.; 11:30 a.m. to 1:30 p.m.. However, the simulation did not stop at the end of the third period to measure the time the last donor leaves the system after completing the donation process, even if it falls after the centre closes. In this way, it was possible to assess whether queuing causes some donors to remain in the system beyond opening hours.

Moreover, the following parameters were taken for the Bernoulli densities: no-show rate of already booked donors $p_{ns} = 0 \cdot 05$; filling rate of pre-allocated slots $p_{fill} = 0 \cdot 9$; deferral rate at the consultation for booked donors equal to $5 \cdot 03 \times 10^{-3}$; deferral rate at the consultation for unbooked donors equal to $4 \cdot 523 \times 10^{-2}$; deferral rate at the haemoglobin testing equal to 5×10^{-3} for both booked and unbooked donors. Deferral rates were chosen to give a total acceptance rate of 99% and 95% for booked and unbooked donors, respectively, as provided by AVIS Milan. These high acceptance rates are associated with the fact that booked donors are usually pre-screened during reservation and with the fact that also unbooked donors are regular donors aware of the acceptance criteria for donating.

Finally, the other parameters used in the DES model are in agreement with the BDAS solution considered: collective nominal physician capacity equal to 450 min for all periods; mean consultation time equal to 20 min.

Key performance indicators

Several KPIs have been identified taking into account the point of view of the three main stakeholders involved: donors, staff and managers of the centre, each one with their own prerogatives. Targets regarding KPIs were discussed with AVIS Milan, but different centres with different needs may personalize their own set of KPIs to reflect the individual needs of the centre.

Two indicators have been identified for donors: total time spent in the system (cycle time, also used in Section 2.4.3 for validation), and time spent in queue. They have also been differentiated between booked and unbooked donors. In particular, cycle time (encompassing all donors) has been our main metric of interest.

Utilization statistics have been considered for resources: three types of personnel (physicians, nurses and clerks) and beds. As for physicians, who are considered the scarce resource, the aim is to limit their utilization, while for the other resources the target is to increase it. This is in agreement with the DBAS scheduler we used.

The main indicator from the management point of view is cost-efficiency, where efficiency is evaluated in terms of donors' cycle times. Moreover, two other indicators have been considered in each period k to evaluate the periodic accumulation of donors in the bottleneck of the system (the queue before consultation): the used physician capacity, and the exit time from consultation of the last donor of the period.

A summary of all KPIs is provided in Table 3.

A non-monetary cost unit (CU) has been associated with all variable resources, ignoring all fixed costs that do not vary with the layout (e.g. structures and physicians, whose number is constant as the layout changes). In particular, the following CUs have been considered, in agreement with Alfonso et al. [36]: clerk salary equal to 5 CUs/workday; nurse salary equal to 10 CUs/workday; the cost of a bed equal to 1 CU/workday. In the AVIS Milan case, 1 CU corresponds to about 20 euros.

Model validation

The DES model was verified and validated in two stages.

First, a face validation and a verification for reasonableness analysis were performed. Face validation was carried out iteratively, comparing model results with feedback from subject-matter experts [41] from AVIS Milan, and adjusting parameters accordingly (the above presented parameters are already those set after validation). As for the verification, the inputs were varied and the adequacy of the system response was verified, for example we checked that queue length and delays increased/decreased as donor arrival rates increased/decreased. System sensitivity to different probability distributions was also checked and found to be low.

Second, and more importantly, a statistical validation of model performance was performed by considering $n = 5$ replications of the baseline DES solution, modelling the current configuration of the centre, and focusing on the main metric of interest, that is the donor cycle time (referred here as T_D). In fact, it is a comprehensive metric, able to define the throughput of the collection centre. AVIS Milan reported that the true value of T_D for their centre is $\mu = 65$ minutes. First, we tested the 5 replications for normality. The sample mean for donor cycle time ($\underline{X}_{T_D}$) was 71·798, and its sample standard deviation (S_{T_D}) was 7·634. Then, we conducted a 2-tailed t-test with significance level $\alpha = 0{\cdot}05$, testing the null hypothesis H_0 that $E(X_{T_D}) = \mu$. The test failed to reject H_0, meaning that the DES adequately models the system. However, failing to reject H_0 is a weak conclusion, unless the power of the test is high. Therefore, we calculated the sample critical difference $\hat{\delta}$ for power analysis:

$$\hat{\delta} = \left|\frac{\underline{X}_{T_D} - \mu}{S_{T_D}}\right| = \left|\frac{71{\cdot}798 - 65}{7{\cdot}634}\right| = 0{\cdot}890$$

Referencing an operating characteristic curve (OC curve), the probability of a type-II error $\beta(\hat{\delta})$ was around 70% with $n = 5$, an unacceptably high value. According to the OC curve, to obtain $\beta(\hat{\delta})$ less than 0·05, $n = 30$ samples were required. Therefore, $n = 30$ replications were used in all experiments to guarantee the significance of results.

Alternative layouts

The following parameters and policies were tested in the experiments:

- Queuing policy: all donors are treated equally (FIFO setting of the baseline scenario) or booked donors are prioritized with respect to unbooked ones. In this case, booked donors are always called before any other unbooked donor already in the queue for the consultation.
- Haemoglobin testing: it is placed within, before or after consultation. In the latter two cases, a designated area for testing must be set up, with personnel dedicated to carrying out the test. The additional unit of personnel is assumed to be a nurse.
- Planned capacity of physicians in each period k: since the considered capacity c_{tk} of 450 min corresponds to 3·75 physicians over a two-hour period, three physicians working for the whole period and one working for an hour and a half are required. This hour and a half can be placed either at the beginning or at the end of the period, resulting in two possible configurations.
- Capacity of other resources not included in the BDAS: the number of clerks was set at 1 or 2, the number of nurses at 2 or 3, and the number of beds at 5, 6 or 7, varying on the baseline of the AVIS Milan case. We assumed that, apart from the physicians, all personnel are present in the blood collection centre throughout the day from the beginning of the first period to the closure.

These are the parameters that can have a significant impact on system performance, both looking at the DES model and from considerations made by the AVIS Milan staff, who suggested the above-mentioned factors and levels.

The combination of these factors produced a total of 144 alternative layouts, as summarized in Table 2. Each layout was tested $n = 30$ times, for a total of 4,320 runs.

Table 2 Alternative parameters and policies tested in the experiments

Parameter	Alternatives			Name
Queuing policy	FIFO			FIFO
	Priority to booked donors			PRTY
Physician timetable	At the beginning			1
	At the end			2
Haemoglobin testing	Within consultation			W
	After consultation			A
	Before consultation			B
Number of resources	*Nurses*	*Clerks*	*Beds*	
	2	1	5	01
	2	1	6	02
	2	1	7	03
	2	2	5	04
	2	2	6	05
	2	2	7	06
	3	1	5	07
	3	1	6	08
	3	1	7	09
	3	2	5	10
	3	2	6	11
	3	2	7	12

The last column reports the name of the alternative, used to compose the name of the scenario.

Repetitions were performed assuming common random numbers as the layouts varied, by repeating the simulations with exactly the same streams of uniforms random numbers. The name of each layout is uniquely identified by a string that includes the name of the alternatives as in Table 2. For example, the layout 'FIFO-1-W-01' uses the FIFO queuing policy, has the fourth physician working at the beginning of each period and the haemoglobin test performed during the consultation, and includes 2 nurses, 1 clerk and 5 beds.

Results

This Section presents the results from both the analysis of alternative layouts and the feedback to the BDAS.

Analysis of alternative layouts

We performed a multivariate ANOVA for the KPIs, including the six factors reported in Table 3 (queuing policy, physician timetable, haemoglobin testing, number of nurses, number of clerks and number of beds) with their respective levels, and all possible two-factor interactions. The cost-efficiency KPI was excluded from this first part of the analysis, as it is a function of cycle time and the cost associated with the number of resources involved. Calculations were made using MATLAB (version R2020a, Mathworks, Natick, MA, USA).

To properly employ ANOVA, the normality of the KPIs over the repetitions was checked by analysing their respective q-q plots. Although some outliers were present, the q-q plots showed that a normal distribution can be assumed for the sampled data, which allowed to correctly use ANOVA for all KPIs. A summary of the statistically significant factors is reported in Table 4, along with the statistically significant two-factor interactions.

Regarding donors, queuing policy was found to have a very strong impact (p-value = 0·0000). The prioritization policy regularized booked donors' cycle times, reducing both their dispersion and mean value (on average, in the order of a minute). However, at the same time, the prioritization policy created a population of unbooked donors for which the cycle time increased to over 2 h. This is undesirable for unbooked donors because a bad service experience is likely to push potential periodical donors out of the system, resulting in a net loss. If strictly judged from the operational point of view, the prioritization policy in this form should be avoided, considering the small difference in booked donors' average cycle time between the alternatives. However, on the one hand, giving equal priority to both types of donors may undermine the scheduling system itself. On the other hand, AVIS Milan observed that too long waiting times discourage new and occasional donors to become regular. Therefore, the decision maker should find a balance between these two aspects. We simulated both scenarios to quantify the impact of prioritizing booked donors.

Experiments also showed that having more physician capacity towards the end of the period (configuration '2') significantly helped to disperse queues more quickly (p-value = 0·0002). In particular, this is true in collection centres where the population of booked donors is prevalent, and no queues before opening hours are observed.

Considering haemoglobin testing, both configurations 'A' and 'B' helped to provide a better service (p-value = 0·000). In fact, although it is more expensive to create a new station which also generates a new queue for donors, these configurations free up physicians, who are considered the bottleneck of the system by the upper-level scheduler. In particular, screening donors before the consultation (configuration 'B') allows to defer anaemic donors before they consume valuable screening resources, in addition to freeing up physician time. As confirmed by AVIS, it may be important to carry out the consultation to donors even when their haemoglobin is low and a donation is not possible on that day. In the case of periodic donors, in fact, a consultation may help them take

Table 3 KPIs analysed in the DES solutions

Stakeholder		KPI	Notes
Donors		Cycle time	Stratified for booked/unbooked
		Queue time	Stratified for booked/unbooked
Resources	Nurses	Utilization	Goal is to saturate
	Clerks	Utilization	Goal is to saturate
	Beds	Utilization	Goal is to saturate
	Physicians	Utilization	Goal is to desaturate
Management		Capacity	Physician capacity actually used, per period
		Last donor	Exit time from consultation, per period
		Cost-efficiency	Efficiency in terms of donors' cycle times

Table 4 p-values from the ANOVA for the significant factors and interactions

			Main effects					Interactions				
			Queueing policy	Physician timetable	Haemoglobin testing	Number of nurses	Number of clerks	Number of beds	Queuing • Haemoglobin	Queuing • N° of beds	Timetable • N° of nurses	Haemoglobin • N° of nurses	N° of nurses • N° of beds
Donors	Cycle time			**0·0002**	**0·0000**		0·0243	**0·0000**					
	Cycle time – booked		**0·0000**	**0·0009**	**0·0000**		*0·0044*	**0·0000**					
	Cycle time – unbooked		**0·0000**	*0·0029*	**0·0000**			0·0196					
	Queue time		**0·0000**	**0·0006**	**0·0000**			**0·0000**	0·0112				
	Queue time – booked		**0·0000**	**0·0006**	**0·0000**			**0·0000**	**0·0000**				
	Queue time – unbooked		**0·0000**		**0·0000**			**0·0000**		**0·0007**			
Resources	Nurses' utilization			**0·0008**	**0·0000**	**0·0000**	0·0235	**0·0000**			**0·0007**	**0·0000**	**0·0000**
	Clerks' utilization						**0·0000**						
	Beds' utilization							**0·0000**					
	Physicians' utilization				**0·0000**								
Management	Capacity	k = 1			**0·0000**								
		k = 2			**0·0000**								
		k = 3			**0·0000**								
	Last donor	k = 1	**0·0000**		**0·0000**								
		k = 2		**0·0006**	**0·0000**								
		k = 3		**0·0000**	**0·0000**								

The different significance levels are highlighted as follows: 0·001 in bold, 0·01 in *italics* and 0·05 without any formatting.

the necessary actions to get well soon and return to donating as soon as possible.

However, regarding resources, even in the best-case layout, physicians are only saturated at approximately 70% of their capacity, a quantity lower than the utilization of nurses. This is due to the fact that the scheduler is focused on optimizing with respect to physicians' capacity. However, it is important to note that the saturation only takes into account physician time directly devoted to consultation. Operations such as compiling paperwork, assisting colleagues or other activities that normally occur in a real collection centre (including breaks) make up the remaining 30%. This is true for the other resources as well. Moreover, not reaching saturation values close to 100% reduces burnout risk and improves workplace satisfaction, so this value is not considered low or undesirable.

In this sense, haemoglobin testing is the only factor significantly impacting physicians' utilization (p-value = 0·0000). Indeed, as expected, performing the haemoglobin tests separately results in less saturated physicians. Concerning the exit time from the consultation of the last donor, queuing policy has an impact only in the first period $k = 1$ (p-value = 0·0000), when queues are still forming, while physicians' timetable configuration '2', which allows queues to disperse better, becomes significant for periods $k = 2$ and $k = 3$ (p-value = 0·0006 and p-value = 0·0000, respectively).

Based on the significant factors highlighted in Table 4 and the above discussions, Table 5 summarizes the best combination of significant factors for each KPI.

As mentioned, cost-efficiency was analysed separately. Figure 3 presents the results of the cost-efficiency analysis, with costs on the x-axis and donor throughput rate (the inverse of cycle time) on the y-axis; moreover, the line represents the linear regression of the data plot. As expected, the system responded with higher throughput rates (lower cycle times) for higher costs, thanks to the greater number of resources invested. However, the R^2 square of the regression is fairly low, qualifying only into a moderate correlation [42]. For a deeper cost-efficiency analysis, we used isocost curves, considering the best-performing configuration at a fixed cost. This allowed us to highlight an interesting pattern, as the best configurations for different isocost levels were similar to each other.

Based on these results, the best-performing layout was identified for each stakeholder: for donors, *FIFO-2-B-10*; for resources, *FIFO-2-B-03* and for management, *PRTY-2-B-02*.

Table 5 Best combination of factors for each KPI, divided by stakeholder, including only the factors with a statistical significance for the KPI and excluding interactions

		Queueing policy	Physician timetable	Haemoglobin testing	Number of nurses	Number of clerks	Number of beds
Donors	Cycle time		2	B or A		2	7 or 6
	Cycle time – booked	PRTY	2	B		2	7 or 6
	Cycle time – unbooked	FIFO	2	B or A			7 or 6
	Queue time	PRTY	2	B or A			5
	Queue time – booked	PRTY	2	B or A			5
	Queue time – unbooked	FIFO		B or A			5
Resources	Nurses' utilization		2	B or W	2	1	7 or 6
	Clerks' utilization					1	
	Beds' utilization						5
	Physicians' utilization			B			
Management	Capacity k = 1			B			
	k = 2			B			
	k = 3			B or A			
	Last donor k = 1	FIFO		A or B			
	k = 2		2	A			
	k = 3		2	A			
	Cost-efficiency	PRTY	2	B	2	1	6 or 7

Feedback

Some sub-optimal behaviours in the DES could be traced back to the fact that the BDAS framework was designed

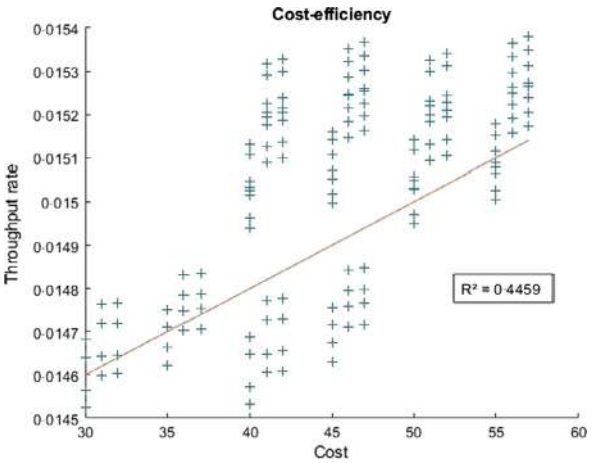

Fig. 3 Cost-efficiency trend: raw data and linear regression trend.[Colour figure can be viewed at wileyonlinelibrary.com]

to take into account only the midterm objectives of the collection centre, without considering the actual implementation of the plans at the operational level. Therefore, parameters and constraints of the BDAS must dynamically adapt to address the main criticalities that could arise in the daily execution of the activities, that is observed in the DES model outputs.

The first consideration was to revise the consultation time r in the BDAS when considering configurations in which the haemoglobin test is performed separately (configurations 'A' and 'B'). More specifically, the value of the standard consultation time (20 min) should be changed to $18 \cdot 33$ min, that is the mean value of the triangular distribution associated with the consultation only. The second feedback from the DES was that the first period $k = 1$, in which the system is still warming up, resulted undercharged. In opposition, both periods $k = 2$ and $k = 3$ were overcharged, exceeding their respective nominal physician capacities (450 min). To change that, it was possible to intervene in the BDAS by changing the values of the period-specific penalties associated with the periodic accumulation of donors. We used the same set of weights of the original solution in the inverse order, to penalize the formation of queues more in the third period and less in the first one. All other parameters were left unchanged.

The analysis of the feedback loop was performed for the three best configurations. The new results from the DES with the modified input were then compared with the original ones. To grant a fair comparison, the BDAS was forced to produce an output with the same total number of donors for each day as the original BDAS solution investigated.

Minimum, average and maximum values of the KPIs were compared between pre- and post-feedback run. Results are reported in Table 6.

Regarding donors, the most evident result is the reduction in the maximum cycle time in all configurations analysed. This reduction is more evident for unbooked donors in the configuration with prioritized queue management (*PRTY-2-B-02*). At the same time, it is true that the minimum and the average cycle time are slightly increased for almost all configurations. However, the regularization of the cycle time with a reduction of the maximum peak is a desirable outcome for this metric. Compatible results were obtained for queue time.

Regarding resources, generally speaking, their saturation is slightly increased. However, the differences are minimal.

From the management's viewpoint, the physicians' used capacity in period $k = 1$ was higher than without feedback. This proved that rescheduling was effective in better filling the first period. Coherently, the increment with respect with the baseline without feedback was lower in period $k = 2$, and the physicians' used capacity was even lower in $k = 3$.

The results concerning the exit time of the last donor were similar to with those of physicians' utilization. With respect to the case without feedback, the exit time was delayed in the first and second periods, where also physicians' utilization was higher, while the exit time was lower in the third second period where physicians' utilization was lower.

Overall, it is possible to conclude that the feedback loop to the BDAS achieved normalization of the results, for almost all KPIs considered, contributing to better management of the system. Further refinements could be achieved by iterating the feedback in a complete optimization-simulation approach. In summary, the coupled use of the scheduler with the DES provides an inexpensive tool to manage a blood collection centre, enabling to combine mid-term tactical decisions with short-term operational ones, and verifying the feasibility of the former iteratively.

Discussion

Even if the approach has been applied only to the case of AVIS Milan, the DES can be easily tailored to model other centres, due to its flexibility and generality, and the outcomes are expected to be confirmed also in these cases. In particular, the DES is built in a parametric form: this feature allows the testing of more scenarios than those included in this work, including (but not limiting to) donor arrival patterns and variations of shift durations. To extend the applicability, it would also be interesting to study how some parameters may evolve over time, such as no-show and deferral rate, and to dynamically readapt the management of donors' appointments in compliance.

Table 6 Results of feedback on the KPIs in the three configurations analysed

			FIFO-2-B-10			FIFO-2-B-10 (Feedback)			FIFO-2-B-03			FIFO-2-B-03 (Feedback)			PRTY-2-B-02			PRTY-2-B-02 (Feedback)		
			Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max	Min	Avg	Max
Donors	Cycle time		61·2	66	78·6	62·2	66·3	72·8	61·0	65·4	77·1	61·3	65·7	71·1	60·9	65·3	77·2	61·3	65·5	70·9
	Cycle time – booked		60·8	66	79·1	61·9	66·4	72·5	60·4	65·5	77·3	61·5	65·8	70·6	60·3	64·8	72·1	61·5	65·6	70·6
	Cycle time – unbooked		60·8	66·2	76·4	60·6	66	77·5	59·3	64·7	76·1	59·9	65·2	73·7	59·1	67·8	120·2	59·9	65·0	72·9
	Queue time		4·0	7·3	15·7	4·6	7·3	12·6	4·3	9·0	22·0	5·2	9·1	17·1	1·6	7·0	21·8	1·0	2·7	4·2
	Queue time – booked		4·0	7·3	16·1	4·5	7·4	12·6	4·2	9·1	22·8	5·0	9·3	17·6	1·7	5·8	13·3	1·0	2·5	3·8
	Queue time – unbooked		3·0	7·1	13·8	3·5	6·6	12·5	2·0	8·7	18·8	2·8	7·8	17·0	0·9	12·5	74·2	0·9	2·0	4·9
Resources	Nurses' utilization		0·62	0·85	1	0·66	0·84	1	0·88	0·96	1·00	0·91	0·97	1·00	0·89	0·96	1·00	0·91	0·97	1·00
	Clerks' utilization		0·11	0·13	0·14	0·12	0·13	0·14	0·23	0·25	0·27	0·23	0·26	0·28	0·23	0·25	0·27	0·23	0·26	0·28
	Beds' utilization		0·54	0·62	0·68	0·55	0·62	0·69	0·38	0·44	0·49	0·39	0·45	0·49	0·45	0·51	0·57	0·46	0·52	0·58
Management	Capacity	k = 1	329	368	414	354	399	452	329	368	414	354	399	452	329	368	414	354	399	452
		k = 2	294	364	443	277	396	479	294	364	443	277	397	479	294	365	443	277	396	479
		k = 3	296	364	438	253	316	401	296	364	438	253	316	401	296	364	438	253	316	401
	Last donor	k = 1	09:30	09:53	10:18	09:36	09:54	10:17	9:30	9:53	10:18	9:37	9:54	10:17	9:30	9:55	11:33	9:36	9:54	10:16
		k = 2	11:28	11:54	12:19	11:37	11:59	12:21	11:28	11:54	12:19	11:37	11:59	12:21	11:28	11:55	12:19	11:37	11:58	12:21
		k = 3	13:34	13:51	14:17	13:24	13:48	14:14	13:34	13:51	14:17	13:24	13:48	14:14	13:34	13:51	14:17	13:24	13:48	14:14

Future research will extend the DES model. For example, we could identify other factors that impact the organization of blood donation centres, and fine-tune the simulation parameters to other specific cases. At the same time, as in this work we only take into account whole blood donations, we will consider apheresis procedures to be incorporated into the model. Additionally, for regarding personnel, our DES model does not take into account differentiated work schedules and assumes that human resources are always available within the period, with no breaks. These two aspects could be integrated into the framework to better study human resource and staffing requirements for collection centres.

Finally, starting from the already implemented feedback from the simulator to the scheduler, we will extend the interaction to obtain a full optimization-simulation scheme.

Conclusions

In this paper, we study the operations in blood collection through a DES model developed to describe a general collection centre in a flexible way. The two goals are: to build a model that enables to study different configurations and work rules and to evaluate the effectiveness and feasibility of schedules defined at higher planning levels. A great effort has been made to make the model consistent with and adaptable to the characteristics of the centres, which is the main contribution of this work, based both on the interaction with AVIS Milan and on the analysis of works present in the literature.

Our DES model includes most of the key features of a collection centre, for example the simultaneous presence of booked and unbooked donors, and an assessment of the perspectives of all three stakeholders involved. With respect to the available literature, in which blood collection is not adequately addressed, we specifically address this step of the BDSC. We also underline that, thanks to the interaction with the BDAS, our DES model represents a crucial element for implementing the goals pursued by the scheduler in the real practice of providers.

Given the context of increasing demand and stationary budget faced by most blood centres, the proposed DES model and the decision framework in which it is included can provide an inexpensive aid for evidence-based decision-making. In this direction, the presented results prove that the tool can be effectively used in practice both to find out the best setting and to give feedback to a higher decision level (i.e. scheduler). In particular, the outcomes of the DES analysis can be beneficial for a series of activities such as centre dimensioning, daily operations improvement and staff shift management, either as a standalone tool or together with a higher level planning system.

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ORIGINAL PAPER

Preservation of neutralizing antibody function in COVID-19 convalescent plasma treated using a riboflavin and ultraviolet light-based pathogen reduction technology

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Vox Sanguinis

Background and objectives Convalescent plasma (CP) has been embraced as a safe therapeutic option for coronavirus disease 2019 (COVID-19), while other treatments are developed. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is not transmissible by transfusion, but bloodborne pathogens remain a risk in regions with high endemic prevalence of disease. Pathogen reduction can mitigate this risk; thus, the objective of this study was to evaluate the effect of riboflavin and ultraviolet light (R + UV) pathogen reduction technology on the functional properties of COVID-19 CP (CCP).

Materials and methods COVID-19 convalescent plasma units (n = 6) from recovered COVID-19 research donors were treated with R + UV. Pre- and post-treatment samples were tested for coagulation factor and immunoglobulin retention. Antibody binding to spike protein receptor-binding domain (RBD), S1 and S2 epitopes of SARS-CoV-2 was assessed by ELISA. Neutralizing antibody (nAb) function was assessed by pseudovirus reporter viral particle neutralization (RVPN) assay and plaque reduction neutralization test (PRNT).

Results Mean retention of coagulation factors was ≥70%, while retention of immunoglobulins was 100%. Starting nAb titres were low, but $PRNT_{50}$ titres did not differ between pre- and post-treatment samples. No statistically significant differences were detected in levels of IgG ($P \geq 0{\cdot}3665$) and IgM ($P \geq 0{\cdot}1208$) antibodies to RBD, S1 and S2 proteins before and after treatment.

Conclusion R + UV PRT effects on coagulation factors were similar to previous reports, but no significant effects were observed on immunoglobulin concentration and antibody function. SARS-CoV-2 nAb function in CCP is conserved following R + UV PRT treatment.

Key words: antibody – function, blood safety, pathogen inactivation, plasma, transfusion therapy.

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Introduction

The coronavirus disease-2019 (COVID-19) pandemic bears testimony to the risk presented by emerging infectious diseases (EID). Few treatment options are available when novel viruses first arise, but the use of convalescent plasma (CP) may be an expedient therapeutic approach until other medical countermeasures become widely available. CP is a treatment in which putatively antibody-

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rich plasma is taken from those recovered from the disease and transfused to provide passive immunity to infected patients or susceptible individuals. Case reports of effective use of CP date back to the 1918 influenza pandemic [1] and more recently to EID outbreaks including severe acute respiratory syndrome (SARS) [2, 3], Middle East respiratory syndrome (MERS) [4], H1N1 influenza [5] and Ebola virus disease (EVD) [6]. In the current pandemic, COVID-19 CP (CCP) has demonstrated safety with minimal side-effects [7], though controlled clinical efficacy data are only beginning to come in [8–10].

While the most effective protocols for treatment with CCP are yet to be defined, plasma transfusion is a routine medical procedure available globally. However, as with any blood product, there is a risk of transmitting bloodborne pathogens with CCP transfusion. The causative agent for COVID-19, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is itself not believed to be transfusion-transmissible [11]. Yet, the possibility of co-infections is present, particularly in regions with a high endemic prevalence of other infectious diseases [12]. Pathogen reduction technology (PRT) treatment of CCP is a measure that can be taken to maintain the safety of the blood supply while providing potential benefits to COVID-19 patients.

Pathogen reduction technology systems have been developed over the past decades as a proactive means to reduce the residual risk of transfusion-transmitted infections that continues to exist despite the implementation of routine blood safety practices such as donor questionnaires, travel deferrals and viral screening tests [13, 14]. Donor infections could escape these blood safety measures for a number of reasons, including a 'window period' donation where the viral load has not yet reached the detection limit of screening tests, a lack of testing capability for particular infectious agents or an unfavourable cost–benefit ratio for continuing to implement more and more tests. PRT provides a broad-spectrum means to reduce pathogen loads and inhibit infectivity by disrupting the micro-organism's ability to replicate. Commercial PRT systems use chemicals, ultraviolet (UV) light or the combination of a photosensitizer and UV light to inactivate pathogens, but pathogen kill must be balanced to preserve the blood product quality [15]. Recently, a PRT system based upon riboflavin and UV light (R+UV) has been reported to be effective in inactivating SARS-CoV-2 [16, 17]; the work described herein evaluates the effect of R + UV treatment on functional properties of CCP.

Methods

COVID-19 convalescent plasma collection

COVID-19 convalescent plasma was provided by an accredited blood centre specializing in biomaterial collections for research (Key Biologics, Memphis, TN, USA). CCP was collected by apheresis under an IRB-approved protocol from donors determined to have recovered from COVID-19 and was shipped to Colorado State University. All products were placed into frozen storage at ≤−20°C upon receipt until needed for further processing.

Riboflavin and UV light pathogen reduction treatment

COVID-19 convalescent plasma units were treated using a R + UV PRT system (Mirasol® Pathogen Reduction Technology, Terumo Blood and Cell Technologies, Lakewood, CO, USA) as previously described [18]. Briefly, thawed CCP units were transferred to an illumination bag and mixed with 35 ml of riboflavin solution (500 μmol/l riboflavin in 0·9% sodium chloride, pH 4·0–5·0 [Terumo Blood and Cell Technologies, Larne, Ireland]). The prepared units were then placed into the UV illumination device (Terumo Blood and Cell Technologies, Lakewood, CO, USA) and exposed to 6·24 J/ml of energy. Samples for analysis were taken prior to the addition of riboflavin solution (Post-Collect), after addition of riboflavin (Pre-Treat) and after UV illumination (Post-Treat). Sample aliquots were stored frozen (≤−20°C) in cryovials until testing. CCP units were analysed for selected coagulation factors, immunoglobulins and SARS-CoV-2 antibody binding and neutralizing activity.

Plasma protein assays

Coagulation factors were tested at Terumo Blood and Cell Technologies (Lakewood, CO, USA) using the STA Compact Max (Diagnostica Stago US, Parsippany, NJ, USA). Chromogenic assays were used to measure factor VIII activity (Chromogenix Coamatic® Factor VIII reagent, DiaPharma Group, Inc., West Chester, OH, USA) and antithrombin III activity (STA®-Stachrom® AT III reagent, Diagnostica Stago). An immuno-turbidimetric method was used to assess von Willebrand factor antigen activity (STA® Liatest® VWF: Ag). Clotting assays included fibrinogen (STA® Fibrinogen 5), Protein C (STA®-Staclot® Protein C) and Protein S (STA®-Staclot® Protein S). The performance of the STA Compact Max instrument has been qualified for intra-run and total precision for all assays performed.

Plasma immunoglobulins and IgG subclasses were measured by standard quantitative nephelometry (IgG, IgA, IgM at UC Health Anschutz, Aurora, CO, USA; IgG subclasses at ARUP Laboratories, Salt Lake City, UT, USA). The reference laboratories performing immunoglobulin analysis are accredited by the College of American Pathologists (CAP) and maintain Clinical Laboratory Improvement Amendments (CLIA) certification.

SARS-CoV-2 functional assays

An enzyme-linked immunosorbent assay (ELISA) was performed at Colorado State University to test CCP samples and a negative control (normal plasma sample) for antibody binding to the SARS-CoV-2 spike protein receptor-binding domain (RBD) and epitopes associated with the spike protein subunits S1 and S2 (catalogue numbers 40592-V08H, 40591-V08H and 40590-V08B, Sino Biological US Inc., Wayne, PA, USA). The protocol for ELISA was adapted from Robbiani et al. [19] with a few modifications. Briefly, high binding 96-half-well microplates (Corning Life Sciences, Tewksbury, MA, USA) were coated with 50 ng S1, S2 or RBD protein prepared in PBS and incubated overnight at 4°C. The next day, the plates were washed five times with 180 µl wash solution (PBS + 0·05% Tween-20) and non-specific interactions were blocked using 180 µl blocking buffer (PBS + 0·05% Tween-20 + 2% BSA + 2% normal goat serum [Jackson ImmunoResearch Inc., West Grove, PA, USA]). After 2 h, the plates were washed and different CCP sample dilutions prepared in blocking buffer were added to the wells and incubated for 1 h. Plates were then washed and incubated for 1 h with horseradish peroxidase (HRP)-conjugated anti-human IgG or anti-human IgM secondary antibodies (Jackson ImmunoResearch Inc.) prepared in blocking buffer (1:10 000 dilution). The colorimetric substrate was developed with the addition of 100 µl TMB substrate (Thermo Fisher Scientific, Rockford, IL, USA), and the reaction was stopped by adding 50 µl 1 M sulphuric acid. Absorbance was measured at 450 nm using a BioTek Synergy 2 plate reader (BioTek Instruments Inc., Winooski, VT, USA).

The neutralizing activity of CCP samples was evaluated by two assays, a pseudovirus reporter viral particle neutralization (RVPN) assay and a plaque reduction neutralization test (PRNT). The RVPN assay was performed at Vitalant Research Institute (VRI, San Francisco, CA, USA) as previously described [20, 21]. In brief, a vesicular stomatitis virus (VSV)-firefly luciferase pseudotype modified to express the SARS-CoV-2 spike protein was mixed with fourfold dilutions of heat inactivated CCP. A positive serum control and a negative serum control were also prepared. After incubation for one hour at 37°C, the preparations were used to infect reporter cells that were plated into black 96-well tissue culture treated plates. The reporter cells were lysed after 24 h at 37°C and removal of the supernatant. Luciferase activity was measured to determine the RVPN result. NT_{50} titres were estimated by calculating percentages of the no serum control and performing non-linear regression. Titres measuring <40 are deemed to lack nAbs.

The PRNT assay was conducted in a biosafety level 3 (BSL-3) laboratory at the Colorado State University Infectious Disease Research Center (Fort Collins, CO, USA). CCP samples were heat inactivated for 30 min at 56 °C, and serial twofold dilutions were prepared in a 96-well plate (Greiner Bio One, Monroe, NC, USA). Viral stock (strain hCoV-19/USA/WA1/2020, BEI Resources, Manassas, VA, USA) containing approximately 200 plaque-forming units (pfu) per 0·1 ml was added to each well containing plasma dilutions. Following an incubation period at 37°C in a 5% CO_2 incubator, 6-well plates (Greiner Bio One) containing recently confluent Vero cells (ATCC, Manassas, VA, USA) were inoculated with the virus–plasma mixtures. After a second incubation period at 37°C, 2 ml of overlay (2× MEM with 4% FBS [Peak Serum, Wellington, CO, USA] and agarose) was added to each well. After 24 h incubation at 37°C, a second overlay containing neutral red (Millipore Sigma, ST. Louis, MO, USA) was dispensed into each well and the number of plaques was counted 48–72 h after initial inoculation. The highest dilution of plasma that inhibited plaque formation by 50% ($PRNT_{50}$) was determined based upon the titre of the viral stock and the number of plaques present at each dilution. Donors with $PRNT_{50}$ titres of less than or equal to 1:20 are considered negative for nAbs.

Statistical analysis

Descriptive statistics including the mean and standard deviation were calculated for all continuous parameters. To assess the effect of R + UV PRT treatment, Pre-Treat samples were used as the basis for comparison rather than Post-Collect samples in order to account for dilution with riboflavin solution. Protein retention percentages were calculated by taking the ratio of Post-Treat to Pre-Treat values for each sample pair and multiplying by 100. ELISA results were analysed by plotting optical density measurements by dilution and calculating the area under the curve (AUC) using the trapezoid method.

Comparisons for parameters passing the Shapiro–Wilk test for normality were performed using a paired, two-tailed *t*-test where statistical significance was defined as $\alpha < 0{\cdot}05$. Data sets exhibiting a non-normal distribution were evaluated non-parametrically using a Wilcoxon matched-pairs signed rank test. Statistical analysis was performed using Prism 8 for Windows (GraphPad Software, Inc., San Diego, CA, USA).

Results

COVID-19 convalescent plasma was collected from 6 donors with demographics as described in Table 1. All 6 units met the incoming product specifications for the

Table 1 COVID-19 convalescent plasma donor characteristics

Unit ID[a]	Age	Gender	Race/ethnicity	Blood type	Days since diagnosis
370020800808	45	F	W	O+	72
370020800898	44	F	W	A+	58
370020800970	35	F	W	O+	75
370020801002	27	M	W	O+	94
370020801095	42	M	W	O+	71
370020801130	29	M	W	A+	118

[a]All units were shipped in liquid form on cold packs except 370020800808, which was shipped frozen.

R + UV PRT process and were successfully treated. Protein retention analysis (Table 2) demonstrated that although there was a statistically significant treatment effect for the coagulation factors, retention was on the order of 70% or better, thus meeting the European Directorate for the Quality of Medicines & HealthCare (EDQM) guideline for fibrinogen retention in PRT-treated fresh-frozen plasma (FFP, ≥60%) [22]. Factor VIII concentrations were below the EDQM standard for PRT-treated FFP (≥50 IU/100 ml), but starting values were also lower than the standard for untreated FFP (≥70 IU/100 ml). Of note is that the immunoglobulin concentrations, including those for IgG subclasses, were unaffected by R+UV treatment as demonstrated by retention remaining at 100%.

All 6 CCP units demonstrated binding to the SARS-CoV-2 RBD as well as the S1 and S2 subunits of the spike protein when assessed by ELISA using anti-IgG and anti-IgM secondary antibodies. The levels of IgM antibodies detected were generally lower and more variable than IgG antibodies, particularly for those targeted against the RBD, but normalized AUC values did not significantly differ between Pre-Treat and Post-Treat time-points for either IgG or IgM at any of the binding sites (Fig. 1 and Fig. S1). Similarly, SARS-CoV-2 neutralizing activity was detected by the PRNT assay in all of the Post-Collect and Pre-Treat CCP samples, though one unit was at the 1:20 threshold. The $PRNT_{50}$ titre for one unit (370020801130) dropped by one dilution between Post-Collect and Pre-Treat, but all CCP units demonstrated stable $PRNT_{50}$ titres when comparing Pre-Treat and Post-Treat samples (Table 3). Two units and one additional Pre-Treat sample tested negative by the RVPN assay, and estimated RVPN NT_{50} titres were variable (Table 4).

Discussion

This study evaluated the effect of R + UV PRT treatment on functional properties of CCP. A treatment effect upon coagulation factors was observed following R + UV treatment, but the reductions seen were consistent with previously published R + UV literature [18, 23–26]. Moreover, all PRT methods are known to degrade plasma proteins to varying degrees [27–30]. Minimal effects upon antibodies were demonstrated, from the very general

Table 2 Protein retention after R + UV PRT treatment of COVID-19 convalescent plasma, mean ± 1 standard deviation (range)

Protein	Pre-treat	Post-treat	*P* value	% retention
Factor VIIIc (%)	58·7 ± 30·6 (25·0–97·0)	42·5 ± 19·3 (16·0–67·0)	0·026	74·5% ± 11·1% (62·9%–90·3%)
Fibrinogen (mg/dl)	219·3 ± 33·4 (174·0–261·0)	154·8 ± 37·1 (103·0–187·0)	< 0·001	69·8% ± 7·8% (59·2%–79·4%)
VWF:Ag (%)	91·7 ± 40·7 (41·0–153·0)	78·0 ± 33·0 (34·0–126·0)	0·009	85·7% ± 3·8% (82·4%–92·7%)
Antithrombin III (%)	76·3 ± 5·9 (66·0–84·0)	70·3 ± 4·8 (64·0–77·0)	0·016	92·3% ± 5·1% (87·2%–98·7%)
Protein C (%)	97·0 ± 7·2 (88·0–105·0)	77·2 ± 6·9 (67·0–86·0)	0·002	79·8% ± 7·5% (71·4%–93·5%)
Protein S (%)	62·8 ± 16·1 (36·0–80·0)	51·2 ± 13·9 (34·0–71·0)	0·040	82·5% ± 13·7% (65·0%–101·4%)
IgG (mg/dl)	711·7 ± 28·9 (676·0–754·0)	715·7 ± 30·2 (678·0–752·0)	0·229	100·6% ± 1·0% (99·1%–101·6%)
IgA (mg/dl)	165·3 ± 65·5 (91·0–242·0)	165·2 ± 64·6 (90·0–238·0)	0·872	100·1% ± 1·6% (98·3%–102·9%)
IgM (mg/dl)	70·2 ± 17·4 (53·0–100·0)	70·2 ± 18·5 (52·0–103·0)	>0·999	99·8% ± 1·9% (97·4%–103·0%)
IgG_1 (mg/dl)	333·3 ± 35·6 (299·0–378·0)	333·8 ± 43·8 (293·0–394·0)	0·899	99·9% ± 2·6% (96·4%–104·2%)
IgG_2 (mg/dl)	237·7 ± 60·0 (202·0–358·0)	242·8 ± 58·1 (214·0–361·0)	0·156[a]	102·5% ± 3·3% (97·9%–106·3%)
IgG_3 (mg/dl)	32·3 ± 21·2 (15·0–60·0)	31·8 ± 21·9 (14·0–62·0)	0·563[a]	97·0% ± 5·5% (92·9%–106·9%)
IgG_4 (mg/dl)	32·2 ± 20·9 (6·0–66·0)	33·3 ± 24·2 (6·0–75·0)	0·504	100·5% ± 7·8% (90·5%–113·6%)

[a]IgG_2 and IgG_3 evaluated non-parametrically.

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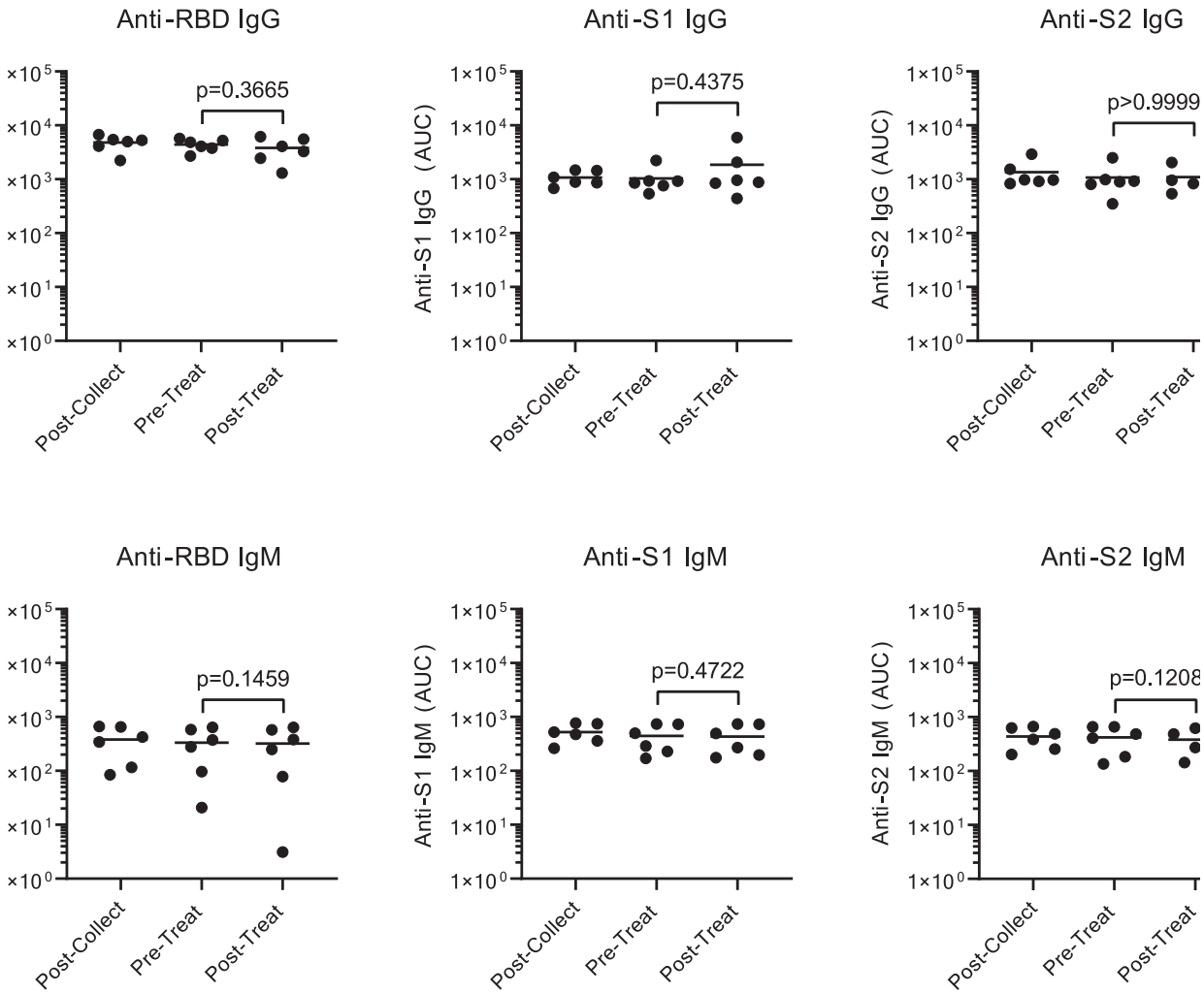

Fig. 1 Plasma antibodies against SARS-CoV-2. ELISA results expressed as area under the curve (AUC) values based upon optical density at 450 nm (OD_{450}) measurements over a range of plasma dilutions (Fig. S1).

immunoglobulin retention percentages to the more specific SARS-CoV-2 epitope binding measurements. Neutralizing antibody activity was similarly well preserved, with the highest RVPN dilutions positive for neutralizing activity remaining the same after treatment and Pre-Treat and Post-Treat $PRNT_{50}$ values being identical for all CCP units. The $PRNT_{50}$ titre for one CCP unit dropped when comparing Post-Collect and Pre-Treat samples, which is likely an artefact of dilution with riboflavin solution

Table 3 SARS-CoV-2 $PRNT_{50}$ limiting dilution titres

Unit ID	Post-collect	Pre-treat	Post-treat
370020800808	1:80	1:80	1:80
370020800898	1:40	1:40	1:40
370020800970	1:40	1:40	1:40
370020801002[a]	1:20	1:20	1:20
370020801095	1:320	1:320	1:320
370020801130	1:80	1:40	1:40

[a]This unit is negative based upon a $PRNT_{50}$ threshold $\leq$ 1:20.

Table 4 SARS-CoV-2 pseudovirus reporter viral particle neutralization (RVPN) assay

	RVPN result		RVPN NT_{50}	
Unit ID	Pre-treat	Post-treat	Pre-treat	Post-treat
370020800808	Positive	Positive	106·73	58·80
370020800898	Positive	Positive	40·44	43·22
370020800970	Negative	Negative	N/A	39·20
370020801002	Negative	Negative	N/A	N/A
370020801095	Positive	Positive	158·92	119·34
370020801130	Negative	Positive	35·89	124·22

during the R+UV PRT treatment process. The stability demonstrated in this study is consistent with previous assessments of antibody function in PRT-treated plasma [31, 32]. These data suggest that PRT treatment does not impair the passive immunity provided by CCP.

Some differences were observed between the results provided by the two assays for nAb activity. An additional unit was deemed negative for nAb activity, and lower titres were reported for two units by the RVPN assay compared to the PRNT assay. The higher sensitivity of the PRNT assay may stem from greater susceptibility of the wild-type virus to a more diverse set of antibodies or quaternary epitopes that cannot be replicated with the pseudovirus [33]. While the PRNT assay has higher sensitivity, working with live SARS-CoV-2 requires BSL-3 containment measures. The RVPN assay was developed to quantitatively measure SARS-CoV-2 neutralization titres safely in laboratory facilities typical of blood centres to select CCP units for therapeutic use [21]. RVPN NT_{50} values were quite variable and most likely were not representative of R+UV PRT treatment effects. Given the low titre of the CCP units evaluated in the study, the non-linear regression used to calculate the titre was based upon a limited non-zero data set, thereby affecting the accuracy of the estimate. This should not be an issue at therapeutic antibody titres.

Importantly, the levels of IgG and IgM antibodies to specific viral proteins in the receptor-binding domain (RBD) and spike proteins (S1 and S2) were maintained following treatment. These antibodies have been shown to have high virus neutralizing capacity. Robbiani et al. [19] demonstrated that despite variations in the levels of overall neutralizing antibodies in donors of convalescent plasma, the presence of these specific subsets of antibodies with potent antiviral activity correlated with improved clinical outcomes in patients receiving the convalescent plasma products. The data imply that maintenance of the level of these subsets of antibodies may correlate with clinical effectiveness more directly than measure of overall neutralizing antibody levels.

CCP is the most readily available source of anti-SARS-CoV-2 antibodies, and its use has been widely embraced as a treatment for COVID-19, while other antiviral therapies and vaccines are in development and can be widely deployed. The ability to safely utilize convalescent plasma in these settings, however, depends on the safety of the product collected from donors who may have experienced a period of immune compromise during acute phases of the disease. Exposure to a variety of transfusion-transmitted diseases during this period or reactivation of latent disease could introduce additional risk into the use of such products for therapeutic applications. PRT treatment of CCP may be seen as a prudent safety measure to mitigate the risk of possible co-infections known to be transmissible by transfusion. The ability to limit the risk of transfusion-transmitted co-infections is of particular importance in areas having a high prevalence of endemic disease, as is the case in many resource-limited settings. Local collection of CCP in these environments may be challenged by the need for apheresis infrastructure and cold chain requirements [34], though success in establishing a CP supply chain to support an EVD clinical trial in Guinea through the collaboration of international research consortia, government agencies, charitable foundations and blood establishments has been described [35]. Since scale-up of such a system to serve the needs of the broader population for the COVID-19 pandemic is likely not feasible, whole blood (WB)-derived CCP or perhaps even convalescent WB may be more plausible where resources are limited. There is precedent for efficacious use of convalescent WB against EVD, and WB collection is far simpler to implement than plasmapheresis [36]. PRT systems to treat WB are available or in development, including the R+UV PRT system used to treat CCP in this study [37]. Although the preservation of antibody function in R+UV-treated WB was not evaluated in this study, R+UV treatment effects on plasma coagulation factors are similar to those reported herein [38].

Limitations of this study include the small sample size and the generally low anti-SARS-CoV-2 titres in the CCP units. In the original Emergency Use Authorization (EUA) for the use of CCP to treat hospitalized COVID-19 patients, the United States Food and Drug Administration defined high-titre CCP to be units with an ID_{50} titre cut-off of 250 using a SARS-CoV-2 neutralization assay similar to the PRNT [39]; subsequent revisions to the EUA have listed qualifying results for additional acceptable assays [40]. The six CCP units evaluated in this study were collected specifically for research at a time when blood centres were urgently calling for therapeutic CCP donations. It is possible that the donors providing research CCP units were unable to donate therapeutic units due to low antibody titres or other donor deferral factors. Despite the low titres, the various antibody assays performed in this study consistently demonstrated stability between pre- and post-treatment samples, whether testing for retention, epitope binding or neutralizing activity.

Conclusions

With the worldwide need for treatment options to address the COVID-19 pandemic, CCP is an expedient therapeutic option that can be implemented globally, whether in resource-rich or resource-limited environments. The addition of PRT may be warranted to address possible co-

infections in regions experiencing a high prevalence of endemic transfusion-transmissible diseases, but conservation of the passive immunity conveyed through CCP must be ensured. Based upon this small study, there is no indication that R+UV PRT treatment compromises SARS-CoV-2 nAb function in COVID-19 convalescent plasma.

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Conflict of interest

S.Y. and S.M. are employees of Terumo Blood and Cell Technologies, the manufacturer of the pathogen reduction technology described in this article. L.H., T.D., M.H.D. and R.G. have no conflicts of interest to declare.

Authors contributions

All authors meet the criteria of the International Committee of Medical Journal Editors (ICMJE) recommendations dated December 2014. S.M. and R.G. conceived the work, while S.Y., L.H., T.D. and M.H.D. participated in the acquisition, analysis and interpretation of data. S.Y. drafted and revised the work based upon critical review from all authors and other subject matter experts. All authors approved the final work and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Fig. S1 Optical density at 450 nm (OD_{450} nm).

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An international comparison of HIV prevalence and incidence in blood donors and general population: a BEST Collaborative study

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Background and Objectives Efficiency in mitigating HIV transmission risk by transfusion may vary internationally. We compared HIV prevalence and incidence in blood donors across different jurisdictions in relation to those rates in the general population and differences in deferral practices.

Materials and Methods Data from 2007 to 2016 were collected in Australia, Brazil (São Paulo), Canada, England, France, Italy, Ireland, Japan, the Netherlands, New Zealand, Norway, Spain (Basque Country), USA (Vitalant) and Wales. For each country/region, the number of HIV antibody-positive donations and nucleic acid testing (NAT)-only-positive donations was broken down according to first-time or repeat donor status, along with the relevant denominators.

Results There is a modest correlation between HIV prevalence among first-time donors and HIV prevalence in the general population. However, rates of HIV-positive donations in repeat donors, a proxy for incidence, do not correlate with incidence rates in the general population. Rates in donors from Italy and Basque Country, where deferral criteria for men having sex with men are less stringent, are higher compared with most other jurisdictions. Rates of NAT-only-positive

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donations are extremely low and do not differ significantly after adjustment for multiple comparisons.

Conclusion Donor HIV rates are only weakly associated with those observed in the general population. Countries with less stringent deferral criteria have higher HIV rates in their donor population, but the rates remain very low.

Key words: donors, blood donation testing, NAT testing, blood safety, epidemiology, transfusion – transmissible infections.

Introduction

The residual risk of HIV transmission by transfusion is currently extremely low, at least in countries with a voluntary non-remunerated donor base with robust quality systems to qualify and test donors. The risk is so low that it is virtually unmeasurable and can only be estimated, for example through the incidence rate/window period model [1]. By country, the current estimates for this residual risk vary but they are in the order of one in a million or less [2–5]. Nevertheless, the risk of acquiring HIV by transfusion is not zero and all jurisdictions strive to make this risk as low as possible.

The current approach to prevent HIV transmission by transfusion is multifaceted [6]. The general public is educated regarding this hazard, and those having risk factors are discouraged from donating. Prospective donors are screened extensively for high-risk activities. All donations are tested for HIV; in a majority of jurisdictions, this is done by serology and nucleic acid testing (NAT). Quality systems are in place to reduce the risk of human errors that could inadvertently lead to the release of contaminated units. If historically, most of the reduction in the HIV transmission risk was achieved by introducing specific donor deferral rules, more recently it is the application of highly sensitive screening tests that have further increased transfusion safety [6].

Even in countries having very similar practices regarding screening for high-risk behaviour and testing for HIV, other subtler factors may also have a significant impact on the very small residual risk of transmission by transfusion. Such countries might have different HIV donor rates simply because of differing levels of HIV incidence and epidemiology in the general population. Also, test-seeking behaviour may differ depending on testing availability or because of differential stigma associated with HIV screening. Finally, there may be cultural differences in the perception of risk that could have an impact on how deferral policies are understood by potential donors and applied by health professionals. Such factors may have a significant impact on the actual magnitude of the very small residual risk of HIV transmission. One way to compare this risk between countries is to look at the prevalence and incidence of HIV in the donor population. In theory, the lower the rates of HIV infection in the donor population, the lower the risk of transmission by transfusion. This is especially true for incident infections among donors, which pose the risk of being undetected because of the early, undetectable phase of infection [7]. Others have compared donor HIV rates in a limited number of countries, but they did not attempt to correlate these findings with HIV rates in the general population or with specific deferral practices applied in these jurisdictions [8]. The purpose of our study is to compare the prevalence and incidence rates of HIV in blood donors and in the general population in several jurisdictions and determine whether the observed differences relate to specific deferral policies or HIV rates in the general population. Finding such differences and understanding their causes may help to identify best practices to further prevent transfusion-associated HIV and possibly other infectious risks.

Design & methods

Data collection

Fourteen blood centre members of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative participated in this study. We obtained data on all whole blood donations over a 10-year period (2007–2016) in the following regions/countries: Australia, Brazil (São Paulo), Canada, England, France, Italy, Ireland, Japan, the Netherlands, New Zealand, Norway, Spain (Basque Country), USA (Vitalant) and Wales. For Brazil and Spain, data were provided by one blood collection agency serving a specific geographic area of the country. Vitalant operates in most but not all regions of the USA. All centres included in the study carried out HIV antibody testing and HIV NAT on all donations, except for Norway, where plasma from nearly all whole blood donations is tested in minipools for HIV NAT prior to the fractionation process. Participating centres provided the following information: (i) denominator data on the number of whole blood

donations during the study period, (ii) numerator data on the number of donations found to be positive by serology for HIV (the few donors who were confirmed positive by serology but negative by NAT were included in the analysis, and the majority of those were taking anti-retroviral therapy), and (iii) numerator data on the number of donations found to be positive for HIV by NAT only. For most countries, the data were available according to first-time/repeat donor status and sex; England, Ireland and Norway only provided aggregated numerator data for first-time and repeat donations. Blood collection agencies also provided information on deferral policies that were applied to men who have sex with men (MSM) during the study period.

Data analysis

For each country, the prevalence and incidence rates of HIV in the general population (15–69 years old) for 2007–2016 were calculated by averaging yearly statistics obtained from Global Health Data Exchange from the University of Washington [9]. For São Paolo, we used the population data for the state and not the whole country. For Basque Country, we used the population data for Spain. We calculated the prevalence of HIV in the donor population as the proportion of first-time donors who tested positive by serology or NAT. As a proxy for HIV incidence in donors, we considered the rate of HIV-positive donations in repeat donors. As a second, more direct measurement of incidence, we used NAT-only-positive donations (in first-time and repeat donors). The mean window period reduction for HIV-1 RNA by pooled sample NAT is approximately 11–15 days relative to antibody testing [10]. Some countries (e.g. Australia, Italy, New Zealand) use a combined HIV antigen/antibody test, which in theory can reduce slightly this period, but not enough to make any meaningful difference in the incidence calculations. Similarly, we did not consider the small differences in the duration of NAT-only-positive period, which is theoretically longer in countries using individual testing; therefore, we used 13 days when calculating the incidence (I) according to the formula I = P/D, where P is the prevalence of NAT-only donations and D is the duration of the NAT-positive, serology-negative period in years (13/365).

We compared rates of HIV-positive (antibody and NAT) and NAT-only-positive donations between jurisdictions, using chi-square statistics and Tukey–Kramer's adjustment for multiple comparisons. We correlated the prevalence of HIV in first-time donors in each jurisdiction with the HIV prevalence in the general population, using robust linear regression, weighing the contribution of each jurisdiction according to the number of observations. Similarly, we correlated the estimated incidence of HIV among donors with the incidence of HIV in the general population. Finally, we compared rates of NAT-only-positive donations, after considering the possible effect of different rates of HIV incidence in the general population. For a given country, we did this by multiplying the observed rate of NAT-only-positive donations by the inverse ratio of the reported HIV incidence in this country, with Australia as the country of reference. We used SAS Enterprise Guide software, version 7.1 for the analyses.

All jurisdictions had a permanent or a temporary deferral for MSM, except for Italy and Basque Country, where MSM donors were evaluated for risk behaviours, such as having more than one sexual partner [11].

Results

Table 1 shows numerator and denominator data of HIV-positive donations in regions/countries included in the study, the overall rates of HIV-positive donations and the prevalence and incidence of HIV in the general population during the study period.

Figure 1 reports HIV prevalence in first-time donations in relation to the reported prevalence in the general population for all regions/countries, with the exception of São Paulo because of the limited representativeness of these donors compared with the whole state. The rates are much lower in donors compared with the general population, with a difference in at least tenfold. Also, rates show a modest but statistically significant correlation with HIV prevalence in the general population ($r^2 = 0{\cdot}68$; $P < 0{\cdot}0001$). Rates for most countries/regions lie close to the regression line, while Italy and, less so, Wales are above the regression line, and Canada is below the line.

When comparing prevalence rates of HIV in first-time donations between regions/countries (Fig. S1), the highest rate was observed in São Paolo at $65{\cdot}3/10^5$ (95% CI: $37{\cdot}1/10^5$–$115{\cdot}1/10^5$), followed by Italy at $15{\cdot}4/10^5$ (95% CI: $13{\cdot}8/10^5$–$17{\cdot}1/10^5$) and Basque Country at $12{\cdot}7/10^5$ (95% CI: $6{\cdot}6/10^5$–$24{\cdot}3/10^5$); they were substantially lower in Australia, Norway, Japan and Canada, between 1 to $2/10^5$ donations. Statistically, São Paulo's rate is significantly higher compared with all other jurisdictions. Italy and Basque Country also have higher rates compared with most other countries, although not statistically different compared with USA ($7{\cdot}91/10^5$), Wales (7·67/105) and Ireland (4·23/105).

Figure 2 shows the lack of correlation between the prevalence of HIV-positive donations among repeat donors and HIV incidence in the general population ($r^2 = 0{\cdot}06$, $P = 0{\cdot}75$) (Again, data from São Paolo are not included in this analysis.). When considering the HIV

Table 1 Distribution of HIV-positive donations, rates of infection in donors and in the general population, in countries or jurisdictions over a ten-year period (2007–2016).

	Australia	Brazil (São Paulo)	Canada	England	France	Ireland	Italy	Japan	The Netherlands	New Zealand	Norway	Spain (Basque Country)	USA (Vitalant)	Wales
Whole blood donations	8 346 654	78 994	11 189 276	19 491 320	29 176 011	1 535 230	13 904 870	51 101 463	5 409 881	1 375 027	2 137 455	651 428	5 403 810	927 691
First-time donations	1 052 583	18 365	1 194 736	1 709 443	4 673 077	118 117	2 129 041	4 273 861	329 200	173 052	169 575	71 095	1 163 187	104 342
Male	478 882	8448	547 366	-	2 169 214	-	1 339 254	2 921 115	109 525	79 969	-	36 397	493 278	46 887
Female	573 701	9917	647 370	-	2 503 863	-	789 787	1 982 746	219 675	93 083	-	34 698	669 909	57 455
Repeat donations	7 294071	60 629	9 994 540	17 781 877	24 502 934	1 417 113	11 775 829	46 197 602	5 080 681	1 201 075	1 967 880	580 333	4 240 623	823 349
Male	3 743 058	37 612	5 598 848	-	12 255 477	-	8 327 980	32 276 530	2 880 513	582 103	-	352 351	1 846 715	433 513
Female	3 551 013	23 017	4 395 692	-	12 247 457	-	3 447 849	13 921 072	2 200 168	619 872	-	227 982	2 393 908	389 836
Antibody positive/ NAT-only positive	38/2	20/0	43/0	137/3	289/16	9/0	823/14	256/15	20/0	5/0	5/0	27/2	145/8	12/0
First-time donations	19/0	12/0	24/0	62/1	126/3	5/0	324/3	64/2	9/0	4/0	3/0	9/0	87/6	8/0
Male	15/0	10/0	18/0	-	91/3	5/0	253/3	64/2	4/0	3/0	-	8/0	64/4	7/0
Female	4/0	2/0	6/0	-	35/0	0/0	71/0	0/0	5/0	1/0	-	1/0	23/2	1/0
Repeat donations	19/2	8/0	19/0	75/2	163/13	4/0	499/11	192/13	11/0	1/0	2/0	18/1	58/2	4/0
Male	15/1	8/0	10/0	-	125/13	3/0	448/10	182/12	8/0	1/0	-	16/1	44/2	4/0
Female	4/1	0/0	9/0	-	38/0	1/0	51/1	10/1	3/0	0/0	-	2/0	14/0	0/0
HIV rates in donations														
First time (per 10^5)	1·81	65·3	2·01	3·69	2·76	4·23	15·4	1·35	2·73	2·31	1·77	12·7	7·91	7·67
Repeat (per 10^5)	0·29	13·2	0·19	0·43	0·72	0·28	4·33	0·44	0·22	0·08	0·10	3·27	1·41	0·49
Overall (per 10^5)	0·48	25·3	0·38	0·72	1·05	0·59	6·02	0·53	0·37	0·36	0·23	4·30	2·81	1·30
HIV rates in the general population														
Prevalence (per 10^5)	80·4	383·0	258·6	152·6	202·3	108·0	376·3	22·2	151·9	112·1	122·8	582·8	545·7	159·2
Incidence (per 10^5)	6·9	29·2	9·7	13·1	9·2	15·0	12·8	1·6	7·1	6·1	6·8	12·5	19·9	18·2

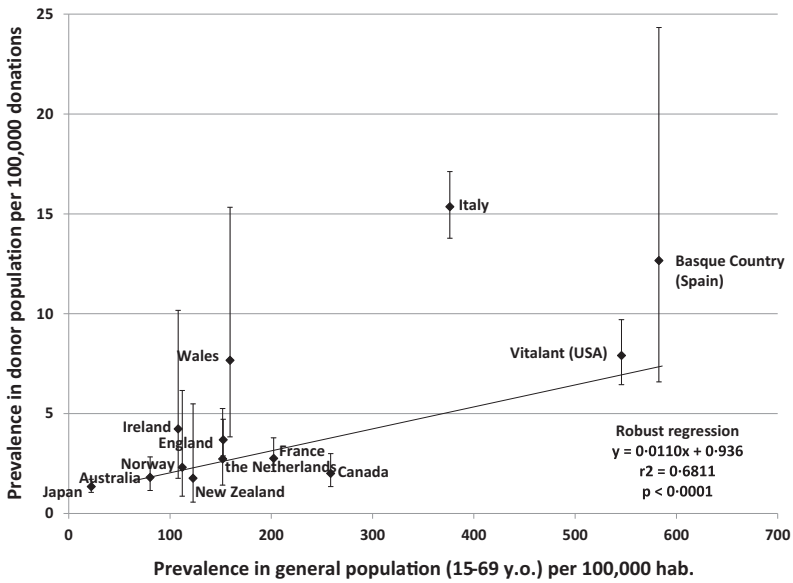

Fig. 1 Relation between prevalence rate in general population 15–69 y.o. and that of first-time donors (without São Paulo). 95% confidence intervals around the rate for donors are shown.

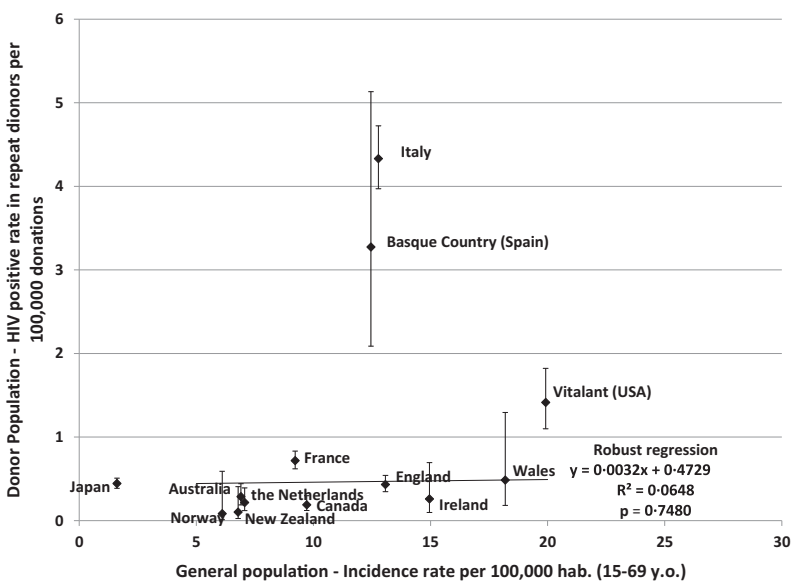

Fig. 2 Relation between incidence rate in general population per 100 000 inhabitants (15–69 y.o.) and HIV-positive rate in repeat donors per 100 000 donations (without São Paulo). 95% confidence intervals around the rate for donors are shown.

incidence in the general population, rates in repeat donors in Italy, Basque Country, France, the USA and Japan were higher in comparison with the other countries.

Prevalence rates among repeat donors do not differ between most countries, with some exceptions. First, prevalence is highest in São Paolo at $13{\cdot}2/10^5$ (not shown in Fig. 2) and significantly different from all other jurisdictions, despite adjustment for multiple comparisons. Also, rates in Italy and Basque Country, at 4·3 and $3{\cdot}3/10^5$, respectively, are statistically significantly higher compared with all other jurisdictions, with the exception of the non-significant difference between Basque County and USA. Finally, prevalence in repeat donors in the USA ($1{\cdot}4/10^5$) is higher compared with all other countries/regions, excluding those already mentioned, even after adjusting for multiple comparisons. Rates in these other countries are below $1/10^5$ donations, the lowest being in New Zealand ($0{\cdot}08/10^5$) and the highest in France ($0{\cdot}72/10^5$) (See Fig. S2.)

Figure 3 shows the relationship between donor HIV incidence, as calculated from HIV NAT-only-positive donations, and general population HIV incidence. Some jurisdictions reported no or very few HIV NAT-only-positive donations. In countries where NAT-only-positive donations were reported, incidence among donors is

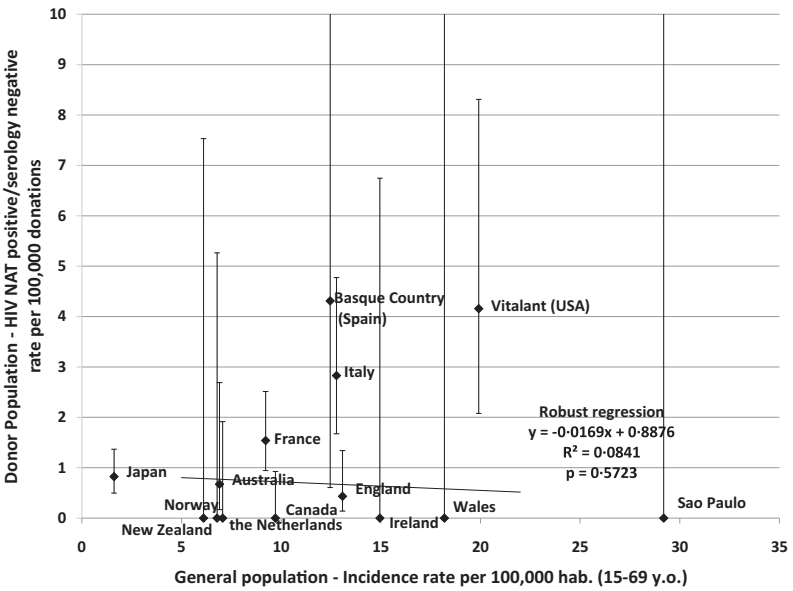

Fig. 3 Relation between incidence rate in general population per 100 000 inhabitants (15–69 y.o.) and incidence in blood donors as derived from HIV NAT-only-positive donations. 95% confidence intervals around the rate for donors are shown; upper bounds for the 95% confidence intervals above 10 are not shown.

always lower compared with the general population, by a factor of at least 2 (Japan), up to 5 or 6 in other countries (e.g. France, USA). There is also no correlation between rates of HIV NAT-only donations and HIV incidence in the general population. Some regions or countries with very similar donor deferral policies have seemingly but non-significantly different rates of NAT-only-positive donations. The USA and France, for example, reported a substantial number of such donations, whereas none or very few were seen in other countries, including Canada, the Netherlands, Norway and Australia, with confidence intervals that, in some cases, do not overlap. However, when comparing these rates between all countries/jurisdictions, accounting for multiple comparisons, none of these differences reach statistical significance (See Fig. S3).

Based on the incidence derived from NAT-only-positive donations and assuming a period of 9 days for the early undetectable phase of infectious donations, the residual risk of transmission varies from zero for those countries without any NAT-only-positive donations to 1/940 951 for Basque Country. When aggregating data from all countries, the risk is in the order of 1/3 125 000 donations.

We also calculated the proportion of all HIV-positive donations that are NAT-only positive, as an indicator of the proportion of very recent infections. This proportion varies from zero per cent in some countries (Canada, the Netherlands, Norway, Wales, São Paulo), to around five per cent in others (France, Australia, USA); however, the observed differences are not statistically significant, mainly due to small numbers and adjustment for multiple comparisons.

Finally, Fig. 4 shows the rates of HIV in repeat donations after adjusting these rates relative to the general population incidence, with Australia as the reference. Compared to unadjusted rates, a higher number thus indicate a rate that is higher than expected given the rate in the general population. Again, these rates are very similar and no jurisdiction has a rate much different compared with its unadjusted rate, with the possible exception of Japan. São Paolo has a significantly higher rate compared with other countries/regions except Italy, Basque Country, Japan and Wales (The small number of observations in Wales is likely the reason for this non-significant difference.) The rate in Italy is significantly higher compared with all other remaining countries, except for Wales (again, likely as a result of small numbers). Based on this adjusted comparison, France and Japan have significantly higher rates compared with Canada and Norway.

Discussion

We hypothesized that one potential determinant of the prevalence and incidence of HIV in the donor population might be the rates observed in the general population. If strong correlations were to be observed, one could conclude that the main determinant of the residual risk is the rate of HIV in a given jurisdiction and no other factors such as donor education and awareness of transfusion risk, donor eligibility criteria or donor selection modalities. If no correlation is observed, or for those appearing as 'outliers' with regard to regression curve, then one may deduce that other factors than population rates of HIV have an impact on the residual HIV transfusion risk. Some of these other factors may be rather obvious such

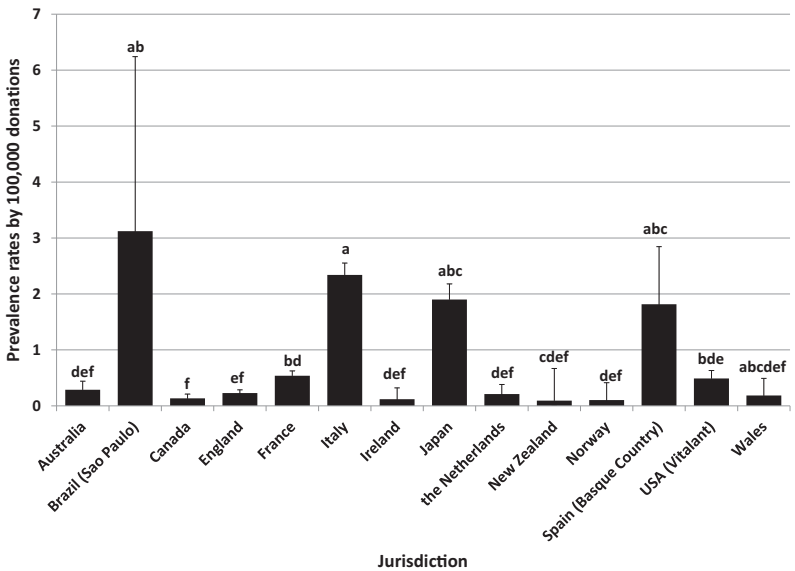

Fig. 4 HIV-positive donations in repeat donors adjusted for the incidence of HIV in the general population (Australia as the reference). 95% confidence intervals are shown. Countries/regions sharing the same letter are not statistically significantly different.

as specific deferral policies for MSM. Other subtler factors, such as test-seeking behaviour or stigma associated with HIV, may also have a significant impact, and a better understanding of such factors might pave the way to 'best practices' regarding further HIV transfusion risk mitigation and possibly other infectious transfusion risks.

Our results show some degree of correlation between the prevalence of HIV among first-time donors and the prevalence in the general population, suggesting that the prevalence of HIV in the general population might, at least in part, determine the prevalence of infection in first-time donors. However, because of the early, undetectable phase of infection (window period), the main determinant and the best indicator of the residual risk of HIV transmission by transfusion is the HIV incidence among donors. The usual manner in which this incidence is measured is by looking at the rate of seroconversion among repeat donors.[1] We did not have access to data that would allow us to do this analysis, and we used instead the prevalence of HIV among repeat donors as a proxy for incidence. A more direct way of measuring HIV incidence is by looking at NAT-only-positive donations. Examining the incidence of HIV among donors whether through the rate among repeat donors or based on NAT-only donations, these rates are not correlated with HIV incidence in the general population. This suggests that other factors could have a significant impact on the incidence of HIV in donors and the magnitude of the residual risk.

Another striking, although expected, feature of these findings is that both the prevalence and the incidence of HIV in blood donors are much lower compared with the general population. The majority of HIV-infected people are aware of their status and will not present to donate blood, which drives down the prevalence in first-time donors. Nevertheless, given that incidence rates are also lower, this is a strong indication that public education, self-exclusion of those with high-risk behaviours and policies and practices regarding eligibility to donate are highly efficacious in decreasing the risk of collecting units from HIV-infected individuals. These policies and practices also appear to have a much stronger effect on donor rates of HIV compared with the influence of HIV rates in the general population.

Although NAT-only-positive donations provide a direct measurement of HIV incidence and residual risk, such donations are extremely rare. It is therefore difficult to have a high level of precision for this estimate, at least in those countries with fewer total numbers of donations. However, the aggregated residual risk for all countries included in the study is very low, on the order of one in three million donations, an estimate that is well aligned with those derived from the classical window period model looking at seroconverting donors [1]. These data are also consistent with those reported in Germany by Fiedler et al. [12], where there were 20 NAT-only-positive donations in a total of around 46·2 million donations between 2008 and 2015, translating into an annual incidence of $1{\cdot}22/10^5$ and a residual risk of 1/3·3 million donations. Admittedly, the incidence rate derived from NAT-only donations will vary slightly depending on whether a given country applied minipool of individual NAT, a factor that was not included in our calculation. However, the rates are so low and the difference in window period duration is so small that such adjustment would not meaningfully influence the incidence estimate and the derived residual risk. The same considerations

apply to the slight differences in window period duration between antibody-only versus combined antigen-antibody screening tests.

For Basque Country and Vitalant, we did not have access to the rates of HIV in the general population for the corresponding geographical areas; we used instead the rates for the whole country, which may not be perfectly representative of the donor catchment area. However, based on published data, it appears that rates of HIV in donors in those regions are fairly similar to rates in other regions of the same country. For example, the prevalence of HIV in Spanish donors for the year 2005 is reported to be $6{\cdot}0/10^5$, whereas this rate for Basque Country in our study is $4{\cdot}3/10^5$ [13]. Similarly, the rate of HIV-positive donations among first-time donors in a recently published study that included 60% of all donations in the USA, covering a period of more than 3 years ending in December 2018, is reported at $8{\cdot}2/10^5$, which is very close to the rate seen at Vitalant in our study ($7{\cdot}7/10^5$) [14]. This suggests that donor HIV rates from these two countries are fairly representative of the total donor population. We were not able to determine whether donors from São Paolo were representative of donors elsewhere in the state, which is the reason for excluding them from the regression analyses.

Most countries included in this study apply a temporary deferral to MSM, with the exception of Spain and Italy, where sexually active MSM are allowed to donate whether they meet criteria for low-risk sexual behaviours applied to all donors [11]. Our data suggest that in comparison with other countries, those two jurisdictions have a higher rate of HIV infection in their donor population. This is possibly the result of allowing sexually active MSM to donate. However, differing patterns of HIV transmission may also contribute to the observed differences. For example, the prevalence of heterosexually acquired HIV infections in the general population and hence in blood donors may be higher in Italy compared with other countries in Europe [15]. It is also noteworthy that the rate of NAT-only-positive donations, which is directly related to incidence and residual risk, may not be discernably higher in Italy and Basque Country, compared to several other countries, for example the USA or France, though the number of HIV NAT-only donations is admittedly too small to conclude that there is no difference in HIV incidence. A policy allowing sexually active MSM to donate might have a limited impact on donor HIV rates if it also includes specific deferrals for higher risk behaviours, such as having more than one sexual partner. In a recently published risk assessment conducted in France, it was projected that allowing sexually active MSM to donate but including such restrictions would indeed have a negligible impact on donor HIV rates [16]. Similar findings in England have resulted in a recent recommendation that MSM donors who have had the same sexual partner in the last 3 months should be eligible to donate [17].

Even in countries having very similar deferral practices and HIV rates in the general population, there are some apparent differences in the rates of NAT-only-positive donations. Compared to Canada, for example, France has a measurable and substantial rate of NAT-only-positive donations; this rate in Canada has been zero during the study period, despite the eligibility criteria for donation, including those for MSM, being virtually identical. The incidence of HIV in the Canadian general population is even slightly higher, yet the incidence of HIV in the donor population appears to be substantially higher in France. This is reflected in the statistically significant difference between these two countries after adjusting for the incidence rates in their general population. The same discrepancy exists between France and Australia or England. On the higher end of the curve, the rate of HIV NAT-only-positive donations in the USA is about the same as the rate observed in Italy and not much lower than the rate in Basque Country. It could therefore be that factors other than eligibility criteria for MSM also have an impact on this very small residual risk. In particular, it could be that the higher rate of HIV NAT-only-positive donations in France compared with Canada is explained, at least in part, by a higher level of noncompliance with donor selection criteria. In France, 0·73% of men subsequently admitted not having complied with MSM criteria, whereas this proportion was 0·24 % in Canada [18,19]. This factor and possibly others that are not readily recognizable might have a significant effect on the very small residual risk of HIV transmission by transfusion. Finally, it should be noted that most countries/jurisdictions included in this study are high income with well-funded blood transfusion services, thus limiting the extrapolation of these findings to other less developed countries.

In conclusion, this international comparison of the rates of HIV among donors shows that most countries have very low rates of HIV infections in their donor population and that these rates are quite comparable. They are also systematically lower and, with regard to prevalence rates, associated with the rates observed in the general population. Countries that allow MSM to donate have higher rates of prevalent and incident HIV in their donor population, but the risk to recipients remains very small and is often comparable to the risk observed in some other countries with more stringent policies. It seems likely that other factors that are not obvious can lead to apparent fluctuations in the very low level of residual risk of HIV transmission by transfusion. Further

dissection of the observed differences between jurisdictions may reveal varying efficacy of mitigation strategies and allow for the identification of best practices.

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Conflicts of interest

None to declare.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1 HIV positive donations in first-time donors per 100,000 donations by jurisdiction between from 2006 to 2017 (without São Paulo).
Fig. S2 HIV positive donations in repeat donors per 100,000 donations by jurisdiction between from 2006 to 2017 (without São Paulo).
Fig. S3 HIV NAT positive/serology negative in donor population per 100 000 donations by jurisdiction between from 2006 to 2017 (without São Paulo).

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West Nile virus and blood transfusion safety: A European perspective

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Vox Sanguinis

Background and Objectives There is a growing concern for the transmission of arboviral infections by blood transfusion in Europe. However, no assessment of the risk of transmission through all European blood supplies has been reported. Risk regulations at a European level should take differences in local transmission risk and the risk of transmission by travelling donors into consideration.

Materials and Methods A risk model and publicly available tool were developed to calculate the risk of transmission by all European blood supplies for arboviral outbreaks within Europe. Data on individual European blood supplies from Council of Europe reports and inter-European travel data from EUROSTAT were used to populate this model.

Results Each neuroinvasive case of WNV reported in Europe will on average result in 0·43 (95%CI: 0·32–0·55) infected blood product by locally infected donors and 0·010 (95%CI: 0·006–0·015) infected products by travelling donors. On basis of the 1373 neuroinvasive human WNV cases reported in the outbreak of 2018, it is estimated that without safety interventions this outbreak would have resulted in 708 (95%CI: 523–922) infected components derived from resident donors. Noncompliance to European regulations, which requires donor deferral or testing of donors who visited WNV-infected areas, would have resulted in 7.4 (95%CI: 4·7–11·1) infected blood components derived from infectious travelling donors exposed in outbreak areas throughout Europe.

Conclusion The risk of WNV transmission by a local outbreak is on average 113 times (95%CI: 95–139), so two orders of magnitude higher than the risk of transmission by travelling donors in Europe.

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Key words: European Transmission risk, reproduction number (R_0) for blood transfusion, travelling donors.

Introduction

Arboviral diseases have been identified as a threat to human health due to their biological complexity, epidemic potential and medical impact [1]. Since the first reported cases of West Nile virus (WNV) transmitted by blood transfusion in the United States in 2002, it has become a concern for blood transfusion safety as well [2,3]. Next to the risk of local transmissions, there is the risk from travelling donors who donate blood in their country of residence after returning from WNV-affected areas [4]. The recognition of donors at risk is challenging due to the high proportion of asymptomatic cases (80%), making it difficult to identify infected donors during the donor selection process, and due to the definition of affected areas which depends on surveillance and timely reporting of spread of the arbovirus, especially in newly affected areas [5,6].

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A large number of European residents travel each year to areas within Europe with arboviral outbreaks. Every year around 155 million European residents have short-term travels to Italy, France, Austria, Greece, Croatia, Czech Republic, Hungary, Bulgaria, Cyprus, Slovenia, Romania and Serbia, which are countries with WNV cases reported in the peak WNV outbreak in 2018 [7,8]. This substantial number of travellers induces a risk for individual European blood supplies which calls for protective measures such as donor deferral for 28 days after leaving an area with circulating West Nile virus, or testing returning donors with an individual nucleic acid test (NAT), as required by European regulations [9].

In 2016, a risk assessment tool (*EUFRAT*) and underlying model were published that allows estimating the risk of transmission of a virus by blood transfusion from donors who travelled to specific outbreak areas [10,11]. The assessment of the risk of an outbreak for individual countries may be sufficient for local decision-making, but insight into the impact for the entire region is necessary to support regulations at a European level. Such an assessment, however, requires data on European travel as well as characteristics of the national blood supply for each of the individual countries. Detailed data on travel between various countries within the European region are difficult to acquire. Therefore, a simplification of the *EUFRAT* model was derived that requires less detailed travel information but allows calculating a conservative estimate of the number of infected blood products throughout Europe from donors who were infected whilst travelling to outbreak-affected areas within Europe. With this model, the number of infected blood products is estimated that are acquired from European donors who visited an outbreak area elsewhere in Europe and donate upon return to their country of residence. The model allows comparing the risk of transmission of infections by donors travelling within Europe and the risk of transmissions by donors living in the outbreak area themselves. The model outcomes may support decision-makers in formulating rational and proportional risk prevention measures for blood safety for various donor groups.

Methods

Risk model

One of the disadvantages of the *EUFRAT* model is that, next to information on the number of visitors to an outbreak region, it also requires information on the length of stay of these visitors in the outbreak area [11]. It is clear that length of stay information is less easily available than the number of visitors. However, the length of stay of a visit is relevant as it provides information on duration of exposure of the visitors. From the original *EUFRAT* model, it can be found that the risk of transmission by travelling donors is limited by both the length of stay and the disease-specific length of the time interval from acquiring the infection to no longer being able to transmit the infection (D) [11]. We realized that for infections like arboviruses, where D is small, the risk might not be overestimated substantially if the risk of transmission would be limited by the interval D only. This risk estimate then conservatively presumes that the length of stay of any visitors would be at least equal to the length of interval D, which in case of WNV adds up to 13 days (2 days incubation time, followed by 11 days of infectivity) [12].

The risk, expressed as the number of infected blood components distributed in the country of origin of the donor per infected individual in the country of the outbreak, is calculated as:

$$R_t = p_o \varphi_o \cdot \frac{f_o}{N_d} \cdot \frac{D_a(D_a/2 + D_i)}{1 - p_u} \quad (1)$$

The definition of each of the parameters from eqn 1 is given in Table 1. Eqn 1 consists of three parts: the first $(p_o \varphi_o)$ expresses the number of blood components issued per inhabitant per day in the country of origin of the donors. The more components issued, the more infected components are likely to be distributed as well. The higher the proportion of donors in a population, the more likely that a donor will become infected during travel. The second term (f_o/N_d) is the number of visitors per day per inhabitant in the outbreak region. This number reflects the relative exposure and therefore the risk of travellers becoming infected relative to residents. The final term $(D_a(D_a/2 + D_i)/(1 - p_u))$ is a disease-specific term which reflects how likely a travelling donor will present to donate, corrected for the number of unobserved infections in the outbreak region.

For the calculation of the risk of residential donors, by which we refer to non-travelling donors being infected in their country of residence by locally circulating WNV, we refer to the original paper on the *EUFRAT* risk model or to the Appendix S1 [11]. The online Appendix S1 also contain a more detailed description on how the formula in Eqn 1 is obtained from the original *EUFRAT* model.

Travel and blood supply data

Data on travel by European residents from 2016 to 2018 were collected from the statistical office of the European Union (EUROSTAT) and consist of reported arrivals at tourist accommodation establishments throughout Europe [7]. The data consist of the number of visitors to and

Table 1 Model parameters and their definitions

Symbol	Dimension	Description
R_t	-	Number of infected blood components per observed infection in the outbreak country
p_o	-	Proportion of donors in the country of origin
f_o	1/day	Number of visitors per day from a particular country of origin to the outbreak region
φ_o	1/day	Number of blood components distributed per blood donor per day in the country of origin
N_d	-	Number of inhabitants in the outbreak region
D_a	Days	Duration of infectivity of an infected individual
D_i	Days	Length of time it takes before an individual becomes infectious
D	Days	Length of the time interval between acquiring the infection and no longer being able to transmit the infection ($D_a + D_i$)
p_u	-	Proportion of unobserved infections

from 39 European countries, of which data from the countries Armenia and Georgia were missing completely, as were data on arrivals in Russia, Turkey and Ukraine, as were data on departures from Montenegro, North Macedonia and Serbia (Fig. 1). In addition, some data were missing for travel between Croatia, Cyprus, France, Iceland, Romania, Turkey and Ukraine. The missing data were imputed as the expected number of travellers given the marginal proportion of arrivals and departures of the countries involved.

Data on blood supply characteristics from European countries (total number of donors, total number of blood components and the proportion of donors in the general population) were obtained from the annual reports from the Council of Europe from 2013 to 2015 [13–15]. This resulted in estimates for the blood supply characteristics in 36 European countries. As data from Slovenia, Turkey and Ukraine were unavailable from the Council of Europe reports, the average donation characteristic derived for all other countries was used instead. Due to the relatively low variation in the average number of blood components per inhabitant per day among European countries between ($\overline{p_o \varphi_o} = 1 \cdot 6E-04$; SD = 5·3E−05), the use of the European average seemed acceptable for estimating

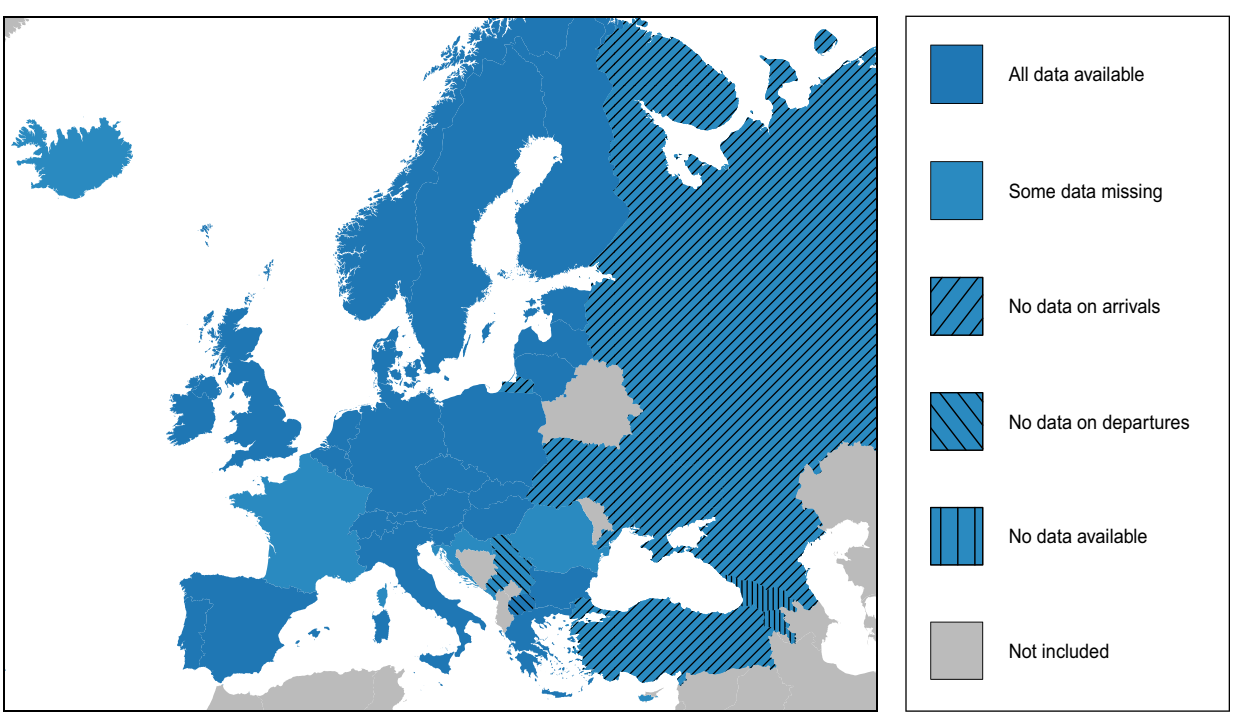

Fig. 1 Availability of travel data for the assessment of the risk of transmission of infectious diseases by travelling donors from European outbreaks.

the risk of transmission for those countries. Travel and blood supply data used can be found in the online Appendix S2 and all imputed values are marked as such.

Application of the model to analyse the impact of the peak outbreak of WNV infections in Europe in 2018

The newly derived risk model was implemented in a MS Excel workbook (the 'European Travelling Donors' Risk Assessment Tool') which is made available by the European Blood Alliance (https://europeanbloodalliance.eu/a-european-perspective-on-emerging-infections-and-blood-safety/). The tool allows quantification of the risk of infectious disease transmission by blood transfusion from both travelling and resident donors.

The risk model was applied to calculate the expected average number of infected blood components based on the total number of locally acquired neuroinvasive WNV cases reported in Europe in 2018 (Table 2). With 1373 cases in 2018, there was a *sevenfold* increase in the number of neuroinvasive WNV cases in Europe as compared to the previous years. With 307 cases in 2019, the increasing trend from the years before (118, 213 and 195 cases in 2015, 2016 and 2017, respectively) seems to be continued.

The total number of WNV infections was estimated under the assumption that each neuroinvasive WNV infection reported represents 245 incident WNV infections ($p_u = 1-1/245$) [16,17]. For the incubation time D_i (the time before an individual becomes infectious), and the duration of infectivity of an infected individual (D_a), 2 and 11 days were used, respectively [12].

Sensitivity and uncertainty assessment

All model input parameters are simulated with a probabilistic distribution to represent the uncertainty in the point estimates used. This allows an evaluation of the uncertainty, expressed in a 95% confidence interval (CI) for each of the estimated outcomes. Each of the outcomes was calculated for 10 000 random draws from the probabilistic input distributions, which was sufficient for a robust estimate of the confidence intervals. This means that the relative deviation of all of the 95%CI estimates was less than 1%. Further details on the simulation (a description of the probabilistic distributions used and their parameters) can be found in Appendix S1. The sensitivity to various model parameters was assessed by determining the first order derivative of the outcome for a relative change in model parameters scaled to the maximum derivative obtained [18]. Sensitivity and uncertainty analyses were performed in *R* (version 3.6.3) [19].

Table 2 Locally acquired neuroinvasive cases of WNV in Europe (1373 in total) reported per country in 2018 (data kindly provided by the ECDC from The European Surveillance System—TESSy)

Country	Neuroinvasive cases reported	Country	Neuroinvasive cases reported
Albania	1	Greece	241
Austria	4	Hungary	152
Bulgaria	13	Italy	243
Croatia	47	Kosovo	12
Cyprus	1	Romania	277
Czech Rep.	3	Serbia	368
France	7	Slovenia	4

Reproduction number R_t for Europe

For each infection in the population, there is a probability that this infection will result in a local or, with a substantially lower probability, foreign transmission by blood transfusion. The ratio between the number of infections transmitted per infection observed is a measure (R_0) that is well known in epidemiology [20]. The reason for its popularity and use is its ease of interpretation: it refers to the number of transmissions per infected case in a population. There is a strong similarity between the R_0 and the R_t from eqn 1 as R_t represents average number of WNV-infected blood products distributed throughout Europe per neuroinvasive infection observed (somewhere) in Europe. Next to a reproduction number for the number of infected blood products distributed throughout the European blood supplies, we also calculated a European reproduction number for the number of infected products obtained from locally infected residential donors. Details on the calculation of both European estimates can be found in Appendix S1. Note that the reproduction numbers here reflect the number of infectious blood products and not the number of infected individuals unless it is (conservatively) presumed that each infected blood product transfused will result in an infection in the transfusion recipient.

Results

The risk from the 1373 neuroinvasive WNV cases observed in the 2018 outbreak was calculated for each country within Europe and summated to obtain an estimate for number of infected products distributed through all European blood supplies. The 13 neuroinvasive WNV cases reported in Kosovo and Albania (0·9% of all cases) were not included in the assessment due to the absence of (travel) data for these countries. Based on the

remaining 1360 neuroinvasive cases of WNV infections reported in Europe, the estimated number of WNV-infected blood components through donations of travelling donors in Europe is 7·4 (95% CI: 4·7–11·1). Note that this estimate refers to the number of infected blood products given that no safety interventions would be in place. It should also be clear that this number reflects 7·4 infected blood components distributed throughout Europe and that these are blood products derived from donations from any (European) visitors to any of the outbreak areas in Europe in 2018. The estimated number of infected blood products derived from locally infected residential donors in one of the 12 outbreak areas included in the analysis is 708 (95% CI: 521–923).

Countries with the highest risk for transfusion-transmitted WNV by travelling donors are Germany, the United Kingdom, France and Italy (Fig. 2). This is primarily the result of the relatively high number of travellers from these countries visiting the outbreak areas. The number of blood components issued per inhabitant per day in the country of origin of the donors ($p_o\varphi_o$), the first term in eqn 1 that determines the transmission risk, varies substantially less between European countries than does the number of travellers.

The reproduction number for Europe for travelling donors is 0·010 (95% CI: 0·006–0·015) per neuroinvasive case of WNV infection, whereas reproduction number for Europe for locally infected donors is 0·43 (95% CI: 0·32–0·55) per neuroinvasive case. This implies that on average 100 (95% CI: 68–158) neuroinvasive WNV cases have to be observed in Europe to distribute one blood component obtained from a travelling donor who has been exposed to a European outbreak. In contrast, on average only 2 (95% CI: 1·8–3·2) neuroinvasive WNV cases are required per WNV-infected blood component from a resident donor in the outbreak region. On average, the risk of an infectious blood product from a local outbreak is 113 (95%CI: 95–139) times higher than that of a remote outbreak, which is completely in line with the findings from the 2018 outbreak.

The number of infected products from travelling donors is most sensitive to relative changes in the duration of length of the infectious period (D_a, 100%), the number of infections per neuroinvasive WNV infection observed (1/(1-p_u), 57%), and the incubation time (D_{ia}, 15%). This makes sense as these parameters have the most generic effect on the risk of transmission. Next are the countries with a large number of infections in combination with a relatively high number of visitors per inhabitant. For the 2018 outbreak, these were Greece, Italy and Croatia, with a sensitivity of 25%, 11% and 10%, respectively. Despite the fact that there were most infections in Romania and Serbia, of all countries considered these two have the lowest number of visitors per inhabitant which limits their contribution to the risk to only 8%. Germany, with a sensitivity of 15%, has by far the largest number of travellers of all European countries and is exposed to 25% of the risk from travelling donors of the 2018 outbreak. Note that 21% of all European travels originate from Germany. The European reproduction number for infected products obtained from travelling donors is equally sensitive to the disease parameters mentioned above (100%, 57% and 15%). Next sensitive is European reproduction number to change in the number of visitors from (15% for Germany and 7% for the United Kingdom) or to (11% for Iceland, 5% for Malta) countries with respectively a high number of travellers or a high number of visitors per inhabitant.

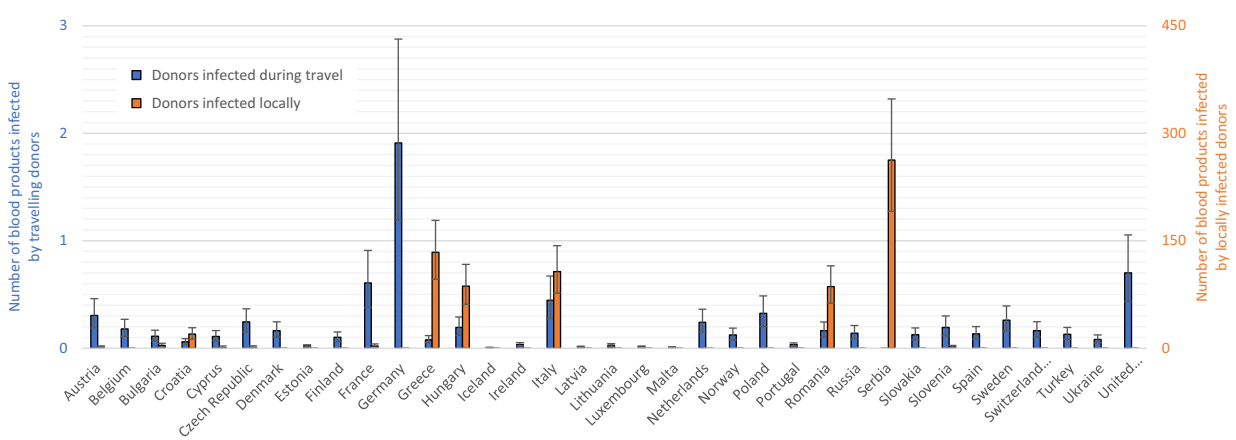

Fig. 2 Expected number of WNV-infected blood components derived from travelling donors (left) and derived from residential donors in outbreak areas (right) in case no safety interventions would have been in place. Calculations are based on 1360 human neuroinvasive cases reported in the European WNV outbreak in 2018. Note that the scale for the infected of blood components by local donors (right-hand side axis) is more than *two orders of magnitude* larger than the scale for the travelling donors (left-hand side axis).

Local infections are equally sensitive to the infectious period (D_a) and the number of infections per neuroinvasive WNV infection observed ($1/(1-p_u)$, both 100%). It is next sensitive to the product of the number of infections in the outbreak country and the number of blood components issued per inhabitant per day ($p_o\varphi_o$) in that country. For the 2018 outbreak, the sensitivity is highest for Serbia, Greece and Italy (37%, 19% and 15%). The sensitivity of the European reproduction number for local outbreaks transmitted by residential donors is again directly affected by a change in the two disease parameters D_a and p_u. Its sensitivity for change in the number of blood components issued per inhabitant per day ($p_o\varphi_o$) is 5% or less.

A full list of sensitivity factors for each of the risks discussed above is provided in Appendix S1.

Discussion

With the popularity of some of the arboviral outbreak areas for European travellers and an increasing number of arboviral infections in humans, an increase in the risk of infection transmission by blood transfusion is expected. As shown in our results (Fig. 2) in countries like Italy, Greece, Romania and Hungary, there is next to the risk of local transmissions by blood transfusion a small supplementary risk from travelling donors from these countries to other risk areas in Europe. Additionally, the increasing number of travellers to outbreak areas create a risk of transmission in areas where no arboviral outbreaks were present locally, as in 2018 in Germany (1·9 infected components from travellers), the United Kingdom (0·7 infected components from travellers) or France (0·6 infected components from travellers).

As described earlier, data on travel by the European residents from 8 out of 39 countries (21%) were partially or completely missing. For 5 of these countries (Armenia, Georgia, Russia, Turkey and Ukraine, see Fig. 1), this lack of data implied that the risk of transmission by travelling donors from these areas could not be assessed. Travel data are too country-specific to justify the use of average travel statistics to estimate the risk for these countries. This leads to a structural underestimation of the risk of transmission within Europe. However, as long as it is clear which countries are excluded in the assessment the interpretation of the results remains valid.

A comparison of the novel approach to assess the risk of transmission to the conventional *EUFRAT* model using the length of stay data available from EUROSTAT shows that conservative risk estimate proposed in this paper is on average overestimating the risk by a factor of 2·4. Given that the *EUFRAT* model would rather require information on the distribution of lengths of stay per country instead of an average value, the estimate obtained with the novel estimation method might in fact be preferable.

The main bias in the risk estimates reported in this paper, however, is caused by the lack of accurate data on travel to the specific outbreak areas. As the data currently used reflect travel to a country rather than travel to a specific outbreak area, these may substantially under- or overestimate actual donor exposure and hence bias the estimated risks. Nonetheless, a previous study showed that the estimates obtained may still provide an acceptable 'order-of-magnitude' estimate of the transmission risk [21]. More representative travel data—which may be difficult to obtain—will always result in better risk estimates. The European Travelling Donors' Risk Assessment Tool therefore allows the user to override the visitors per inhabitant ratio based on national statistics provided by the tool in case specific travel data for an outbreak region are available.

The risk assessment tool for each outbreak automatically provides estimates for the number of infected blood products derived from residential donors that were infected by a local outbreak as well as the number of infected blood products from donors who visited other outbreak areas within Europe. These estimates provide decision-makers (regulators and/or blood operators) with information that may support the management of a safe and sufficient blood supply [22,23]. According to the Commission Directive 2014/110/EU, temporary deferral for 28 days or donation screening is required for travellers after leaving a risk area of locally acquired WNV [9]. Our results show that the average risk of transmission by a travelling donor within Europe is 0·010 per neuroinvasive case of WNV observed. This implies that without the proposed blood safety measures, there would be a 1% chance of a WNV-infected component from an infected travelling donor being transfused in Europe per neuroinvasive case of WNV. This is equivalent to one infected component distributed for every 100 neuroinvasive cases observed. Whether this justifies the precautionary measures currently required is arguable, as the risk from travelling donors in Europe is on average 113 times lower than the risk of a local outbreak. Nonetheless, the risk estimates provided in this paper put the risk of transmission by travelling donors in a proper perspective. The fact that our estimate for the travellers' risk (one infected blood product in $1360/7{\cdot}4 = 183$ neuroinvasive cases) is lower than the reproduction number for Europe (one per 100 neuroinvasive cases) is due to the fact that the countries with the highest number of infections have a relatively low number of visitors per inhabitant.

Our current model conservatively presumes that the length of stay equals the sum of incubation time and infectious period, which for WNV is 13 days. Diseases with longer infectious periods can be assessed using the

original *EUFRAT* model but their risk may be overestimated substantially by the model described in this paper. Such an assessment would, in addition to estimates on the number of visitors to all European countries, also require estimates for the length of stay of these visitors during their travel.

The travellers' risk model presented in this paper can be applied to assess the risk of transmission by all arboviral infectious diseases with short viraemic phases, and its application was demonstrated in the European WNV outbreaks in 2018. The availability of the tool allows decision-makers to obtain rapid and up-to-date risk estimates to support rational management and response to emerging outbreaks. In addition, the estimated 100 neuroinvasive WNV cases on average per infected blood product distributed in Europe provides a rough but realistic order of magnitude estimate for the combined risk of transmission of WNV infections by blood transfusion in Europe.

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Conflict of interests

The authors declare no conflict of interests.

Contributors

RGJ initiated the study, carried out the analyses and wrote the draft manuscript; RLW participated in the study design and coordination and helped writing the final manuscript; MPJ was responsible for the conception of the research, coordination of the study, checking formulas and results and reviewing the final manuscript.

Disclaimers

Data provided by ECDC extracted from The European Surveillance System—TESSy and by Albania, Austria, Bulgaria, Croatia, Cyprus, Czech Republic, France, Greece, Hungary, Italy, Kosovo, Romania, Serbia and Slovenia and released by ECDC. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the ECDC. The accuracy of the authors' statistical analysis and the findings they report are not the responsibility of ECDC. ECDC is not responsible for conclusions or opinions drawn from the data provided. ECDC is not responsible for the correctness of the data and for data management, data merging and data collation after provision of the data. ECDC shall not be held liable for improper or incorrect use of the data. Copyright notice: ©European Union, 1995-2019 Reuse is authorized, provided the source is acknowledged. The reuse policy is implemented by the Commission Decision of 12 December 2011—reuse of Commission documents (2011/833/EU).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Appendix S1: Formulas, parameter values and sensitivity analyses
Appendix S2: Data blood use & travel data used

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SHORT REPORT

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Anaemia in elderly patients at discharge from intensive care and hospital

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Vox Sanguinis

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Background and objectives Anaemia is common in the elderly and is recognized as a risk factor for several adverse outcomes in older adults, including hospitalization, morbidity and mortality. The study aims were to examine the prevalence of anaemia in elderly patients at discharge from the intensive care unit (ICU) and hospital.

Materials and methods Patient randomized under the INFORM trial and with an ICU admission were included. Two cohorts, Cohort 1 patients who were alive on discharge from ICU and Cohort 2 patients who were discharged alive from hospital to home. Prevalence of significant anaemia defined as haemoglobin levels, less than 100 g/l was measured at ICU and hospital discharge.

Results Overall, 76·5% (683/893) of elderly admissions in Cohort 1 had a haemoglobin <100 g/l, and 44·1% (395/893) had a haemoglobin <90 g/l on ICU discharge. Nadir haemoglobin during ICU stay, length of stay in ICU and transfusion during ICU stay was associated with significant anaemia at ICU discharge. At hospital discharge, in Cohort 2, 54·8% (263/480) of elderly ICU admissions had Hb < 100 g/l, and 23·4% (112/480) had Hb < 90 g/l. Male gender, haemoglobin level at ICU discharge, and length of stay and nadir Hb between ICU and hospital discharge were associated with anaemia at hospital discharge.

Conclusions Significant anaemia is highly prevalent in elderly patients on discharge from ICU and to a lesser degree at hospital discharge.

Introduction

Anaemia is common in the elderly and is recognized as a risk factor for several adverse outcomes, including hospitalization, morbidity and mortality [1–6]. Approximately 10% of elderly people (age > 65) are anaemic (haemoglobin (Hb) <120 g/l in women and <130 g/l in men) [7]. By age 85, the overall prevalence of anaemia increases to 20% and is associated with a twofold increase in all-cause mortality [7].

Elderly trauma patients have been found to be anaemic on hospital admission and on discharge [8]. Lower admission Hb levels among elderly patients may reflect the impact of nutritional deficiencies, polypharmacy and myelodysplasia [8]. The prevalence of anaemia in the elderly may be magnified during hospitalization commonly resulting from bleeding, repeated phlebotomy and decreased erythropoiesis.

There are several studies that have documented the prevalence of anaemia on admission to and during ICU stay [9, 10]; however, there is limited information particularly in the elderly on anaemia during the recovery phase of critical illness [11].

Our study aims were to examine the prevalence of anaemia in elderly patients at discharge from the intensive care unit (ICU) and hospital.

Methods

The INFORM trial was a pragmatic, randomized, controlled trial conducted at six hospitals in four countries (Australia, Canada, Israel and United States), which

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assigned patients to receive transfusion of the freshest red cells (short-term storage group) in inventory or the oldest available red cells (long-term storage group) in a 1:2 ratio [12]. Flinders Medical Centre (FMC) was one of the participating sites of the INFORM trial. For this subgroup analysis, all adult patients (≥65 years) who received at least one blood component during their ICU admission were included.

Data were extracted from the INFORM, ICU and laboratory databases to identify patients ≥65 years including their demographic information, transfusion status while in ICU and nature of separation (discharged or died). A total of 7276 patients were randomized in the INFORM study at FMC, and 5783 patients were included in this study. Of the 5783 patients, 1352 elderly patients were admitted to ICU, amounting to 1530 ICU admissions for analysis.

All haemoglobins (Hb) from ICU admission to hospital discharge, at ICU discharge, nadir Hb during ICU stay, between ICU and hospital discharge and at hospital discharge (last recorded Hb within 24 h prior to hospital discharge) were collected. Anaemia prevalence at Hb level <100 g/l and 90 g/l at ICU and home discharge was determined.

Intensive care unit data were collected from the Australian and New Zealand Adult Patient (ADP) database. ICU admission date and time, APACHE II score, APACHE II diagnosis (code and description) was collected.

To examine anaemia at ICU and hospital discharge, all ICU admissions discharged alive from ICU to the ward and where ICU patients were discharged home directly to the community were included.

Our primary outcome was the prevalence of significant anaemia with haemoglobin values <100 g/l and <90 g/l at ICU and hospital discharge and factors that may be associated with the prevalence of significant anaemia of Hb <100 g/l at discharge.

Continuous data were presented as medians and interquartile ranges (IQRs). Nadir Hb and discharge Hb across the length of ICU stay were compared by use of the Kruskal–Wallis test. Proportion of patients with significant anaemia at ICU and hospital discharge was compared by the chi-square test. Nadir Hb (g/l), ICU length of stay, status of transfusion and APACHE II score were included in a binary regression model to identify the risk factors associated with significant anaemia at ICU discharge. Similarly, Hb at ICU discharge, gender and length of stay and nadir Hb (g/l) between ICU and hospital discharge were included in binary regression model to identify the risk factors associated with significant anaemia at hospital discharge. Statistical significance was $p < 0{\cdot}05$. Analyses were performed by Stata version 16.

Results

Of the 1530 ICU admissions, 1454 admissions were discharged alive from ICU. Of those, 38 were discharged to another acute hospital and 523 admissions had missing data. The remaining 893 admissions formed Cohort 1 which included 77 admissions where patients were discharged from ICU but died in hospital and 308 admissions where patients were transferred to another acute hospital prior to discharge home including 508 admissions that were discharged home from hospital which formed Cohort 2. Patients transferred to another hospital had similar demographics to Cohort 2 patients, apart from a slightly higher number of cardiac (31·5% vs. 29·5%), gastrointestinal (21·8% vs. 17·5%), neurology (4·5% vs. 1·8%), renal (6·8% vs. 5·1%) and trauma (4·2% vs. 1·2%) admissions.

Cohort 1 Cohort

The median age in Cohort 1 was 76 (70–82) years with 57% male patients. The overall length of stay was 92 (47–183) hours with almost 75% of admissions spending less than 7 days in ICU. A total of 393 admissions received no transfusion compared to 500 admissions who received red cells.

76·5% (95% CI 73·6–79·2) (683/893) of elderly ICU admissions had a haemoglobin <100 g/l, and 44·1% (95% CI 40·9–47·6) (395/893) had a haemoglobin <90 g/l on discharge from ICU.

The nadir median haemoglobin level was 87 (80–94) g/l vs. 80 (75–85) g/l, $p < 0{\cdot}01$ between transfused and non-transfused admissions. ICU admissions longer than 1 week had a significant fall in haemoglobin to a median nadir of 78 (73–84) g/l with a transfusion rate of 76·8% compared to a median nadir of 88 (82–94) g/l in admissions of ≤1 day.

Variance in haemoglobin levels at discharge between transfused and non-transfused admissions was 91 (85–100) g/l vs. 90 (84–97), $p = 0{\cdot}19$; however, the prevalence of anaemia (Hb < 100 g/L) was significantly higher in transfused admissions at ICU discharge, [400/500, 80% (95% CI 76·2–83·4) vs. 283/393, 72% (95% CI 67·3–76·4), $p = 0{\cdot}005$].

Nadir haemoglobin during ICU stay, length of stay in ICU and transfusion during ICU stay were independently associated with haemoglobin <100 g/l at ICU discharge (Table 1a). When regression model was repeated without including nadir haemoglobin, transfusion during ICU stay was the only factor associated with haemoglobin less than 100 g/l at ICU discharge (Table 1b).

Cohort 2

Of the 508 admissions that were discharged home, data were available for 480 ICU admissions. Cohort 2 patients were similar to Cohort 1 patients, 45% of the admissions were in the hospital for more than 7 days following ICU discharge.

At hospital discharge, 54·8% (95% CI 73·9–79·6) (263/480) of elderly ICU admissions had Hb <100 g/l, and 23·4% (95% CI 19·6–27·4) (112/480) had Hb <90 g/l. Of the 375 admissions discharged with Hb <100 g/l, the median overall change (increase) in Hb between ICU discharge and hospital discharge was 9 (1–17) g/l. 73 (19·5%) admissions had a decrease in hospital discharge Hb.

Male gender, haemoglobin level at ICU discharge, and length of stay and nadir Hb between ICU and hospital discharge were associated with anaemia at hospital discharge (Table 2).

Discussion

This study demonstrated that anaemia remains highly prevalent in elderly patients discharged from ICU. Nearly, 77% of discharged ICU admissions had a haemoglobin <100 g/l and 44% had haemoglobin <90 g/l. At hospital discharge, this prevalence was 54·8% and 23·4%, respectively. In a Scottish multicentre study [9], 50% of ICU patients had a haemoglobin <100 g/l and 25% had haemoglobin <90 g/l at ICU discharge and at hospital discharge; this prevalence was 33% and 11%, respectively.

Intensive care unit discharge haemoglobin was strongly associated with anaemia at hospital discharge. There was a small haemoglobin recovery following ICU discharge during hospital stay, and however, 19–20% of ICU admissions also had a further decrease of haemoglobin at hospital discharge. Elderly patients in our study possibly had pre-existing anaemia, the aetiology of which was not available in this study. The recovery of haemoglobin following critical illness may be longer in elderly patients, most patients discharged from hospital have been shown to have normochromic normocytic anaemia consistent with anaemia of chronic disease [13]. This may have been the case in elderly patients in our study but was not studied.

It is possible that targeted anaemia treatment in ICU and during post-ICU period, for example with erythropoietin and iron therapy could impact on long-term outcomes.

In a recent randomized controlled trial, patients admitted to the ICU that were anaemic, had intravenous iron compared with placebo. This did not result in a significant lowering of red blood cell transfusion requirement during hospital stay; however, patients who received intravenous iron had a significantly higher haemoglobin concentration at hospital discharge [14]. It is likely that patients in our study may have received anaemia treatment in the form of iron therapy during hospital stay or at discharge.

Further studies in this patient group examining the impact of targeted therapy during ICU and post-ICU stay is warranted. Future research into both available and newer functional markers of iron deficiency such as reticulocyte haemoglobin content, introduction of hepcidin measurement on automated laboratory platforms including introduction of novel therapies for functional that inhibit hepcidin production, directly targets hepcidin itself or promote erythropoietin production and iron support have been recommended [15].

Our cohort was an unselected mixed general ICU population with a high illness severity and varied case mix. Since it is a subgroup analysis of INFORM trial that included hospital admissions that were transfused, a small proportion of admissions that were not transfused and still had an ICU admission may have been missed. The authors do acknowledge that the data used in this study is old and may not reflect current practice.

In conclusion, significant anaemia is highly prevalent in elderly patients on discharge from ICU and to a slightly lesser degree on discharge from hospital. Elderly patients may benefit from higher haemoglobin on discharge from ICU which may be achieved by reducing the length of stay and maintaining higher nadir haemoglobin.

Table 1a Factors associated with significant anaemia (haemoglobin <100 g/l) at ICU discharge.

	p value	Odds ratio	95% confidence interval
Nadir Hb (g/l)	<0·001	0·877	0·857–0·897
ICU Length of stay (days)	<0·001	0·959	0·942–0·977
Transfused in ICU	0·001	0·526	0·356–0·778
APACHE II Score	0·62	0·992	0·960–1·024

Table 1b ICU factors excluding nadir haemoglobin associated with significant anaemia (haemoglobin <100 g/l) at ICU discharge

	p value	Odds ratio	95% confidence interval
Gender	0·25	1·206	0·875–1·664
ICU Length of stay (days)	0·054	0·986	0·972–1·000
Transfused in ICU	0·005	1·592	1·151–2·201
APACHE II Score	0·05	1·030	1·000–1·061

Table 2 Factors associated with significant anaemia (haemoglobin <100 g/l) at discharge from hospital

	p value	Odds ratio	95% confidence interval
Gender	0·02	1·702	1·074–2·700
ICU discharge Hb >100 g/l	0·02	0·972	0·947–0·997
Length of stay between ICU and hospital discharge	0·011	0·982	0·968–0·996
Nadir Hb between ICU and Hosp discharge	<0·001	0·930	0·903–0·958

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Conflict of interests

No conflict of interest identified.

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ORIGINAL PAPER

A fluorometric erythrophagocytosis assay using differentiated monocytic THP-1 cells to assess the clinical significance of antibodies to red blood cells

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Background and Objectives The significance of antibodies to red blood cells (RBCs) is variable and cannot be predicted solely by serological testing. A flow cytometry-based erythrophagocytosis assay was established using phorbol 12-myristate 13-acetate (PMA)-treated THP-1 cells and RBCs labelled with PKH26 to assess allo- and autoantibodies to RBCs.

Materials and Methods THP-1 cells were differentiated into macrophage-like cells by treatment with PMA. RBC samples coated with alloantibodies or autoantibodies were obtained from 16 patients with autoimmune haemolytic anaemia of warm type (wAIHA) as well as from five pregnant women with warm autoantibodies. RBCs from healthy blood donors were used as controls. RBCs were labelled with the red lipophilic fluorescent dye PKH26 and incubated with PMA-treated THP-1 cells. After removal of nonadherent RBCs by washing and haemolysis of adherent RBCs, erythrophagocytosis was quantified by flow cytometry.

Results We observed significant phagocytosis of RBCs coated with clinically relevant alloantibodies (i.e. anti-D and anti-K) or autoantibodies from patients with active wAIHA, but not of those coated with alloantibodies (anti-Ch) or autoantibodies from patients and pregnant women without haemolysis.

Conclusion The flow cytometry-based erythrophagocytosis test described here is quantitative, highly reliable, and may be helpful for the assessment of the clinical significance of antibodies to RBCs.

Key words: AIHA, alloantibodies, autoantibodies, flow cytometry, macrophages, phagocytosis, THP-1 cells.

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Introduction

Antibodies to red blood cells (RBCs) may show various effects *in vivo* on circulating RBCs compared with their reactivity with RBCs in serological testing. In addition, their clinical significance often depends on the clinical condition of individual patients. Indeed, some patients may develop a severe haemolytic transfusion reaction (HTR) due to an incompatible RBC transfusion, whereas other patients may develop only mild or no reactions under identical serological findings [1, 2]. Therefore, the clinical significance of detectable antibodies cannot always be determined using standard serological testing.

During the last decades, numerous methods have been developed to measure or predict RBC survival. Currently, chromium-51 or biotin-labelled tests for survival measurement [3, 4] are most reliable; however, they cannot be used routinely. Alternative techniques, including the

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biological cross-match, antibody-dependent cellular cytotoxicity (ADCC) assay [5], chemiluminescence test and the monocyte monolayer assay (MMA) [6], are often predictive but do not invariably exclude HTRs. The MMA is the most widely used assay to evaluate the clinical significance of alloantibodies to RBCs. This test is based on the use of autologous or allogeneic monocytes and RBCs opsonized with recipient serum. It is yet not unanimously clear if the outcome of patients transfused with serologically incompatible RBCs demonstrates a sound correlation for all antibodies with results of the MMA. Since patients with alloantibodies potentially causing clinically relevant haemolysis are usually not transfused with incompatible blood, data correlating with results of the MMA with haemolysis parameters are difficult to obtain. A recent study [7] used a MMA to cross-match 61 RBC alloantibodies with RBC units. Thirty-one out of 61 patients with no or variable significant antibodies were transfused successfully with RBC units and negative MMA cross-match. Unlike macrophages, circulating monocytes are not known to exhibit erythrophagocytosis in alloantibody-mediated haemolytic anaemia [6]. Hence, it remained questionable whether the use of macrophages might more accurately reflect the *in vivo* outcome than the use of monocytes [6,8,9].

The human leukemic cell line THP-1 has several advantages over human peripheral monocytes and is commonly used for investigating the function and regulation of monocytes and macrophages. THP-1 cells express FcRI and FcRII receptors [10]. THP-1 monocytes can convert to macrophage-mimicking cells in the presence of phorbol 12-myristate 13-acetate (PMA) [11, 12]. With increasing cell adherence, FcRI and FcRII receptor expression is reduced [10]. Interestingly, phagocytosis of IgG-coated sheep RBCs increases much stronger than that of uncoated RBCs (64 % vs. 35 %) [12]. In a previous study that applied the fluorometric quantitative erythrophagocytosis assay using human THP-1 cells and PKH26-labelled RBCs, high sensitivity and good reproducibility were demonstrated [13]. We modified this assay by using PMA-treated THP-1 cells and PKH26-labelled RBCs in a shaking assay. Thus, we used adherent macrophage-like cells instead of monocytes, and gentle shaking may favour antibody-mediated interaction between macrophage-like cells and RBCs. Accordingly, a trypsinization step was needed. All other steps and analysis of the data were done as described by Healey *et al.* [13]. The test was initially used to assess the clinical relevance of several known alloantibodies. To demonstrate a correlation between the assay and antibody-dependent haemolysis, RBCs from three groups of patients were analysed as follows: patients with clinically significant AIHA, patients with AIHA in remission and pregnant women with detectable autoantibodies to RBCs.

Materials and methods

Patients

The research was approved by the local ethics committee (No EA2/058/12). Sixteen patients with AIHA of warm type were included. Routine haemolysis parameters (haemoglobin, LDH, haptoglobin, reticulocytes) were determined. In addition, five pregnant women with detectable autoantibodies without haemolysis were investigated. EDTA blood samples for the control group were obtained from healthy blood donors.

Serological testing

Serological testing, including antibody screening and the monospecific direct antiglobulin test (DAT), was performed using standard gel technology as described elsewhere [14,15]. Acid eluates were performed according to the manufacturer's recommendation (BAG Health Care GmbH, Lich, Germany).

THP-1 cells culture

THP-1 human monocytic leukaemia cells (TIB-202, American Type Culture Collection (ATCC), Manassas, VA, USA) were cultured at a density of 1–5 $\times$ 10^5 cells/ml in RPMI-1640 medium (GIBCO, Grand Island, NY, USA) containing 10% fetal bovine serum (FCS; GIBCO, Invitrogen, Carlsbad, California, USA), and 1% penicillin 100 U/ml and 1% streptomycin 100 μg/ml (GIBCO Life Technologies, Carlsbad, California, USA). Cells were maintained at 37°C in a humidified atmosphere with 5% CO_2. Cell suspensions were pelleted at 900 *g* for 2 min, resuspended and transferred at a density of 1 $\times$ 10^5 cells/ml in refreshed medium twice weekly.

Differentiation of THP-1 cells to macrophage-mimicking cells

THP-1 cells were resuspended at a density of 1 $\times$ 10^6 cells/ml in fresh growth medium and PMA (Calbiochem, Darmstadt, Germany, hazardous substance, please acknowledge the safety data sheet) to a final concentration of 10^{-8} M [16]. Five hundred microlitres of the suspension was transferred into each well of 6-well culture plates (Thermo Fisher Scientific, Waltham, MA, USA) and incubated at 37°C in a humidified atmosphere with 5% CO_2. Differentiation medium with 10^{-8} M PMA was refreshed after 24 h. Following 48 h, the medium was

replaced with fresh medium without PMA and incubated for a further 3 h. The adherent, macrophage-like THP-1 cells were almost confluent before use in the phagocytosis assay.

Verification of THP-1 cell differentiation by flow cytometry

After a 10-min trypsinization period (GibcoTM TrypLE Express), PMA-treated THP-1 cells were resuspended in cell culture medium. PMA-treated and non-treated THP-1 cells ($0{\cdot}5 \times 10^6$) were diluted in 200 µl PBS and stained with 10 µl of the fluorescent labelled anti-human CD11b / MAC-1 (FITC) or anti-human HLA-DR (APC) antibodies (both from BD Biosciences Pharmingen TM). Cells were subsequently analysed by flow cytometry [16] with MACSQuant® Flow Cytometer (Miltenyi Biotech, Bergisch Gladbac, Germany).

RBC preparation and labelling by PKH26 and the phagocytosis assay

RBCs from patients with AIHA, pregnant women with detectable autoantibodies and from healthy donors were washed three times with saline (0·9% NaCl) and pelleted at 900 *g* for 2 min before PKH26 staining. In contrast, patients' plasma containing alloantibodies (anti-D, anti-K, anti-Lu(b), anti-Yt(a), anti-Ch (200µl)) were incubated with 50 µl pelleted donor RBCs (expressing the correspondent antigen) for 2 h at 37°C and then washed three times with saline (0·9% NaCl). The alloantibody-coated RBCs were further tested in an identical way as patients' and healthy donors' RBC, respectively.

All RBCs were membrane-stained using PKH26 according to the manufacturer's recommendations (Sigma-Aldrich, St. Louis, MO, USA). Briefly, 10 µl of RBCs from pellet was diluted in 125 µl diluent C (supplied with the PKH26 kit), added to 0·5 µl PKH26 in 125 µl diluent C, followed by immediate mixing and incubation at room temperature for 4 min. The reaction was terminated by the addition of 100 µl of human AB plasma. After 1 min, 2 ml of cell culture medium was added. Cells were pelleted at 1700 *g* for 10 min, resuspended in 100 µl of cell culture medium and added to PMA-differentiated THP-1 cells in a 6-well plate. Subsequently, plates were incubated at 37°C for 45 min under soft continuous agitation.

Flow cytometry

After incubation at 37°C for 45 min, non-phagocytic RBCs were removed by washing three times with 2 ml of ice-cold isotonic saline (0·9% NaCl). Residual RBCs were haemolysed with ice-cold hypotonic saline (0·2% NaCl) for 2 min, followed by the addition of 2 ml of ice-cold hypertonic saline (1·6% NaCl) for 2 min to restore isotonicity [13,17]. Subsequently, the supernatant was removed and the adherent macrophage cells were detached by trypsin. Before and after trypsinization, THP cells were checked microscopically for remaining RBCs or detritus THP cells were diluted with 500 µl PBS (1×10^6 cells/ml) and kept on ice until analysis by flow cytometry (MACSQuant® Flow Cytometer, Miltenyi Biotech). At least 10 000 events were collected for each sample. Data were analysed using the FlowJo® software (FlowJo LLC, Ashland, OR, USA). The mean fluorescence and percentage of cells that were strongly positively stained for PKH26-red-labelled RBCs compared with control group were calculated. Controls using RBCs from two or three healthy blood donors with no selection of age, sex or blood group were included in each experiment.

Analysis and statistics

Clinical relevance of allo- or autoantibodies was determined by comparing patients' results (MFI or % phagocytosis) with arithmetic means ± standard deviation (SD) of the same day control group. As a cut-off, antibodies generating results (MFI or % phagocytosis) higher than arithmetic means + 3 SD of controls were considered clinically relevant.

For statistical analysis of patient groups and control groups, data were calculated as arithmetic means ± standard error of the mean (SEM). Statistical analysis was performed using Microsoft Excel and SSPS. Significance between two groups was determined using Mann–Whitney U-test.

Results

Serological and clinical data

In total, RBCs from 16 patients with long-term AIHA were studied. All patients had a positive eluate (Table 1). Fourteen of 16 patients showed IgG-positive DAT and 11 patients C3d-positive DAT. One patient predominantly had an IgM-wAIHA (Pat. W7). Another patient (Pat. W12) had both warm and cold autoantibodies (mixed-type IgM-AIHA). Based on haemoglobin, reticulocyte, lactate dehydrogenase and haptoglobin levels, as well as the clinical picture, 12 patients showed clear signs of haemolysis. All five pregnant women had an IgG-positive DAT and detectable autoantibodies in the eluate (Table 1). There were no signs of haemolysis in the pregnant cohort [18].

Table 1 Most relevant data of patients with AIHA

Patient No.	Age (years)	DAT					Eluate	Hb (g/dl)	LDH (U/l)	Hp (mg/dl)	RETR 10E3/µl	Medication
		Sex	IgG	C3d	IgM	IgA						
W1	8	M	4+	–	(+)	1+	P	10·9	281	nd	nd	Rituximab / Prednisolone
W2	64	F	4+	2+	–	–	P	13·4	583	<5·8	nd	Prednisolone 1 mg/d
W3	49	F	4+	4+	–	–	P	10	443	<5·8	140	Cyclosporin A 300 mg /d, Prednisolone 10 mg/d, Darbepoetin alfa
W4	82	F	3+	3+	–	–	P	11·4	348	122	50	Prednisolone 5 mg/d, Darbepoetin alfa
W5	48	F	4+	(+)	–	2+	P	8·4	1326	<5·8	454	Prednisolone 30 mg/d, *Cyclophosphamide* 150 mg/d
W6	60	F	4+	4+	–	–	P	13·0	483	<5·8	nd	Prednisolone 5 mg/d
W7	36	M	(+)	4+	4+	(+)	P	12·4	281	<5·8	169	Prednisolone 75 mg/d
W8	43	M	4+	3+	–	–	P	10·4	460	<5·8	112	Prednisolone 10 mg/d, Azathioprine 200 mg/d
W9	68	F	4+	3+	2+	–	P	8·6	1214	nd	419	Mycophenolic acid; 2000 mg/d, Prednisolone 10 mg
W10	50	M	4+	–	–	–	P	12·4	397	<5·8	nd	Prednisolone 7·5 mg/d, Cyclophosphamide 150 mg/d
W11	27	M	3+	(+)	–	–	P	12·9	252	<5·8	nd	–
W12	51	F	–	4+	–	–	P	12·5	314	nd	nd	–
W13	55	F	4+	1+	–	(+)	P	11·5	227	116	nd	Prednisolone 10 mg/d, Cyclophosphamide 150 mg/d
W14	73	F	1+	–	–	–	P	11·3	234	149	101	Cyclophosphamide 25 mg/d
W15	27	F	4+	–	–	–	P	14·8	235	95·6	79	Prednisolone 10 mg/d, Azathioprine 200 mg/d
W16	56	M	–	–	–	–	P	13·9	242	110	81	Prednisolone 2·5 mg/d
G1	36	F	4+	–	(+)	–	P					
G2	36	F	4+	2+	–	–	P					
G3	29	F	3+	–	–	–	P					
G4	32	F	1+	–	–	–	P					
G5	40	F	1+	–	–	–	P					

DAT, direct antiglobulin test; G, pregnancy with autoantibodies of warm type; Hb, haemoglobin; Hp, haptoglobin; LDH, lactate dehydrogenase; nd, no data; P, positive; RETR, reticulocytes; W, patient with AIHA of warm type.

PMA-treated THP-1 cells transform into macrophage-like cells

Phorbol 12-myristate 13-acetate-differentiated THP cells were adherent, demonstrating increasing CD11b expression, as well as a downregulation of HLA-DR (Fig. 1a). The strongest changes in CD11b/HLA-DR expression were observed from 48 to 72 h. Therefore, THP cells were used after 48 h of differentiation in further experiments. Scatter plots of THP-1 cells and differentiated THP-1 cells (macrophage-like cells; blank control) were similar and showed only background fluorescence in channel B2 B585 (Fig. 1b) and a very low number of cells in region P3 (<0·1 %). After the phagocytosis assay, trypsinated THP-1 cells were checked for remaining RBCs by the use of Hayem's solution and the improved Neubauer haemocytometer showing an almost complete haemolysis of non-phagocyted RBCs (data not shown). Figure 1c shows an overlay of the histograms of PMA-treated THP-1 cells and PKH26-stained RBCs from healthy donors. After incubation with PKH26-stained RBCs, PMA-treated THP-1 cells showed increased fluorescence from ingested PKH26-stained RBCs (control group; Fig. 1d). This reflects already published results showing that non-opsonized RBCs were also digested in larger amounts [13,17].

RBCs coated with alloantibodies

RBCs loaded with significant (anti-D, anti-K) or potentially significant alloantibodies for haemolysis (anti-Lu(b), anti-Yt(a)) were observed to further enhance phagocytosis by PMA-treated THP-1 cells (Fig. 2a–j, m). However, two of three anti-Yt(a) did not enhance phagocytosis significantly by more than mean ± 3 SD of the control group, which corresponds to the clinical experience with this antibody. In contrast, pre-incubation of RBCs with the non-haemolytic alloantibody anti-Ch did not affect phagocytosis (Fig. 2k,l,m).

Enhanced phagocytosis of RBCs from patients with active wAIHA

A typical result obtained from patients with significant wAIHA is depicted in Fig. 3a–c using the example of patient no. W9 (Table 2). Erythrophagocytosis was significantly increased (> average mean of control + 3 SD) in patients with active AIHA compared with healthy donors (control group). This significant increase in the mean fluorescence of all THP-1 cells and percentage of strongly positive stained cells in region P3 indicates a strong avidity of macrophages to patient RBCs. In comparison, erythrophagocytosis of RBCs from a pregnant woman (G4, Table 2) was not increased (Fig. 3d–f). Patients with clinically relevant AIHA of warm type showed a highly significant enhanced erythrophagocytosis as shown by mean fluorescence (Fig. 3g). In contrast, a similar erythrophagocytosis was observed in patients with AIHA of warm type in remission or patients with autoantibodies due to pregnancy and healthy blood donors, respectively (Fig. 3g). Further analysis did not demonstrate a correlation with the amount of bound IgG or C3d (Tables 1 and 2).

Positivity of haemolysis parameter (LDH/haptoglobin) was found to correlate strongly positive with erythrophagocytosis. Using our assay, RBCs of all patients with significant haemolysis showed increased phagocytosis by THP-1 cells. Increased phagocytosis was not observed of RBCs from three AIHA patients without haemolysis (W14-W16) and all pregnant women with warm autoantibodies (G1–G5; Table 2).

Discussion

The MMA has been shown to correlate well with the significance of alloantibodies [6]. However, numerous technical variables may affect assay results. These include the selection of monocytes, RBCs, culture conditions and analytical method [6]. In addition, compared to abounding MMA results about the significance of alloantibodies there is little information available on the use of the MMA to determine the significance of autoantibodies to RBCs. In one study, it has been shown that the amount of IgG1 autoantibodies on ^{51}Cr-labelled RBCs corresponds well with the phagocytosis by monocytes and the haemolysis *in vivo* [19]. In the work of Gallagher *et al.* [20], all of the 16 patients with AIHA and clinical evidence of haemolysis showed an enhanced phagocytic index (PRBC) in the MMA, whereas six non-haemolysing patients showed a normal PRBC.

In the present study, we focused on further optimization of the MMA and on assessing the relevance of autoantibodies rather than alloantibodies to RBCs. The assay described here is both quantitative and highly reliable. Furthermore, it has some advantages as compared to previously described assays. First, the macrophage-like cells are derived from the immortalized monoclonal cell line THP-1 after differentiation with PMA. The cells are easily accessible, and their phagocytic activity is highly reproducible compared with monocytes from healthy donors. As in most other MMA, only phagocytosed RBCs are measured since free or adherent erythrocytes are removed quantitatively by washing and RBC lysis. Second, measurement by flow cytometry is easier and allows for a more precise quantification through higher numbers of counted cells compared with microscopic analysis. One notable disadvantage lies in the necessity of staining the

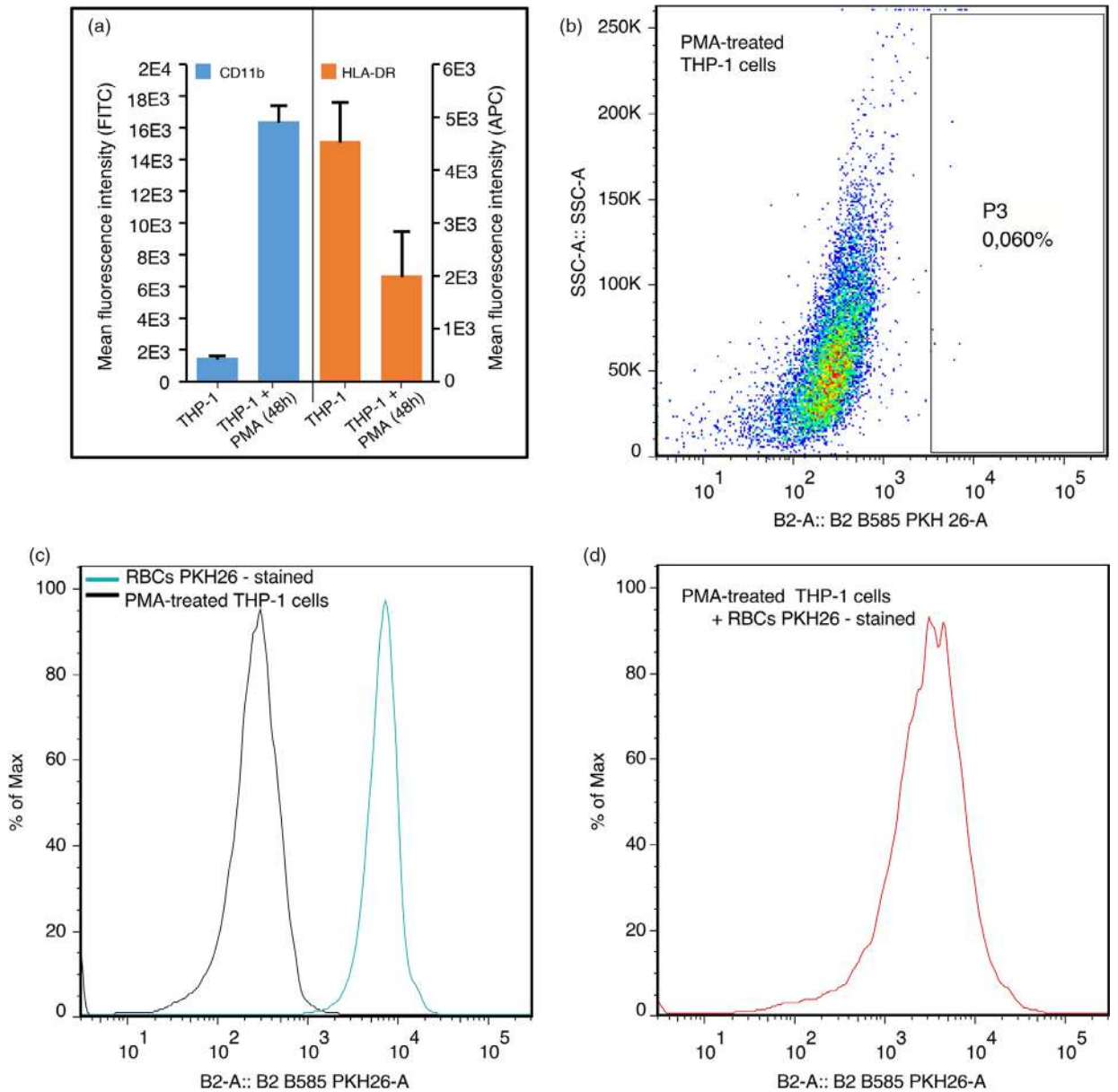

Fig. 1 Characterization of PMA-treated THP-1 cells. (a) Effect of PMA differentiation on CD11b (left) and HLA-DR (right) expression in THP-1 cells at different times (0 and 48 h). (b) Scatter plot of PMA-treated THP-1 cells (blank control). Region P3 has been selected as contains ≤ 0·1% of events. (c) Histogram overlay of PMA-treated THP-1 cells (black) and PKH26-stained RBCs (blue). (d) Histogram of PMA-treated THP-1 cells after phagocytosis of PKH26 stained RBCs from healthy blood donors (control group). [Colour figure can be viewed at wileyonlinelibrary.com]

RBCs; however, it is required for fluorocytometric analysis and is easy to perform. Another drawback may be the permanent culturing of the THP-1 cells and the time-consuming differentiation of 48 h.

Initially, we determined the reliability by testing well-characterized alloantibodies known to induce phagocytosis. Two such antibodies, anti-D and anti-K, led to significant phagocytosis, whereas there was no enhanced phagocytosis observed for the insignificant antibody anti-Ch. Two other alloantibodies, anti-Yt(a) and anti-Lu(b), were able to induce enhanced phagocytosis, albeit two of three anti-Yt(a) not significantly. The results indicate a potential clinical significance, which is, however, variable [21–23]. Our findings also correlate with the results of a retrospective study that analysed MMA data from 46 patients over 20 years to predict the clinical significance of alloantibodies [24]. Therefore, the assay could be used to select compatible RBCs in alloimmunized patients

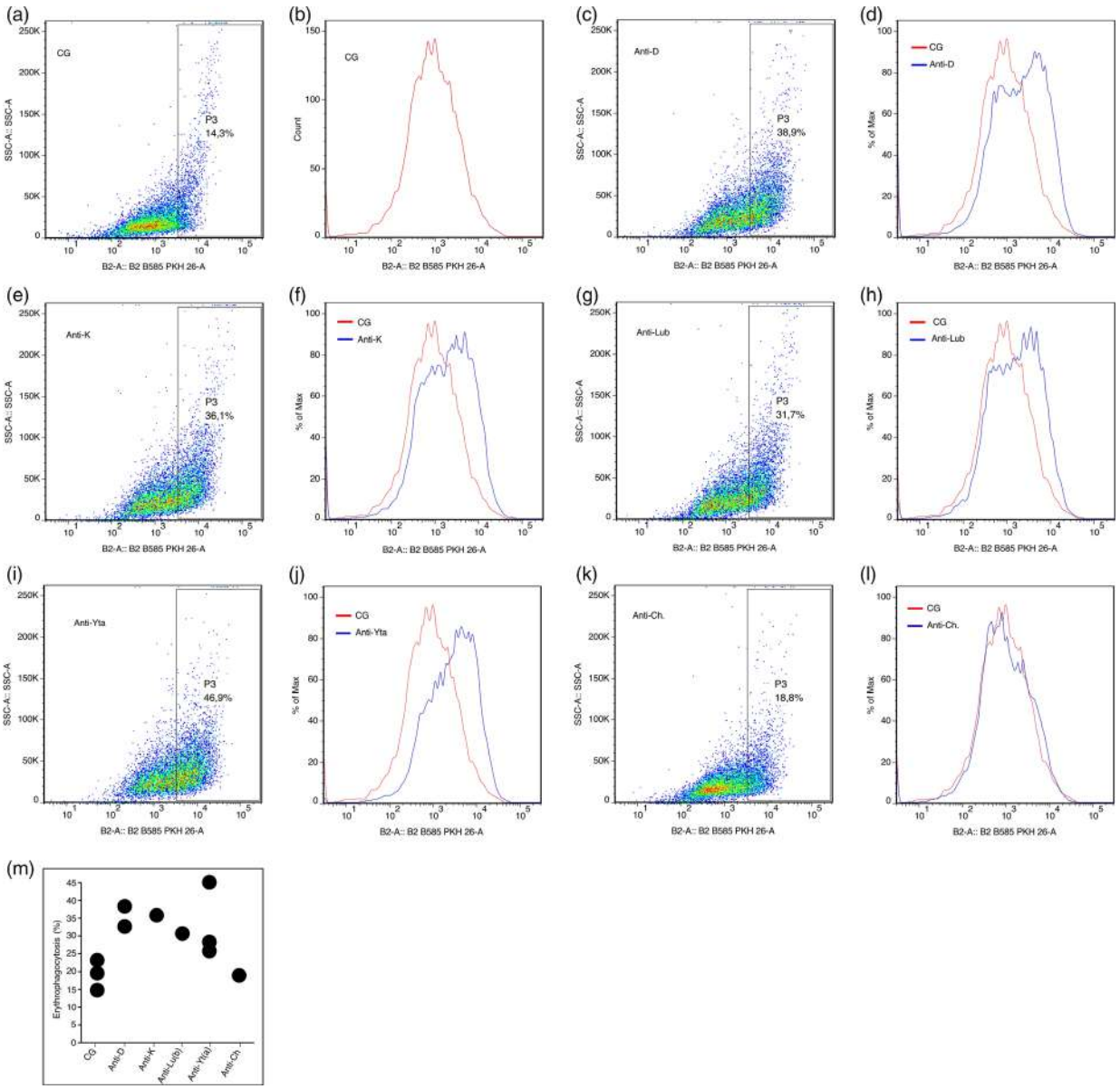

Fig. 2 Erythrophagocytosis of RBCs coated with alloantibodies. (a-l) Scatter plot (a) and histogram (b) of PMA-treated THP-1 cells after phagocytosis of RBCs from a healthy blood donor (control group). Scatter plots (c, e, g, i, k) and overlay of histograms with a control group (d, f, h, j, l) of PMA-treated THP-1 cells after phagocytosis of alloantibody-coated RBCs [anti-D, anti-K, anti-Lu(b), anti-Yt(a) and anti-Ch (blue).]. (m) Results of THP-1 phagocytosis of RBCs pre-incubated with alloantibodies in comparison with control group RBCs. [Colour figure can be viewed at wileyonlinelibrary.com]

requiring blood transfusion in cases where all cross-matched units are serologically incompatible. Noumsi et al. used the Monocyte index (MI) of <5 % in MMA for cross-matching sera with alloantibodies to select RBC units for patients [7]. The evaluation of Noumsi's test is difficult due to the fact that also clinically relevant antibodies (anti-s, anti-e, anti-hrS, anti-Fy3, anti-Jkb) showed no significant phagocytosis in MMA. Nevertheless, patient with these alloantibodies has not been transfused. Therefore, the reliability of this assay for assessing the clinical relevance of these antibodies remains unclear.

In some cases, the cause of anaemia cannot be completely attributed to the presence of autoantibodies, even when affected patients were previously or currently afflicted with AIHA. Though rarely observed, some patients may have diseases associated with mild haemolysis such as hereditary or acquired non-immune haemolytic anaemia [25]. For example, eryptosis, the programmed

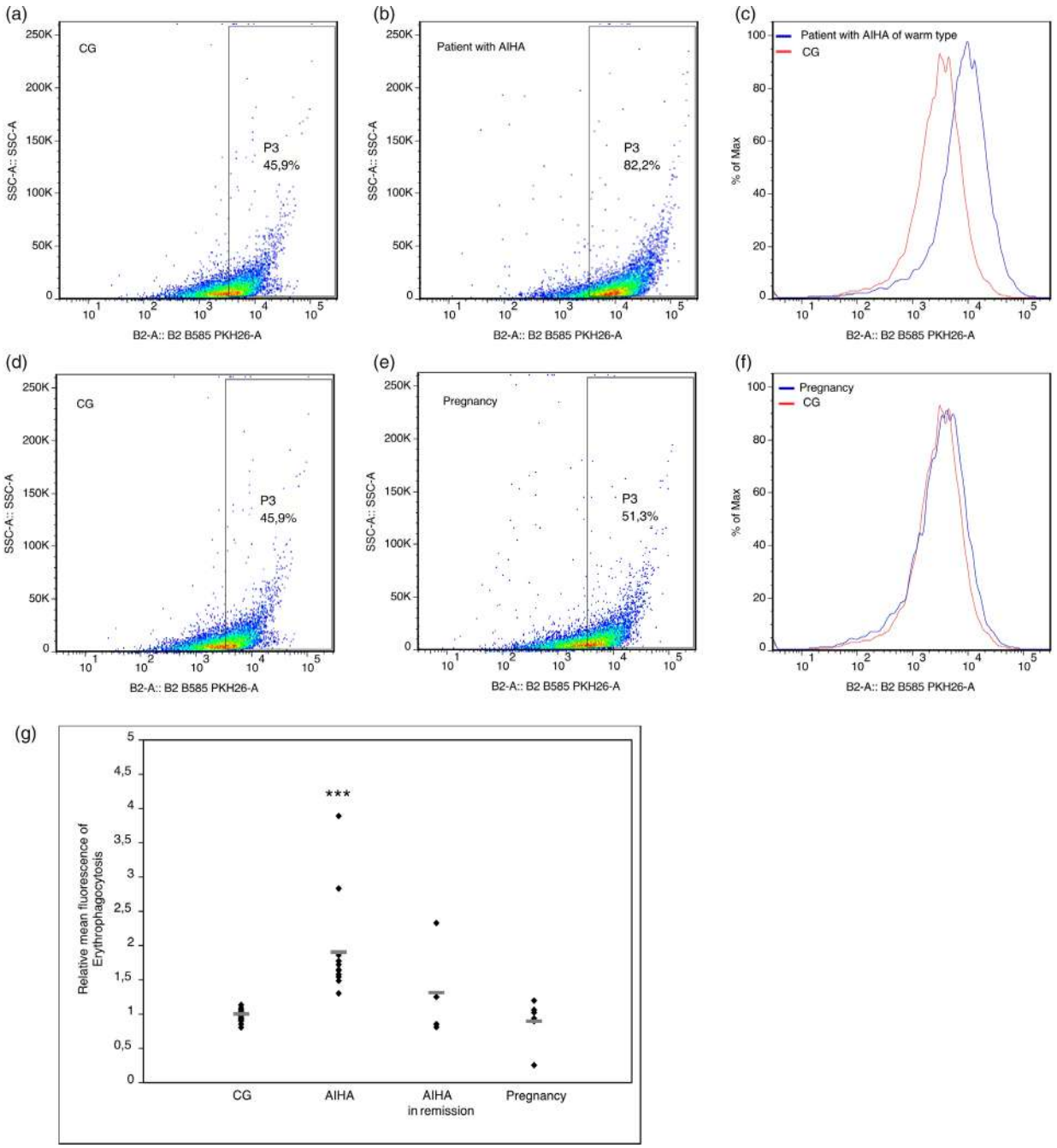

Fig. 3 Phagocytosis of RBCs from a patient with AIHA and a pregnant woman with warm autoantibodies. Scatter plots and overlay histogram of PMA-treated THP-1 cells after phagocytosis of RBCs from a healthy blood donor (a, c: red line) and of RBCs from a patient with clinically relevant AIHA (b, c: blue line). Scatter plots and overlay histogram of PMA-treated THP-1 cells after phagocytosis of RBCs from a healthy blood donor (d, f: red line) and of RBCs from a pregnant woman with warm autoantibodies (e, f: blue line). (g) Comparison of THP-1 phagocytosis of RBCs from healthy blood donors (CG, first column), patients with significant wAIHA (second column), patients with wAIHA in complete remission (third column) and pregnant patients with autoantibodies (fourth column). Each point indicates one THP-1 sample after incubation with the respective RBCs. The grey lines represent the mean of each group. The mean fluorescence for the CG was normalized at 1. ***$P < 0{\cdot}001$ significant difference from control group. [Colour figure can be viewed at wileyonlinelibrary.com]

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Table 2 In vitro erythrophagocytosis by PMA-treated THP-1 cells

Patient no	Haemolysis[a] Yes/No	MFI		% Phagocytosis	
		Patient	CG (mean ± SD)	Patient	CG (mean ± SD)
W1	Yes	**3271**	2119 ± 195	**37**	19·5 ± 3·5
W2	Yes	**3943**	2119 ± 195	**42**	19·5 ± 3·5
W3	Yes	**2745**	1738 ± 51	**28**	14·3 ± 0·6
W4	Yes	**3540**	2148 ± 188	**33**	16·3 ± 1·5
W5	Yes	**5908**	3431 ± 169	**60**	33·7 ± 5·9
W6	Yes	**4301**	2723 ± 44	**34**	20·5 ± 1·2
W7	Yes	**6774**	4140 ± 585	**63**	42·0 ± 4·2
W8	Yes	**5096**	3431 ± 169	**56**	33·7 ± 5·9
W9	Yes	**12032**	4247 ± 596	**82**	45·7 ± 7·5
W10	Yes	**3513**	2700 ± 186	**41**	27·3 ± 3·2
W11	Yes	**1912**	1076 ± 192	**41**	20·3 ± 6·7
W12	Yes	**4189**	1076 ± 192	**70**	20·3 ± 6·7
W13	No	**2507**	1076 ± 192	**55**	20·3 ± 6·7
W14	No	5176	4140 ± 585	54	42·0 ± 4·2
W15	No	1822	2248 ± 141	12	19·7 ± 2·5
W16	No	2324	2723 ± 448	15	20·5 ± 2·1
G1	No	3218	3431 ± 169	36	33·7 ± 5·9
G2	No	3644	3431 ± 169	40	33·7 ± 5·9
G3	No	5084	4247 ± 596	51	45·7 ± 7·5
G4	No	4467	4981 ± 557	47	58·5 ± 6·4
G5	No	5091	4981 ± 557	55	58·5 ± 6·4

Bold MFI or % **Phagocytosis** indication of a significant result, higher than mean ± 3 SD of the control group (CG).

[a]Haemolysis from laboratory findings and as judged by the treating physician; MFI, mean fluorescence intensity of the THP-1 cells after phagocytosis; % phagocytosis, proportion of THP-1 cells that contained fluorescence signal in p3 area; CG, control group; SD, standard deviation.

death of RBCs, has been described to occur in a numerous clinical conditions including sickle cell anaemia, thalassemia, glucose-6-phosphate dehydrogenase deficiency, hereditary spherocytosis, paroxysmal haemoglobinuria, myelodysplastic syndrome, phosphate depletion, iron deficiency, sepsis, haemolytic–uremic syndrome, renal insufficiency, diabetes mellitus, malaria, mycoplasma infection and Wilson disease [26, 27]. Importantly, eryptosis has been described recently in AIHA related to IgA or IgM autoantibodies [28]. The clinical significance of autoantibodies in AIHA is reflected usually by the rate of ongoing haemolysis. Nevertheless, confusion may arise in a number of cases due to co-morbidities associated with anaemia and/or non-immune haemolytic anaemia [25]. However, the results obtained by the DAT, as the main marker for the presence of AIHA, are of little value without sufficient clinical information [25]. The phenomenon related to the long-term persistence of detectable autoantibodies in patients whose AIHA had entered into complete remission is intriguing. It is unclear whether the causative autoantibodies and/or macrophages in these patients have acquired a new character following treatment. In fact, the finding in patient no. 13 reflects that these patients' autoantibodies appear to be clinically relevant as has been demonstrated by our test. This may indicate that the macrophages of affected patients were incapable to phagocyte the opsonized RBCs, probably, due to treatment with immunosuppressive drugs. This observation has been shown before [19, 20]. Similarly, it remains unknown why pregnancy-induced autoantibodies to RBCs do not appear to cause significant haemolysis [18, 29].

The present study analysed heterogeneous autoantibodies to RBCs. The results obtained largely reflect conditions *in vivo*. RBCs from patients with positive DAT and recognizable haemolysis were preferentially digested from macrophage-like cells in contrast to RBCs from patients without signs of haemolysis and pregnant women.

Therefore, a positive result in the described assay strongly indicates haemolysis *in vivo*. This finding may be helpful in the management of patients who had been successfully treated or are still under treatment for AIHA but cannot compensate for their anaemia. While *in vitro* phagocytosis of patient RBCs supports the clinical significance of the detectable autoantibodies, a negative result may indicate that the anaemia is related to another disease such as an infection, tumour or renal or cardiac failure. In addition, autoantibodies are frequently associated

with HTRs due to alloantibodies [30, 31]. It remains unknown whether these autoantibodies are also involved in RBC destruction and our assay may be useful to clarify that question.

Finally, the question of whether pregnancy-induced autoantibodies [18, 29] and persisting autoantibodies in AIHA following complete remission do not lead to phagocytosis has not yet been completely resolved. The results obtained in this study support our hypothesis that these autoantibodies are incapable of causing haemolysis. Based on our findings, this phenomenon cannot be either explained by IgG subclasses, the number of antibodies attached to the RBCs or autologous macrophages. A possible explanation might be related to the IgG Fc region structure, which is determined by a specific glycosylation to modify signal pathways targeted by the macrophages [32–34]. Further studies are required to clarify this phenomenon.

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Conflict of interests

The authors declare no conflict of interests.

Author contributions

AS, TB and AB designed the experiments. BM and AS treated the patients. AB and TB performed the experiments. TB and AB analysed the data. AS wrote the paper with input from all other authors. All authors reviewed the manuscript and approved the final version.

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INTERNATIONAL FORUM

International Forum on the Collection and Use of COVID-19 Convalescent Plasma: Protocols, Challenges and Lessons Learned: Summary

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Introduction

Coronavirus disease-2019 (COVID-19), caused by severe acute respiratory coronavirus 2(SARS-CoV-2), has rapidly spread since its first declaration by the World Health Organization (WHO) as a global pandemic in March 2020 [1]. Until vaccination has started to be rolled out in an increasing number of countries, different modalities of treatment were deployed in an attempt to combat this deadly virus but with limited efficacy so far [2]. Collection and use of convalescent plasma for coronavirus disease-2019 (COVID-19) (CCP) treatment for passive immunotherapy had gained interest worldwide and still is considered as a potentially effective therapeutic option when containing high-titre antibodies and administered early in the course of the infection [3,4] including against SARS-CoV-2 variants [5]. Transfusion of CCP itself has been conducted either within a framework of clinical trials or on a compassionate basis in patients with active SARS-CoV-2 infection. CCP may also be fractionated into hyperimmune immunoglobulins for treatment of patients or alternatively for prophylaxis in high-risk individuals such as healthcare providers or individuals who have the underlying risk factors, such as an exposure to persons with confirmed COVID-19 infection.

The medical rationale for transfusing CCP is based on historical perspectives that demonstrated the clinical benefit of transfusing convalescent plasma from recovered individuals in respiratory infections caused by other coronaviruses [6] as well as diseases such as Argentine haemorrhagic fever [7]. The advantages of CCP include its almost immediate availability (once safe recovered donors can be identified) as a local resource in all affected countries worldwide, while specific treatments and vaccines are under development and evaluation. The relative ease of access to CCP from recovered donors, and potential for deployment in different settings, including low- and middle-income countries, made it attractive especially in early stages of the pandemic. Soon after the start of the pandemic, recommendations and 'points to consider' have therefore been published by the International Society of Blood Transfusion (ISBT) to establish and share at a global level to ensure quality and safety, as well as respect of ethical principles, in the collection and use of CCP [8–11]. An emphasis was given to the fact that CCP transfusion was to be considered as an experimental therapy that, whenever possible, should be evaluated within the scope of controlled clinical studies to maximize the knowledge gained, with optimal monitoring of (1) convalescent donors, (2) CCP characteristics and (3) patients outcomes [9].

In addition, there was recognition of variations and gaps existing at a global level in the practices applied to the collection, testing and preparation of CCP [8,12]. ISBT initiated a multidisciplinary working group with representation from all six continents with the aim of reviewing existing practices on CCP preparation and use. It was felt that such information would be invaluable not only to document strategies implemented in CCP collection, but also as a tool for better preparedness against future pandemics.

This Vox Sanguinis International Forum aimed to gather information on the practice and challenges of

collection of CCP on an international level and to draw lessons learned from establishing a CCP collection programme for blood establishments and hospital-based blood services. This international forum was only intended for institutions that collect CCP. Participants were invited to participate in this international forum on 9 December 2020 and were asked to describe the CCP collection programme in their institutions or countries. Responses were collected up to 17 February 2021. The current document is a summary of the findings that have been collected and analysed for practices in place during the specified timeframe.

Participants

Thirty-eight participants from 34 countries were invited to participate in the international forum. We aimed to cover all WHO regions and include both large national blood suppliers and smaller blood centres. We received 32 responses reflecting practice in 35 centres in 25 countries from around the world (Table 1).

Responses

Describe your institution COVID-19 convalescent plasma (CCP) collection programme

Q1: Demographics

The majority of the institutions had apheresis services, including the hospital-based centres. All hospital-based institutions were in hospitals that treated adults and children and had medical and surgical services. These hospitals ranged in size from 100 beds up to ~2000 beds.

Q2: What type of CCP donation does your institution perform? How many times is a donor allowed to donate CCP by apheresis, and over what time period? Is the frequency different from routine plasma donation by apheresis? If whole blood (WB) is collected, are red blood cells and/or platelets derived from the WB donation used for standard transfusion?

All respondents indicated that CCP is collected in their institution using plasmapheresis (Table 2). The frequency of plasmapheresis was variable between institutions. While 20 respondents indicated that the frequency did not differ from that of routine plasmapheresis, five institutions had CCP collection made at a higher frequency than what is permitted routinely. Two respondents indicated that plasmapheresis was an infrequent procedure in their institutions, only used for specific indications (e.g. collection of plasma for IgA-deficient patients) or was only established for CCP collection.

The majority of the institutions (n = 16) allowed plasmapheresis every 2 weeks, while six allowed every week with variable maximum allowable donations per donor. The highest donation frequency was every 48 h reported by three centres. In Héma-Québec (Canada), CCP collection was initially performed at a frequency identical to that of standard plasmapheresis procedures (every 6 days, up to 12 weeks after first donation) for early CCP donations, but decreased to a maximum of 6 weeks after resolution of symptoms, considering the reported decline in the SARS-CoV-2 antibody titre with time [13–15]. In Singapore, the CCP plasmapheresis was initially conducted at the standard plasmapheresis procedure frequency and was subsequently increased to every 2 weeks to allow more frequent donation.

Eight institutions collected WB and plasmapheresis if the donor/units were found to have anti-SARS-CoV-2 antibodies, and donors meet all other donor eligibility criteria. Considering that these donors fulfilled blood donation qualifications and testing for transfusion-transmitted infections, red blood cells and/or platelet components derived from such donated WB were labelled for clinical use. This decision necessitated approval by the institutional ethics committee in Argentina. Two blood centres in the USA (American Red Cross and OneBlood) applied a minimum deferral period of 14 days from the time of resolution of COVID-19 infection for CCP and WB donation. Plasma components from qualified blood donors were labelled as CCP if they met the necessary levels of anti-SARS-CoV-2 antibodies. In Italy, WB collection from recovered COVID-19 individuals was introduced at later stages of the pandemic, and donors were allowed to donate blood after 10 days of recovery. In Norway, the decision to use derived red blood cells (RBCs) and platelets for standard transfusion was based on the European Center for Disease Prevention and Control and European Blood Alliance guidelines recommending accepting WB donors after 28 days following recovery. The same rule was applied in Australia and Israel. In Singapore, only male blood donors who had made standard WB donation with a history of COVID-19 within 6 months before the donation had their samples tested for SARS-CoV-2 neutralizing antibodies. Based on the antibody level present, a decision was made to label the WB-derived plasma as CCP. Derived RBCs and platelet components were labelled for clinical use. Some respondents indicated that the decision of labelling units as for standard components was made based on a lack of evidence of SARS-CoV-2 transfusion transmission (Norway, Australia).

Q3: Is collected CCP intended for transfusion, prophylaxis, or fractionation purposes? If collected for transfusion, kindly indicate if collected for compassionate-use, trial-use or both?

All respondents indicated that CCP is collected for transfusion purposes, and one also used it for preparation

Table 1 Demographics of participating institutions

Country, Institution	Type of institution	Hospital Beds	RBCs transfused/ year	Approximate number of collections/year
Americas				
Argentina	Hospital-based BTS/BB	421	587	WB: 600
Brazil	Hospital-based BS	510	6000	WB: 5500; Apheresis: 1750
Canada, *Héma-Québec*	National BE	-	-	WB: 220 000; Plateletpheresis: 40 000
Canada, *CBS*	National BS	-	-	WB: 763 319; Plateletpheresis: 11 339
United States, *Vitalant*	Regional blood collector/TS	-	-	RBCs: 87 000; PLT: 25 000
United States, *ARC*	National BE	-	-	WB: 4·4 million; Apheresis: 1·4 million
United States, *OneBlood*	Regional BS/BC	-	-	WB: 790 700; Plateletpheresis:48 507
Africa				
South Africa, *SANBS*	Regional BS/BC	-	-	WB: 900 000; Plateletpheresis: 18 138
South Africa, *UCT*	Regional BS/BC	-	-	WB: 147 684; PLT: 9265
Eastern Mediterranean Region				
Egypt	Hospital-based BS	100	25 000	Data not provided
Israel	National BE	-	-	WB: 265 000, Plateletpheresis:550
Oman	Hospital-based BS	450	18 500	WB: 12 500, Plateletpheresis: on demand
Saudi Arabia	Hospital-based BS	780	12 000	WB: 12 500; Plateletpheresis: 100
Europe				
Belgium	National BE	-	-	RBCs: 160 000; PLT: 11 000
Finland	National BE	-	-	WB: 200 000; Plateletpheresis: 2500
France	National BE	-	-	WB: 2·5 million; Apheresis: 440 000
Germany	Regional BS/BC	-	-	WB: 5849; Plateletpheresis: 379
Italy	National BC	-	-	WB and apheresis: 2 996 264
Norway	Hospital-based BS/BC	-	-	WB: ~175 000; Plateletpheresis: 5000
The Netherlands	National BE	-	-	WB: 413 653; Apheresis 313 811
Turkey, *BUU*	Hospital-based BS	900	22 000	WB: 20 000; Plateletpheresis: 1700
Turkey, *TRC*	National BS/BC	-	-	WB: 2 766 581; Apheresis: 42 656
Turkey, *AHGH*	Hospital-based BTS/BB	2129/16 hospitals	44 465	WB: 1080; Apheresis: 5800
United Kingdom	National BE	-	-	RBCs: 1·4 million; PLT: 255 000
South-East Asia				
India, *AIIMS*	Hospital-based BTS/BB	2000	75 000	WB: 80 000; Apheresis: 2000
India, *PGIMER*	Hospital-based BTS/BB	1740	135 685	WB: 57 842
Indonesia	National BS/BC	-	-	WB: 3 523 982
Western Pacific				
Australia	National BS/BC	-	-	WB: 690 115; Plateletpheresis 27 024
China, *BRCBC, WHBC, SXBC*	Regional BS/BC	-	-	*BRCBC:* WB: 450 000*;* Plateletpheresis: 68 000 *WHBC:* WB: 350 000*;* Plateletpheresis: 70 000 *SXBC:* WB:32 000*;* Plateletpheresis: 40 000
Hong Kong, China	Regional BS/BC	-	-	WB: ~215 000; Apheresis: ~10 000
Singapore, *HAS, TTSH*	National BE (*HAS)* Hospital-based BTS/BB (*TTSH)*	>1700	15 000	WB: 117 000; Apheresis: 8000
South Korea	Hospital-based BTS/BB	2437	50 200	NA

AHGH, Acıbadem Health Group Hospitals; AIIMS, All India Institute of Medical Sciences; ARC, American Red Cross; BB, blood bank; BC, blood centre; BE, blood establishment; BRCBC, Beijing Red Cross Blood Center; BS, blood service; BTS, blood transfusion service; BUU, Bursa Uludağ University; CBS, Canadian Blood Services; HAS, Health Sciences Authority; PGIMER, Post Graduate Institute of Medical Education and Research; PLT, platelets; RBC, red blood cell; SANBS, South African National Blood Service; SXBC, Shaanxi Blood Center; TRC, Turkish Red Crescent; TS, transfusion service; TTSH, Tan Tock Seng Hospital; UCT, University of Cape Town; WB, whole blood; WHBC, Wuhan Blood Center.

Table 2 Convalescent plasma donation programmes

Country, Institution	Intended use of CCP	Method of CCP collection	Frequency of CCP Plasmapheresis	Frequency different from local standard plasmapheresis?	Components used from whole blood?
Americas					
Argentina	Clinical trials; Compassionate use	Plasmapheresis Whole blood	Every 2 weeks, maximum 1 l/week, 15 l/year; Maximum 600 ml per session	No	Yes
Brazil	Clinical trials; Compassionate use	Plasmapheresis	Maximum 4 over 2 months Shorter inter-donation interval can be allowed in specific circumstances Maximum 600 ml per session	No	NA
Canada, *Héma-Québec*	Clinical trials	Plasmapheresis	Every 6 days[a]	No	NA
Canada, *CBS*	Clinical trials	Plasmapheresis	Every 7 days	No	NA
United States, *Vitalant*	Clinical trials; Compassionate use	Plasmapheresis	Medical director discretion, up to every 48 h	No	NA
United States, *ARC*	Clinical trials; Compassionate use	Plasmapheresis Whole blood	Every 7 days, maximum of 8 over 3 months	No	Yes
United States, *OneBlood*	Clinical trials; Compassionate use	Plasmapheresis Whole blood	Medical director discretion, up to every 48 h	No	Yes
Africa					
South Africa, *SANBS*	Clinical trials; Compassionate use; Fractionation	Plasmapheresis	Every 2 weeks, maximum of 24 donations/year	No	NA
South Africa, *UCT*	Clinical trials; Fractionation	Plasmapheresis	Every 2 weeks, maximum of 24 donations/year	Not specified	NA
Eastern Mediterranean Region					
Egypt	Clinical trials; Fractionation	Plasmapheresis	Every 2 weeks	No	NA
Israel	Clinical trials; Compassionate use	PlasmapheresisWhole blood	Every 2 weeks, maximum of 6 per 10 days	Yes	Yes
Oman	Clinical trials	Plasmapheresis	Every 7 days, maximum of 4	NA	NA
Saudi Arabia	Clinical trials	Plasmapheresis	Every 7 days, maximum of 2	Not specified	NA
Europe					
Belgium	Clinical trials; Compassionate use	Plasmapheresis	Maximum 2 l/month, 23/year, and 15 l/year Maximum 650 ml per session	No	NA
Finland	Clinical trials	Plasmapheresis	Every 2 weeks, maximum of 5	NA	NA
France	Clinical trials; Compassionate use	Plasmapheresis	Every 2 weeks, maximum of 24/year	No	NA
Germany	Clinical trials; Compassionate use	Plasmapheresis	Every 2 days (48 h), maximum of 60/year	No	NA
Italy	Clinical trials; Compassionate use; Fractionation	PlasmapheresisWhole blood	Every 2 weeks, maximum of 12 l/year 600–700 ml per session	No	Not specified
Norway			Maximum 4 donations over 4 weeks	Yes	Yes

Table 2 (Continued)

Country, Institution	Intended use of CCP	Method of CCP collection	Frequency of CCP Plasmapheresis	Frequency different from local standard plasmapheresis?	Components used from whole blood?
	Clinical trials; Compassionate use	PlasmapheresisWhole blood			
The Netherlands	Clinical trials; Compassionate use; Fractionation	Plasmapheresis	Maximum of 26 donations and 25 l/year Maximum 750 ml per session	No	NA
Turkey, *BUU*	Compassionate use	Plasmapheresis	Every 10 days, maximum of 8 in 3 months	Yes	NA
Turkey, *TRC*	Per direction of Ministry of Health	Plasmapheresis	Every 10 days, maximum of 3 in a month; 1·8 l/month and 8 per 3 months period[b]	Not specified	NA
Turkey, *AHGH*	Clinical trials; Compassionate use	Plasmapheresis	Every 10 days, maximum 3 in a month, maximum 8 per 3 month period[b]	Not specified	NA
United Kingdom	Clinical trials	Plasmapheresis	Every 7 days, maximum of 24/year	No	NA
South-East Asia					
India, *AIIMS*	Clinical trials; Compassionate use	Plasmapheresis	Every 2 weeks Maximum 500 ml per session, 1 l per month	No	NA
India, *PGIMER*	Clinical trials; Compassionate use	Plasmapheresis	Every 2 weeks	No	NA
Indonesia	Clinical trials	Plasmapheresis	Every 2 weeks, between 3–6 donations	Not specified	NA
Western Pacific					
Australia	Clinical trials; Fractionation	PlasmapheresisWhole blood	Every 7 days, up to 12 donations	Yes	Yes
China, *BRCBC, WHBC, SXBC*	Clinical trials; Compassionate use	Plasmapheresis	Every 2 weeks, maximum of 24 times/year	No	NA
Hong Kong, China	Clinical trials; Compassionate use	Plasmapheresis	Every 2 weeks, maximum of 6 donations	No	NA
Singapore, *HAS, TTSH*	Compassionate use	PlasmapheresisWhole blood	Every 2 weeks[c]	Yes	Yes
South Korea	Clinical trials	Plasmapheresis	Every 2 weeks	No	NA

AHGH, Acıbadem Health Group Hospitals; AIIMS, All India Institute of Medical Sciences; ARC, American Red Cross; BRCBC, Beijing Red Cross Blood Center; BUU, Bursa Uludağ University; CBS, Canadian Blood Services; HAS, Health Sciences Authority, NA; not applicable; PGIMER, Post Graduate Institute of Medical Education and Research; SANBS, South African National Blood Service; SXBC, Shaanxi Blood Center; TRC, Turkish Red Crescent; TTSH, Tan Tock Seng Hospital; UCT, University of Cape Town; WHBC, Wuhan Blood Center.

[a]initially allowed for up to 12 weeks after their first donation. Later reduced to up to 6 weeks after resolution of symptoms.

[b]allowed for up to 3 months after recovery.

[c]initially started at allowable frequency as for standard plasmapheresis. Higher frequency is allowed provided donors' serum albumin and globulin levels before each donation was in the reference range.

of minipool CoV immunoglobulin (CoVIg). A total of 20 institutions collected CCP for clinical trials and compassionate use. Of these, four institutions had CCP initially collected for use in clinical trials but later was provided on a compassionate basis (India; Hong Kong; China, in Wuhan; Italy; Table 2).

Eight institutions had CCP collected for transfusion in clinical trials only. Three respondents indicated that the CCP had been used in rare cases on a compassionate basis outside a clinical trial setting (Saudi Arabia; Australia; and the UK). A regional institution in the USA had CCP initially provided for use in both compassionate and study protocols, but since the Mayo Clinic investigational new drug trial was completed, CCP was provided as an investigational new drug.

In Brazil, CCP was collected mostly for use within the setting of clinical trials for patients with severe pneumonia, and to a lesser extent, for compassionate use depending on the physician's request. Two institutions collected CCP solely for compassionate use (Singapore; one in Turkey). In the Netherlands, the compassionate use of CCP was offered for immune-compromised hospitalized patients with persistent and/or severe COVID-19 disease. CCP was also collected for clinical trials and as a source plasma for CoVIg production.

Six institutions have been collecting CCP for fractionation purposes. The use of CCP for fractionation was under consideration in Canada, Norway and France at the time of response to this international forum.

Q4: Describe the CCP donor eligibility and recovery criteria used in your institution? Do they need to have a confirmatory test result status of past COVID-19 infection before CCP donation? What type of test?

Laboratory confirmation of COVID-19 infection

The donor eligibility criteria varied dramatically between countries (Table 3). All institutions, except the French and Australian, required proof of a previous COVID-19 infection, either in the form of a positive SARS-CoV-2 polymerase chain reaction (PCR) test (29 respondents) or anti-SARS-CoV-2 antibodies (17 respondents). In France, during the first peak of COVID-19 pandemic, patients with mild clinical symptoms were not systematically tested by SARS-CoV-2 polymerase chain reaction (PCR). The diagnosis of COVID-19 was made presumptively based on patients' symptoms. In Australia, donors had to report that they had a 'laboratory-confirmed COVID-19 infection' as per the national guidelines for a confirmed case (tested positive by RNA or cell culture with PCR confirmation or showed evidence of seroconversion). However, donors were not required to provide the diagnostic report when presenting to donate CCP.

Time to donate and confirmatory negative tests

The vast majority of respondents indicated that donors were accepted for CCP donation 28 days after the resolution of signs/symptoms without any testing. In Italy, this deferral period however needed to be accompanied by a negative SARS-CoV-2 PCR. Alternatively, donors with no prior history of hospitalization could be accepted if SARS-CoV-2 PCR was performed at least 10 days after the onset of symptoms, with negative test results obtained at least 3 days from resolution of signs/symptoms. In Hong Kong, this deferral period was accompanied by a negative SARS-CoV-2 PCR on both nasopharyngeal swab and blood serum within 1 week prior to CCP donation.

Ten respondents indicated that donors were accepted for CCP donation 14 days after the resolution of signs and symptoms without testing. There were some institutions accepting the CCP donor 14 days after resolution of signs/symptoms if they had one negative SARS-CoV-2 PCR on nasopharyngeal swab. Other institutions required two negative test results. In Brazil, donors who remained reactive by the PCR were invited to perform a third PCR test after an additional deferral period of 14 days. If the PCR test remained positive, the donor was deferred from the CCP collection programme [16]. In China and one institution in India, the two SARS-CoV-2 PCR tests had to be performed 24 h apart. The regional blood services/centres in China required a PCR test performed at a minimum of 3 weeks after the onset of symptoms. In the national blood centre and one hospital-based blood service in Turkey, one of the SARS-CoV-2 PCR tests had to be performed within 48 h prior to CCP donation.

Three respondents indicated an acceptance criterion different from the above. In Egypt, donors were accepted after 10 days or more from the resolution of symptoms or if they had two negative PCR tests minimum 24 h apart. In South Korea, CCP collection is allowed 14 days after quarantine release (if no signs and symptoms for 10 days upon confirming the infection). Donors were also tested twice, at least 24 h apart, 14–28 days from quarantine release before donation. In Belgium, donors were accepted 17 days after recovery. In a regional blood centre in Hong Kong, donors had to have a documented SARS-CoV-2 PCR test on nasopharyngeal swab 4 weeks before donation, and a negative SARS-CoV-2 PCR on nasopharyngeal swab and serum within 1 week prior to CCP donation.

Evolution of eligibility criteria

The eligibility criteria evolved in some countries due to testing limitations at certain phases of the pandemic or changes in the definition of a confirmed case. For

Table 3 CCP donor eligibility and testing

Country, Institution	Confirmatory test of past COVID-19?		CCP donor eligibility criteria*					
	SARS-Cov-2 PCR	Anti-SARS-Cov-2 Antibody	14 days after resolution of symptoms	14 days after resolution of symptoms + 1 negative PCR	14 days after resolution of symptoms + 2 negative PCR	28 days after resolution symptoms/ recovery	Pre-donation SARS-CoV-2 testing	Pre-donation anti-SARS-CoV-2 antibody testing
Americas								
Argentina	Yes	No	No	Yes[a]	No	Yes[a]	Yes	Yes
Brazil	Yes	No	No	Yes	No	No	Yes	Yes
Canada, *Héma-Québec*	Yes[b]	No	Yes	No	No	No	No	No
Canada, *CBS*	Yes[b]	No	No	No	No	Yes	No	No
United States, *Vitalant*	Yes	Yes	No	No	No	Yes	No	Yes[c]
United States, *ARC*	Yes	Yes	Yes	No	No	No	No	Yes[c]
United States, *OneBlood*	Yes	Yes	Yes[d]	No	No	No	No	Yes[c]
Africa								
South Africa, *SANBS*	Yes	Yes	Yes[d]	No	No	Yes	No	Yes
South Africa, *UCT*	Yes	Yes	Yes	No	No	No	No	Yes[c]
Eastern Mediterranean Region								
Egypt	Yes	Yes	No	No	No	No	No	Yes
Israel	Yes	No	No	No	Yes	No	No	Yes[c]
Oman	Yes	Yes	No	Yes	No	Yes	Yes	Yes
Saudi Arabia	Yes	No	Yes	No	No	No	No	Yes[c]
Europe								
Belgium	Yes	Yes	No	No	No	No	No	Yes[c]
Finland	Yes	No	Yes[d]	No	No	No	No	Yes
France	No	No	Yes	No	No	No	No	No
Germany	Yes	Yes	No	No	No	Yes	Yes	Yes[c]
Italy	Yes	No	No	No	No	Yes[e]	No	Yes
Norway	Yes	Yes	No	No	No	Yes	No	Yes[c]
The Netherlands	Yes	No	Yes	No	No	No	No	No
Turkey, *BUU*	Yes	Yes	No	No	Yes[f]	Yes	No	Yes
Turkey, *TRC*	Yes	Yes	No	No	Yes[f]	Yes	No	No
Turkey, *AHGH*	Yes	Yes	No	No	No	Yes[g]	Yes	Yes
United Kingdom	Yes	Yes	No	No	No	Yes	No	Yes[h]
South-East Asia								
India, *AIIMS*	Yes	Yes[i]	Yes	No	No	No	No	Yes
India, *PGIMER*	Yes	No	No	No	Yes[j]	Yes	No	Yes
Indonesia	Yes	No	No	Yes	No	No	No	Yes

Table 3 (Continued)

Country, Institution	Confirmatory test of past COVID-19?		CCP donor eligibility criteria*					
	SARS-Cov-2 PCR	Anti-SARS-Cov-2 Antibody	14 days after resolution of symptoms	14 days after resolution of symptoms + 1 negative PCR	14 days after resolution of symptoms + 2 negative PCR	28 days after resolution symptoms/ recovery	Pre-donation SARS-CoV-2 testing	Pre-donation anti-SARS-CoV-2 antibody testing
Western Pacific								
Australia	No	No	No	No	No	Yes	No	No
China, *BRCBC, WHBC, SXBC*	Yes	Yes	No	No	Yes[j,k]	Yes[l]	Yes[m]	Yes[m]
Hong Kong, China	Yes	Yes	No	No	No	Yes[n]	No	Yes
Singapore, *HAS, TTSH*	Yes	No	No	No	No	Yes[o]	No	Yes
South Korea	No	No	No	No	No	No	No	No

AHGH, Acıbadem Health Group Hospitals; AIIMS, All India Institute of Medical Sciences; ARC, American Red Cross; BRCBC, Beijing Red Cross Blood Center; BUU, Bursa Uludağ University; CBS, Canadian Blood Services; HAS, Health Sciences Authority, NA, not applicable; PGIMER, Post Graduate Institute of Medical Education and Research; PCR, polymerase chain reaction; SANBS, South African National Blood Service; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; SXBC, Shaanxi Blood Center; TRC, Turkish Red Crescent; TTSH, Tan Tock Seng Hospital; UCT, University of Cape Town; WHBC, Wuhan Blood Center.

[a]Initially required negative SARS-CoV-2 PCR on 2 tests, but then accept one test or 28 days post full recovery.

[b]Or presumptive positive.

[c]Testing is done at time of donation.

[d]Initially required negative SARS-CoV-2 PCR, but then accepts 14 days post full recovery without testing.

[e]Along with a negative SARS-CoV-2 PCR (hospitalized patients), or if performed at least 10 days after the onset of symptoms with a negative SARS-CoV-2 PCR at least 3 days from resolution of symptoms (for other symptomatic patients).

[f]One PCR test should have been done within 48 h of donation.

[g]Along with 2 negative PCR tests.

[h]Except for men over 50 years of age (whites) and over 35 years of age (black, Asians and minority ethnic).

[i]By an immunoassay or rapid antigen test.

[j]Minimum 24 h apart.

[k]At least 3 weeks since onset of the symptoms and 2 weeks from time of discharge from hospital.

[l]One centre.

[m]One centre performs SARS-CoV-2 mini-pool or ID-NAT on plasma samples, and tests for anti-SARS-CoV-2 antibodies in-house. Other 2 centres receive test results from recruiting hospitals or perform testing in an external certified laboratory.

[n]Along with a negative nasopharyngeal swab and serum PCR for SARS-CoV-2 within 1 week of the donation.

[o]initially required negative SARS-CoV-2 PCR, but then accepts 28 days post full recovery without testing.

*Other eligibility criteria are applied in Egypt, Belgium and South Korea.

instance, in Norway and France, testing by SARS-CoV-2 PCR was limited at some stages of the pandemic; therefore, presumably recovered patients were allowed to donate CCP and were tested for the presence of antibodies. This criterion was added to the donor eligibility criteria to include donors if a laboratory-confirmed diagnosis was not available. In the Netherlands, a confirmatory SARS-CoV-2 PCR of past infection was required for all donors at the initial stages. This requirement was temporarily removed for CoVIg source plasma donors before it was re-introduced when a lower proportion of these donors were found to have anti-SARS-CoV-2 antibodies compared with apheresis donors (60% vs. 85%).

In the USA, South Africa and Finland, testing was initially required to confirm non-reactivity of SARS-CoV-2 PCR before CCP donation, but this requirement was later removed. In the USA, donors were initially required to have proof of infection in the form of a positive SARS-CoV-2 PCR test and/or antibody test results and be 28 days from infection and symptom-free. The latter criterion was changed to complete resolution of symptoms at least 14 days before the donation [17]. In Argentina, the eligibility criteria initially required negative SARS-CoV-2 PCR on two tests performed 24 h apart, 14 days after resolution of signs/symptoms. This latter criterion was changed to accept donors at least 14 days after symptoms resolution with 1–2 negative PCR tests. Later on, the eligibility criteria included acceptance of donors 28 days post-resolution of symptoms without additional testing. In Singapore, the definition of clinical recovery has evolved with the evolution of the criteria for release from quarantine. Initially, it was defined by the resolution of fever and any clinical symptoms for at least 24 h, along with a negative SARS-CoV-2 PCR from 2 separate nasopharyngeal swabs taken 24 h apart. Since May 2020, non-immune compromised patients could de-isolate 21 days after onset of symptoms if feeling well without further testing. Hence, donors were later accepted 28 days after recovery without testing.

Definition of recovery

The definition of recovery was variable between countries. The majority of the institutions used a complete resolution of symptoms. Other institutions required confirmation of negative SARS-CoV-2 testing and the absence of symptoms. Some institutions relied on the regulatory authorities' definition (e.g. Ministry of Health in Singapore; China; Argentina; and Italy). In the Australian and Norwegian blood centres, donors with residual symptoms (e.g. residual loss of smell and fatigue) could be accepted for CCP donation after medical assessment.

Other eligibility criteria

A few respondents indicated other eligibility criteria for CCP donation. In six countries (Canada; India; Oman; Italy; Egypt; and South Africa), male and nulliparous female donors with no history of pregnancy were accepted as CCP donors. In Italy, other donors were accepted for CCP donation for fractionation purposes. In Finland and the UK, both male donors and female donors tested for anti-HLA-antibodies were accepted. In the UK, potential female donors also had to be tested for anti-HNA antibodies. In the USA, at time of this international forum, individuals who had received the SARS CoV-2 vaccine were accepted to donate CCP if they have had symptoms of COVID-19 and a positive test result from a diagnostic test approved, cleared or authorized by FDA, received the COVID-19 vaccine after diagnosis of COVID-19 and were within six months after complete resolution of COVID-19 symptoms [17].

Q5: Does your institution test the CCP donor for SARS-CoV-2 by PCR before donation to confirm clearance of the infection?

The vast majority of the institutions did not perform SARS-CoV-2 PCR testing on the donation, or on the donor, prior to donation to confirm the clearance of the infection (Table 3). However, some specificities were noted in institutions that perform testing. In Brazil, donors should have had a negative SARS-CoV-2 PCR either on nasopharyngeal swab or serum. In Germany, donors were tested for SARS-CoV-2 by PCR during a pre-donation visit (typically 14 days prior to donation). A donation was possible 14 days after a negative PCR test, at the earliest. In regional blood services/centres in China and Hong Kong, testing was performed in the recruiting hospitals. One centre in China performed SARS-CoV-2 NAT test either on a pool of 6–8 plasma samples or ID-NAT [18]. In Hong Kong, donor eligibility criteria required negativity for SARS-CoV-2 on nasopharyngeal swab and serum within 1 week of donation. In Singapore, donor blood samples needed to be tested and confirmed negative for SARS-CoV-2 PCR before CCP donation. However, this requirement was later removed.

Q6: Does your institution test the CCP donor for anti-SARS-CoV-2 antibodies before donation?

The practice of testing CCP donors for anti-SARS-CoV-2 antibodies before the donation was variable (Table 3). Sixteen institutions from 13 countries required antibody testing on the donor before the donation, and only those with a predetermined cut-off level were accepted to donate CCP. The system in the regional blood services/centres in China was dependent on the recruiting hospitals in performing testing before referral of the donor to the blood centres for CCP donation. One centre

however performed the testing in-house. Institutions in other countries either performed testing in-house or in an external laboratory. This infers that donors underwent a screening before being accepted for donation. In the institution from Brazil, both SARS-CoV-2 PCR results and the virus-neutralizing antibodies data were made available before medical screening for assessing the donor's eligibility for donation. A total of nine respondents indicated that testing was instead performed at the time of donation. In Singapore, testing was performed at time of donor screening and repeated on the day of donation.

Testing for anti-SARS-CoV-2 antibodies was performed using different testing platforms such as the enzyme-linked immunosorbent assay (ELISA), chemiluminescent assay (CLIA), chemiluminescent microparticle immunoassay (CMIA) and the virus neutralization test (Table 4). A few institutions relied on a rapid screen test of donors for the presence of anti-SARS-CoV-2 antibodies on the day of donation. Some centres used two different testing methods to screen the donors, either using different testing platforms or different manufacturers. In Brazil, both the ELISA and virus neutralization tests were done, but the decision to accept the donation depended on the neutralization test results. In a blood centre in the USA (OneBlood), two CLIA methods were used: a screening test (to detect anti-spike protein) and a confirmatory assay (to detect anti-nucleocapsid protein). If the signal-to-cut-off (S/C) ratio on the screening test was 10 or greater, this result was confirmed by the confirmatory test. In Israel, a rapid test was first performed to screen all donors. Donors with negative results on the rapid test were further tested using a CMIA method.

Q7: Does your institution test the CCP unit for anti-SARS-CoV-2 antibodies? Are samples collected from the CCP unit and freezed/archived for future assessment?

A total of 23 institutions tested the CCP units for anti-SARS-CoV-2 antibodies, eleven tested the donor on the donation day (Table 4). Some centres pre-screened the donors by an antibody testing before donation, as described above, and repeated the testing on the unit on the day of donation using the same or additional testing methods. Different test methods were used in qualifying the units, and the cut-off criteria to accept the units varied among centres and testing methodology used, with some institutions using the manufacturer's cut-offs, others a higher cut-off. The specificity of the ELISA, CMIA and CLIA tests used was variable, including viral spike protein, nucleocapsid protein or receptor-binding domain. Moreover, some of these tests were IgG specific, while others were reactive for other antibodies.

Thirteen institutions indicated a virus neutralization test performed on a sample collected on the day of donation &t/or the collected unit (Brazil; Canada; one centre in South Africa; Saudi Arabia; Belgium; Finland; France; Germany; Italy; Norway; Australia; Hong Kong and Singapore). The acceptable level of virus neutralization titre ranged from >1:20 up to >1:320. In Finland and Norway, no cut-off has been set in accepting the donation. In Singapore, the viral neutralization test that is based on antibody-mediated blockage of angiotensin-converting enzyme (ACE)-2 spike protein interaction was done at the time of donor screening using an in-house SARS-CoV-2 surrogate virus [19]. In France, the virus neutralization test was assessed using live SARS-CoV-2 virus, as described by Gallian *et al.* [20].

The criteria to accept donated CCP units for use varies between centres and few centres utilized more than one test or relied on a step-wise approach in qualifying the units. Four institutions decided on diverting the unit to transfusion or fractionation based on titre levels (Australia; one centre in South Africa; Israel; Egypt). Twenty-seven correspondents indicated that samples from the units are archived for future testing.

Q8: Is CCP subjected to a pathogen-reduction treatment?

A majority of the institutions (20), located in both high-income countries (HIC) or low- and middle-income countries (LMIC), did not apply any pathogen reduction treatment on the CCP units (Table 4). Among the institutions which performed pathogen reduction, five used the Intercept® Blood system (Cerus Corporation), three used the Mirasol® Pathogen Reduction Technology (Terumo BCT) and three used a methylene blue treatment, including two institutions in China. One institution in Egypt reported the use of caprylic acid, a purification and virus inactivation agent employed in a process to prepare mini-pool CoVIg [21].

Q9: Does your institution accept recipients of CCP for convalescent plasma donation?

Nine institutions accepted recipients of CCP as being themselves a donor of CCP (Table 5). Sixteen institutions indicated that these CCP recipients were not allowed to donate CCP (unless as part of a study in one institution in Egypt). In some of these countries, the reason was related to existing donor deferral period after transfusion which exceeds the maximum period allowable for CCP donation as per local protocols. For instance, in Canada, all plasma recipients (including CCP) are deferred from donating blood products for a period of 6 months. As a result, these individuals will not be accepted for CCP donations because of the long delay, which exceeds the 6 weeks post-recovery timeframe for CCP donation. Other respondents indicated that considering that the deferral period for transfusion recipients before allowing them to donate blood is 12 months, recipients of CCP cannot be accepted for convalescent plasma donation (Brazil; India; Turkey; Australia; China; and Singapore). Institutions that allowed

Table 4 Antibody testing methods and CCP component modification

Country, Institution	Component antibody testing?	Anti-SARS-CoV-2 antibodies testing methods		Pathogen inactivation?
		Method	Cut-off criteria to qualify donor/donation	
Americas				
Argentina	No[a]	ELISA (COVIDAR)	>800	No
Brazil	Yes	VNT ELISA	≥1:160NA	INTERCEPT®
Canada, *Héma-Québec*	Yes	ELISA[b]	>cut-off at 1:100 plasma dilution	No
Canada, *CBS*	Yes	VNT	$PRNT_{50}$ titre of ≥1:160	No
United States, *Vitalant*	No[a]	ELISA (Euroimmun)	>cut-off at 1:100 plasma dilution	No
United States, *ARC*	Yes	CLIA (VITROS)	S/C ≥ 1·0	No
United States, *OneBlood*	Yes	CLIA (VITROS) *(screening)* CLIA (Elecsys) *(confirmatory)*	S/C ≥ 10	No
Africa				
South Africa, *SANBS*	Yes	ELISA[c]	OD ≥ 1·0 *(transfusion)*No cut-off *(fractionation)*	INTERCEPT®
South Africa, *UCT*	Yes	VNT	≥1:160 *(transfusion)*	Mirasol®
Eastern Mediterranean Region				
Egypt	Yes	Rapid Test (AMEDA) CLIA (MAGLUMI) CLIA (Elecsys)	Reactive ≥ 1·0 AU/ml *(transfusion)* ≥10·0 AU/ml *(fractionation)* S/C ≥1 *(transfusion)* S/C ≥10 *(fractionation)*	Lipid enveloped virus inactivation by Caprylic Acid for immunoglobulin production
Israel	Yes	Rapid Test (PharmaAct) CMIA (Architect)	Reactive S/C > 4 *(transfusion)* S/C > 1·4 *(fractionation)*	No
Oman	Yes	ELISA (EUROIMMUN)	OD ≥ 2	Mirasol®
Saudi Arabia	No[a]	VNT	1:80	INTERCEPT®
Europe				
Belgium	No[a]	VNT	>1:320	Methylene blue
Finland	Yes	VNT	None	No
France	Yes	ELISA (EUROIMMUN) VNT[d]	OD ≥ 8≥ 1:80	INTERCEPT®
Germany	No[a]	VNT	>1:20	No
Italy	Yes	VNT	≥1:160	Yes (type not specified)
Norway	Yes[e]	Anti-SARS-CoV-2 commercial tests, In-house multiplex tests, VNT	Not established	No

Table 4 (Continued)

Country, Institution	Component antibody testing?	Anti-SARS-CoV-2 antibodies testing methods		Pathogen inactivation?
		Method	Cut-off criteria to qualify donor/donation	
The Netherlands	Yes	ELISA	OD ≥ 0·1	No
Turkey, *BUU*	No[a]	CMIA (Architect)	S/C > 1·4	No
Turkey, *TRC*	Yes	ELISA (EUROIMMUN) CLIA (Elecsys)	Undisclosed	Mirasol®
Turkey, *AHGH*	No[a]	Rapid test	Reactive	INTERCEPT®
United Kingdom	Yes	ELISA (EUROIMMUN)	OD ≥ 6	No
South-East Asia				
India, *AIIMS*	No[a]	CMIA (Architect)	S/C ≥ 1·4	No
India, *PGIMER*	No[a]	CLIA (VITROS)	S/C ≥ 13·0	No
Indonesia	No[a]	Rapid test (Assure Fastep)	≥1:80	No
Western Pacific				
Australia	Yes[f]	CMIA (Architect) *(screening)* ELISA (EUROIMMUN) *(screening)*	S/C ≥ 1·4 *OD* ≥ 1	No
		VNT	≥1:80 *(transfusion)* ≥1:40 *(fractionation)*	
China, *BRCBC, WHBC, SXBC*	Yes	ELISA (IgG) ELISA (Total)	IgG ≥ 1:160 Total ≥ 1:320	Variable Methylene blue
Hong Kong, China	Yes	VNT	≥1:80	No
Singapore, *HAS, TTSH*	No[a]	VNT	>1: 80 (Apheresis); >1:40 (WB)	No
South Korea	Yes	Rapid test (AFIAS)	COI > 1·0	No

AHGH, Acıbadem Health Group Hospitals; AIIMS, All India Institute of Medical Sciences; ARC, American Red Cross; AU, absorbance unit/ml; BRCBC, Beijing Red Cross Blood Center; BUU, Bursa Uludağ University; CBS, Canadian Blood Services; CLIA, chemiluminescence enzyme immunoassay; CMIA, Chemiluminescent microparticle immunoassay; ELISA, enzyme-linked immunosorbent assay; HAS, Health Sciences Authority, NA, not applicable; OD, optical density ratio; PGIMER, Post Graduate Institute of Medical Education and Research; SANBS, South African National Blood Service; SARS-CoV-2, severe acute respiratory syndrome coronavirus-2; S/C, signal to cut-off index; SXBC, Shaanxi Blood Center; TRC, Turkish Red Crescent; TTSH, Tan Tock Seng Hospital; UCT, University of Cape Town; VNT, virus neutralization test; WB, whole blood; WHBC, Wuhan Blood Center.

[a]Testing is done on the donor sample.

[b]Anti-SARS CoV-2 antibodies against total spike protein, neutralization assay, antibody-dependent cell-mediated cytotoxicity assay are done at external collaborators' laboratories.

[c]Initially, samples with sufficiently high OD on ELISA test were tested for neutralizing antibodies, however the later was discontinued.

[d]Neutralizing antibody test is done for CCP units with OD ratio≥ 1·6 (earlier 1·1) and < 8 (earlier 5·6) on Euroimmun anti-SARS-CoV-2 ELISA assay.

[e]For transfusion, the donation must be positive on one of the two screening tests and have a neutralizing antibody titre ≥1:80.

[f]For release for clinical use, the donation must be positive on one of the two screening tests and have a neutralizing antibody titre ≥1:80.

Table 5 COVID-related modification of blood donor eligibility criteria

Country, Institution	Recovered individuals allowed to donate blood?	Minimum deferral criteria for donating blood	CCP recipients allowed to donate CCP?	Minimum deferral criteria for a CCP recipient before CCP donation	CCP recipients allowed to donate blood?	Minimum deferral criteria for a CCP recipient before blood donation
Americas						
Argentina	Yes	14 days from resolution of symptoms with negative SARS-CoV-2 PCR -*Or*- 28 days from recovery	No	-	Yes	12 months
Brazil	Yes	60 days after resolution of symptoms[a]	No	-	Yes	12 months
Canada, *Héma-Québec*	Yes	14 days after resolution of symptoms	No	-	Yes	6 months
Canada, *CBS*	Yes	21 days after resolution of symptoms	No	-	Yes	6 smonths
United States, *Vitalant*	Yes	28 days after resolution of symptoms	No	-	No	-
United States, *ARC*	Yes	14 days after resolution of symptoms	Yes	3 months	Yes	3 months
United States, *OneBlood*	Yes	14 days after resolution of symptoms	Yes	Not specified	Yes	3 months
Africa						
South Africa, *SANBS*	Yes	14 days after resolution of symptoms	Yes	3 months	Yes	3 months
South Africa, *UCT*	Yes	14 days after resolution of symptoms	Yes	3 months	Yes	3 months
Eastern Mediterranean Region						
Egypt	Yes	14 days from recovery	No[b]	-	No policy	-
Israel	Yes	28 days after resolution of symptoms	Yes	6 months	Yes	6 months
Oman	Yes	28 days after resolution of symptoms	No	-	Yes	12 months
Saudi Arabia	Yes	28 days after resolution of symptoms, -*Or*- 28 days after the date of the positive swab, if asymptomatic	Undecided	-	Yes	3 months
Europe						
Belgium	Yes	28 days from recovery	Yes	4 months	Yes	4 months
Finland	Yes	28 days from recovery[a] 3 months for hospitalized patients 3 months for hospitalized patients	NA[c]	NA	Yes	4 months
France	Yes	28 ays after resolution of symptoms; 4 months for hospitalized patients	No[d]	NA	No	-
Germany	Yes	4 weeks from recovery	No	-	Yes	12 months
Italy	Yes	10 days from recovery[e]	See note[f]	4 months	See note[f]	4 months
Norway	Yes	28 days from recovery	Yes	6 months	Yes	6 months
The Netherlands	Yes	14 days from recovery	No[d]	-	No	-
Turkey, *BUU*	Yes	28 days from recovery	No	-	Yes	12 months
Turkey, *TRC*	Yes	28 days from recovery	No[d]	-	No	-
Turkey, *AHGH*	Yes	28 days from recovery	No	-	Yes	12 months
United Kingdom	Yes	28 days from recovery	Yes	28 days from recovery	No	-

Table 5 (Continued)

Country, Institution	Recovered individuals allowed to donate blood?	Minimum deferral criteria for donating blood	CCP recipients allowed to donate CCP?	Minimum deferral criteria for a CCP recipient before CCP donation	CCP recipients allowed to donate blood?	Minimum deferral criteria for a CCP recipient before blood donation
South-East Asia						
India, *AIIMS*	Yes	28 days from recovery	No	-	Yes	12 months
India, *PGIMER*	Yes	28 days from recovery	No	-	Yes	12 months
Indonesia	Yes	14 days after resolution of symptoms	Yes	Not specified	Yes	12 months
Western Pacific						
Australia	Yes	28 days from recovery	No	-	Yes	12 months
China, *BRCBC, WHBC, SXBC*	Yes	6 months from recovery	No	-	Yes	Minimum 5 years
Hong Kong, China	Yes	180 days from recovery	Undecided	-	Yes	12 months
Singapore, *HAS, TTSH*	Yes	28 days from recovery	No	-	Yes	12 months
South Korea	Yes	3 months from recovery	No	-	Yes	12 months

AHGH, Acibadem Health Group Hospitals; AIIMS, All India Institute of Medical Sciences; ARC, American Red Cross; BRCBC, Beijing Red Cross Blood Center; BUU, Bursa Uludağ University; CBS, Canadian Blood Services; HAS, Health Sciences Authority, NA, not applicable; PGIMER, Post Graduate Institute of Medical Education and Research; SANBS, South African National Blood Service; SXBC, Shaanxi Blood Center; TRC, Turkish Red Crescent; TTSH, Tan Tock Seng Hospital; UCT, University of Cape Town; WHBC, Wuhan Blood Center.

[a]If they had a mild form of the disease.

[b]Unless if part of a study.

[c]CCP transfusion has not yet started in the country.

[d]Transfusion recipients are not allowed to donate blood.

[e]Including a negative SARS-CoV-2 RT-PCR on nasopharyngeal swab.

[f]Only plasma for fractionation.

CCP recipients to donate CCP had a shorter deferral period post-transfusion (e.g. 3 months in South Africa, 4 months in Belgium). A few institutions do not accept recipients of CCP to donate CCP although they accept other recipients of transfusions (Germany and South Korea). On the other hand, in the UK recipients of CCP are accepted for convalescent plasma donation, although they are not accepted as donors for any other blood components. Three institutions mentioned that no decision was made yet, and one institution (Italy) used the plasma collected from those donors for fractionation purposes only, in line with practice with other blood donors as a means of mitigating the risk of transfusion-related acute lung injury.

Q10: Does your institution accept individuals recovered from COVID-19 infection for standard WB or apheresis (platelet or plasma) donation? What is the minimum deferral period after recovery before WB or apheresis donation?

All institutions indicated that they accepted individuals who recovered from COVID-19 for blood donation (Table 5). There were substantial variations in the deferral period applied that ranged from 10 days to 180 days. A majority of institutions implemented a deferral of 14 to 28 days after recovery or resolution of signs and symptoms. In a few institutions, deferral periods of 10 or 14 days were combined with a confirmation of the resolution of the infection by a PCR test on nasopharyngeal swab (in Italy and Argentina, respectively). In two institutions, a longer deferral period of 3 or 4 months was applied for individuals who experienced severe COVID-19 disease or were hospitalized (in Finland and France, respectively).

Q11: Does your institution accept recipients of CCP for standard WB or apheresis (platelet or plasma) donation? What is the minimum deferral period after CCP transfusion before WB or apheresis donation can be made?

Most institutions (27; including 3 institutions in China) responded that they accepted recipients of CCP for WB or plasma donations (Table 5). Among these, substantial differences in the duration of the deferral period were noticed, probably reflecting differential perception of the possible risks, if any, to the recipient. The deferral period ranged from 3 months (five institutions), 4 months (three institutions), 6 months (four institutions) and 12 months (thirteen institutions). Three regional blood services/centres in China indicated a period of at least 5 years.

By contrast, five institutions (one regional institution in the USA and the national blood establishments and centres of France; the Netherlands; Turkey; and the UK) had a different regulation and deferred these donors; as part of a general regulation to exclude recipients on blood products as blood donors for some (France and the Netherlands). In Italy, plasma from such donors was used for fractionation, while one institution in Egypt mentioned the lack of a national policy. Institutions located in some countries (USA and Turkey) had different policies in that regard.

Q12: Describe any challenges faced and lessons learned from establishing a CCP collection programme in your institution?

The CCP programmes described herein range from single centre-based collection and transfusion up to national programmes. Several institutions faced many challenges in initiating a CCP programme, ranging from operational challenges to communication with the public and the community. Two respondents did not disclose challenges and lessons learned in their facilities.

Operations

Setting up a CCP collection programme as a new service during the pandemic and integrating CCP production planning into operations was reported as a challenge. CCP programmes had to be deployed rapidly early in the pandemic despite the lack of evidence from high-quality clinical trials on its efficacy and safety. Starting a CCP collection programme required test development and/or acquisition, validation, protocol set-up and continuous training. This occurred while many centres were busy maintaining blood inventory and implementing COVID-19-related safety precautions and social distancing, which slowed the facility's operations. In one blood centre in the USA, the challenge was further augmented with the difficulties in predicting the pandemic's effect on the blood supply and the regional and national needs. For such a programme to be deployed, some institutions had to develop specific set-ups involving collaboration with external institutions, thereby bringing additional specific challenges. For instance, in Singapore, the CCP collection was set up in a hospital that was not a blood supplier. The lack of an administrative relationship and service agreement between this institution and the treating hospitals was a challenge. In the USA, the lack of coordination and preparation at the blood centre/hospital level with the national CCP programmes was challenging. Issuing high-titre CCP in South Africa required many operational procedures and system modifications to ensure lower titre convalescent plasma was not issued to patients.

Early and ongoing collaboration between all parties involved in this multi-disciplinary project was a key to overcome these challenges early. This involves the national blood services, hospital blood banks, treating hospitals and the authorities. Respondents highlighted the

need for ensuring early interest within the organization and acceptance of various internal stakeholders to work together to achieve rapid acquisition. Adhesion to the programme underlined a clear understanding and joined efforts to achieve the project's goals from every service involved. The staff's commitment to adapt to the rapid and continuous change in the operations during the pandemic was vital. Enhancing the operations' flexibility during the pandemic to adapt to the new and rapidly changing public health context (e.g. lockdown and social distancing) was stressed. Substantial coordination between hospitals, testing sites and blood establishments/centres was required to facilitate the referral of recovered COVID-19 patients. It was vital to maintain ongoing communication between the cooperating hospitals and the clinicians and blood centre physicians.

Resources

Different challenges regarding scarce resources, especially during the early stages of the pandemic, were described. These included providing additional personal protective equipment (PPE) at times of national shortages and severe supply chain shortages of disinfectants, paper goods and apheresis kits. In one blood centre in the USA, communication challenges in the prioritization of resources were faced. Dependence on single-source suppliers and 'just-in-time' inventory practices added to the challenge of replenishing supplies. Limited apheresis kits and apheresis equipment was faced in different institutions (Finland; the Netherlands; South Africa; and India). Moreover, scarcity in human resources was challenging with staff losses due to COVID-19 illness or mandatory isolation due to case contacts. Another difficulty described was the implementation of a dedicated information technology (IT) supported process for CCP collection, manufacturing and testing (France). One respondent indicated that manual systems during the early development of the programme caused delays and frustration.

Six institutions described occasional competition on the existing resources between WB and apheresis collections, including the staff and space available for donation to maintain social distancing. In South Africa, this led to specific periods of the pandemic requiring careful assessment for the need of CCP collections vs. WB collections. The blood centres in Norway created a separate workflow for handling CCP donors while institutions in India initiated an appointment system for sample collection and donation. An institution in Oman extended the working hours of the blood bank to accommodate CCP donors. In the UK, the CCP national collection programme was supported by 20 new donor centres that were opened to enable the collection of CCP on a national scale.

One of the most important lessons learned is that the rapid implementation of such a programme requires a coordinated effort from every service (such as operation, quality assurance, IT and medical affairs). One large blood centre in the USA (OneBlood) reported that the pre-existence of a robust implementation support structure before the pandemic's onset greatly enhanced the blood centre's responsiveness to the CCP project challenges, and proved to be critical in disaster management. However, there was a need to enhance existing networks and communications pathways at all levels and keep them open. The involvement of research and development staff members was found to be beneficial at the Canadian national blood services. One respondent highlighted the need to be equipped with standardized and homogeneous risk assessment tools at national and international levels and increase the capacity to build up blood components collection programmes in emergencies.

Protocol development, testing and product characterization

Another challenge described was the need to develop a study protocol for CCP collection and use in a short time and define the product specification and therapeutic indications, dose and frequency of administration for COVID-19-infected patients. This was especially the case with the frequently changing donor selection criteria, patient eligibility criteria to receive CCP and the lack of high-quality clinical trials to demonstrate the efficacy of CCP therapy. Delays in ethical board approvals to set up clinical trials was a challenge in some countries.

Another challenge faced by a couple of institutions was the lack of anti-SARS-CoV-2 antibodies licensed tests for donor screening and CCP product characterization, especially at early stages of the pandemic. The development of an in-house test was challenging and time-consuming. Access to neutralizing antibody assays was limited to few institutions. In addition, there was a lack of clear consensus on the cut-off of anti-SARS-CoV-2 antibodies on the different antibody testing platforms and the correlation with the in vitro virus neutralization tests. In Australia, collecting CCP for fractionation before fractionators determined what would likely be a standard level of neutralizing antibody titre required was a challenge.

One participant indicated that communication with international counterparts was invaluable to learn and assess practices and adapt what works best for locals setting in developing CCP collection protocols. The value of collection and storage of donor/product samples for future testing and research was highlighted. Two respondents recommended having trained staff to check updates

on approved tests and identify commercially available serological tests that correlate with in vitro neutralization as they are developed. Clinical trials should be established early in the pandemic before the number of cases starts to drop.

Education and training

Several institutions faced challenges with the limited number of trained staff and lack of experience in collecting CCP, as its collection programme needed to be established quickly. Several institutions reported dependence on the clinicians and trial coordinators in the hospitals to recruit donors from recovered patients, assess and consent patients for CCP in the trial environment. However, the unproven safety and effectiveness of CCP coupled with a lack of other therapeutic modalities for COVID-19 resulted in a level of uncertainty around the availability and use of the product. In the USA, the unfamiliarity of the clinicians with CCP, and with the paths of use under an emergency investigational new drug or expanded access programme to some non-research-based hospitals and blood centres was a challenge. One respondent indicated that educating the ordering physicians about clinical criteria to consent patients was challenging given the wide range of physicians who treated COVID-19 patients.

There was a need for continuous training, especially with the successive changes in the CCP donor eligibility criteria. Training a small group of hospital-based clinicians to obtain consent from the patients was found to be useful in this setting.

Donor recruitment and eligibility

Donor recruitment was a challenge reported by 11 of the respondents, especially during the extended summer holiday and lockdown as reported in South Africa. The lack of access to qualified CCP donor lists due to regulations in some countries to protect patient privacy resulted in inhibition of the hospitals and the health department from sharing needed information and made it challenging to reach recovered patients to donate CCP. One institution in India described the lack of knowledge on CCP donation among COVID-19 patients, while another provided donors with hospital transportation during lockdown. Managing requests of CCP for both clinical trials and compassionate use, and recruitment of donors of specific blood groups to fulfil hospital requests was a challenge in some institutions. An institution in Turkey described that most of the CCP donors were replacement donors due to difficulties in recruiting voluntary donors.

On the contrary to the above, one respondent from South Africa indicated that recovered COVID-19 patients were eager to donate CCP, even if they were not regular blood donors after realizing the possible clinical benefit of CCP. In Argentina, the commitment of the patients to donate CCP was supportive to maintain supplies. The centres in China indicated that CCP donors were very enthusiastic, and almost half became loyal repeat donors. As the media platforms in the Netherlands (social media, messaging services, press releases and TV/radio) were heavily engaged in donor recruitment, this resulted in long waiting lists of donors waiting for the first medical examination and donation.

Nine respondents indicated that donors' unfulfilment of general and CCP donor eligibility criteria was a challenge. This was especially triggered due to many of the recovered COVID-19 patients being new to blood or plasma donations. In addition, the frequent change in donor eligibility criteria for CCP collection further challenged some blood centres to keep up with their donor recruitment efforts. In the early stages of the pandemic in the USA, the FDA required a 28-day donor deferral period after recovery before CCP donation, limiting the number of recruitable donors. In Singapore, the initial requirement of two negative SARS-CoV-2 PCR results resulted in delays in CCP donation. The antibody titres' variable kinetics upon repeat donations in different donors was another challenge. Recruiting donors with the required high anti-SARS-CoV-2 titre was a challenge in the second wave in Canada and South Africa. As community spread level started to decline in Singapore, most recovered patients who were referred for CCP donation were migrants originating from malaria-endemic areas, which posed an additional challenge in finding eligible donors meeting the donor selection criteria in place.

Several respondents reported involving physicians, treating hospitals and public health departments to recruit recently recovered COVID-19 patients. This included educating patients on discharge from the hospital, providing them with information on the importance of CCP donation and streamlining the referral of potential donors. This collaboration should be initiated early and maintained throughout. Respondents expressed the importance of correct marketing to ensure an adequate CCP supply and have a proactive approach in controlling messages to the public using social media, emails and traditional media. One learning lesson was to have a dedicated donor recruitment unit if this does not exist.

The utility of performing point-of-care rapid tests for anti-SARS-CoV-2 antibodies was highlighted to avoid the collection of CCP from people with no or low levels of antibodies. Some institutions further stratified donors for CCP donation, with more data being made available from clinical studies on the dynamics of anti-SARS-CoV-2 antibodies and its relationship with donor factors. Others

focused on recruiting hospitalized patients as they were likely to have higher antibodies titres. A respondent from India applied relaxation of some donor eligibility criteria after careful analysis of risk vs. benefit to enhance the CCP donor pool. In Australia, the use of the same time-frame of acceptance post-recovery for CCP and WB donors allowed the utilization of donated CCP units for regular transfusion, if not meeting the required criteria for CCP.

Donor, staff and society concerns

Insufficient knowledge of COVID-19 and protection measurement required for the collecting staff was reported as a challenge. Inconsistent national messaging regarding disease risk and mitigation, blood donation safety and the need for PPE leading to confusion of donors and staff were described. Several correspondents reported the apprehensiveness of the collection staff to manage CCP donors, especially in the early stages of the pandemic (South Africa; Belgium; the Netherlands; and Hong Kong).

Several respondents expressed concerns of CCP donors on their health post-COVID-19 illness and their worries that CCP donations could affect their immunity to reinfection (South Africa; India; Hong Kong). An institution in India indicated donors' fears of acquiring infections (COVID-19 and non-COVID-19) due to weakened immunity because of COVID-19 illness, resulting in donor's hesitation to donate CCP in hospital-based blood facilities. One respondent from Indonesia reported difficulties in donor recruitment due to social stigma and the inability to meet patients and their families' expectations from the benefit from CCP transfusion. One respondent from South Africa reported some donors concerns from coming to the collection sites, while others used CCP donation to get a SARS-CoV-2 antibody level test rather than being altruistic. Handling fake news on social media was described as a challenge in Argentina.

Donation by apheresis was not a standard procedure in some facilities and was limited to platelet apheresis in others. An institution in India described the hesitation of recovered patients towards CCP donation by apheresis, since plasmapheresis was not routinely practised. Moreover, there were other challenges faced in recruiting recovered patients who were eligible and willing to donate CCP due to the impact of COVID-19 on donor lives and careers.

One respondent indicated that the importance of consistent messaging to the public that the blood centres followed all recommended guidelines for protective measures for the safety of donation. Educating the patients and society on the importance of CCP donation, on the apheresis procedures, and the collection staff on the safety of handling recovered patients was found to be useful. The motivation and continuous reassurance of the recovered patients to donate the CCP by the treating physicians they trust were highlighted.

Conclusion

Early and rapid deployment of a CCP collection programme during COVID-19 pandemic, at a time when the blood establishments were struggling in meeting blood supply, has brought up unique challenges at different levels in both HIC and LMICs. These challenges spanned a wide range from the lack of resources, short supplies, personnel loss, to operational challenges and the need for inter-organizational collaboration. The challenges also included recruitment of recovered patients who are eligible to donate blood and CCP, while handling staff, donors and society concerns. The World Health Organization recommended that blood services should take steps to assess, plan and respond to the emerging challenges appropriately and proportionately after undertaking a data-driven risk assessment [22]. The role of professional organizations in sharing experiences and providing guidance and recommendations is paramount. We summarize here information on practice of collection of CCP and lessons learned on establishing a CCP collection programme on an international level that can be utilized in developing a framework in facing similar pandemics in the future.

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INTERNATIONAL FORUM

International Forum on the Collection and Use of COVID-19 Convalescent Plasma: Responses

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United States – Vitalant

Mark Yazer & Darrell Triulzi

Question 1

Vitalant in Pittsburgh administers a centralized transfusion service that provides transfusion medicine expertise and blood products to about 24 area hospitals. Between these hospitals, virtually all medical and surgical services are provided from neonatal to geriatric care, including several Level 1 trauma hospitals. Vitalant Pittsburgh is also the regional blood collector, collecting approximately 87 000 RBCs, 25 000 platelets, and 31 000 plasma units per year.

Question 2

CCP is collected at Vitalant by apheresis. Our collection of CCP is the same as for routine plasma apheresis donations in that, according to the US FDA, plasma can be donated every 28 days, up to 13 times per year. While the FDA allows donors to give plasma more frequently, as soon as 48 h apart, these are considered frequent plasma donors and require close monitoring. The FDA waived the frequent donor requirements temporarily during the pandemic. The Vitalant blood donor medical director can give medical approval for donation more frequently than 28 days on a donor-by-donor basis.

Question 3

CCP is collected for transfusion to patients with dyspnoea and a positive COVID-19 diagnosis. Initially, we provided CCP for use in both compassionate and study protocols, in particular the Mayo Clinic study. However, since the Mayo Clinic IND was completed, all of the CCP is provided as an investigational new drug and the physician who orders it is required to explain the investigational nature of the product to the patient and document the patient's understanding of this fact in the consent note.

Question 4

Yes, a donor must have a positive test indicating that they were infected with COVID-19. We accept the results of either the PCR or the antibody test as proof that they were infected.

The donor must wait 28 days after resolution of their last sign or symptom before they can donate CCP.

Question 5

No.

Question 6

As a service to the community, and as a means of identifying potential CCP donors, Vitalant tests all donors who

donate whole blood or apheresis products for these antibodies. This testing is performed after the donation is made and the donor can check their results on the website. Similarly, potential donors who are referred to the blood centre by the hospital to donate CCP once they have recovered from their infection are also tested for antibodies after the donation is made. Thus, having a high titre antibody is not an exclusion criteria as the titre is determined after the donation is made.

Question 7

Vitalant uses the Euroimmun IgG/IgA immunoassay, with the donor plasma diluted 1:100.

Yes, samples are frozen for future assessment.

Question 8

No, our CCP does not undergo pathogen inactivation.

Question 9

No.

Question 10

Yes, individuals who have recovered from COVID-19 can donate standard blood products 28 days after resolution of their last sign or symptom.

Question 11

No.

Question 12

1. Substantial coordination was needed between hospitals, testing sites, in order to streamline the referral of potential donors to the blood centre.
2. Donor recruitment is still a challenge
3. Educating the ordering physicians about clinical criteria, dose, and what is required in order to obtain informed consent is a challenge given the wide range of physicians who treat COVID-19 patients. We found it best to have a small group of hospital-based clinicians who were trained in the appropriate and up-to-date use of, and consent for, CCP approve its use and ensure that the proper documentation has been obtained on each patient.

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Turkey – Bursa Transfusion Centre

Levent Tufan Kumaş

Question 1

- Type of institution
  - ***Hospital-based Blood Services*** (a hospital unit performing the functions of blood establishment and transfusion services at a hospital level)
- Institution demographics

For hospital
- Number of in-patient beds: 900
- Approximate number of RBCs transfused/year: 22 000
- Age group of patients treated: Neonates, paediatrics <18 years, adults >18 years.
- Type of patients treated: Both medical and surgical.

For blood establishments and blood services
- Whole blood donation number: 20 000/year
- Apheresis donation number for platelets: 1700/year (3300 units of apheresis platelet concentrates prepared totally)

Question 2

We perform *plasmapheresis* for collection of CCP.
Donation frequency permitted for routine plasma donation by apheresis: 48 h (Max. twice a week, totally 33 donations/year).
Donation frequency permitted for CCP donation by apheresis: 10 days (Max. 8 donations in a 3 months period).

Question 3

CCP is collected for *transfusion*, and for compassionate use.

Question 4

Yes. Diagnosis of COVID-19 infection by a laboratory test result (Either PCR test positivity studied from the

nasopharynx swab sample or serologically test positivity for SARS-CoV-2 antibodies) is considered acceptable.
In addition to general blood donor selection criteria:
28 days after complete cessation of COVID-19 symptoms donors considered to be acceptable for CCP donation
Or, 14 days after complete cessation of COVID-19 symptoms with 2 negative PCR test results (One of the tests must have been done within the last 48 h).

Question 5

No.

Question 6

Yes. SARS-CoV-2 IgG assay (Abbott Architect). Chemiluminescent microparticle immunoassay. Predefined index value threshold of 1·4 signal-to-cut-off (S/C) ratio for seropositivity. (Tests are run just before each donation process).

Question 7

No. The samples are collected from the CCP unit and freezed/archived for future assessment.

Question 8

No.

Question 9

No.

Question 10

Yes. The minimum deferral period after recovery before whole blood or apheresis donation is 28 days.

Question 11

No. According to our regulations; these people are considered to be people who have received a blood transfusion, so they cannot donate blood or plasma at least 12 months.

Question 12

- In the first step, we had difficulties in supplying an antibody test that meets the national guideline requirements to be used in our institution to select available donors for CCP apheresis.
- At the beginning of the pandemic (April to June), the donor organization and the referral of suitable donors to our blood centre was done by the local health authority. However, as this organization is not maintained and there are not enough volunteer donors at the moment, most of our CCP donors are replacement donors.
- High donor deferral rate (General blood donor selection criteria, negative anti-SARS-CoV-2 antibody test results, and deferral of female donors due to pregnancy history) and short donation period (Max. 3 months) make it difficult to prepare sufficient number of products.
- Another challenge is not measuring the neutralizing antibody titre in the prepared products before transfusion.

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Turkey – Turkish Red Crescent

Levent Sağdur

Question 1

- Type of institution
- ***National Blood Services/Blood Centre*** (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a national level)
- Institution demographics
  - *Year 2019:*
    - *Blood Donation Number: 2·766·581*
    - *Apheresis Donation Number: 42·656*
    - *RBC production: 2·738·117*
    - *Fresh Frozen Plasma (FFP)Production: 1·390·223*
    - *Pooled platelet production: 281·327*
    - *Apheresis Platelet Production: 44·726*
  - *Year 2020 (11 month):*
    - *Blood Donation Number: 2·126·279*
    - *Apheresis Donation Number: 55·247*
    - *Convalescent Plasma (CP): 35·383*
    - *RBC production: 2·110·140*
    - *Fresh Frozen Plasma (FFP)Production: 1·098·723*
    - *Pooled platelet production: 244·554*
    - *Apheresis Platelet Production: 77·429*
    - *Apheresis CP Production: 63·145*

Question 2

- *Immune plasma donation can be done within a minimum of 14 days and a maximum of 3 (three) months after recovery. The date of the first donation is accepted as the starting date and it can be made 8 times in 3 months with a minimum of 10 days intervals and a maximum of 3 times in a month. A maximum of 1800 ml of plasma can be collected from a donor in one month.*
- *No*

Question 3

- *Turkish Red Crescent is responsible for procurement. Indications for use should be taken from the Ministry of Health or from faults.*

Question 4

- *In order for individuals caught and recovered from COVID-19 virus infection to become donors:*
  - *The diagnosis of COVID-19 infection was made according to the results of the laboratory test (PCR test positivity from the nasopharynx swab sample or the test positivity of SARS-CoV-2 antibodies serologically) AND*
  - *At least 14 days have passed since clinical recovery (cough, fever, shortness of breath, weakness, etc.) AND*
  - *At least 2 PCR test results from nasopharyngeal swab samples must be negative (one of the tests must have been done in the last 48 h).*
  - *If 28 days have passed since clinical recovery, PCR test negativity is not required. The records of these people should be complete, documented and traceable.*

Question 5

No

Question 6

No

Question 7

Yes. ELISA (17 Regional Blood Center) and CLIA (1 regional blood center) (Euroimmun/Roche)
If yes/no, are samples collected from the CCP unit and freezed/archived for future assessment? YES

Question 8

Yes. Mirasol® Pathogen Reduction Technology - Terumo BCT

Question 9

No. These people are considered to be people who have received a blood transfusion, so they cannot donate blood or plasma

Question 10

Yes. 28 days.

Question 11

No. These people are considered to be people who have received a blood transfusion, so they cannot donate blood or plasma.

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Turkey – Acıbadem Health Group Hospitals

Nil Banu Pelit

Question 1

- Type of institution
- ***Hospital-based blood transfusion service/blood bank*** (a hospital unit responsible for pre-transfusion and compatibility testing, and issuing blood for clinical transfusion exclusively for use within hospital facilities)
- Institution demographics
  - For hospital
    - Number of in-patient beds: 2129/16 hospitals
    - Approximate number of RBCs transfused/year: 44 465 between Nov 2019–2020
    - Age group of patients treated (neonates, paediatrics <18 years, adults >18 years): <18 y: 19 500 >18 y: 72 000
    - Type of patients treated (medical or surgical): M:68500; S: 23000
  - For blood establishments and blood services
    - Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable): WB: 1080; Aph: 5800

Question 2

Immune plasma donation can be done within a minimum of 28 days and a maximum of 3 (three) months after recovery. The date of the first donation is accepted as the starting date and it can be made eight times in 3 months with a minimum of 10 days intervals and a maximum of three times in a month.

Question 3

Both.

Question 4

The diagnosis of COVID-19 infection was made according to the results of the laboratory test (PCR test positivity from the nasopharynx swab sample or the test positivity of SARS-CoV-2 antibodies serologically) AND.
At least 28 days have passed since clinical recovery (cough, fever, shortness of breath, weakness, etc.) AND.
At least 2 PCR test results from nasopharyngeal swab samples must be negative (one of the tests must have been done just before donation) AND.
The result of Anti-SARS-Cov-2 antibodies is IgG Positive but IgM Negative.
If there is no symptoms (weakness, cough, shortness of breath, etc.), he/she is accepted for donation.

Question 5

Yes, patients with negative nasopharyngeal swab test were accepted as a suitable donor.

Question 6

Yes, the donors are screened with rapid card tests for anti-SARS-CoV-2 IgG and IgM in donor serum/plasma. The donors who are IgG positive and IgM negative were accepted as donors.

Question 7

No.
Yes.

Question 8

Yes: Cerus Corporation, Intercept Blood System.

Question 9

No. According to our regulations, these people are considered to be people who have received a blood transfusion, so they cannot donate blood or plasma at least 12 months.

Question 10

Yes, at least 28 days have passed since clinical recovery (cough, fever, shortness of breath, weakness, etc.)

Question 11

No. According to our regulations, these people are considered to be people who have received a blood transfusion, so they cannot donate blood or plasma at least 12 months.

Question 12

We had developed a project for CCP collection soon after first case in Turkey was defined. In addition to the described criteria, we also prepared cryopoor plasma and pooled plasma from them in order to decrease the coagulation factors and the effect of antibody-dependent enhancement of infection. We screened the isohemagglutinin titres before pooling. We pooled 8 units in order to have the titres below 1:32. We divided this pooled plasma into 200 ml bags. By this way, we made a composition that can be used by each patient without blood group match. We will soon publish the results of this project. At the beginning of the pandemic the CCP was used in ICU patients who had pneumonia according the MoH guidelines, however the guideline was reviewed and CCP was advised during the first 7–10 days of the infection. We think that immune plasma is more useful at initial days in order to prevent the development of pneumonia or in patients in risk groups such as medical staff and close contacts to infected people.

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Canada – Héma-Québec

Renée Bazin

Question 1

- Type of institution
  - Héma-Québec is the National blood establishment for the Province of Quebec, Canada
- Institution demographics
  - For blood establishments and blood services
    - Approximate number of whole blood donations/year: 220 000

- Approximate number of RBC apheresis collections/year: 2000
- Approximate number of platelet apheresis collections/year: 40 000
- Approximate number of plasma apheresis (750 ml) collections/year: 25 000
- Approximate number of plasma (250 ml) apheresis collections (multiple components)/year: 2500

Question 2

○ CCP donation is done by plasmapheresis only at our institution. We collected CCP between April and July 2020 and will resume collection in January 2021. Donors are allowed to donate every 6 days, in accordance with our standard procedures for regular plasma donations. Between April and July, donors were allowed to donate for up to 12 weeks after their first donation. Starting in January 2021, donors will be allowed to donate for a maximum 6 weeks after resolution of their symptoms. This change is introduced to reflect the waning of antibodies to SARS-CoV-2 (both total and neutralizing antibodies) occurring rapidly after symptom onset. We observed this decline during the characterization of CCP collected from repeat donors at our institution and reported it in several scientific publications [1-3].

Question 3

○ CCP collected between April and July 2020 is used exclusively in one of the three clinical trials for the treatment of COVID-19 patients currently underway in Canada. Plasma collected from January 2021 will also be dedicated to clinical trials but with the possibility of redirecting units not qualified for clinical trials to other uses such as fractionation or research projects, provided that donors have consented to these potential uses.

Question 4

○ Recovered COVID-19 patients are recruited for CCP donation mostly following self-identification and through social media. All participants must have received a diagnosis of COVID-19 by the Québec Provincial Health Authority through either PCR or epidemiologic contact. They also have to meet the donor selection criteria for plasma donation in use at our institution. They are allowed to donate plasma at least 14 days after complete resolution of COVID-19 symptoms (fever, cough), which is the same criterion applied for recovered COVID-19 patients who choose to donate regular blood donations instead of CCP.

○ Males and females with no history of pregnancy meeting the above criteria are invited to donate CCP after informed consent.

Question 5

○ No test to confirm clearance of the infection is required before donation at our institution. The same rule applies to regular blood donations, in compliance with Health Canada regulations.

Question 6

○ Donors are not tested for the presence of anti-SARS-CoV-2 in their plasma prior to donation. Plasma is collected from recovered individuals who meet the criteria for CCP donation and then tested for the presence of anti-SARS-CoV-2 antibodies within one week of donation using an in-house RBD ELISA test.

Question 7

○ All CCP units are tested at our institution using an in-house SARS-CoV-2 RBD ELISA. Seropositivity is defined as an ELISA result above the cut-off value at a 1:100 plasma dilution [1]. This value was calculated using the mean OD + 3 SD of COVID-negative plasma samples (collected before the outbreak of SARS-CoV-2) plus a 15% inter-assay coefficient of variation. Additional tests to further characterize CCP are performed at our external collaborators' laboratories (total spike antibodies, neutralization and ADCC assays).

○ Retention samples for all CCP are kept in case of need for future assessment.

Question 8

○ CCP is not subjected to a pathogen reduction treatment at our institution.

Question 9

○ At our institution, all plasma recipients are deferred from donating blood products for a period of 6 months. The same criterion is applicable to CCP recipients. As a result, these individuals will not be accepted for CCP donations after the deferral period because of the long delay after symptom resolution, which exceeds the 6 weeks donation period set for CCP donations.

Question 10

○ Our institution accepts individuals recovered from COVID-19 infection for standard whole blood or apheresis donations, after a minimum deferral period of 13 days after recovery. In other words, donors are accepted 14 days after complete resolution of their COVID-19 symptoms.

Question 11

○ As for all other recipients of blood products, recipients of CCP are deferred from donating blood products for a period of 6 months after which period they are welcomed to donate blood products.

Question 12

○ When setting up the CCP collection program at our institution, we faced many challenges such as the need to rapidly recruit donors, collect and test CCP while maintaining adequate inventories of other blood products, especially those collected using the same equipment (competition for apheresis machines). Other challenges included the frequent changes in regulatory requirements regarding donor eligibility during program initiation, which led to several adjustments in a short time. In addition, the need to rapidly develop and validate an anti-SARS-CoV-2 assay given the uncertainty over the short-term availability of commercial assays in April 2020. One of the most important lessons learned is that the rapid implementation of a new activity such as CCP program requires coordinated efforts from every service (particularly Operations, QA, Regulatory affairs, IT and Medical Affairs) as well as a clear and collective understanding of the objectives underlying the project. Establishing a CCP collection program in our institution turned out to be a very motivating endeavour for all participants, allowing them to make a difference in the efforts to fight COVID-19.

Section References

1 Perreault J, Tremblay T, Fournier M-J, et al. Waning of SARS-CoV-2 RBD antibodies in longitudinal convalescent plasma samples within four months after symptom onset. Blood 2020;136:2588–91.
2 Beaudoin-Bussières G, Laumaea A, Anand SP, et al. Decline of humoral responses against SARS-CoV-2 spike in convalescent individuals. MBio 2020;11:e02590-20.
3 Prévost J, Gasser R, Beaudoin-Bussières G, Richard J, Duerr R, Laumaea A, et al. Cross-sectional evaluation of humoral responses against SARS-CoV-2 spike. Cell Reports Med 2020;1:100126.

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Saudi Arabia

Salwa I. Hindawi & Maha A. Badawi

Question 1

- Hospital-based blood services
- Institution demographics:
  - Number of in-patient beds: 780 beds
  - Approximate number of RBCs transfused/year: 12 000
  - Age group of patients treated (neonates, paediatrics < 18 years, adults > 18 years) All age groups
  - Type of patients treated (medical or surgical): Medical and surgical for all age groups but without transplant or trauma.
  - Approximate number of whole blood and apheresis collections made/year for whole blood: 12 500, which processed to blood components, apheresis platelets donation around 100

Question 2

Type of CCP donations in our institution is performed through plasmapheresis and donor can donate once or twice one week apart.

Question 3

Collected CCP is intended for transfusion of COVID-19 patients only under clinical trial and only few have been used as compassionate use.

Question 4

The CCP donor eligibility criteria used in our institution are as follow:
Donor should match routine donor eligibility criteria; donors should have history of SARS-CoV-2 infection with positive confirmation for SARS-CoV-2 by PCR testing and complete recovery from symptoms of at least 2 weeks before donation.

There is no need to have a confirmatory test result status of past COVID-19 infection before CCP donation but anti-SARS-CoV-2 neutralizing antibodies titre regularly done for all donors at time of donation.

Question 5

No need to test the CCP donor for SARS-CoV-2 by PCR before donation as he should have complete clinical recovery for 2 weeks before CCP donation.

Question 6

To date, we are testing all donors for SARS-CoV-2 neutralizing antibodies titre before donation through in-house neutralization test using live virus. No other antibody testing is done.

Question 7

We are testing only donors at time of CCP donation for SARS-CoV-2 neutralizing antibody titre testing.

Question 8

At our institution, CCP subjected to a pathogen reduction treatment and we use Intercept system (Amotosalen and UV light treatment).

Question 9

At the current time, the protocol does not clearly exclude recipients of CCP from CCP donation. However, we did not have come across any recipients of CCP whom like to donate. At our institution recipients of blood or blood components are deferred from donating any blood components for 3 months (new policy).

Question 10

Individuals recovered from COVID-19 infection can donate if 28 days have passed since resolution of symptoms, or 28 days after the date of the positive swab if they were asymptomatic.

Question 11

Recipients of blood or blood components (including recipient of CCP) are deferred from donating any blood components for 3 months. This change from the previous policy of deferral for 12 months was based on FDA guidance https://www.fda.gov/media/92490/download

Question 12

We faced few challenges as convincing recovered patients to donate CCP after their discharged from the hospital, as they seem to be worried about their health and contracting infection again. The other challenges are the transfusion protocol, dosing and frequency of treatment.
An important lesson we learned that the recruitment of CCP donors should be started shortly after diagnosis by healthcare professionals in contact with the patient at that early stage and before discharge from the hospital. Awareness and education to the patients and society on the importance of CCP donation and its implication through distribution of educational materials and discussion with patients, through donor campaigns and social media are essential. We should be prepared with effective plan for future pandemics if any.

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India – New Delhi

Gopal K Patidar, Hem Chandra Pandey & Rahul Chaurasia

Question 1

We are a hospital-based blood transfusion service within All India Institute of Medical Sciences (AIIMS), New Delhi, India, that is one of the largest tertiary care academic hospital in North India. Our hospital has more than 2000 in-patient's hospital beds, catering to patients of all age groups i.e. neonates, paediatrics, adults and geriatrics, across all medical and surgical specialties. We received approximately 1 50 000 blood component transfusion requests form annually from various departments out of them around 75 000 red blood cell units issued annually for transfusion. At our institution, there are three blood centres under the purview of Department of Transfusion Medicine, which collects more than 80 000 of whole blood units annually and fully process it into blood components like red blood cells, platelet concentrates and plasma. We also perform pre-transfusion testing and issue the blood components within our hospital and outsource excess plasma for fractionation. We also have apheresis facilities which mainly focuses on platelet (apheresis)

collection and peripheral blood stem collection accounting for collection of 2000 apheresis units. Therapeutic procedures such as plasma exchanges are also quite frequent (400–500 plasma exchanges are performed annually). Apheresis plasma collection was not performed routinely in the pre-COVID-19 period.

Question 2

At our institution CCP donation is done by plasmapheresis, as per the regulations mandated under the Drugs and Cosmetic Act (second amendment) 2020, India [1]. The act recommends "The quantity of plasma separated from the blood of donor shall not exceed 500 ml per sitting and once in a fortnight or shall not exceed 1000 ml per month". Thus, allowing a CCP donor to donate twice a month with maximum collection of 500 ml in each donation.

Question 3

At our institute, CCP collected is intended for transfusions only. During the initial phases of COVID-19 pandemic, CCP transfusions were performed as a part of clinical trial to evaluate its safety and efficacy. Later on, its use was restricted for off label indication or transfused on compassionate grounds.

Question 4

CCP donor eligibility criteria at our institution were in accordance with national guidelines issued by Indian Council of Medical Research (ICMR) [2] and National Blood Transfusion Council (NBTC) [3], India.

Our donor eligibility criteria are as following:

1. Donor age should be 18–60 years.
2. Males or nulliparous female donors of weight >50 Kg.
3. Laboratory confirmed COVID-19 either by RT-PCR *OR* Rapid antigen test.
4. Recovered patient (CCP donor) preferably symptomatic during illness (fever, cold, cough, etc.) and demonstrable anti-SARS-CoV-2 IgG antibodies by available immunoassay.
5. Complete resolution of symptoms at least 14 days prior to donation, RT-PCR negative report is not mandated in this situation. If RT-PCR negative report is available, then the period can be reduced to 14 days from the negative RT-PCR report.
6. Asymptomatic donors may be accepted, if anti-SARS-CoV-2 IgG antibodies are present and verified by two different approved test.
7. In addition, donor eligibility criteria for whole blood donation is being followed in accordance with Drugs and Cosmetics (Second Amendment) Rules, 2020 [1].
8. Complete blood count including Haemoglobin (Hb), Haematocrit (HCT), Platelet count, Total leucocyte count (TLC) and differential leucocyte count (DLC). Donors with Hb > 12·5 g/dl, platelet count >1 50 000 per microlitre of blood and TLC within normal limits are accepted.
9. Donors negative for screening test of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and malaria are accepted.
10. Donors with total serum protein >6 gm/dl are accepted.
11. Titration of anti-SARS-CoV-2 neutralizing antibodies may be done depending on availability of facilities at the time of testing. Unavailability of antibody titres is not preclude convalescent plasma transfusion. Desired titres for IgG antibodies is 1:640 by enzyme-linked immunosorbent assay (ELISA) method *OR* 13 arbitrary unit (AU) by chemiluminescence method *OR* for neutralizing antibodies titre of 1:80 (Plaque reduction neutralization test (PRNT)/microneutralization *test* (MNT).
12. Blood group (ABO grouping and Rh phenotyping) and antibody screening for clinically significant antibodies (Extended Rh, Kell, Duffy, Kidd, MNS) – Antibody screen positive donors are deferred.

Question 5

At our institution CCP donors are not tested for SARS-CoV-2 by PCR method to confirm clearance of the infection before donation.

Question 6

Yes, we do test the CCP donors for presence of IgG anti-SARS-CoV-2 antibodies using chemiluminescence assay in Abbott platform (i1000SR) before donation. We were using all the CCP above the cut-off recommended by the manufacturer (1·4) for differentiating positive and negative samples.

Question 7

Pre-donation testing of blood donors for presence of IgG anti-SARS-CoV-2 antibodies is being done and repeat testing of the collected units is not done. The unit is collected either on the same day of testing or the next day. As we did not have facility for testing neutralizing titre, we do store the frozen sample of CCP for testing in future.

Question 8

Pathogen reduction treatment for plasma is still not in use in our country, so collected CCP units were not subjected to pathogen reduction treatment.

Question 9

As per the regulations laid out in the Drug and Cosmetic Act (Second Amendment), 2020 [1], any blood and blood component recipient should be deferred for any donation until 12 months. Accordingly, we also do not accept recipients of CCP for convalescent plasma donation.

Question 10

At our institution, we accept whole blood or apheresis donation after 28 days of complete recovery after COVID-19 infection as per the guidelines issued by the National Blood Transfusion Council, India [3].

Question 11

As per the regulations laid out in the Drug and Cosmetic Act (Second Amendment), 2020 [1], any blood and blood component recipient should be deferred for any donation until 12 months. Accordingly, we also do not accept recipients of CCP for whole blood and apheresis donation until 12 months after receipt of transfusion.

Question 12

Blood collection by apheresis is not a routine procedure in our country and is limited to only platelet collections at most of the blood centres. Thus, general public in our country is less aware and motivated towards blood component donations through apheresis procedure. Motivating individuals who have recently recovered from severe mental stress due to COVID-19 for apheresis donation was a daunting task. This was subdued by continuous motivation of potential donors, especially by treating clinicians during treatment or recovery and follow-up by the blood centre staff.

Since recruiting donors for CCP donation was difficult amid the national lockdown, we had to modify our approach towards recruitment and screening of the blood donors. A telephonic screening of potential donors by asking the health related questions or sending questionnaire by digital means, allowed only the eligible donors to come at the blood centre. This not only reduced the hardships faced by the donors during travel restrictions but also helped in maintaining social distance. At times, incentivizing the donor in the form of transportation through hospital/medical vehicle, also aided them to come and donate CCP.

Since plasma donation was new procedure for most donors, the total time taken for CCP donation, including waiting time required for testing and due to the limited apheresis equipment was also a demotivating factor for many donors. This was improved by allotting time slots for sample collection and procedure to donors and demarcating dedicated space and equipment for CCP donation.

Apart from donor-related issues, we also faced issues related to the availability of quality antibody assays to screen donors for presence of SARS-CoV-2 antibodies. The availability of neutralizing assays remains a challenge.

However, stringent CCP donor selection criterion remained a major hurdle in the overall process as it reduced the number of eligible donors significantly. A careful analysis of risk vs. benefit of certain less innocuous conditions in the donors and relaxing them, during the pandemic situation can further improve the overall recruitment of donors for CCP.

Section References

1 Drugs and Cosmetics Act 1940 and Rules 1945, (second amendment 11 March 2020), Ministry of Health and Family Welfare, Government of India. (accessed on 19 December 2020) (https://cdsco.gov.in/opencms/opencms/system/modules/CDSCO.WEB/elements/download_file_division.jsp?num_id=NTc2MQ==).

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Brazil

Roberta Maria Fachini, Patrícia Scuracchio & Silvano Wendel

Question 1

- Type of institution: Hospital-based blood service
- Institution demographics:

The Hospital Sírio-Libanês is a not for profit private hospital with 510 in-patient beds that transfuses an average of 6000 red blood cells (RBCs)/year. The hospital receives paediatric and adult patients affected by clinical or surgical diseases. It is a national reference institution for oncological and critical patients, including those that need to be submitted to bone marrow transplantation, solid organ transplants, cardiovascular, orthopaedic, neurological and extensive oncological surgeries.
The Hospital Sírio-Libanês Blood Bank collects approximately 5500 whole blood units and 1750 apheresis, rendering additional 2300 components per year.

Question 2

As no specific single therapeutic measure has been proven efficient for COVID-19 treatment yet, the transfusion of SARS-CoV-2 convalescent plasma (CCP) has been studied through clinical trials.
Only male candidates, who had a moderate/mild disease, and full clinical recovery at least for ≥14 days, with a previous positive RT-PCR, age ranging from 18 to 60 years and body weight >55 kg were accepted as potential CCP donors, according to national guidelines [1,2]. Those approved by a medical interview, were submitted to a second RT-PCR (whose result had to be negative), and had their neutralizing antibodies (nAb) tested by a virus neutralization test (VNT), and anti-nucleocapsid (NP) SARS-CoV-2 IgM, IgG and IgA ELISAs, as described elsewhere [3]. CCP units were collected only via plasmapheresis with a standard 600 mL plasma collection. According to Brazilian guidelines, each apheresis donor is allowed to donate plasma up to four times by apheresis in a two-month period; however, in specific situations, they can donate in shorter period in order to supply the transfusion demand for special patients, as COVID-19 [2].

Question 3

All the collected CCP is intended for transfusion, mostly for clinical trials, and in lesser amount as compassionate use, depending on the medical request. For clinical trial use, admitted patients were confirmed by RT-PCR, being ≥18 years old, and with criteria for severe pneumonia (defined by respiratory distress: oxygen saturation of 93% or less on room air, respiratory rate >30 breaths/min and/or arterial partial pressure of oxygen (PaO_2)/fraction of inspired oxygen (FiO_2) of 300 or less). Patients with preexisting history of anaphylactic transfusion reaction, pregnant or lactating women were excluded from trials [4]. No CCP units were intended as prophylaxis or fractionation use at this moment.

Question 4

Donors had to present a previous positive molecular test (SARS-CoV-2 RT-PCR) collected by nasopharyngeal swab at the time of the diagnosis, and full clinical recovery for at least 14 days after the beginning of their symptoms.

Question 5

Donors approved by the medical examination underwent a second molecular test (SARS-CoV-2 RT-PCR by nasopharyngeal swab), which had to be non-reactive in order to be accepted for donation. Donors who remain reactive by the RT-PCR were invited to perform a third RT-PCR test after additional 14 days. If the subsequent RT-PCR test is non-reactive, the donor was accepted; otherwise, rejected from the CCP collection program [3].
Additionally, donors were tested for blood typing (ABO and RhD), irregular antibodies to red blood cell antigens (immunohematologic tests), and infectious diseases markers (hepatitis B and C virus, human immunodeficiency virus, human T-lymphotropic virus-1/2, syphilis and Chagas disease).

Question 6

Serum samples were collected for a cytopathic effect (CPE)-based virus neutralization test (VNT) carried out with SARS-CoV-2 (Gen-Bank: MT MT350282) and for immunoglobulins (IgA, IgM and IgG) nucleocapsid protein (NP)-based SARS-CoV-2 enzyme-linked immune-sorbent assays (ELISA) [3] at the moment of the first medical interview, together with the second RT-PCR. Only donors with nAb titre ≥160 and a negative RT-PCR were later accepted for donation, regardless of anti-NP results.

Question 7

Likewise, the same tests for anti-SARS-CoV-2 antibodies (nAbs and immunoglobulins) were carried out on donated CCP units, using the same criteria described above.

Question 8

All CCP units were treated with INTERCEPT® (Cerus Corporation, Concord, USA), according to manufacturer's instructions, either individually or pooled two by two.

After treatment, units are separated into 200 ml doses. Pre- and post-treatment samples were tested for nAb titres and specific anti-NP antibodies, with no considerable change both in nAb or anti-NP levels before or after pathogen reduction [5].

Question 9

The answer is no, based on our current protocol, which establishes that only individuals who had mild/moderate SARS-CoV-2 infections can be candidate for donations. In addition, the Brazilian legislation [2], defines that anyone who has received a transfusion of any blood component, could donate blood only after a minimum period of 1 year.

Question 10

Yes, we accept individuals recovered from COVID-19 infection for standard whole blood or apheresis donation, as long as they have had the mild form of the disease and only after 60 days of complete recovery of all symptoms.

Question 11

See question 9.

Question 12

The implementation of a CCP collection program in our blood service was a very important action in order to provide specific immunological support therapy during the pandemic and to analyse the strengths and weaknesses of this program.

One of the main concerns observed was the long persistence of RT-PCR positive for more than 28-day period in 30% of the CCP donors [3], which led us to decide extending the current 28-day to 60-day period for temporarily donor rejection after being infected by SARS-CoV-2. We have also observed that specific IgG antibodies might correlate with high nAb titres as well as an interesting direct relation between body mass index (BMI) and donor nAb titres, suggesting that overweight or obese donors have more capacity to produce higher nAb levels [6].

Another issue to be highlighted was the pathogen reduction treatment (PRT) in all CCP units, whose method was already implemented in our service. To date, PRT is not a national routine in Brazil, although considered an important method for coronavirus inactivation in blood components.

The main challenge is still to define which patients would certainly benefit from this kind of therapy, since large RCT are quite difficult to be implemented in the country at this moment, despite the high number of COVID cases.

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Singapore

Ai Leen Ang & Kiat Hoe Ong

Question 1

Singapore's COVID-19 Convalescent Plasma (CCP) Programme was jointly developed by the National Centre for Infectious Diseases (NCID), Tan Tock Seng Hospital (TTSH) and Health Sciences Authority (HSA). HSA is a National Blood Establishment and TTSH is a tertiary hospital that operates a hospital-based blood transfusion and therapeutic apheresis service.

While TTSH is not usually involved in blood collection, it has been specifically licensed to collect CCP for Singapore due to logistical reasons as it has close affiliations with NCID (Singapore's main referral centre for COVID-19). TTSH is responsible for the selection of suitable CCP donors referred by NCID and the collection, storage and distribution of CCP in Singapore. HSA is responsible for establishing the general blood donation criteria (non-specific for CCP but still need to be fulfilled by CCP donors), freezing and standard donation testing (non-specific for CCP) of the CCP collected by TTSH.

- Institution demographics
  - For hospital (TTSH)
    - Number of in-patient beds: >1700 beds
    - Approximate number of RBCs transfused/year: About 15 000 units/year
    - Age group of patients treated: Adults of >18 years old.
    - Type of patients treated: Medical and surgical
  - For blood establishments and blood services (HSA)
    - Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable): About 117 000 whole blood collections and about 8000 apheresis collections (majority for platelets) per year.

Question 2

TTSH collects CCP by plasmapheresis. The donation frequency for CCP was initially aligned to that of routine plasma donation by apheresis at HSA, which was a maximum of 13 donations per year with at least 4-weeks interval between donations. To allow more frequent CCP collections from donors with high SARS-CoV-2 neutralizing antibody titre, the minimum inter-donation interval was subsequently changed to 2 weeks, provided that the donors' serum albumin and globulin levels before each donation were in the reference range. Despite these changes, most CCP donors were only eligible to donate once or at most twice, mostly due to decreasing neutralizing antibody titres. Only one donor had antibody titres high enough to donate thrice.

HSA has also established a process to convert suitable standard whole blood-derived plasma to CCP. This is meant to supplement the bulk of the CCP collected by plasmapheresis at TTSH. Male blood donors who have made standard whole blood donations will have their blood samples tested for SARS-CoV-2 neutralizing antibody titre if they declare a history of COVID-19 infection within the past 6 months. If their neutralizing antibody titres meet the minimum target, HSA will label their whole blood-derived plasma as CCP. The red cells and platelets from their whole blood donation will still be used for routine transfusion since they are accepted as standard whole blood donors for their donations. So far, there had not been any whole blood-derived plasma suitable for conversion to CCP as the neutralizing antibody titres tested were below the target level. These plasma units were labelled as standard frozen plasma and stored for routine transfusion.

Question 3

The CCP collected in Singapore is only for therapeutic transfusion as part of a monitored expanded access programme. It is not sent for fractionation.

Question 4

Besides the standard blood donation criteria, other donation criteria specific for CCP are:

1. History of laboratory confirmed COVID-19 infection.
   - All diagnoses of COVID-19 infections in Singapore are confirmed by laboratory tests ordered by the clinics or hospitals. This is most commonly performed by SARS-CoV-2 PCR on nasopharyngeal swabs.
   - The above test results are accessible to the NCID team who refer potential CCP donors to TTSH. The team at TTSH would also have access to the relevant COVID-19 diagnostic test results.
2. At least 28 days after clinical recovery from COVID-19.
   - The definition of clinical recovery is aligned to prevailing definition of the Ministry of Health (MOH).
   - Initially, clinical recovery was defined by resolution of fever and all clinical symptoms for at least 24 h and negative SARS-CoV-2 PCR from 2 separate nasopharyngeal swabs taken 24 h apart. This was consistent with the initial criteria for persons with history of COVID-19 infections to be de-isolated in Singapore.

- Subsequently this is changed to a time-based discharge criteria. Since end May 2020, non-immunocompromised persons with history of COVID-19 infections can be de-isolated 21 days after onset of illness without further nasopharyngeal swab PCR, if they are well. Our definition for clinical recovery was also changed accordingly to the time when they could be de-isolated without the need for negative nasopharyngeal swab PCR.

3. SARS-CoV-2 surrogate neutralizing antibody titre of at least 40. This was subsequently increased to at least 80 in early May 2020 when there were more potential CCP donors.

(Initially as a precaution, the donor's blood sample needs to be negative for SARS-CoV-2 PCR before CCP is collected from them. There were plans to remove this requirement but no further CCP donors were screened when the number of eligible donors decreased dramatically since Sep 2020 due to the low number of new COVID-19 infections in Singapore. This requirement will be removed should there be subsequent screening of new CCP donors.)

Question 5

We currently do not test the CCP donor for SARS-CoV-2 by PCR before donation to confirm clearance of the infection.

Question 6

CCP donors would be tested for SARS-CoV-2 neutralizing antibody titre to ensure that they meet the minimum antibody titre before they are accepted for CCP donation by plasmapheresis at TTSH. The titre is performed by a lab at an academic institution (DUKE-NUS Medical School) which has developed the SARS-CoV-2 surrogate virus neutralization test (sVNT) based on antibody-mediated blockage of ACE2-spike protein–protein interaction [1]. The minimum surrogate neutralizing antibody titre was initially set as 40, but this was subsequently increased to 80 in early May 2020 when there were more potential CCP donors.

Question 7

At the time of CCP donation by plasmapheresis at TTSH, the donors' blood samples are sent for SARS-CoV-2 neutralizing antibody titre again using the same method by the same lab stated in response to question 6. The CCP units collected would still be stored for future use even if the neutralizing antibody has fallen below the minimum titre of 80 at the time of donation. However, CCP units with higher titres of 80 and above would be prioritized for use. Samples from the CCP donation are archived for future assessment.

For the conversion of suitable standard whole blood-derived plasma to CCP (see response to question 2 for details), the neutralizing antibody titre for SARS-CoV-2 is also determined using the same method by the same lab as stated in response to question 6. The minimum neutralizing antibody titre is 40 for the whole blood-derived plasma to qualify for conversion to CCP. As a routine practice, all of HSA's standard blood donations would have an archived sample kept for up to 12 months post-donation.

Question 8

The CCP in Singapore is not pathogen reduced. Pathogen reduction technology is currently also not applied to the standard plasma in Singapore.

Question 9

Those who have received any type of blood transfusion (including CCP) would need to wait for 12 months before they can make any blood donation (including CCP). This is aligned to the standard blood donation criteria. Therefore, most patients would likely not qualify as CCP donor if they have received CCP since they would need to be deferred for 12 months after their CCP transfusion, by which time their neutralizing antibody titres will likely fall too low for them to be CCP donors.

Question 10

HSA accepts individuals who have recovered from COVID-19 infection for standard whole blood or apheresis donation, at least 28 days from clinical recovery. The definition of clinical recovery is the same as that for CCP donors (please see response to question 4 for details.

Question 11

HSA accepts recipients of CCP for standard whole blood or apheresis donation at least 12 months from their CCP transfusion. This is aligned to the standard blood donation criteria, which require a deferral of 12 months from any type of blood transfusion, including CCP.

Question 12

The collection of CCP by TTSH, which is closely affiliated with the main referral centre for COVID-19 in Singapore (NCID), helps to overcome several logistical challenges,

such as identifying and recruiting suitable CCP donors. Close collaboration was needed between TTSH and HSA to establish the necessary processes and protocols to facilitate the relevant approvals for TTSH to start CCP collection in a timely manner, when the number of infections was rising in Singapore.

One challenge is in finding donors who meet the target SARS-CoV-2 neutralizing antibody titre. Only about 21% of the donors screened had SARS-CoV-2 neutralizing antibody titre of at least 80. This could be due to the mild infection in these donors who are mostly young and healthy. Prior to revising to a time-based discharge criterion for infected persons, it might also be due to the longer interval from their infection when they could be screened for CCP donation, as they initially need to meet the more stringent de-isolation criteria of two negative nasopharyngeal swab PCRs. This might have contributed to a lower neutralizing antibody titre in some of the potential CCP donors. In the later part of the pandemic, most of the COVID-19 infections affected migrants who originated from malaria-endemic areas. This posed additional challenges in finding donors who were eligible to donate CCP.

An unexpected administrative difficulty that was faced initially was the lack of a service level agreement or memorandum of understanding between TTSH as a CCP supplier and other institutions with ill COVID-19 patients deemed appropriate for CCP infusion, as TTSH is not the blood establishment that supplies routine blood products to these institutions. All the stakeholders subsequently agreed that the CCP supplied by TTSH should not be subjected to the standard administrative requirements for routine blood products and would be best regarded as an urgent blood product needed for a serious life-threatening infection.

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United States – American Red Cross

Pampee Young

Question 1

- Type of institution
  - ***National Blood Establishment*** (responsible for any aspect of the collection, testing, processing, storage, release and/or distribution of human blood or blood components). Including recruitment of donors, screening and selecting blood donors.
  - Institution demographics
    - Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable). American Red Cross collects 4·4 million WB, 1·4 million apheresis collections

Question 2

Every 7 days for a maximum of 8 times over 3 months.
Routine plasma apheresis donors can donate every 28 days. There is no maximum number of donations for them over the year but their plasma volume loss is tracked and we have an upper limit for that.

Yes.
They are labelled as routine. These are our general donors who are coming in to donate because they fully qualify as a blood donor. If we were not doing COVID-19 antibody testing we would not even know these people had SARS-CoV-2 antibodies. Thus, the RBC products manufactured are labelled similar to any manufactured RBC from WB donation.

Question 3

Transfusion for both compassionate use and clinical trials.

Question 4

Yes. PCR, antigen and SARS-CoV-2 antibodies all qualify. 2 weeks from last day of symptoms associated with COVID-19

Question 5

No.

Question 6

If *yes*, please describe the test method and the cut-off criteria for accepting the donor for donation. Ortho total Ig test. A reactive test qualifies the unit.

Question 7

Ortho total Ig for spike 1 protein. A reactive test is permissible for labelling as CCP.
Yes.

Question 8

No.

Question 9

Not for 3 months following transfusion.

Question 10

Yes. Two weeks from last date of symptoms.

Question 11

Three months after transfusion

Question 12

The challenge has always been recruiting and retaining appropriately qualified donors. Most hospitals want to transfuse ABO matched plasma. Finding sufficient AB and B donors has been challenging.

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Finland

Jarkko Ihalainen & Antti Vierikko

Question 1

Finnish Red Cross Blood Service is the only Blood Establishment in Finland. Our laboratory and production are located in Helsinki where also our sole apheresis clinic resides. We collect and process whole blood from the whole country with 11 permanent offices and mobile drives.
Blood collection in Finland is being adjusted according to demand of red cells primarily and it has been diminishing slowly for the last 10 years. Currently we perform little less than 200 000 whole blood collections/year. We do not collect plasma with apheresis with the exception of a few units of IgA deficient plasma/year. Platelet apheresis is performed for HLA/HPA typed units and to maintain stocks in special situations. The number of thrombapheresis/year is ca. 2500, little more than 10% of collections.

Question 2

We only collect CCP with apheresis. We set up the procedure based on the plasmapheresis for IgA deficient plasma on our Terumo BCT Trima Accel v6.0 machines. We also configured the CCP product in our newly implemented Microsoft CRM/AX based blood establishment IT system with ICCBBA/ISBT codes, which were slightly adjusted, to our process needs. The products are labelled as CCP COVID-19 and in addition they are labelled for "research use only" because we only deliver to a clinical trial which has its own specifications for the products.
Our donors come from Helsinki University Central Hospital/University of Helsinki clinical research protocols for COVID-19 follow-up, i.e. all our donors are clinical trial participants as well as blood donors. We have set the maximal number of donations to five and the minimum interval between donations to 2 weeks (14 days). Only one donor so far has donated four times and the donation interval has generally been above two weeks. Since we only perform 1–3 plasmapheresis per year in normal circumstances all of this was new.

Question 3

Our CCP programme was built together with a clinical research group in Helsinki University Central Hospital. There has not been significant interest in CCP from other hospitals in Finland. Combined with the recommendations from the EU Commission, our local Competent Authority (Fimea) and WHO this cooperation made it clear that we only provide CCP for a clinical trial, which has not yet been registered to EU Clinical Trials Register. No compassionate or monitored use has occurred in Finland. We do neither currently have an agreement with industry on providing plasma for hyper-Ig production.

Question 4

Our CCP donors need to be eligible for donation according to our routine blood donor criteria. In addition, all of them come from follow-up cohorts who have had a PCR-confirmed COVID-19 infection and their recovery has been controlled and documented. We accept both female and male donors; the females are tested for anti-HLA-antibodies before donation.

Question 5

At first, we required negative COVID-19 PCR for eligibility but later this was changed to 14 days post full recovery. This is practicable since the donors come from follow-up cohorts so the timeline of recovery has been documented.

Question 6

Our research collaborators tests the recovered patient cohorts and they target the invitations to participate in CCP donation research according to nAb levels. The research group laboratory uses an in-house microneutralization assay [14] [1].

Question 7

In addition, we send aliquots from the donated CCP units to be tested by our collaborators and re-invite persons who have maintained adequate nAb levels. However, nAb levels are not used as a release criteria for our units as long as they only go to the clinical research project which is randomized and double-blinded for no plasma or high or low nAb concentrations.

Question 8

Pathogen reduction/pathogen inactivation has not been our routine and we have not implemented it for CCP.

Question 9

So far, no CCP transfusions have taken place in Finland so this question has not been completely solved. In principle, normal rules for donor health and transfusion requirements would be followed in addition to the CCP donor requirements.

Question 10

We accept donors recovered from COVID-19 after 28 days in mild cases and 3 months after hospital treatment if they fulfil the other criteria. Quarantine after transfusion is 4 months so in practice this would be the limiting factor in these cases.

Question 11

As in question 10, we accept the recipients of CCP according to the normal donor rules, in practice at a minimum of 4 months after the transfusion.

Question 12

Early in the pandemic when we started the CCP project we had problems in getting apheresis collection sets from the manufacturer. In addition, we were in the final stages of implementation of our new blood establishment IT system, which took a significant part of the personnel resources. Later we have had problems with the two-way clinical trial setting since both our donors and the recipients of CCP are participants in clinical trials. This has meant delays with several updates and Ethical Board applications for the protocols according to the cumulating clinical data and research findings. Finding an adequate number of donors in all blood groups is still a challenge. We have decided to stick to the ABO compatibility rules even though also cross-ABO protocols have been published (Focosi and Farrugia 2020) [2].

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China

Yan Qiu, Ru Yang & Hua Xu

Question 1

- Type of institution
  - ◯ ***Regional Blood Services/Blood Centres***
- Institution demographics

The WB collection requirements for blood donor are the same all around the nation. The volume of WB collection can be 200ml, 300ml and 400ml, excluding anticoagulant and samples for test, which be selected by WB donors voluntarily during the pre-donation questionnaire. In China, the volume of 1 Unit (U) WB is 200 ml, namely 1 U = 200 ml.

According to the national regulation, the apheresis mainly collects the single donor platelets, while RBC and plasma are barely collected by apheresis in China. But 1 platelet apheresis procedure may produce 1 therapeutic unit (TU, 1TU = $2{\cdot}5 \times 10^{11}$ plts) PLTs, 1·5 TU PLTs, 1TU PLTs plus 2 U plasma or 2TU PLTs. The PLTs apheresis strategies vary among blood services with the equipment used.

- BRCBC
  - WB collections was approximate 450 000 U/year and apheresis collections was 68 000 TU/year for platelet and 3000 U for plasma.
- WHBC
  - WB collections was approximate 350 000 U/year and apheresis platelet was 70 000 TU/year.
- SXBC
  - WB collections was approximate 320 000 U/year and apheresis platelet was 40 000 TU/year

In the early February 2020, the national health authority issued a notice on Treatment COVID-19 patients with CCP affiliated a guideline for CCP collection. The guideline noted that the local COVID-19 patient designated hospitals were responsible for mobilization and recruiting CCP donors. The local blood centre implemented the CCP collection and preparation [1]. The detail of process varied among blood centres. For BRCBC and SXBC, the CCP collection were carried out in a blood donation mobile which equipped to ensure the safety of CCP donor, staff and the public. For WHBC, the CCP collection performed at 5 separated sites from the voluntary blood donation sites. The personal protection, disinfection and operation were taken strictly following the recommendations.

Question 2

Only *plasmapheresis* is collected in China. CCP collection was performed following the plasma collection instruction of plasmapheresis procedure. The CCP donation interval is no less than 14 days and no more than 24 times a year, which is the same as the mandatory requirements of platelets apheresis by national guide for voluntary donors. In fact, the ratio of the repeat CCP donors is lower than that of regular blood donors from BRCBC and SXBC.

Question 3

Generally the CCP was collected intend for *transfusion*. BRCBC and SXBC collected CCP only for prescription treatment-use. WHBC for trial use in the early stage, later for prescription treatment-use.

Question 4

The CCP donor eligibility criteria:

(1) diagnosed with COVID-19;
(2) met the criteria of both disisolation and hospital discharge, including temperature returned to normal more than 3 days, respiratory symptoms improved significantly, pulmonary imaging showed significant improvement in acute exudative lesions, negative nucleic acid test (NAT) for two consecutive respiratory specimens (nasopharyngeal swab sampling time at least 24 h. apart);
(3) had recovered from COVID-19, no COVID-19 symptoms and had been discharged from the hospital for more than 2 weeks;
(4) it is at least 3 weeks after the onset of symptoms prior to CCP donation [2];
(5) qualification for blood donation health examination requirements, such as aged 18–55 years; weight should be more than 50 kg for male, and 45 kg for female. Alanine transaminase (ALT) testing result was below 40 IU/L. HIV Ag/Ab, anti-HCV, anti-syphilis, and HBsAg were all negative; NAT for HIV, HBV, and HCV were negative [1].

In addition for WHBC, they preferred to select CCP donors who had a fever lasting more than 3 days or a body temperature exceeding 38·5°C (101·3°F), and who intended to donate 4 weeks after the onset of symptoms [3,4].

Yes, SARS-CoV-2 ID-NAT must be negative for blood sample; anti-SARS-CoV-2 IgG antibody titre $\geq$ 1:160 or the qualitative test of the anti-SARS-CoV-2 total antibodies in serum/plasma is reactive and the test is still positive after the 320-fold dilution according to "Clinical treatment plan of convalescent plasma from recovered COVID-19 patients" [1].

The definition of recovery for donor eligibility criteria were stipulated in the "Diagnosis and Treatment of New Coronavirus Pneumonia" [2], and clinicians make judgments based on these regulations.

Question 5

Not for BRCBC and SXBC which had no capability to conduct SARS-CoV-2 NAT which must be carried out in qualification Lab approved by certificate authority. Before the blood donation registration, the clinical hospital recruited the CCP donor and tested SARS-CoV-2 through PCR to confirm clearance of the infection and conveyed the results to blood centre.

But WHBC performed real-time reverse transcription PCR (RT-PCR) for SARS-CoV-2 RNA by using MultiScreen Pro RT-PCR assay (SYM-BIO LifeScience, they performed pool testing by mixing 6–8 plasma samples or individual testing by using 1·6 ml of plasma, eluting 100 μl of nucleic acid template and added 40 μl of it to the RT-PCR mix [5].

Question 6

The answer is no for both BRCBC and SXBC. BRCBC had send the samples to cooperated certificated Lab to detect SARS-CoV-2 NAT and anti-SARS-CoV-2. SXBC accepted the tested results from the hospitals, which recruited the

CCP donors for blood centre to collect plasma. Yes for WHBC.
The anti-SARS-CoV-2 antibodies were detected by enzyme-linked immunosorbent assay (ELISA), which the 96 well microplate coated with recombinant RBD or N polypeptides per well. The samples were 20 times diluted by the phosphate-buffered saline, after incubation, washing, enzymed, incubation, washing, then adding chromogenic reagent and termination solution, finally we observed the plates for the horseradish peroxidase reaction. The OD values were calculated by measuring the change in the absorbance at 450 and 630 nm using an automatic microplate reader. Results were reported as the S/CO value, calculated as the ratio of the OD value to the cut-off value. The sample S/CO value ≥cut-off value was considered reactive, otherwise non-reactive. Titres were reported as the highest dilution when the ELISA assay was still reactive [6].

Question 7

Yes.
The ELISA to measure the anti-SARS-CoV-2 antibodies. The anti-SARS-CoV-2 IgG titre ≥ 1:160 or the total antibodies titre ≥ 1:320 as baseline for accepting for transfusion.
BRCBC, WHBC and SXBC collected the samples at the end of apheresis for future assessment and study.

Question 8

Not for BRCBC and Yes for WHBC and SXBC.
In China, there is only Methylene blue (MB) light treated plasma disposal set get the license from SDA. The pathogen reduction treatment for plasma is still optional.

Question 9

No. We all do not. According to national "health examination requirements for blood donor", those who have received whole blood and blood components within 1 year cannot donate blood again.

Question 10

Yes.
We accept individuals who had recovered from COVID-19 and had been discharged from the hospital for more than 6 months for standard whole blood or platelet apheresis donation.

Question 11

Not yet at present.

As mentioned in answer to question 9, one year deferral for blood transfused recipients. Another reason is that it will take a long time to evaluate whether individuals recovering from COVID-19 infection have other potential health impairments, and whether they can recover or how long it will take to recover to normal. Therefore, it is still too early to discuss whether to accept individuals recovered from COVID-19 infection as normal blood donors. At present, according to the regulations, we will not accept it for at least five years.

Question 12

Lack of enough background knowledge of COVID-19 and protection measurement that are vital for staff.
No experience in collection and training of COVID-19 CCP in a short period time.
Prepare all the equipment, facilities under contingency situation.
The relative guideline updated frequently.
Set up rapidly good communication with the cooperated hospitals and other institutes.
It is very encouraging that the CCP donors in WHBC are very enthusiasm and half of them become loyal repeat blood donors. WHBC had a donor who gave 13 consecutive donations!
Most of the CCP donors in WHBC's pilot program had an S-RBD-specific IgG titre of ≥1:160. As time goes on, the antibody titres of different donors show differences. Some donors lost their antibodies very quickly; some of them lasted for more than half a year.
Whether the CCP effect will be reduced by the pathogen reduction treatment?
We all believe that the problem was resolved and the process was smooth under the great effort of blood people.

Section References

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Israel

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Question 1

- Respondent demographics
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Magen David Adom is a *National Blood Establishment* (responsible for any aspect of the collection, testing, processing, storage, release and/or distribution of human blood or blood components).

- The approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (based on 2019):
  - 265 000 whole blood units, from them: 150 000 random donor platelet units were separated and 90 000 fresh frozen plasma units were separated.
  - 1750 apheresis plasma units (each divided into 3 units of plasma).
  - 550 units single donor apheresis platelet units.

Question 2

2a. The donor is allowed to donate CCP every 2 weeks till anti N antibodies (Abbott, USA) level drops below S/C0 of 4. No more than 6 times in a 100 days period.
2b. This differs from donation frequency for routine plasma by apheresis. Routine plasma donors are invited every 4 weeks.
2c. The plasma from Whole blood donors can be used as CCP if 28 days elapsed from the full recovery from COVID-19. The cellular components are labelled as regular components. This decision is based on the FDA/AABB and the Israeli Ministry of Health regulations.

Question 3

CCP is collected both for compassionate use and for trial use.

Question 4

4a. SARS-CoV-2 PCR test is acceptable for CCP donor eligibility.
4b. Donor eligibility for CCP is 14 days following recovery with 2 negative SARS-CoV-2 PCR test results or a letter from the treating physician stating full recovery.

Question 5

No, the donor is not tested for SARS-CoV-2 by PCR before donation to confirm clearance of the infection.

Question 6

Yes. We perform a Point of Care rapid test by lateral flow that detects anti S antibodies (PharmaAct, Germany) to avoid collection of CCP with donors with no-detectable antibodies.

Question 7

7a. We use Abbott SARS-CoV-2 anti N on each donor. The CO is 1·4. Only units with antibodies levels above S/CO of 4 are used for transfusion and units with antibody levels above 1·4 are used for fractionation.

7b. We also archive samples for future tests.
7a. We use Abbott SARS-CoV-2 anti N on each donor. The CO is 1·4. Only units with antibodies levels above S/CO of 4 are used for transfusion and units with antibody levels above 1·4 are used for fractionation.
7b. We archive samples for future tests.

Question 8

No, CCP is not subjected to a pathogen reduction treatment.

Question 9

Recipients of CCP for convalescent plasma donation are accepted only 6 months post-transfusion.

Question 10

Individuals recovered from COVID-19 infection are accepted for standard whole blood or apheresis (platelet or plasma) donation after 28 days.

Question 11

Recipients of CCP for standard whole blood or apheresis (platelet or plasma) donation are accepted after 6 months post-transfusion.

Question 12

The challenges faced and lessons learned from establishing a CCP collection program are to:

1. Set up a multi-disciplinary project, involving the Authorities, treating physicians, national blood services, hospital blood banks.
2. Provide potential donors with the information of the importance of donations of CCP, for the treatment of patients with moderate (and sometimes severe) COVID-19.
3. Perform POC quick antibodies test to avoid collection of CCP from people with no or low level of antibodies.
4. Provide CCP ASAP to the patients (no later than 72 h of physician request).

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Argentina

Carlos Alberto Gonzalez, David Martin Ferrari & Paula Verónica Cini

Question 1

We work on a Hospital-based blood transfusion service with blood bank (a hospital unit responsible for blood collections, pre-transfusion and compatibility testing, and issuing blood for clinical transfusion exclusively for use within hospital facilities). Hospital de Infecciosas Francisco Javier Muñiz is a monovalent hospital; it serves only patients with infectious diseases.
The hospital has 421 beds, there are approximately 587 RBC's transfused/year (without pandemic) attending to both adult and paediatric patients. Patients admitted for medical or surgical reasons are treated. Our blood bank collects 600 blood donors per year.

Question 2

Our Institution performs CCP donation for plasmapheresis and whole blood, following the guidelines issued by our Ministry of Health of the Nation [1]. There are no differences in frequency regarding routine plasma donation by plasmapheresis.
A donor may be subjected to plasmapheresis up to 1 time every 2 weeks. In any case, the volume of plasma extracted per session should not exceed 600 ml (not counting the anticoagulant, i.e. about 650 ml of anticoagulated plasma), 1000 ml in a week and 15 litres in a year.
Red blood cells concentrates and platelets obtained from whole blood donation from convalescent donors were used for standard transfusions because of the following main reasons. First, according to our donor eligibility criteria and literature, SARS-CoV-2 transmission by transfusion has not been demonstrated. Secondly, our recipients were all patients with COVID-19. Finally, the protocol was approved by our Institutional Ethics Committee.
These components are labelled as convalescent donation.
We always try that patients receiving multiple components receive those components from the same donor.

Question 3

From the beginning of the pandemic in our country, our institution has collected CCP for an institutional clinical trial (NCT04468009) [2] and an extended access protocol.

Questions 4, 5, 10

The CCP donor eligibility criteria used in our institution changed according to the guidelines by the Ministry of Health of the Nation.
Since April 4th, people with a full resolution of symptoms at least 14 days prior to donation and 2 negative PCR SARS-CoV-2 tests performed with an interval of at least 24 h from nasopharyngeal sampling were accepted as donors. Since May 12th, people with a full resolution of symptom at least 14 days prior to donation with negative PCR SARS-CoV-2 (1 or 2) tests from nasopharyngeal sampling performed with an interval of at least 24–48 h were accepted as donors. Since July 2nd, people can be accepted to donate 28 days after symptom resolution (if a test has not been performed); at the same time, they can be accepted to donate 14 days after symptom resolution with a negative PCR SARS-CoV-2 test.

Questions 6 and 7

For detection and titration of IgG anti-SARS-Co-V-2 antibodies in blood donors, a manual enzyme immunoassay in microplate was performed at our institution. This assay (COVIDAR IgG, Conicet-Leloir Institute) uses the spike and RBD (receptor-binding domain) proteins as antigenic source corresponding to SARS-CoV-2 expressed in cell culture. The cut-off criterion was >160 until 19th October; since then, a title >800 or R.P. > 4·0

Question 8

We never used a pathogen reduction methodology in CCP.

Questions 9 and 11

According to our national standards [1], all transfusion recipients can be accepted as blood donors after 1 year of being transfused.

Question 12

The current pandemic is an extraordinaire and unexpected event for blood services and has allowed us to learn some lessons.
In a very short time, we had to support the continuous training of physicians and technicians in biosecurity, ethics, good clinical practices and teleworking. According to the evolution of the pandemic and successive changes in the definitions of the Ministry of Health, the donor interview and the clinical trial CCP were regularly updated.
We had to learn how to make the operation of the service more flexible in order to adapt to a new context of public health. The changes that were usually implemented in a long time, we had to implement them in days or hours. In Argentina and because of the effects of the pandemic, the ASPO (Preventive and Mandatory Social Isolation) began on 20th March, which reduced the concurrence of blood donors by more than 90% in June and we managed to recover quickly in July by reaching and tripling the historical level of blood donation to our PCC-collection program. This was achieved by coordinating with other services of our institution that treat patients with COVID-19 so that once they recover, they invite them to donate plasma. More importantly, to facilitate the concurrence of donors an email address and cell phone ad hoc and social networks contact were implemented.
On the other hand, the pandemic shows the worst and the best of society. The worst accounts for the mass media and fake news. The best, the solidarity of our patients who spontaneously came to donate plasma, achieving a quick change of reposition donor (majority in our environment) to voluntary convalescent donor.
Finally, it is clearly that human resource is a critical and essential component in the management of health service. These continuous changes were possible in the light of the commitment of the members of the hemotherapy service.

Section References

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2 https://clinicaltrials.gov/ct2/show/NCT04468009

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Indonesia

Robby Nur Aditya

Question 1

- Type of institution
  - ◯ ***National Blood Services/Blood Centre*** (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a national level)
- Institution demographics
  - ◯ For blood establishments and blood services
  - ■ Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable)

We have collected 3 523 982 Whole Blood Donation in 2019

Question 2

Plasmapheresis.
If *plasmapheresis* is collected, how many times is a donor allowed to donate CCP, and over what time period?
Donor allowed to donate CCP between 3-6 times, every 2 weeks, and will depends on the antibody titre.

Question 3

For transfusion only.
Trial use.

Question 4

Requirements are:
- 18–60 years old
- Body Weight ≥ 55 kg
- History of positive swab with at least 1 negative result
- 14 days free of symptoms

SARS-CoV-2 PCR with Swab test.
14 days with no symptoms and negative result for PCR

Question 5

No.

Question 6

Yes, the test method is Rapid test with cut-off criteria is 1/80.

Question 7

Same with question 6.

Yes, samples will archived for future assessment.

Question 8

No.

Question 9

Yes.

Question 10

Yes.
14 days with no symptoms

Question 11

Yes.
1 year

Question 12

- Lack of donor to donate CCP due to social stigma
- 'Big hope' from patients and family for CCP treatments
- More education, information and socialization about CCP to community
- Apheresis or plasmapheresis more better known as an alternative donation for public

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India – Chandigarh

Ratti Ram Sharma, Suchet Sachdev, Rekha Hans & Divjot Singh Lamba.

Question 1

Type of institution.
Hospital-based Blood Transfusion Service/Blood Bank (a hospital unit responsible for pre-transfusion and compatibility testing, and issuing blood for clinical transfusion exclusively for use within hospital facilities).

Institution demographics.

For hospital (2019–2020).
Number of in-patient beds: 1740.
Approximate number of RBCs transfused/year: 1 35 685.
Age group of patients treated (neonates, paediatrics <18 years, adults >18 years): All age groups.

Type of patients treated (medical or surgical): Medical and Surgical.

For blood establishments and blood services (2019–2020). Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable).
Whole blood collection: 57 842.
RBCs: 56 962.
Platelets: 26 460.
Plasma: 51 411.

Question 2

Our institution performed Plasmapheresis for CCP donation.

- ○ Most of the donors donated for single time, however few donors donated twice or thrice (one donor only) after an interval of more than 14 days of previous donation. The donation frequency did not differ from that permitted for routine plasma donation by apheresis.
- ○ Whole blood was not collected for CCP donation at our centre.

Question 3

CCP collected is intended for transfusion to the patients fulfilling criteria for convalescent plasma (Off Label) under Investigational therapies (Version 4, 27·6·2020) Ministry of Health and family Welfare, India notification, or as updated from time to time. Initially, it was done as a part of PLACID trial (Clinical Trial Registry of India CTRI/2020/04/024775) and later for compassionate use also. Our institute started facility of plasma bank to support COVID-19 convalescent plasma therapy to the patients admitted in COVID-19 hospital facility of our institute as well as patients admitted in hospitals outside our institute.

Question 4

Eligible donors are men or nulliparous women with age between 18 and 65 years, weight more than 50 kg, were diagnosed COVID-19 by confirmed RT-PCR test result and had experienced symptoms of COVID-19 with at least fever and cough. Additionally, the symptoms must have completely resolved for 28 consecutive days before donation or a period of 14 days before donation with two negative RT-PCR test results for SARS-CoV-2 from nasopharyngeal swabs collected 24 h apart. All routine screening tests, including ABO blood grouping and antibody screening; Rh D phenotype; complete blood counts; screening for HIV, hepatitis B or C virus, syphilis, and malaria; and total serum protein were conducted as per Indian Council of Medical Research protocol for convalescent plasma use in COVID-19 patients version 1·5 dated 11th May 2020 being followed and donor eligibility criteria for whole blood donations in accordance to the Drugs & Cosmetics Act 1940 and rules 1945 therein (as amended till March 2020 [1]).

Yes, our donors need to have a confirmatory test result status of past COVID-19 infection before CCP donation.

- ○ RT-PCR test results for SARS-CoV-2 from nasopharyngeal swab is considered acceptable.
- ○ Complete resolution of symptoms for 28 consecutive days before donation or a period of 14 days before donation with two negative RT-PCR test results for SARS-CoV-2 from nasopharyngeal swabs collected 24 h apart and reactivity for SARS-CoV-2 IgG by Chemiluminescence method was tested for recovery in our donors.

Question 5

No, our institution does not test the CCP donor for SARS-CoV-2 by PCR before donation to confirm clearance of the infection.

Question 6

Testing of SARS-CoV-2 antibody by Chemiluminescence method (Ortho Clinical Diagnostics) with a minimum acceptable signal-to cut-off ratio of ≥13·0 as per guidelines of Indian council of medical research (ICMR), under Ministry of Health and Family Welfare, Govt. of India and protocol for convalescent plasma use in COVID-19 patients version 1.5 dated 11th May 2020 being followed and subsequent Evidence Based Advisory to address Inappropriate Use of Convalescent Plasma in COVID-19 Patients by ICMR dated 17/11/2020.

Question 7

No, not tested as of now but the samples are being collected from CCP unit and being frozen for future assessments.

Question 8

CCP was not subjected to any pathogen reduction.

Question 9

No, until 1 year, CCP/Blood or Blood component recipients are deferred from routine blood donations and convalescent plasma donations as per ICMR protocol for

convalescent plasma use in COVID-19 patients version 1.5 dated 11th May 2020 being followed and donor eligibility criteria for whole blood donations in accordance to the Drugs & Cosmetics Act 1940 and rules 1945 therein (as amended till March 2020 [1]).

Question 10

Yes, they are accepted for whole blood and platelet donations after they have completely recovered from COVID-19 infection. Minimum deferral period after recovery is 28 days.

Question 11

No, until 1 year, CCP/Blood and blood component recipients are deferred from routine blood donations as per ICMR protocol for convalescent plasma use in COVID-19 patients version 1.5 dated 11th May 2020 being followed and donor eligibility criteria for whole blood donations in accordance to the Drugs & Cosmetics Act 1940 and rules 1945 therein (as amended till March 2020 [1]).

Question 12

1. The information, education and communication to motivate convalescent plasma donors was a challenging task for the Transfusion services that were already grappling with issues of motivating and recruiting enough whole blood donors to meet the blood and blood component demands of patients coming to us with non-COVID indications such thalassaemia, pregnant women, cancers and other medical and surgical emergencies.
2. The arrangements of logistics for blood donors transport, critical supplies for testing blood and the extra measures that were instituted for maintain safety of the blood donors and the staff.
3. The donors were not having knowledge about convalescent plasma donation when they were under medical supervision in designated hospitals or quarantine centre's or when they were being tele-counselled about the do's and don'ts about the corona virus disease 2019 (COVID-19).
4. Donors were hesitant to come to hospital-based blood centres for the plasma donation, as they were apprehensive about the fact that their immunity will be weakened, and may be susceptible to infections (COVID and Non-COVID).
5. Donors had residual issues after recovering from COVID-19, in form of generalized weakness, muscle pains, residual cough, shortness of breath on mild exertion etc. Donors having any of these symptoms were excluded from the CCP Donation.
6. Many COVID-19 recovered patients were asymptomatic and did not have explicit symptoms suggesting a systemic immune response like fever. Whereas others of age less than 18 or greater than 60 years and multiparous females were not included as potential plasma donors.
7. Donors, who were initially willing, were finally not available as they were mostly struggling to step up daily routine due to the financial and business disruption caused by the pandemic.
8. Many potential donors were not fit to be included in the program due to reasons like uncontrolled DM/HTN, low haemoglobin, deranged Liver Function Tests and significant weight loss.
9. Initially there was no clear consensus on IgG antibodies cut-off for the different testing platforms (ELISA, CLIA, Enhanced CLIA, Electrochemiluminescence), later the national guidelines provided signal-to-cut-off ratio of ≥13·0 for prospective CCP donor eligibility.
10. The lesson learnt was the need for a dedicated donor recruitment cell in the blood centre with tele calling facility to motivate whole blood and plasma donors as part of the disaster management plan of the blood centre.

Section References

1 Government of India. Drugs and Cosmetics Act 1940 and Rules 1945, amended. 2020. https://cdsco.gov.in/opencms/opencms/system/modules/CDSCO.WEB/elements/download_file_division.jsp?num_id=NTc2MQ==(accessed 10 Apr 2020).

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Norway

Lise Sofie H. Nissen-Meyer.

Question 1

- In Norway, a national cooperation (NORPLASMA COVID-19 [1]) uniting all the major regional/hospital-based blood services was established in April 2020 in an effort to provide CCP to patients. The project applied national rules in coherence with European guidelines, for collection, testing and storage of CCP. Recently, an initiative to test anti-SARS-CoV-2 for all collected units on one platform has been taken. In addition to production and testing of CCP, the project also includes design of clinical studies to evaluate effect of CCP treatment in patients. The description provided here thus includes all blood centres participating in the national project.

○ Institution demographics

Altogether, Norwegian blood banks collect ~175 000 whole blood units per year (2018) and perform 16 000 apheresis procedures (2000 RBC, 5,000 TRC, 9000 plasma). We serve all the hospitals in the country and all types of patients are treated.

Question 2

Norwegian blood banks collect CCP through plasmapheresis, thrombapheresis procedures and from whole blood.

Collection by plasmapheresis is allowed 4 times over at least 4 weeks as long as the donor is eligible for donation. This is more often than our regular plasma donation frequency (monthly or twice a month).

When plasma is collected from whole blood donations, the derived RBCs and platelets are used for standard transfusions. This was decided based on the guidelines from ECDC/EBA, recommending that all donors are quarantined >4 weeks following recovering from COVID-19 illness or positive PCR test before allowed to donate whole blood. Since no transmission of SARS-CoV-2 has been shown, this is considered safe. The products (apart from plasma) are not labelled in any specific way.

Question 3

In Norway, collected CCP is intended for transfusion if it contains a sufficient amount of antibodies, and if not, the plan is to use the plasma for fractionation.

The intention for collection of CCP is both compassionate use and trial use. In Norway, we have not succeeded in obtaining financial support for an RCT, therefore, the use we have had this far is only compassionate use. However, all patients are asked to join a monitoring study to collect data to evaluate the treatment, as recommended in EU/EBA guidelines.

Question 4

All CCP donors have to fulfil the eligibility criteria for ordinary blood donors in Norway.

Towards the end of the year, the donors need to have a confirmatory test result before CCP donation. In the springtime when testing was limited to very strict criteria, we collected plasma from some donors based on anamnestic information, and a majority of these had antibodies.

At present, either a positive PCR test at the time of infection, or a positive antibody test later, is considered acceptable. Antibody levels are being measured at every donation.

Recovery is defined by the absence of acute symptoms including fever, cough, other upper airway symptoms, headaches. Prolonged loss of smell/taste or intermittent, short episodes of fatigue are not deferral reasons in itself, if the donor feels generally recovered and is back in normal routines.

Question 5

In Norway, we do not test the donor by PCR before donation to confirm clearance of SARS-CoV-2 infection. All donors are quarantined for 28 days following recovery as described in Q4.

Question 6

In Norwegian blood centres, some donors are tested for anti-SARS-CoV-2 antibody levels before donation, but most are tested in connection with donation and the decision to use CCP is taken later.

We have recently agreed to use an in-house test showing inhibition of binding to the ACE2-receptor as the national test method for CCP, with a cut-off which has not been finally calculated yet but most likely will be somewhere around 60%.

Question 7

CCP units are tested for anti-SARS-CoV-2 antibodies using at least two of the following: total antibody testing (Commercial tests from different providers), in-house multiplex tests developed with peptides to bind anti-RBD and anti-nucleocapsid protein, inhibition of binding to ACE2 and conventional neutralization testing of selected units. Final cut-off values have yet not been established. In the initial phase, we have used a combination of

antibodies against RBD and nucleocapsid as release criterion. When data from inhibition and neutralization assays now is available, the release criterion will be changed accordingly. In addition, samples collected from all CCP units are freezed/archived for future assessment.

Question 8

CCPs are not subjected to a pathogen reduction treatment. The prevalence of transfusion-transmitted infectious agents is very low in Norway, and the use of only approved blood donors further contributes to product safety.

Question 9

No recipients of CCP have offered to donate convalescent plasma yet, but the decision whether such person could donate plasma would depend upon the general eligibility for blood donation of this person (see question 11).

Question 10

Norwegian blood banks accept individuals recovered from COVID-19 infection for standard whole blood or apheresis (platelet or plasma) donation following a recovery time of at least 28 days.

Question 11

Recipients of CCP can be accepted for donation of standard whole blood or apheresis if they are otherwise eligible as regular blood donors. However, according to Norwegian rules, receiving an allogeneic transfusion elicits a minimum deferral period of 6 months.

Question 12

The most challenging part of establishing a CCP collection program has been to develop and validate testing methods. This is time-consuming and depends on strong collaboration with the laboratory developing the tests. Also, the handling and separate workflow concerning these donors have been challenging, as all requirements for regular blood donation and transfusions had to be met. Lastly, the blood banks did not receive additional resources to implement the CCP program. However, knowledge and experience have been gained and we are probably better prepared for a new pandemic.

Section References

1 NORPLASMA covid-19. Available at: https://www.ous-research.no/NORPLASMA

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Dana V. Devine.

Question 1

- Type of institution
  - ◯ ***National Blood Service***
- Institution demographics
  - ◯ For blood establishments and blood services
    - ■ Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable): In fiscal year 2019–2020, Canadian Blood Services collected 763 319 whole blood units, 126 277 platelet doses of which 21 493 were apheresis platelet collections in 11 339 individual apheresis procedures, and 48 610 plasma collections made either as specific apheresis plasma collections or collected concurrently with a plateletpheresis procedure.

Question 2

Canadian Blood Services stood up its CCP program in April 2020. Plasma is collected only by plasmapheresis. Donors who have appropriately high titres of neutralizing antibodies are requested to donate as frequently as once a week. This is the same donation frequency permitted for routine plasma donation.

Question 3

In Canada, CCP is collected primarily for use in three clinical trials. The regulatory authority, Health Canada, has provided a mechanism by which a physician could request an '$n = 1$' clinical trial to treat an individual patient. Perhaps due to the paperwork burden of obtaining a clinical trials authorization for a single patient, there have been no requests for '$n = 1$' studies, and all CCP collected has been used in national clinical trials. At this point, all CCP is intended for transfusion although discussions about supporting hyperimmune globulin production are ongoing.

Question 4

In addition to meeting all donor eligibility criteria that are applied to regular plasma donors, CCP donors must

have evidence of a RT-PCR positive SARS-CoV-2 test or be presumptive positive. The latter means that the donor resides in a household with someone who tested positive by RT-PCR and was sick with COVID symptoms at the same time. In addition, donors must be 28 days past resolution of symptoms prior to donation.

Question 5

Donors to the Canadian Blood Services CCP program are not tested for virus as there is little evidence that viral material is still in the circulation 28 days after resolution of symptoms, and no evidence to date that virus in blood is infectious.

Question 6

Donors are not tested for antibodies prior to donation; rather we rely on a confirmed virus test or presumptive positivity based on symptoms and cohabitation with an individual who was test positive.

Question 7

All donations are tested for anti-SARS-CoV-2 neutralizing antibodies. The test is a plaque reduction neutralization test (PRNT) using Vero E6 cell cultures and live SARS-CoV-2. Those donations with a $PRNT_{50}$ titre of $\geq$1:160 are released for clinical trial use. Donors with titres of 1:80 are encouraged to return for a subsequent donation; owing to the variability in the PRNT assay subsequent donations may return to 1:160 or greater. Samples are retained for subsequent testing on various antibody testing platforms.

Question 8

CCP used for clinical trials is Canada is not subjected to any pathogen inactivation technology. This is also true of regular transfusion plasma.

Question 9

We have not faced this particular situation; however, given the relatively short time that donors maintain adequate antibody titre, it would be unlikely. Any recipient of a blood product is deferred for 6 months.

Question 10

Yes, donors who have recovered from COVID-19 may donate blood or plasma as long as they are at least 21 days past resolution of their symptoms.

Question 11

Donors who had received CCP would be accepted for donation, but only after a 6-month deferral for receipt of a transfusion product.

Question 12

The main challenge in setting up a new program to collect COVID convalescent plasma was doing so in the midst of the pandemic when our organization was trying to cope with the installation of COVID safety precautions including masking and physical distancing which slowed down clinic operations. The need to maintain the blood supply is paramount, so the CCP program set up had considerable involvement from research & development staff members who could be diverted to this work. The main challenge in ongoing program operation has been recruitment of donors. Many of the recovered COVID patients are new to blood or plasma donation; only 4% of eligible Canadians donate blood so there is minimal overlap between the population of blood donors and the population of COVID patients. While we had good media coverage of CCP early in the pandemic (March-May), it has been difficult to attract sufficient donors with high titre antibodies during the second wave. This has led to challenges supporting the national clinical trials of CCP that are ongoing in Canada. Novel recruiting strategies and the involvement of institutions and physicians caring for COVID patients has been key to the recruitment of new donors.

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Hong Kong

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Question 1

Regional Blood Services/Blood Centre (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a regional level)

- Institution demographics

Around 215 000 whole blood collection and 10 000 apheresis collections for platelets and plasma per year

Question 2

Eligible donors are invited to donate up to 6 times of plasmapheresis.
The donation frequency is the same as that for routine plasma donation i.e. with donation interval of at least 14 days apart.

Question 3

Both, for clinical study use at the beginning, mostly for compassionate use now.

Question 4

Yes, all recovered patients must have SARS-CoV-2 PCR confirmed positive at diagnosis and recovered with at least two SARS-CoV-2 PCR negative before discharge. In addition, they must have neutralizing antibodies titre ≥1:80 before referral for convalescent plasma donation.
All donors have to be fully recovered from the SARS-CoV-2 infection as documented by negative NPS RT-PCR for SARS-CoV-2 four weeks before donation and negative NPS and serum RT-PCR for SARS-CoV-2 within 1 week of the donation.

Question 5

No, as response to Question 4, recovered patients were confirmed full recovery from COVID-19 and demonstrated to have sufficient neutralization antibodies titres.

Question 6

Yes, sufficient titre of neutralization antibodies were required to be eligible.

Question 7

An in-house live virus neutralizing antibody assay.
Samples are collected from the CCP unit and sent to the university for further assessment.

Question 8

No.

Question 9

Not decided but unlikely as (1) most CCP recipients were elder and (2) the number of patients received were still small locally.

Question 10

Recovered patient would only be accepted for whole blood or apheresis donation at 180 days after he/she completely recovered from COVID-19 infection and passed the health screening assessment required by our institution.

Question 11

Only at 12 months after the last transfusion event.

Question 12

CCP donors
It is observed that majority of referred CCP donors never donate blood before and they have little idea on blood donation and virtually none on apheresis donation. The frontline staff have to spend more time to introduce the apheresis donation and explain the procedures in detail.
As the CCP donors were just recovered from the COVID-19, they most have concerns on their health after the COVID-19 infection. Some also expressed their worries and myths about apheresis plasma donation, such as plasma donation would reduce their antibody titre to a level not enough to provide protection against subsequent infection. The professional staff has to reassure them to ease their concerns and explain how they could help other critical patients. Nevertheless, some CCP donors accepted and joined the program but some still had hesitation and withdrew.

Infection control concerns
Initially some staff and public have concerns of contamination from the potential reactivation of COVID-19 infection of the CCP donors. A separated room was then prepared for the CCP donation. As the CCP donors have repeatedly negative SARS-CoV-2 PCR results and with reassurance given to staff and public, they were donated at one end of the same apheresis suite.

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France

Pierre Tiberghien, Pierre Gallian & Pascal Morel

Question 1

- Type of institution
  - ***National Blood Establishment***
  - 2019: 2·5 million whole blood donations, 440 000 apheresis (340 000 plasma apheresis, 99 000 plasma/platelet apheresis, 1000 other apheresis)

Question 2

It does not differ. Policy for standard plasma apheresis is applied (at least 2 weeks between 2 plasma donations, and no more than 24 donations by year).

Whole blood is not collected for convalescent plasma collection.

Question 3

CPP is collected for transfusion: both trial use and compassionate use. Collection of CCP for plasma fractionation is being considered.

Question 4

No. Of note, during the first COVID-19 peak in March and April 2020 patients with mild clinical signs were not systemically tested by RT-PCR and diagnosis was essentially symptomatic.

Absence of clinical signs since at least 14 days.

Question 5

No.

Question 6

No.

Question 7

All CCP are screened by an ELISA assay for the detection of IgG anti-SARS-CoV-2 according to the manufacturer instruction (ELISA SARS-CoV-2 (IgG); Euroimmun). CP units with a ratio ≥8 (earlier ≥5,6) are accepted for use as convalescent plasma. CP units with a ratio ≥1,6 (earlier 1,1) and <8 (earlier 5,6) are further tested for neutralizing activity and accepted for use as convalescent plasma in the presence of a titre ≥80 (earlier ≥40). Neutralizing activity is assessed using live SARS-CoV-2 virus, as described Gallian et al. [1].

Yes.

In addition, CCP are qualified according the usual requirements for blood products. Also, individual HEV NAT screening is performed.

Question 8

Yes, all CCP are treated by amotosalen + UVA (Intercept Blood System) according to the manufacturer instruction.

Question 9

No. French regulations do not allow collecting blood from individuals with history of transfusion.

Question 10

Individuals who recovered from COVID-19 are accepted for blood donation 28 days after the end of clinical signs. In addition, patients with history of hospitalization in intensive care unit are deferred 4 months.

Question 11

No. French regulations does not allow to collect blood from individuals with history of transfusion.

Question 12

Strengths included donor implication, EFS personal mobilization, reactive interactions with regulatory authorities, efficient messaging towards the donors, strong productive interactions with academic/research teams, plasma apheresis availability in most fixed collection sites, availability of pathogen reduction technology, and supportive European institutions (ECDC, DG Santé/European Commission).

Weakness and challenges included difficulties to develop a dedicated IT-supported process for CCP collection, manufacturing and testing, occasional competition for resources between whole collection and convalescent plasma collection, introducing novel (and evolving) testing as well as occasional limited personal availability in the midst of an evolving crisis.

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Oman

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Question 1

- Type of institution
  - ***Hospital-based Blood Services*** (a hospital unit performing the functions of blood establishment and transfusion services at a hospital level)
- Institution demographics
  - For hospital
    - Number of in-patient beds: 450
    - Approximate number of RBCs transfused/year: 18 500
    - Age group of patients treated (neonates, paediatrics <18 years, adults >18 years): all
    - Type of patients treated (medical or surgical): both
  - For blood establishments and blood services
    - Whole blood collection: 12 500
    - Platelet apheresis collection: on-demand; approximately 50 platelet apheresis procedures/year

Question 2

Our institution has established a CCP donation program by plasmapheresis only.

Donors are allowed to donate CCP by plasmapheresis up to four times; seven days apart, if tolerated the procedure well.

Our institution did not collect plasmapheresis from regular donors before the establishment of the CCP collection program.

Our institution does not collect CCP from whole blood donations.

Question 3

CCP collected in our institution for transfusion purposes for trial use only.

Question 4

Yes. A confirmatory status of past COVID-19 infection (SARS-CoV-2 RNA by RT-PCR or anti-SARS-CoV-2 IgG antibody) in the donor is a pre-requisite. In addition, all CCP donors undergo testing for anti-SARS-CoV-2 IgG antibody levels before scheduling an appointment for plasmapheresis.

Recovery is defined as:
Resolution of fevers for > 72 h, and
Improvement in respiratory symptoms, and
Passage of >7 days since onset of the symptoms.

Question 5

Yes, donors who have complete resolution of symptoms by at least 14 days and before 28 days are tested for SARS-CoV-2 RNA by RT-PCR on a nasopharyngeal swab specimen at the time of donor screening.

If a donor presented for donation beyond 28 days from the time of complete resolution of symptoms, testing for SARS-CoV-2 RNA PCR is not performed.

Donors are allowed to donate CCP if any of the following:

- Complete resolution of symptoms at least 14 days before donation, AND negative results for SARS-CoV-2 RNA on a nasopharyngeal swab specimen.
- OR
- Complete resolution of symptoms at least 28 days before donation without SARS-CoV-2 RNA testing
- The plasmapheresis appointment is decided based on the time of resolution of symptoms, and the results of the SARS-CoV-2 RNA RT-PCR at the time of donor screening (as above)

Question 6

Yes, donors are tested for anti-SARS-CoV-2 IgG antibodies at the time of donor screening before plasmapheresis. Testing is performed using EUROIMMUN® ELISA assay (Lübeck, Germany). A signal-to-cut-off ratio ≥2 is considered acceptable for donation.

Question 7

Yes. Testing of anti-SARS-CoV-2 antibodies on the CCP units is performed using EUROIMMUN® ELISA assay (Lubeck, Germany). A signal-to-cut-off ratio ≥2 is considered acceptable for unit transfusion/use.

Yes. Additional samples are collected from the CCP unit and freezed for future testing for neutralizing antibody titres.

Question 8

Yes, Collected CCP is subjected to pathogen inactivation via the Mirasol Pathogen Reduction Technology System (Terumo BCT).

Question 9

No. Recipients of CCP are temporarily deferred from all types of blood donation including CCP donation for a minimum of one year. Hence, they will not be eligible to donate CCP within the timeframe of the conducted trial.

Question 10

Yes. Our institution accepts individuals who recovered from COVID-19 infection for blood donation minimum of 28 days after a resolution of symptoms and cessation of therapy.

Question 11

Yes. Our blood services accept recipients of CCP for standard whole blood and apheresis donation if they meet other donation criteria and after a temporary deferral period of a minimum of 1 year.

Question 12

Challenges faced:

Managing a new apheresis service at the time of shortage of staff during the pandemic.

Accommodating CCP donors along with the regular blood donors with existing space limitation in the donation centre, and at the time of undertaking COVID precautionary measures including social distancing. We, therefore, opted to perform CCP plasmapheresis procedures outside the regular blood service's working hours.

Recruiting CCP donors of certain blood groups such as group A, B and AB, considering that group O is the commonest blood group in the population.

Lessons learned:

To initiate clinical trials early in the pandemic to enable recruitment of the required number of patients and before the decline in number of cases.

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Netherlands – Sanquin

Hans Vrielink & Cynthia So-Osman

Question 1

- Type of institution
  - **Sanquin Blood Supply:** ***National Blood Establishment*** of the Netherlands (responsible for any aspect of the collection, testing, processing, storage, release and/or distribution of human blood or blood components)
- Institution demographics
  - For blood establishments and blood services in 2019
    - Whole blood donations 413 653 and apheresis 313 811

Question 2

COVID-19 convalescent plasma (CCP) is solely being collected by plasmapheresis. There are no differences between CCP donors and routine plasmapheresis donors, meaning that in a consecutive period of 12 months, a donor is permitted to donate maximally 26 times, and collection is not allowed to exceed 25 litres of plasma (excluding anticoagulant). The donation volume is based on gender, height and body weight, but maximized to 750 ml of plasma excluding anticoagulant.

Question 3

In the Netherlands, CCP is collected for two reasons. In March 2020, we started with the collection of CCP. Mid of May 2020, also with the collection of source plasma for the production of COVID-19 was started. As of December 31, 2020 approximately 4500 collections for

CCP and 50 500 collections for SARS-CoV-2 immunoglobulins (COVIg) source plasma were performed. In the Netherlands, the fresh frozen plasma for transfusion is preferably used in clinical trials (e.g. the COV-EARLY trial, a study for pre-hospital infected patients, and the REMAP-CAP trial, a study for hospitalized infected patients) and for compassionate use in immune compromised hospitalized patients with persistent and/or severe COVID-19 disease.

Question 4

Starting with CCP collections in March 2020, in all donors COVID-19 infection needed to be proven by a positive SARS-CoV-2 PCR in the symptomatic period. (nose and/or throat swab). In June – August 2020, this eligibility criterion was not mandatory anymore for COVIg source plasma donors. However, since in source plasma donors only 60% had SARs-CoV-19 antibodies versus 85% in FFP donors, this criterion was re-introduced. The donor should be at least 14 days symptom-free from symptoms such as a cold, runny nose, sneezing, sore throat, coughing complaints, increased temperature and fever.

Question 5

No.

Question 6

No, not in general. Some of the donors are found positive in prevalence studies performed by Sanquin. These studies were performed with a SARS-CoV-2 total antibody ELISA (Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China) [1].

Question 7

Starting July 2020, all CCP donations are routinely tested for SARS-CoV-2 IgG antibodies with a sensitive total Ab bridging assays for detection of SARS-CoV-2 Abs to the receptor-binding domain (RBD) and nucleocapsid protein in ELISA format. By using a cut-off value of 0·1 nOD, it is anticipated to provide $\sim$99% specificity [2].
Prior to July 2020, two samples of 5 ml of EDTA whole blood were collected during donation together with samples for blood type and tests for transfusion transmittable infections from the diversion pouch of the apheresis disposable. After centrifuging of the EDTA tubes, the plasma was aliquoted and archived frozen at lower temperatures of −20°C until routine anti-SARS-CoV-2 testing was started.

Question 8

In the Netherlands, the majority of the plasma for transfusion is pooled, solvent-detergent (SD)-treated, prion reduced plasma (Octapharma GmbH, Germany). A minority of the plasma is quarantined. Since there wasn't sufficient plasma collected for pooled SD treatment, no validated single donation SD treatment methods and no time for quarantining, no pathogen reduction to CCP for transfusion is applied which is according to the recommendations of ECDC [3].

Question 9

In the Netherlands, all donors who received one or more blood components since January 1980 are deferred permanently. Therefore, recipients of CCP are not accepted as donors.

Question 10

Similar to other infectious diseases, individuals recovered from COVID-19 are accepted as donors, irrespective of whole blood or apheresis, with a minimal interval of 14 days after recovery.

Question 11

As answered in question 9, in the Netherlands, all donors who received one or more blood components since January 1980 are deferred permanently. Therefore, recipients of CCP are not accepted as donors.

Question 12

One of the first challenges was recruitment of sufficient donors without having sufficient knowledge of the virus and test capacity for SARS-CoV-2 antibodies. Media platforms as social media, messaging applications, national papers, news programs, and radio/TV were initially largely overwhelmed by the notification of recently recovered COVID-19 patients. This lead to a completely full reservoir of potential donors, resulting in long waiting lists for the first medical examination and donation. Unfortunately, despite major effort, only relatively low percentages of the registered persons could be accepted as donors because of medical or other reasons. We found out that the highest success rate to recruit CCP donors was by requesting physicians, hospitals, laboratories, and public health departments to inform recently recovered COVID-19 patients to become CCP donors.

Because our staff at the donor centres occasionally expressed concern on the potentially infectious status of "recovered patients" a thorough information of all staff was needed.

Another challenge was the need for additional spacing between collection beds. Social distancing of 1·5 metres reduced the collection capacity for approximately 40%. As a result, there was an additional competition with collecting of whole blood and especially source plasma since the same apheresis equipment was applied.

Challenging was also to work together with academic hospitals to draw up a study protocol for the use of CCP for COVID-19 infected patients in an amazing short time period. Since Sanquin Blood Supply is the only blood establishment in the Netherlands and thus the only legal provider of CCP, it was essential to become part of the study group. We also felt that the best way to issue CCP was for its use in clinical trials when evidence on the efficacy is still lacking. This resulted in the first randomized controlled study, which started recruiting patients in April 2020 [4].

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Italy

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Question 1

The Italian National Blood Centre is the Italian blood and blood component competent authority operating under the aegis of the Ministry of Health. It coordinates and supervises the 21 Regional Coordinating Blood Transfusion Centres, with the aim of guaranteeing homogeneous standards of quality and safety throughout the blood system. Standards for CCP have been defined by the Italian National Blood Centre in a recent note of guidance (October 2020) [1]. In 2019, a total 2 996 264 collection procedures (whole blood and apheresis procedures) have been carried out in Italy.

Question 2

In the initial phase of the emergency related to the COVID-19, the national policy adopted has included only the donation of plasma in apheresis from convalescent former patients. The latter may donate more than once according to the frequency for routine plasma donations by apheresis and depending on the criteria stated by law in order to protect the donors' health. At a later stage, the possibility of also collecting whole blood from donors with anti-SARS-CoV-2 antibodies was introduced. The donors (many of them are probably already regular blood donors) may donate more than once according to the frequency for routine whole blood or plasma donation by apheresis stated by the law in force [2]. Each unit of collected CCP must be clearly labelled as "Plasma unit collected from a convalescent patient–donor with a virologically documented diagnosis of COVID-19" and must report the titre of the neutralizing antibodies.

Question 3

In Italy, the CCP, which is obtained from a convalescent COVID-19 donor, is intended only for transfusion and fractionation purposes. Although the Italian National Blood Centre recommended the use of CCP in clinical trials, possibly randomized, it has been transfused in emergency/compassionate situations. The Italian National Blood Centre is in charge of a regular monitoring of the CCP collection and transfusion. Up-to-now, a prevalent utilization in clinical trials has been detected.

Question 4

Donors shall be eligible according to selection criteria stated by national law. Careful clinical evaluation is recommended with the focus on the apheresis criteria to protect the health of donor. Only male donors or nulliparous

females with a negative history of blood component transfusion are eligible to CCP for transfusion use; the others, can donate to fractionation purposes. Donors shall have a virologically documented diagnosis of COVID-19 based on a positive SARS-CoV-2 molecular swab healing test. Donors shall be completely recovered from the symptoms of COVID-19 and result negative in one SARS-CoV-2 molecular swab healing test. Plasma collection (donation) can occur 28 days after the negative nasopharyngeal swab in case of previous hospitalization; in other cases, at least 10 days after the onset of symptoms with a negative molecular test performed after at least 3 days without symptoms (only for symptomatic patients).

Question 5

No blood/serum SARS-CoV-2 molecular tests are required. To assess viral clearance, CCP donors must have a documented negative molecular swab healing test performed by laboratories competent for COVID-19 testing.

Question 6

The national policy states that the preliminary selection of CCP donors is based on a clinical history of disease evidence of recovery confirmed by a documented molecular swab healing test. Serological test for anti-SARS-CoV-2 antibodies can be performed either before or after donation. In Italy, several serological methods are currently available, each of them with a cut-off provided by the manufacturer. No serological cut-off criteria are still applied for accepting the donor for donation.

Question 7

The presence of anti-SARS-CoV-2 neutralizing antibodies shall be documented on donor/donation at the time of collection of CCP units intended for transfusion purposes (clinical trials). The national policy recommended to apply the cut-off defined in the specific clinical trials (e.g. the national "TSUNAMI study" - *TranSfUsion of coNvalescent plAsma for the early treatment of pneuMonIa due to SARS-CoV-2*-requested CCP units with titre of at least 1:160), as well as to collect and store CCP unit samples for future assessment.

Question 8

The application of a pathogen inactivation method of recognized efficacy on SARS-CoV-2 was recommended as an additional safety measure in the national "TSUNAMI Study" (*TranSfUsion of coNvalescent plAsma for the early treatment of pneuMonIa due to SARS-CoV-2)* and, in general, for the clinical use of CCP in COVID-19 patients. The thawed and inactivated CCP can be stored at 4°C for up to 5 days [1].

Question 9

In order to prevent the Transfusion-Related Acute Lung Injury (TRALI), donors with a history of blood component transfusion, including CCP transfusion, are not eligible to donate CCP for clinical use. Plasma from recipients of CCP may be collected for fractionation purposes.

Question 10

Individuals are eligible for standard whole blood or apheresis (platelet or plasma) donation if they are completely recovered from COVID-19 infection and tested negative for SARS-CoV-2 molecular swab healing test. The potential donors must be compliant to the selection criteria stated by law in force [2]. The minimum deferral period after recovery before whole blood or apheresis donation is 10 days.

Question 11

According to the selection criteria stated by law in force [2], recipients of CCP are eligible for standard whole blood or apheresis donation. In order to prevent the Transfusion-Related Acute Lung Injury (TRALI), donors with a history of blood component transfusion, including CCP transfusion, are eligible to donate plasma only for fractionation purposes.

Question 12

The SARS-CoV-2 stressed our capacity to respond to an infectious disease outbreak. The need to equip ourselves with standardized and homogeneous risk assessment tools at national and international level has become increasingly evident so to increase the necessity to build-up blood components collection programmes in emergency situations and from former patients (convalescent subjects).

In addition, more evidence from high quality clinical trials is required to fully demonstrate the efficacy of CCP therapy and to design a single protocol that defines the product specifications in terms of quality, neutralizing antibody titre and therapeutic indications.

Among those on the market, it is also advisable to identify a serological test for the detection of neutralizing antibodies, which correlates with the results obtained by means of *in vitro* neutralization tests.

Section References

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Belgium

Michel Toungouz Nevessignsky

Question 1

- Type of institution
  - ◯ ***National Blood Establishment*** (responsible for any aspect of the collection, testing, processing, storage, release and/or distribution of human blood or blood components)
- Institution demographics
  - ◯ For blood establishments and blood services
    - ■ Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable):
    - ■ 160·000 RBC, 11·000 PLT, 25·000 Plasmas collections/year

Question 2

Same as usual plasma donation: 23/year (15 litres/year, 2 litres/month taking into account 650 ml per plasmapheresis) with the limitation of a defined titre of neutralizing anti-SARS-CoV-2 antibodies.

No.

Question 3

Both.

Question 4

Yes, both.

Absence of symptoms.

Question 5

No.

Question 6

No. The test is performed at the time of donation and a defined titre of neutralizing antibodies is used as a release criteria.

Question 7

No.

Yes.

Question 8

Yes.

Yes, methylene blue.

Question 9

Theoretically yes with a temporary exclusion of 4 months.

Question 10

Yes.

28 days

Question 11

Yes.

Question 12

Donor recruitment, performing the neutralization assay and getting the results on time, convincing the collecting staff to welcome convalescent donors, managing physician pressure related to compassionate use and being able to provide enough plasma units for the studies.

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Egypt

Magdy El Ekiaby

Question 1

- Type of institution

Hospital-based blood services (a hospital unit performing the functions of blood establishment and transfusion services at a hospital level)

- Institution demographics
  - For hospital
    - Number of in-patient beds 100
    - Approximate number of RBCs transfused/year 25 000
    - Age group of patients treated (neonates, paediatrics <18 years, adults >18 years) All ages
    - Type of patients treated (medical or surgical) Both

COVID-19 donor pre-selection criteria

- Physical examination of the donor to establish health status and suitability for plasma donation according to Egypt donor selection criteria (lower haemoglobin level down to 11 gm/dl for males and 10·5 gm/dl for females might be allowed)
- Confirmation of previous infection with SARS-CoV-2 by previous positive PCR swab
- Confirmation of the resolution of the infection through demonstration of two repetitively (at least 24 h interval) non-reactive PCR swab or 10 days after disappearance of symptoms
- Male donors or nulliparous female donors
- Selected donor should test positive to SARS-CoV-2 antibodies (IgG or combined IgM and IgG antibodies)
- Approximative date of COVID-19 infection, symptoms and treatment received should be documented and traceable

Plasma collection:

Question 2

Once every 2 weeks. In practice, donors were not regular donors so they donated only one or two times.
Not different.

We did not collect whole blood.

Question 3

Trial use.

Question 4

Yes. Anti-SARS-CoV-2 antibodies.
Disappearance of symptoms for 10 days or more + presence of anti-SARS-CoV-2 antibodies.

Question 5

No.

Question 6

Yes using anti-SARS-CoV-2 rapid test from AMEDA Labor Diagnostika, Austria.

Question 7

Anti-SARS-CoV-2 antibody test IgM & IgG using Maglumi Chemiluminescence assay from Snibe China (cut-off 1) and Chemiluminescence assay on Centaur from Roche.
Yes for neutralizing antibody assay.

Question 8

We are processing Convalescent COVID-19 plasma into anti-SARS-CoV-2 hyperimmunoglobulin, which includes lipid enveloped virus inactivation step by Caprylic Acid.

Question 9

Only within a clinical study.

Question 10

We do not have a specific question about COVID-19 previous infection during medical screening for regular blood or apheresis donation.
If donor states that he/she had recent COVID-19 infection we temporary defer.

Question 11

So far there is no policy.

Question 12

National standards are still not set.

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Australia

James Daly and Veronica Hoad

Question 1

- Type of institution
  - **National Blood Services/Blood Centre** (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a national level)
  - **Also supply plasma for fractionation to fractionator**
- Institution demographics
  - Approximate number of whole blood and apheresis collections made/year for RBCs, platelets and plasma (as applicable):
  - 2019/2020 financial year collections 1 527 089
  - Collections: Whole blood 690 115, plasmapheresis 809 910, Platelets 27 024
  - Components: Red cells 630 993, clinical plasma 238 541 platelets (pooled and apheresis) 138 629 and plasma for fractionation 802 630 kg

Question 2

COVID-19 Convalescent plasma donors may donate by plasmapheresis as frequently as weekly for up to 12 donations. This is more frequent than standard plasmapheresis donations but the same maximum limit for a 12 month period is applied which is up to 33 per year.
We introduced additional monitoring for total protein and Immunoglobulin levels for frequent donors.
Yes, donors are encouraged to make apheresis plasma donations but Whole blood donations are accepted. Because donors are required to meet all other donor eligibility criteria and the collection and processing of the donation is the same as standard donations, the red cells from the whole blood donation are labelled as standard red cells and the platelets may be pooled as standard platelets.

Question 3

Convalescent plasma donations from male donors with a neutralizing antibody titre at or greater than 1:80 are suitable to be used for transfusion in clinical trials. This was used for clinical trials for symptomatic COVID-19 infection in hospitalized patients (ASCOT trial) and patients admitted to Intensive care (REMAP-CAP).
Convalescent plasma donations with a neutralizing antibody titre at or greater than 1:40 were suitable for fractionation to manufacture of COVID-19 immunoglobulin. This product has not yet been used in clinical trials.
COVID-19 Convalescent plasma was approved to be issued for use in clinical trials (ASCOT and REMAP-CAP trials). The initial intention was not to support compassionate use because there was insufficient evidence of benefit from convalescent plasma. Only 2 requests for compassionate access to COVID-19 convalescent plasma were approved – both were for immunosuppressed patients unable to mount an antibody response and with prolonged COVID symptoms/PCR positivity.

Question 4

No. Donors must report that they had 'laboratory confirmed COVID-19 infection' as per our National guidelines for a confirmed case. A copy of the diagnostic report was not required to be provided.
The vast majority were positive by RNA but the confirmed case definition also includes seroconversion and a rise in IgG. For reference the current case definition is:
(i) tested positive to a validated specific SARS-CoV-2 nucleic acid test, or
(iI) had the virus isolated in cell culture, with PCR confirmation using a validated method, or
(iii) undergone a seroconversion to or has a significant rise in SARS-CoV-2 neutralizing or IgG antibody level (e.g. four-fold or greater rise in titre).
Note: Serological confirmation was added to the national case definition on 13 May 2020, so this only applies to donors who have had their diagnosis confirmed by serology after this date. For the diagnosis to be confirmed by this method usually this requires additional evidence to undergo the testing, such as symptoms consistent with COVID-19 where they have tested negative on PCR or an epidemiological link to a confirmed case.
COVID-19 convalescent plasma donors had to have laboratory confirmed COVID-19, and be at least 28 days form recovery of symptoms, and meet all other standard donor eligibility criteria. Minor residual symptoms are allowed after Medical Officer Assessment (e.g. some residual loss of smell).
Only male donors were eligible for clinical plasma donation (as per existing TRALI risk reduction measures for plasma donors).
Donations with a Neutralizing antibody titre greater or equal to 1:80 were suitable for clinical plasma.

Donations with a Neutralizing antibody titre greater or equal to 1:40 were suitable for manufacture of COVID-19 immunoglobulin.

Question 5

No.

Question 6

No, this is done at the time of donation for every donation to determine if the donation is suitable for release as a convalescent plasma product as described below.

Question 7

Each donation is tested by a reference laboratory using 3 separate tests, the Abbott Architect SARS-CoV-2 IgG CMIA, the Euroimmun anti-SARS-CoV-2 ELISA and a virus microneutralization assay using Vero E6 cells. The results were reported as per the product inserts. The microneutralization description can be found here: https://www.medrxiv.org/content/10.1101/2020.12.07.20245696v1

Evaluation of the assays determined that low-level neutralization antibody positive donations would be missed if the donation did not progress to the neutralization antibody test if testing was ceased with a negative or equivocal screening antibody test. The neutralization assay was considered the definitive release test.

However, for a donation to be considered suitable for clinical convalescent plasma the donation must test positive on one of the two screening tests **and** have a Neutralizing antibody titre greater or equal to 1:80.

For a donation to be suitable for COVID-19 Immunoglobulin production the donation required a neutralizing antibody titre greater or equal to 1:40 as the sole test.

Yes, we collected 4 tubes and 2 were used for preliminary testing with an additional 2 tubes saved for further testing/research purposes.

Question 8

No. No clinical plasma in Australia has additional pathogen reduction.

Question 9

No. Transfusion of blood products is an existing exclusion criterion for blood donors (for 12 months since the transfusion). All Convalescent plasma donors must meet all existing donor eligibility criteria.

We have not had significant usage of convalescent plasma in the clinical trials and therefore there are no issues with this policy, which is longstanding. However, there is no regulatory requirement for this deferral so if we were in the situation of other countries where many patients had received convalescent plasma and there was a sufficiency issue, we would reconsider.

Question 10

Yes, donors are eligible for standard blood component donations if they are at least 28 days form recovery of symptomatic COVID-19 and meet all other donor eligibility criteria. Donors that have had laboratory confirmed COVID-19 would be encouraged to make a convalescent plasma donation by apheresis. Those donations that do not meet the minimum 1:40 neutralizing antibody titre are used as standard donations.

Question 11

No. Transfusion of blood products is an existing exclusion criterion for blood donors (for 12 months since the transfusion). All Convalescent plasma donors must meet all existing donor eligibility criteria.

Question 12

Government approval was required before we could commence collections and therefore important to engage and continue the dialogue early as the procedures take time to set up and in the end it was difficult to manage a large program in a small timeframe.

Set up before any evidence on possible effectiveness or before fractionators determined what would likely be a standard level of neutralizing antibody required. It is difficult to change cut-off criteria without definitive evidence on effectiveness but retrospectively would have used higher cut-off for acceptable titre.

Despite having a reasonable sized second wave as we are the collectors and not the users we were reliant on the clinicians and trial coordinators in the hospitals to assess and consent patients for convalescent plasma in the trial environment. Many patients were not suitable and to some degree there was not optimal usage when patients were in hospital during the second wave, which was successfully suppressed that limited further enrolment.

Very grateful that we used exactly the same criteria for existing donation so our donations will not go to waste if CP is determined to be ineffective or only high titre units are acceptable, as they can be used as regular FFP or for plasma for fractionation. That is an important message and outcome to all CP donors who have donated. Their

donations will still be used to help patients in need, no matter what.

Extra information about CP donations

As at January 11th 2021 we have 809 convalescent plasma donors with 3160 collections. Of all donations tested only 5·9% of donations were negative, 40·7% were low-level positive (1:40) and 53·4% were positive with a level ≥1:80. We used 46 clinical plasma units in clinical trials and for compassionate use and have 480 in stock with an inventory aim of 500. We have sent 1377 kg of fractionated plasma to CSL Behring.

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South Korea

Sinyoung Kim

Question 1

- Type of institution;
  - Hospital-based Blood Transfusion Service/Blood Bank
- Institution demographics
  - Tertiary referral hospital
    - 2437 in-patient beds
    - 50 200 RBCs transfused/year
    - All age group of patients treated
    - All type of patients treated

Question 2

Plasmapheresis only

CCP donations are allowed every 2 weeks. This cycle is the same as routine plasma donation in South Korea.

Question 3

CCP is collected for transfusion, especially for COVID-19 patients participating in clinical trial.

Question 4

CCP collection is possible for donors 14 days after quarantine release. However, confirmatory PCR testing should be performed for donors between 14 and 28 days of quarantine release.

Confirmed patients can be released from quarantine if they do not show any symptoms for 10 days upon confirmation, or test negative on PCR test twice in a row with at least a 24-h interval.

Question 5

No.

Question 6

No.

Question 7

Yes. Every CCP unit was tested for anti-SARS-CoV-2 antibodies using AFIAS COVID-19 IgG Ab (Boditech Med., Korea). CCP unit showing positive COVID-19 IgG Ab result (cut-off index >1·0) can be supplied for transfusion.

Samples from the CCP unit have been stored for future study.

Question 8

Pathogen inactivation technique is not available in South Korea.

Question 9

No.

Question 10

Yes, the deferral period is 3 months for blood donation.

Question 11

Yes. Recipient of blood and blood products is deferred for 12 months following transfusion including CCP.

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South Africa

Karin van den Berg, Marion Vermeulen & Tanya Nadia Glatt

Question 1

Regional Blood Service
Whole blood collections per annum: 900 000
Apheresis platelet collections per annum: 18 138
Apheresis source plasma collections: 32 000
RBC issued per annum: 810 000
Pooled platelet issued per annum: 33 000
Apheresis platelets issued per annum: 32 000
Fresh frozen plasma issued per annum: 123 000

Question 2

To date, SANBS has been collecting CCP through plasmapheresis. Donors are allowed to donate once every 2 weeks with a maximum of 24 procedures per annum. This donation frequency is the same as the source plasma program.

Our CCP program is based on the recently implemented source plasma program. This enabled us to rapidly develop and implement a CCP collection program with a near-national footprint. SANBS have source plasma collection equipment and trained staff in most of the major cities and towns in South Africa. Piggy-backing on this program significantly decreased the set-up time we would otherwise have needed.

We recently started a SARS-CoV-2 seroprevalence study among our blood donors. The unexpectedly high prevalence of SARS-CoV-2 antibodies have raised the potential for us to use whole blood recovered plasma for CCP, but this process is not yet fully developed. However, this would certainly be a consideration for other LMIC blood services.

Question 3

Currently, we have two programs for which we are collecting CCP. Firstly, we are collecting high titre CCP for a double blind randomized phase III clinical trial assessing the efficacy of CCP in the treatment of hospitalized patients with moderate to severe COVID-19. Secondly, we are collecting CCP (regardless of titre) for our national fractionator, the National Bioproducts Institute, who is participating in the CoVIg-19 program and preparing a test, not-for-human-use batch of SARS-CoV-2 immunoglobulin.

In addition, we recently started a directed CCP program, which enables clinicians to arrange for the collection of CCP from donors they have identified and to which the patient has agreed. This is a compassionate use type program where SANBS's role is limited to the collection of the plasma.

Question 4

All CCP donors (who donate for the clinical trial or the fractionation program) must have a confirm test result of past COVID-19 infection prior to being accepted as CCP donors. We accept either a SARS-CoV-2 RT-PCR or Ag test or the presence of SARS-CoV-2 antibodies.

Eligibility criteria for our CCP donors include [1]:
At least 28 days since last symptoms OR 14 days since test, with 2 negative results at least 24 h apart. This was subsequently amended to 14 days post last symptoms
Must be between the ages of 18 and 65
Males and nulliparous females only
Must meet general blood donor criteria

Question 5

No, we do not test prospective CCP donors for SARS-CoV-2 prior to accepting them for donation.

We request a copy of their SARS-CoV-2 test and confirm that they meet the "recovery" criteria as mentioned above.

Question 6

Currently, donors donating for the clinical trial and fractionation programs undergo a "pre-test" prior to their first donation. This pre-test includes the full set of routine donation screening tests (HIV, Hepatitis B and C as well as Syphilis) as well as SARS-CoV-2 ELISA antibody test. Initially all donors with detectable SARS-CoV-2 antibodies were accepted, but following publications suggesting that efficacy of CCP may be influenced by antibody titres [2], we only accepted donors with a set cut-off for further donations.

(The National Institute of Communicable Diseases perform the antibody ELISA using a method based on the test developed by Kramer and colleagues [3]. An arbitrary cut-off for a positive was an optical density (OD) of 0·4. However this was later increased to 1·0 for inclusion into the CCP program.

Question 7

Yes, all CCP units collected at SANBS are tested for anti-SARS-CoV-2 antibodies. Donors are tested before they donate their first unit and then at each subsequent donation. Currently, the antibody testing is performed by the South African National Institute of Communicable Diseases. Samples are first tested using an ELISA test and then those with a sufficiently high OD are tested for neutralizing antibodies. Recently however, following

publications suggesting that ELISA tests are likely sufficient to identify high titre plasma [4], we have decided to discontinue the neutralizing antibody testing.
Yes, we are collecting samples from the CCP units for future research purposes. These samples are frozen and archived. Use of these samples for projects other than the current projects is at the discretion of the PI of our CCP donor program.

Question 8

Products intended for the clinical trial (therefore for human use) are pathogen reduced using the Cerus Intercept system.
Perhaps important to note that SANBS currently do not perform pathogen reduction on any of our routine blood products. The decision to implement pathogen reduction for these products is based on the fact that we employ a "donor retested quarantine" program for plasma products intended for patient use. This is part of our blood safety strategy in a country with the biggest HIV epidemic in the world. We were not able to set-up a quarantine program for the CCP donations. Considering the large volume plasma transfused and the high proportion first time donors among the CCP donors, we decided to implement pathogen reduction for the CCP program.

Question 9

SANBS currently have a 3-month deferral period for recipients of blood products. This would therefore apply for recipients of CCP. Other than this, we do not currently have specific exclusions for recipients of CCP. If they meet all the eligibility criteria, including having a sufficiently high SARS-CoV-2 antibody titre and have waited out their 3 month deferral period we would likely accept them. We have, to date, not been confronted with this situation.

Question 10

Yes, we do accept individuals recovered from COVID-19 infection for standard whole blood and apheresis donation as long as they have fully recovered and have been symptom-free for 14 days prior to presenting to donate.

Question 11

Yes, we do accept CCP recipients for routine blood donations, both whole blood and apheresis.
These donors are subject to the SANBS general deferral of 3 months following the receipt of blood or blood products.

Question 12

- Challenges:
  - South African legislation prohibits paid-plasma programs, which significantly hampered the rapid scale-up of this program.
  - SANBS has a limited footprint of trained staff and equipment for the collection of apheresis plasma. This, in combination with the high levels of poverty in the country meant that most COVID recovered patients were not able to donate plasma, as there were no apheresis units located near these sections of the population.
  - The lockdown levels in South Africa with the requirement for anyone who can to work from home to do so, significantly hampered our ability to recruit donors in traditional ways and places.
  - South Africa does not allow payment of blood donors, not even for plasma collections intended for fractionation. This further limited our ability to collect plasma and it meant that we would probably not be able to collect sufficient CCP for large-scale routine use.
  - During certain periods of the epidemic SANBS face severe blood shortages. The CCP program competed with the routine blood collections not only for donors but also for staff. SANBS staff was severely affected during the second wave of the epidemic in South Africa and we had to carefully assess the need for CCP collections versus whole blood collections.
  - Some SANBS staff at CCP collection sites were apprehensive to manage CCP donors for fear of them still being infectious. This required appropriate, ongoing education and training.
  - Some CCP donors were eager to donate but were concerned about the effect CCP donations would have on their own immunity to reinfection. This required appropriate education.
  - It was difficult to maintain CCP donations during the annual extended summer holiday when many donors went on holiday and missed at least one donation.
  - Routine SARS-CoV-2 antibody testing was not available for the first few months of our CCP collections. There were some donors who may have been using their CCP donation to get a SARS-CoV-2 antibody result instead of being completely altruistic.
  - Another challenge was the lower than expected frequency of high titre anti-SARS-CoV-2 antibody levels. This meant that more donors than were

expected were required to meet the clinical arm of the study and many operational procedures and system modifications were required to ensure lower titre plasma was not issued to patients.

- Lessons Learned:
  - Early, ongoing collaboration is key. Locally, collaborations with clinicians and academics meant that we were responsive to the "real-world" needs and challenges faced by those caring for COVID-19 patients. Working side-by-side with our National Institute of Communicable Diseases (NICD) again enabled us to keep informed of new developments in the South African epidemic, but also provided the NICD with ready access to samples for ongoing research. Internationally, the collaboration with the ISBT Working Party has been invaluable. We learned so much from our international counterparts and were able to assess what others were doing and seeing what would work best for our local setting.
  - Rapidly developing a new product program requires various internal stakeholders to work together in a seamless fashion all geared towards achieving a common goal. Ensuring early buy-in and excitement within the organization is key to achieving rapid change in an organization, which is generally quite change and risk averse.
  - Correct marketing is key to ensuring an adequate CCP supply. Recovered COVID-19 patients are eager to donate CCP, even if they are not regular blood donors, when they realize the possible effect their CCP can have. Although we are not allowed to call potential CCP donors directly, an effective way to acquire donors is to ask clinicians to educate patients about CCP donation on discharge from hospital. Recovered patients trust their clinician and are likely to want to become donors. This enables us to get CCP donors who were hospitalized, and likely to have higher antibody titres. Ineligible donors, such as those too young or too old, would not be recruited by clinicians reducing the time required by our staff to manage these ineligible donors.

Section References

1 World Health Organisation, Guidance on maintaining a safe and adequate blood supply during the coronavirus disease 2019 (COVID-19) pandemic and on the collection of COVID-19 convalescent plasma. 2020 [Available from: https://apps.who.int/iris/bitstream/handle/10665/333182/WHO-2019-nCoV-BloodSupply-2020.2-eng.pdf?sequence=1&isAllowed=y (Accessed 17.01.2021).

2 Joyner MJ, Senefeld JW, Klassen SA, Mills JR, Johnson PW, Theel ES, et al. Effect of convalescent plasma on mortality among hospitalized patients with COVID-19: initial three-month experience. medRxiv. 2020.

3 Amanat F, Stadlbauer D, Strohmeier S, Nguyen THO, Chromikova V, McMahon M, et al. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nat Med 2020;26:1033–6.

4 Wouters E, Steenhuis M, Schrezenmeier H, Tiberghien P, Harvala H, Feys HB, et al. Evaluation of SARS-CoV-2 antibody titers and potency for convalescent plasma donation: a brief commentary. Vox Sang 2020.

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Germany

Richard Schäfer

Question 1

Type of institution.

- ***Regional Blood Services/Blood Centre*** (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a regional level)

Institution demographics.

Whole blood donations: 5849/year.

Apheresis: platelets: 379; plasma: 23; RBCs: very rare (<5).

Question 2

CCP is collected exclusively by plasmapheresis:

The allowed frequency is 60 plasma donations per year (same as routine plasma donation by apheresis) with a minimum of 2 calendar days between 2 donations.

Plasma from whole blood donations is not used for CCP.

Question 3

The collected CCP is intended for transfusion for both compassionate use and trial use.

Question 4

Yes.
Both SARS-Cov-2 PCR, or anti-SARS-Cov-2 antibodies can be accepted.
4 weeks without symptoms; no (long-term) sequelae

Question 5

Yes.
The PCR sample is collected during the pre-donation visit (typically 14 days prior to donation). A donation is possible earliest 14 days after negative PCR testing.

Question 6

Yes.
An in-house plaque reduction neutralization test (PRNT) is used to detect and quantify neutralizing antibodies; titres >1:20 are acceptable.

Question 7

Anti-SARS-CoV-2 antibodies are tested at each donation from donor blood samples (not the CCP unit).

Question 8

No.

Question 9

No.

Question 10

Currently, the minimum deferral period after recovery from COVID-19 is 4 weeks.

Question 11

Currently, the minimum deferral period after blood component transfusion is 12 months.

Question 12

The major challenge was having enough donors getting cleared for CCP donation because of substantial dropouts due to unacceptable medications, low Hb and positive testing for HLA antibodies.

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United States – OneBlood

Rita Reik, Richard Gammon & Melissa Lopez

Question 1

- Type of institution
  - ◯ ***Regional Blood Services/Blood Centre*** (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a regional level)
- Institution demographics

Please see the table for calendar year 2020 data.

Actuals– Current Year: 01/01/2020 to 12/31/2020

	Autologous	Allogeneic	Directed	Therapeutic	HH	TT	Total	s
2 units RBC		51 014	35				**51 049**	**5.44%**
Granulocytes			34				**34**	**0.00%**
Plasma Apheresis		10 434	2				**10 436**	**1.12%**
Platelet Apheresis		48 361	146				**48 507**	**5.17%**
Platelets and Concurrent Plasma		29 437	65				**29 502**	**3.14%**
RBC with Plasma		4641	3				**4644**	**0.49%**
RBC with Platelets		280	3				**283**	**0.03%**
RBC with Platelets and Plasma		839	8				**847**	**0.09%**
Single Unit Recovery		2696	2				**2698**	**0.29%**
Stem Cells			13				**13**	**0.00%**
Whole Blood	362	750 423	1215	14,697	6329	17 674	**790 700**	**84.23%**
Total	**362**	**393 175**	**1526**	**14 697**	**6329**	**17 674**	938 763	**100.00%**
%	**0.04%**	**95.68%**	**0.16%**	**1.57%**	**0.67%**	**1.88%**	100.00%	

Question 2

Donation frequency is generally a minimum of 28 days or the same as standard plasmapheresis donation. The US Food and Drug Administration (FDA) considers these to be infrequent plasma donors and allows an exemption from additional history, physical and testing requirements of those who donate more frequently [1]. Contingent upon need, a reduced deferral period can be determined at the Medical Director's discretion, and depends on the type of donation, and specific donor characteristics, donation history and urgency of the situation.

When the Medical Director uses his or her discretion, this differs from the donation frequency permitted for routine plasma donation.

Yes. The rationale is based upon the fact that these products are derived from persons who have met all the usual blood donor eligibility criteria [2,3] and testing for relevant transfusion-transmitted infections [4] must be performed and the donation must be found suitable [5]. In fact, at OneBlood, CCP may be prepared from all qualified traditional blood donors who test positive for SARS-CoV-2 antibodies using orthogonal testing to confirm naturally-acquired immunity. Red blood cells and platelets are labelled as per standard operating procedure in these instances.

Question 3

CCP is collected for compassionate use as well as clinical trial use. CCP is not currently collected at OneBlood for fractionation purposes. The CCP collected is primarily intended to be used immediately to meet patient needs, however, effective efforts have been made to build a stockpile of CCP inventory for future use and as a backup plasma supply as needed.

Question 4

The criteria have evolved over time. Initially the donors were required to have proof of infection in the form of a positive polymerase chain reaction (PCR) test and/or antibody test, and be 28 days from infection and symptom-free. The latter criterion was changed to complete resolution of symptoms at least 14 days before the donation [6]. Currently there are two sources of CCP: (1) volunteer CCP donors with a history of COVID-19 who are now symptom-free and (2) traditional blood donors with an unknown history, who upon testing their donation sample, are found to have antibodies to both Spike and Nucleocapsid proteins ("orthogonal" testing). At present, the BC requires the CCP donors to be symptom-free for 14 days and test positive for both Spike and Nucleocapsid antibodies at the time of donation. Individuals who have received the SARS-CoV-2 vaccine may donate CCP if they had symptoms of COVID-19 and a positive test result from a diagnostic test approved, cleared, or authorized by FDA, and received the COVID-19 vaccine after diagnosis of COVID-19, and are within 6 months after complete resolution of COVID-19 symptoms [6].

Recovery is defined as the absence of symptoms such as cough, fever, body aches, neurologic or other unusual symptoms for at least 14 days for those donors with a history of COVID-19.

Question 5

No.

Question 6

Both CCP and traditional donors' blood samples are collected at the time of donation and tested for SARS-CoV-2 antibodies. (Cut-off criteria listed in question 7).

Question 7

Two test methodologies are used. The screening assay is the Ortho VITROS® SARS-CoV-2 Total (detects anti-Spike protein). If the signal-to-cut-off (S/C) ratio is 10 or greater, then this result is confirmed by the Roche Elecsys anti-SARS-CoV-2 assay (detects anti-Nucleocapsid protein). (Note: Per the manufacturer a S/C ratio of one is considered positive on the Ortho test but at OneBlood we use a higher S/C ratio to qualify a unit for CCP. The rationale is that correlation testing has confirmed that a higher S/C ratio corresponds to higher titres of SARS-CoV-2 antibody levels). If both test results are positive, then the unit qualifies for the manufacturing of CCP. The US Food and Drug Administration (FDA) will be requiring plasma donations to be tested by the Ortho VITROS SARS-CoV-2 IgG test and found to have a signal-to-cut-off (S/C) ratio of 12 or greater to qualify as a High Titre CCP, effective on 1 June 2021 [6,7].

Both serum and plasma samples are collected if needed for future assessment.

Question 8

No.

Question 9

Yes.

Question 10

OneBlood follows the US FDA requirements for deferral of CCP, which is complete resolution of symptoms at least 14 days before the donation [6]. This is for the safety of the donor collection staff.

Question 11

There is a three-month deferral required by the US FDA. This is consistent with the deferral required for transfusion recipients of Whole Blood or blood components such as packed red blood cells, platelets or plasma [8].

Question 12

In reflecting on lessons learned and challenges throughout the process, the following key areas were identified [9]:

1. Data

Comprehensive data was lacking regarding the regional and national blood supply, the number of units available in hospitals, and hospital usage patterns. This impaired the ability of the BC to predict the effect of the pandemic on the blood supply. As it impacted a large portion of the nation's BCs simultaneously, it affected the BCs ability to move blood from unimpacted to impacted areas. Additionally, lack of data impaired the BCs ability to predict the effect of cancellation of elective procedures or the regional need for blood products, a capability that would have proven invaluable for integrating CCP production planning into operations.

2. Communication and Coordination

There were issues with communication at all levels, particularly nationally; highlighting the need for enhancing existing networks and communications pathways and keeping them open. Various parts of the nation were impacted in different ways and times by the pandemic, resulting in disjointed communications between regions and national organizations regarding prioritization of need for resources. The FDA and other national and regional organizations compensated for these gaps by making themselves available at all times. At the regional level, the BC Communication team's proactive approach proved effective in controlling messaging to employees and the public.

3. Donor recruitment

CCP donor recruitment challenges were mainly attributable to the BC lack of access to qualified CCP donor lists. This resulted from regulations intended to protect patient privacy but had the unintended consequence of inhibiting the hospitals and health departments from sharing needed information about individuals who had recovered. This resulted in weeks of delay in CCP inventory development. Another donor recruitment lesson learned was related to timing. Initially, FDA required a 28-day deferral period after a CCP patient became asymptomatic. Since the epidemic was in its early stages in the US, many COVID-19 convalescent individuals had only recently recovered from their illness. Therefore, the 28-day wait period significantly limited the size of the eligible donor base. Another ongoing donor-related challenge was inconsistent national messaging regarding disease risk and mitigation.

4. Testing

The lack of licensed tests for SARS-CoV-2 presented challenges to donor recruitment and CCP product characterization. There was a plethora of new serologic and molecular tests on the market for SARS-CoV-2 that had received emergency use authorization (EUA) by FDA, however lack of test availability remained an issue throughout the two phases. Trained staff were required staff to check the FDA website of EUA approved tests to determine if the test qualified [10]. This process was an impediment to rapid donor intake and processing. Since there were no widely available antibody titre tests to verify CCP product efficacy the FDA recommended additional donor samples were to be collected and stored for future titre testing. The availability of extra sample tubes proved critical for later titre testing and research.

5. Personal Protective Equipment (PPE) and other supplies

The need for additional PPE put a strain on the BC at a time when there were national shortages. Severe supply chain shortages of PPE, disinfectants, paper goods and apheresis kits persisted throughout the project. This created pressure on BC administration to find needed supplies, an effort that was hampered by lack of prior relationships with vendors. Single source suppliers and just-in-time inventory practices, initially designed to reduce BC expenditures, exacerbated the situation.

6. Messaging

Inconsistent national messaging regarding risk, safety of blood donation and need for PPE resulted in ongoing confusion of donors and staff as to what social distancing measures were appropriate and resulted in the loss of significant numbers of donors and drive cancellations. Proactive communication strategies using social media, emails and traditional media helped to maintain open lines of dialogue with employees and the public, who were reassured by consistent messaging that the BC followed all recommended FDA and Centers for Disease Control and Prevention (CDC) guidelines for protective measures and that it was safe to donate.

7. Hospitals and Clinicians

Clinicians were unfamiliar with CCP resulting in hospitals and physicians being inadequately prepared for the roll-out of the product. The unproven safety and effectiveness of CCP coupled with a lack of other therapeutic modalities for COVID-19 resulted in a level of uncertainty around availability and use of the product. This required ongoing communication between clinicians and BC physicians. Convalescent plasma had been used in previous viral outbreaks for decades, yet only in March of 2020 did FDA authorize it for BCs to collect and physicians to use under an emergency investigational new drug (eIND) or expanded access program (EAP) for treatment of COVID-19. The EAP and eIND pathways were unfamiliar to some non-research-based hospitals and BCs, which found the requirements cumbersome. The lack of coordination and preparation at the BC/hospital level with the national programs (eIND and EAP) and the need for use of manual systems during the early development of the program caused delays and frustration. Physician and public lack of education regarding the challenges faced in implementing a CCP program led to public relations issues for the BC, which was expected to be able to produce CCP on demand, like other blood products.

Another lesson learned was that while a CCP donor may present, there is no guarantee that a successful collection will occur. Even if CCP is obtained, ABO incompatibility might make the unit unsuitable. This was also a point of education for the ordering physicians who recruited family members to provide directed donations. Situations occurred in which family members were assured that the unit would be obtainable shortly after the donation only to discover later that the unit was unusable for various quality reasons.

8. BC Supporting Infrastructure

The pre-existence of a strong implementation support structure including the Project Management Office (PMO), in-house Information Technology (IT)/Business Intelligence (BI) and Business Continuity Plan (BCP) teams prior to the onset of the pandemic greatly enhanced BC responsiveness to the CCP project challenges and proved to be critical in disaster management. The PMO at the BC consisted of a dedicated, experienced team of professionals who were rapidly deployed on the project to develop and revise processes and training in response to frequent changes in FDA requirements. This department played a pivotal role in ensuring the success of the project.

IT/BI was instrumental in streamlining and automating the intake and distribution aspects of the process. The BI team tracked and transformed complex data into highly functional dashboards and reports that allowed real time assessment and strategy development.

The BCP team performed daily horizon scanning on a global level and kept BC Leadership apprised of the progression of the pandemic and any additional threats. They gathered the CCP implementation team together daily for updates to facilitate and maintain communication in an extremely fluid environment.

Section References

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Question 1

NHS Blood and Transplant (NHSBT) is a national blood establishment that provides blood components for all hospitals in England. Until March 2020 NHSBT did not have a plasmapheresis service, this has been set up to provide convalescent plasma to the two trials that are being conducted in the UK (RECOVERY and REMAP-CAP). During 2019 we issued 1·4 million RBCs, 255 000 platelet units and 260 000 plasma units. Only platelet components were collected by both apheresis and whole blood donations during 2019. In 2020 we have established a plasmapheresis service for convalescent plasma and have collected over 30 000 units of plasma.

Describe your institution: COVID-19 convalescent plasma (CCP) collection program

Question 2

Convalescent plasma is collected via plasmapheresis. Donors can donate up to every week up to a maximum of 24 times annually. A donor can donate plasma as long as their antibody levels remain at high or medium levels (Euroimmun $\geq$3), however we test total protein levels after every 8 donations to ensure protein levels remain normal. Some of our donors have donated up to 20 times.

We do not have a standard plasmapheresis service for collection of plasma other than convalescent plasma at the present time.

Question 3

CCP is collected for transfusion. We are using CCP within two trials RECOVERY (https://www.recoverytrial.net/) and REMAP-CAP (https://www.remapcap.org/). A few units have been issued outside of the trial for patients who are ineligible for either trial. Data on these patients will be collected as part of the CLEARANCE registry. We are not fractionating plasma, any change to this policy will require UK government authorization.

Question 4

Donors for CCP donation have to meet our standard blood donor criteria. The only exception to this is that previous CCP recipients can become CCP donors but they cannot become standard whole blood or platelet apheresis donors. Potential female donors also have to have negative HLA and HNA antibody tests.

Donors may donate 28 days after recovery. Our definition of recovery from SARS-CoV-2 infection is when the donor is generally well and back to normal activities and no longer has significant fatigue which affects daily activities, fever, respiratory symptoms, cardiac or other symptoms such as headaches or dizziness.

Question 5

No. We do not require donors to be tested for SARS-CoV-2 by PCR before donation. However, we do insist it has to be at least 28 days since recovery from their symptoms prior to donating plasma.

Question 6

We do not test antibodies levels prior to donation for men who have had a previous positive PCR test for

anti-SARS-CoV-2 prior to donation. This is because we have found that 25% of these donors will have high antibody levels (Euroimmun ≥6).
We test all other potential donors with antibody tests prior to donation. The cut-off for accepting the potential donor for donation is a Euroimmun of 6 or above.

Question 7
Yes. We test all units prior to issue for anti-SARS-CoV-2 antibodies. The cut-off for use within the trials is a Euroimmun of 6 or above. All other units with positive antibody tests that do not meet this criterion are currently being stored. Additional samples on the CCP issued for use have been archived in case a better test for assessing the quality of a convalescent plasma sample is developed in the future.

Question 8
No. We do not use any pathogen reduction treatments on our convalescent plasma components or our plasma components.

Question 9
Yes. We started accepting recipients of CCP as convalescent plasma donors but not as donors for any other blood components. Our first donor who has received CCP has donated and had high anti-SARS-CoV-2 antibody levels (Euroimmun 20).

Question 10
Yes. We accept individuals who have recovered from COVID-19 infection as standard whole blood or platelet apheresis donors. They must have recovered from COVID-19 at least 28 days prior to donating.

Question 11
No. Individuals who have received CCP during their illness are unable to become standard whole blood or platelet apheresis donors.

Question 12
We have achieved a lot over the last few months. Rolling out a national convalescent plasma programme has required a huge amount of work by a large number of participants. We have opened 20 new donor centres over the last year to enable collection of convalescent plasma.

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South Africa – Western Cape Blood Service

Vernon Louw

Question 1

Type of institution.
○ Regional Blood Services/Blood Centre (responsible for recruiting donors; screening and selecting blood donors; blood collection; testing and processing blood units; transporting; receiving and storage of blood units; pre-transfusion testing, and issuing blood for clinical transfusion at a regional level)
Institution demographics.
Whole blood donations 147 684, Platelets 9265, Plasma 38 000.

Question 2

Only plasmapheresis.
Every 2 weeks, i.e. 24 × per year.

Question 3

Only for trial use.

Question 4

SARS-CoV-2 PCR and/or antigen test and/or SARS-CoV-2 Ab positive.
14 days post last symptoms or asymptomatic since test

Question 5

No.

Question 6

Yes, but donation allowed before results available.

Question 7

Yes.

Question 8

Yes, Terumo technique (riboflavin)

Question 9

Yes, in theory, no such rules, but 3-month deferral.

Question 10

Yes.

Question 11

Yes, after 3-month deferral.

Question 12

Concerns about the risk of attending a blood collection centre.
Many staff members off-sick from COVID during this period, adding to the high workloads.
Putting in place protocols to ensure staff and donor safety.
Outbreaks of COVID-19 among staff or exposure of staff to COVID patients leading to quarantining and being off work.
Management of staff working remotely.
Decrease in blood drives and blood stocks.

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DIARY OF EVENTS

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See also http://www.isbtweb.org/congresses/

4.5.2021	IPFA/PEI – The International Workshop on Surveillance and Screening of Blood-borne Pathogens
13–15.5.2021	The Canadian Society for Transfusion Medicine (CSTM) are holding their annual scientific conference virtually in 2021.
26–27.05.21	21st Congress of the European Society for Hemapheresis
5–9.6.2021	ISBT In Focus, the 31st regional congress of the ISBT, will be a virtual event in 2021
17.9.2021	11th BIC International Conference – Advances in Haemostasis and Bleeding Disorders
22–24.9.2021	Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie e.V.
23–26.9.2021	16th International Congress on Myelodysplastic Syndromes (MDS 2021)
13–16.11.2021	32nd Regional congress of ISBT, Brisbane, Australia