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Aims and Scope

Vox Sanguinis reports on all issues related to transfusion medicine, from donor vein to recipient vein, including cellular therapies. Comments, reviews, original articles, short reports and international fora are published, grouped into six main sections:

1. Donors and Donations: donor recruitment and retention; donor selection; donor health (vigilance, side effects of donation)
2. Blood Component Collection and Production: blood collection methods and devices (including apheresis); blood component preparation; inventory management; collection and storage of tissues; quality management and good manufacturing practice; automation and information technology; plasma fractionation techniques and plasma derivatives
3. Transfusion-transmitted Disease and its Prevention: identification and epidemiology of infectious pathogens transmissible by blood; donor testing for transfusion-transmissible infectious pathogens; bacterial contamination of blood components; pathogen inactivation
4. Immunohaematology and Immunogenetics: autoimmunity in haematology; alloimmunity of blood; pre-transfusion testing; complement in immunohaematology; blood phenotyping and genotyping; genetic markers of blood cells and serum proteins: polymorphisms and function; parentage testing and forensic immunohaematology
5. Transfusion Medicine : transfusion practice, thresholds and audits; transfusion efficacy assessment, clinical trials; haemovigilance; non-infectious transfusion adverse events; therapeutic apheresis
6. Cellular Therapy: cell-based therapies; CAR T-cell therapies; genetically modified cell therapies; cellular therapy (sources; products; processing and storage); stem cells; cell-based regenerative medicine; cellular immunotherapy; molecular therapy

This comprehensive coverage has made the journal essential reading for a wide range of specialists interested in the present state of transfusion research and practice.

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REVIEW

ABO blood group and COVID-19: a review on behalf of the ISBT COVID-19 Working Group

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Abstract

Growing evidence suggests that ABO blood group may play a role in the immunopathogenesis of SARS-CoV-2 infection, with group O individuals less likely to test positive and group A conferring a higher susceptibility to infection and propensity to severe disease. The level of evidence supporting an association between ABO type and SARS-CoV-2/COVID-19 ranges from small observational studies, to genome-wide-association-analyses and country-level meta-regression analyses. ABO blood group antigens are oligosaccharides expressed on red cells and other tissues (notably endothelium). There are several hypotheses to explain the differences in SARS-CoV-2 infection by ABO type. For example, anti-A and/

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or anti-B antibodies (e.g. present in group O individuals) could bind to corresponding antigens on the viral envelope and contribute to viral neutralization, thereby preventing target cell infection. The SARS-CoV-2 virus and SARS-CoV spike (S) proteins may be bound by anti-A isoagglutinins (e.g. present in group O and group B individuals), which may block interactions between virus and angiotensin-converting-enzyme-2-receptor, thereby preventing entry into lung epithelial cells. ABO type-associated variations in angiotensin-converting enzyme-1 activity and levels of von Willebrand factor (VWF) and factor VIII could also influence adverse outcomes, notably in group A individuals who express high VWF levels. In conclusion, group O may be associated with a lower risk of SARS-CoV-2 infection and group A may be associated with a higher risk of SARS-CoV-2 infection along with severe disease. However, prospective and mechanistic studies are needed to verify several of the proposed associations. Based on the strength of available studies, there are insufficient data for guiding policy in this regard.

Key words: COVID-19, SARS-CoV-2, ABO blood groups, disease susceptibility, disease severity.

Introduction

The COVID-19 pandemic spurred a crisis that is unprecedented in modern times [1]. The disease course varies substantially among individuals, from mild or even subclinical infection to severe disease [2]. Indeed, more than 1 million COVID-19-related deaths have been reported globally. There is interest in potential risk factors that affect susceptibility to infection and disease progression. Multiple medical (e.g. diabetes, hypertension) and sociodemographic (e.g. sex, age and race/ethnicity) risk factors for severe outcomes were already established [2]. Growing evidence suggests that the ABO blood group may also play a role in the immunopathogenesis of SARS-CoV-2 infection, with group O being protective and group A conferring risks of higher disease susceptibility and severity [3–7].

An international group of experts in transfusion medicine and haematology were assembled by the International Society of Blood Transfusion (ISBT) to review and summarize the literature with a view of offering recommendations pertaining to ABO type and COVID-19. To this end, we provide an overview of the ABO blood group system, ABO population frequencies and distributions, its role as a histo-blood group antigen, not just a blood group antigen, and the known associations between ABO type and various infectious and non-infectious diseases. Finally, we present a scoping review of the literature on the associations of ABO type with COVID-19 and propose mechanistic pathways that could potentially explain these observations.

Search strategy and selection criteria

A combination of searching using Medline's controlled vocabulary/indexing, [Mesh]/[Supplemental Concept], and keyword searching titles and abstracts [tiab] features was used to search articles on ABO blood group and infectious diseases and ABO blood group and COVID-19 or SARS-CoV-2 to (a) pull articles either specifically indexed about our topic/concept as well as (b) pull articles wherever our topic/concept of interest was mentioned. Furthermore, we also searched all the Web of Science (WOS) databases using the same keyword searching as for PubMed/Medline. WOS in addition supports the NEAR operator (searches for words that appear near one another in the text without requiring an exact phrase match). The search was confined to English-language articles that were published prior to 10 November 2020. Pre-print articles were also included on a case-by-case basis.

ABO blood group overview

Of the 39 blood group systems and 350 antigens recognized by the ISBT [8], the ABO system is clinically the most important [9]. The A and B antigens are inherited co-dominantly over O [10]. The ABH antigens (H antigen defines the O blood type) are oligosaccharides exposed on RBCs and other cells; they are also found in body secretions. The A and B antigens are determined by allelic genes encoding glycosyltransferases that transfer monosaccharides to the non-reducing ends of specific glycans on glycoproteins and glycolipids. For A and B,

this monosaccharide is N-acetyl-D-galactosamine and D-galactose, respectively. In group O individuals, the corresponding A and B glycosyltransferases are either not present or have been inactivated by one of various polymorphisms, such that the non-reducing ends of the corresponding glycans express the H antigen.

Antibodies in this system (i.e. anti-A and anti-B) develop in the first few months of life; they are typically 'naturally occurring' antibodies produced after contact with non-self A and/or B antigens, often found in food and micro-organisms, notably the gut microbiota [11]. Anti-A and anti-B, typically of the IgM isotype, circulate in almost all healthy individuals who lack the corresponding antigen; IgG anti-A,B is often found in group O individuals [12–14]. Transfusion of ABO-incompatible RBCs can provoke acute haemolytic transfusion reactions because the corresponding IgM antibodies bind complement efficiently, causing intravascular hemolysis of the transfused RBCs and activation of coagulation. IgG antibodies can also cause severe intravascular hemolysis in this setting, because of the very high density of ABH antigens on RBCs, leading to close proximity of anti-A and/or anti-B IgG molecules on the RBC surface with subsequent complement activation [15, 16]. If untreated, this medical emergency can induce acute renal failure, disseminated intravascular coagulation and death. Therefore, always transfusing ABO-compatible RBCs is a central focus of modern blood banking processes and procedures.

Population frequency of ABO blood groups

ABO blood group frequencies vary among human populations, suggesting that migration and the selective advantage of particular blood groups, perhaps relating to exposure to specific pathogens, might have contributed to these variations (Table 1). For example, group O is the most common globally, followed by A, then B, and then AB. Group O may have originated in Africa before early human migration, because it may have provided a selective advantage against malaria [17]. In contrast, group O individuals are at higher risk of developing severe cholera, perhaps explaining the lower prevalence of group O and higher prevalence of group B, in the Ganges Delta region of Bangladesh [18].

Indigenous populations show widely divergent ABO blood group distributions. For example, those in Australia and North and South America almost completely lack group B, whereas those in Asia show the highest rates of group B. In addition, group A is almost non-existent in indigenous populations of South and Central America, but more prevalent (>30%) in Canada, Scandinavia and Central Europe, perhaps due to selective pressure provided by smallpox [18]. In contrast, ABO distributions of *current populations* in these same countries demonstrate the impact of migration; for example, although group A is virtually absent in indigenous populations in Central and South America, its current overall population frequency is as high as 30% [18].

ABO is not just a blood group antigen

Each RBC expresses ~2 million copies of its genetically encoded ABH blood group antigens on its surface, although the density varies by antigen type. Other blood cells (e.g. platelets and lymphocytes) also adsorb ABH-expressing glycosphingolipids from plasma, where they circulate attached to lipoproteins. In addition, ABH antigens are synthesized and expressed on endothelial cells and certain epithelial cells. Thus, although some blood group antigens are only on RBCs, ABH antigens are in various cells, body fluids and secretions. Therefore, they are more correctly denoted as 'histo-blood group antigens' (HBGA), not solely as blood group antigens [11, 19–23].

In addition to serving as antigenic barriers during transfusion, transplantation and pregnancy, ABH oligosaccharides physiologically influence hemostasis and, therefore, confer disease risks in this setting. For example, A and B glycosyltransferases modify H-active glycans on von Willebrand factor (VWF) [24]. Interestingly, VWF in group O individuals has a shorter half-life, accompanied by 25–30% reduced VWF and Factor VIII levels, as compared to group A or B individuals. However, independent of the ABO blood group, glycosyltransferase activity was also decreased in patients with venous thromboembolism, as compared to healthy controls [25]. In addition, higher VWF and factor VIII levels are associated with increased risks for coronary heart disease, arterial thrombosis and venous thrombosis [26, 27]. Therefore, perhaps not surprisingly, recent genome-wide association studies (GWAS) demonstrated that ABO locus variants correlate with increased plasma lipid and inflammatory marker levels [25, 28].

ABO expression may also not be stable over time, with lower levels in healthy children <2 years old [29] and changes in various diseases (e.g. necrotizing infection, thalassemia, malignancy) [20, 30]. In addition, as compared to group O, group A individuals have a higher prevalence of gastric cancer, and group A, AB or B individuals have a higher prevalence of pancreatic cancer; possible mechanisms include ABO blood group influences on regulating proinflammatory [31] and adhesion

Table 1 Table of ABO Geographic Distributions for the native and contemporary populations

Region		Native population			Current population		
		Type A %	Type O %	Type B %	Type A %	Type O %	Type B %
North America	Canada	Up to 40	80–100	0–5	40+	40+	9
	United States	0–15*	80–100	0–5	40+	40+	~10
Central and South America		Absent	90–100	0–5	10–30	50–80	~10
Greenland		Up to 40+					
Australia		Up to 40+[†]	60–80 (North)	0–5	38	49	~10
Africa		15–20	60–80	10–20	~20–25[¥]	Up to 60	West > 20
Middle East		15–20	60–80	5–15	~25	>40	>20
Europe	Scandinavia	25–40[‡]	50–70	0–10	40+	~40	10
	Western Europe	25–30	60–70	5–10	30–40	30–40	~10
	Eastern Europe	25–30	50–60	10–20	30–40	30–40	~10
Russia		15–20	50–60	15–30	~35	~35	~10
Asia	China	20–25	60–70	15–25	~30	~50	~20
	Japan	15–25	50–70	10–15	40	30	20
	Pacific	15–20	60–70	15–25	25–30	>40	~30
India		15–20	56–60	Up to 30	22	29	38

*Blackfoot of Montana: 30–35%.

[†]Aboriginal Australians: 40–53%.

[‡]Lapp: 50–90%.

[¥]Cameroon 38, Uganda 39, South Africa 32%.

Table 2 Potential mechanisms for relationships between histo-blood group antigen (HBGA) and infection

Action as a receptor or co-receptor for a given pathogen
Functions as a receptor for a virulence factor, toxin, or other pathogenic product
Interaction with a pathogen that is limited to a specific strain, specific organ system or disease state
Modification of a key target cell surface glycoprotein or glycolipid, thereby affecting important cellular functions (e.g. endocytosis, phagocytosis, signal transduction) in response to infection

molecules [32, 33], and the role of VWF in angiogenesis and apoptosis [22, 25, 34, 35].

Associations between blood groups and infectious diseases

HBGAs are implicated in the pathogenesis of multiple infections. In particular, the ABO blood type has been associated with, for example, tuberculosis, malaria, cholera, norovirus, retrovirus, Chikungunya virus, *Helicobacter pylori (H. pylori)* and *Escherichia coli* [36, 37]. The underlying mechanisms range from simple (e.g. receptor-ligand interactions) to complex and may be limited to a specific pathogenic product, strain or disease state. For ABO, possible explanations include ABH antigens as receptors for pathogens, natural antibodies and lectins as inhibitors, and molecular mimicry by blood group antigens between pathogen and host.

One specific example involves the P antigen in the Globoside blood group. This antigen is necessary, but not sufficient [38], for parvovirus B19 entry into RBCs, requiring a co-receptor for infection [39, 40]. The distribution of P antigen, including relatively high expression by RBCs and their precursors, is consistent with parvovirus B19 clinical syndromes, including aplastic anaemia [41]. Furthermore, individuals lacking the P antigen (i.e. the p phenotype) are resistant to this infection [42].

For other infections, HBGAs can be receptors for toxins, virulence factors or other pathogenic products without directly binding the implicated pathogen itself. In addition, HBGAs in secretions, body fluids or non-erythroid tissues can contribute to pathogenesis. For example, adhesion and colonization by *H. pylori*, the aetiologic agent of peptic ulcer disease and some forms of gastric cancer, are facilitated by Le^b/H oligosaccharides on gastric epithelial mucins [43, 44] with certain strains

having higher affinity for Le^b/H [45]; in addition, group O individuals, by expressing more H antigen, are more likely to be infected [46].

In summary, the relationship between HBGAs and a specific infection should ideally satisfy Koch's postulates. Nonetheless, this is often difficult to document fully in human studies. Typically, one can only show that individuals expressing a specific blood group or HBGA are more susceptible to infection, whereas individuals without it are completely resistant or, at least, protected from severe disease. Multiple examples demonstrate that HBGAs can interact with pathogens at initial infection, or alter disease progression/severity, or affect clinical presentation (Table 2).

ABO blood group and susceptibility to SARS-CoV-2

During the severe acute respiratory syndrome coronavirus (SARS-CoV-1) epidemic, several observations suggested that ABO type may contribute to disease, with less susceptibility in group O individuals [47]. This was also observed for SARS-CoV-2 (Table 3). Most studies identified a higher proportion of group A, and a lower proportion of group O, among COVID-19 patients, as compared to healthy controls [5, 7, 12, 48–52]. These studies included patients with SARS-CoV-2 pneumonia ranging in severity from mild to critically ill requiring mechanical ventilation or intensive care unit admission [48, 51]. For example, in one study, the proportion of group A infected patients was significantly higher than in healthy controls (38% vs. 32·2 %, $P < 0{\cdot}001$), whereas group O was significantly lower (25·7% vs. 33·8%, $P < 0{\cdot}001$); however, group A patients had higher frequencies of underlying comorbidities [7]. Another retrospective study had similar findings, but did not describe comorbidities [52]. Another study described a higher rate of infection in group AB patients and a lower rate in group O patients [50]. In contrast, an additional study did not find any correlation between group A status and COVID-19; nonetheless, group O individuals had a lower risk of COVID-19 and group B and AB individuals had a higher risk [6]. One potential reason for these varying results is that many such studies did not account for various confounders (e.g. age), including comorbidities. Another potential confounder for some of the studies could be the use of randomly selected volunteer blood donors as controls, because of the risk of group O epidemiological predominance due to blood collectors selectively recruiting group O donors. Importantly, volunteer blood donors are not necessarily representative of general populations; although convenient, their use as a control group is not optimal [53, 54].

It has also been hypothesized that anti-A and anti-B antibodies could interfere with virus–cell interactions. In a secondary analysis of data from ~1900 patients with COVID-19, subjects with circulating anti-A were significantly less represented in the disease group as compared to those lacking anti-A. In addition, anti-A in group O individuals was more protective than anti-A in group B individuals; this may relate to the increased presence of IgG anti-A,B in group O plasma [13].

One study attempted a meta-regression analysis of 101 nations using their known blood group distributions, including ~9-million COVID-19 cases and ~450 000 deaths in a total population of ~7 billion. Although there was no association of group A or B with overall mortality, group O significantly correlated with lower mortality ($p = 0{\cdot}02$). The authors proposed that COVID-19 mortality was lower in nations with higher group O prevalence because overall population ABO blood group prevalence was analysed as the control [55].

Studies have also examined the relationship between the Rhesus blood group (e.g. Rh(D) type) and COVID-19. One study suggested that Rh(D)-positive individuals were more likely to test positive for SARS-CoV-2 [6]. Another study found significant associations between Rh(D) blood group status, group B, and SARS-CoV-2 [56].

In summary, these mixed findings may be ascribed to the different populations, the controls that were used for comparison, the geographical locations and the confounders that were considered. The latter include age, comorbidities and using volunteer blood donors as controls.

ABO and COVID-19 disease severity

The effect of ABO type on COVID-19 disease severity also warrants analysis. As such, in one study, group A patients had a higher risk of hospitalization for SARS-CoV-2 infection, whereas group O patients were at lower risk [7]. However, the group A patients had more comorbid risk factors for severe disease, which were not adjusted for using a multivariate analysis. Another group performed a GWAS of COVID-19 patients with respiratory failure [57], detecting a statistically significant cross-replicating association at 9q34. The 9q34 signal was located at the *ABO* blood group locus and a blood type-specific analysis showed a higher risk of severe COVID-19 with respiratory failure for group A individuals and a protective effect for group O.

As another example, in a nested prospective observational study of critically ill patients with COVID-19 in Canada, using a multivariable adjustment of various risk factors, patients with blood group A or AB had an increased risk of requiring mechanical ventilation,

Table 3 Summary of reported studies assessing the association between blood groups and SARS-CoV-2 infection

Ref. Author (country)	COVID-19 study population	Controls(if applicable)	% group A patients (vs. control) P value (when applicable)	% group O patients (vs. control) P value (when applicable)	Blood group susceptibility to SARS-CoV-2 infection	Association with clinical outcomes and risk of death
Zhao J et al. [5] China	1775 patients	3694 normal individuals	37·75 (32·16) *P < 0·001*	25·80 (33·84) *P* < 0·001	Yes, group A	Group A associated with higher risk of mortality than non group A
Li J et al. [7] China	2153 patients with COVID-19 pneumonia	3694 healthy controls	38·0 (32·2) *P* < 0·001	25·7 (33·8) *P* < 0·001	Yes, group A	Group A patients at higher risk of hospitalization following SARS-CoV-2 infection. Association with risk of mortality not assessed.
Zeng X. et al. [51] China	137 patients with mild pneumonia 97 patients with severe pneumonia	Nil, Chinese population data used for comparison	35·76 (28·39) 39·22 (28·39)	32·45 (33·20) 26·47(33·20)	Yes, group A	Blood group A more susceptible to SARS-CoV-2. Blood groups not relevant to acute respiratory distress syndrome, acute kidney injury and mortality.
Zietz M & Tatonetti [56] USA	Observational data on 14,112 individuals tested for SARS-CoV-2	None	32·7 (32·7)	46·9 (48·2)	Yes, group B and Rh(D)	Risk of intubation decreased among group A and increased among groups AB and B. Risk of morality increased for group AB and decreased for groups A and B. Rh-negative blood type protective for mortality.
GÖKER H, et al . [49] Turkey	186 patients	1881 healthy controls	57 (38) *P* < 0·001	24·8 (37·2) *P* = 0·001	Yes, group A	No significant effect of ABO and RhD on clinical outcomes including intubation, ICU stay and mortality
Wu et al. [52] China	187 patients	1991 non-COVID-19 hospitalized patients	36·9 (27·47) *P* = 0·006	21·92(30·19) *P* = 0·018	Yes, group A	Group A influenced clinical outcomes but no association with mortality
Leaf RK et al. [48] USA	561 critically ill patients.	Nil, local population data used for comparison	45·1 (39·8)	37·8 (45·2)	Yes, group A	No association with any ABO phenotype and mortality
Latz CA et al. [6] USA	1289 patients	Nil	34·2 (NA)	45·5 (NA)	Yes, positive correlation with group B , AB & Rh(D)Negative with group O	No association with risk of intubation, peak of inflammatory markers and death
Gerard C et al. [12]	1175 patients	3694 controls	37·7 (32·2) *P* < 0·001	49·4 (57·6)	Yes, presence of anti-A antibodies in serum and more specifically IgG anti-Aassociated	Mortality risk not assessed

Table 3 (Continued)

Ref. Author (country)	COVID-19 study population	Controls(if applicable)	% group A patients (vs. control) P value (when applicable)	% group O patients (vs. control) P value (when applicable)	Blood group susceptibility to SARS-CoV-2 infection	Association with clinical outcomes and risk of death
					with higher susceptibility to SARS-CoV-2 infection.	
Abdollahi A et al. [50] Iran	397 patients	500 normal controls	40·3 (36) $P = 0·19$	28 (38) $P = 0·002$	Yes, group AB with higher susceptibility than other groups.	No association of ABO or RHD phenotype with severity of disease. No association of ABO or RHD with mortality assessed.
Hoiland et al. [4] Canada	125 critically ill patients admitted to ICU	Nil, Comparison of blood group distributions between blood donor data was performed.	37 (35) p-0·60 No difference from blood donors	Group O 43% ($n = 41$) No difference from blood donors	Yes, group A and AB	Higher proportion of COVID-19 patients with blood group A or AB required mechanical ventilation, and continous renal replacement therapy and had longer ICU stay compared with patients with blood group O or B.
Barnkob et al. [3] Denmark	7422 COVID positive patients among 473 654 individuals tested	466 232 COVID-negative individuals	More A (P <0·001), B ($P = 0·011$), and AB ($P = 0·091$) individuals were COVID positive.	38·41% (95% CI, 37·30 –39·50) group O compared with 41·70% (41·60–41·80) in controls	Yes, Decreased infection risk in group O	ABO blood group as a risk factor for SARS-CoV-2 infection but not for hospitalization or death from COVID-19.

continuous renal replacement therapy and prolonged intensive care unit admission, as compared to group O or B patients [4]. Another recent retrospective cohort analysis including nearly half a million Danish individuals tested for SARS-CoV-2, also showed reduced prevalence of SARS-CoV-2 infection in blood group O individuals. This study identified ABO blood group as a risk factor for SARS-CoV-2 infection but not for hospitalization or death from COVID-19 [3].

Taken together, these studies suggest that the risk of infection with SARS-CoV-2 and the risk of severe COVID-19 disease may be lower in group O individuals than non-group O individuals. Nonetheless, these results are not definitive and further studies are warranted.

Mechanisms for associations between ABO blood group and COVID-19

Several pathophysiological mechanisms were proposed to explain the association between ABO type and SARS-CoV-2 infection (Fig. 1, Table 4). Anti-A and/or anti-B antibodies might bind to A and/or B antigens expressed on the viral envelope, thereby preventing infection of target cells; that is, these naturally occurring antibodies could function as viral neutralizing antibodies. If true, this would help explain differences in initial susceptibility for SARS-CoV-2 infection. For example, an anti-A viral neutralizing antibody in a potentially susceptible group O host would bind the A antigen on virus produced by, and inhaled from, an infected group A (or group AB) host [58]. Why this mechanism would be relevant to disease severity *per se* is less obvious, because subsequent rounds of viral proliferation in a group O host would produce virus expressing the H antigen on its envelope. However, assuming that disease severity relates to the size of the infecting inoculum and yielding the subsequent viral load, a neutralizing isoagglutinin (e.g. anti-A) could attenuate infection, if not preventing infection altogether. Finally, the entry barrier for this virus is the epithelium of the respiratory tract and, possibly, the digestive tract. Thus, to prevent infection, circulating antibodies may need to reach these cell surfaces; although, presumably, the most effective antibodies for this purpose are of the secretory IgA isotype, to date, no data are available about the IgA isotype for either anti-A and/or anti-B in this regard.

Glycan structures at various N-glycosylation sites of the SARS-CoV S protein were previously described [59–61]. In addition, N-glycans of recombinant SARS-CoV-2 S protein were recently characterized [62]; although ABH antigen structures were not described, this may be due to the cell line used to produce the recombinant protein. Interestingly, the receptor-binding domains of the SARS-CoV-2 and SARS-CoV S proteins are structurally nearly identical [63]; in addition, glycosylation yields S trimers in which the receptor-binding domains are covered by N-glycans. Thus, it is conceivable that SARS-CoV-2 S protein could be specifically bound by human anti-A antibodies, which could then block the interaction between the virus and the angiotensin-converting enzyme 2 receptor (ACE2R), thereby preventing entry into the lung epithelium. Relevant to this hypothesis, monoclonal or naturally occurring anti-A antibodies dose-dependently inhibited interaction between SARS-CoV S protein and ACE2R, in a model where the A antigen was associated with S protein [64]. Thus, if this is also true for SARS-CoV-2, it would support the hypothesis that anti-A blocks viral attachment and/or entry [65]. Although not definitive, currently available data could support the above mechanism. There is also emerging evidence that receptor-binding domain (RBD) of SARS-CoV-2 may share sequence similarity to an ancient lectin family known to bind blood group antigens. SARS-CoV-2 RBD binds the blood group A expressed on respiratory epithelial cells, which could explain the linkage between blood group A and SARS-CoV-2 [66].

Another potential mechanism for explaining an association between group A and severe COVID-19 is an increase in angiotensin-converting enzyme 1 (ACE-1) activity, with a predisposition to cardiovascular complications. Severe outcomes could also be explained by higher levels of VWF and factor VIII in group A individuals. Furthermore, VWF is an acute phase reactant with infection inducing even higher levels in group A individuals. Given that anti-A and anti-B antibody titres are highly variable among individuals [67, 68], the potential neutralizing effect of such antibodies is also expected to be highly variable [69], possibly obscuring the 'signal' in large population studies. This variability may be further compounded by the significantly higher binding affinity of SARS-CoV-2 S protein for ACE2R, as compared to SARS-CoV [70].

Studies to confirm this hypothesis *in vitro* would involve producing recombinant SARS-CoV-2 S protein in cell lines that can synthesize terminal ABH oligosaccharides to determine whether these are, indeed, on the recombinant protein. In addition, it is important to culture SARS-CoV-2 in cell lines capable of synthesizing ABH oligosaccharides, isolate the virus and determine whether anti-A and anti-B prevent infection of cell lines that do not express the corresponding ABH antigen. If successful, these experiments would also allow testing whether IgM, IgA or IgG anti-A (or anti-B) antibodies were equally effective [71]. To our knowledge, there are no published studies to date that address this.

Other potential mechanisms may explain the epidemiological results. For example, if ABH oligosaccharides are

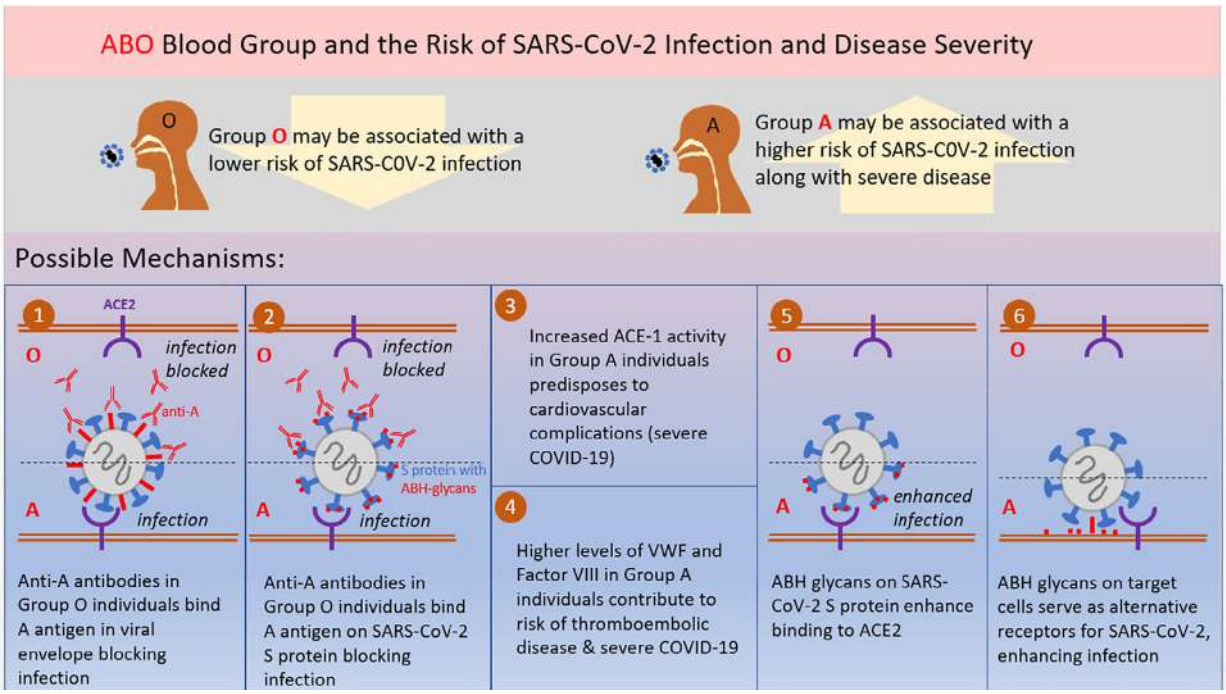

Fig. 1 Graphical summary of proposed mechanisms for association Between ABO blood groups and SARS-CoV-2 infection.

on SARS-CoV-2 S protein, they may modify the affinity of SARS-CoV-2 for ACE2R, its cellular receptor. This could be evaluated formally by producing recombinant S protein in otherwise identical host cell lines (e.g. by transfecting in the relevant glycosyltransferases) and then quantifying the affinity of the purified proteins for their receptor. Analogously, if the virus could be produced *in vitro* in these ABH-expressing cell lines and purified, the infectivity of a given target cell line could then be quantified. Given the published human population data, one might expect 'group O virions' to be less infectious in these experiments, thereby correlating with decreased COVID-19 disease severity.

A different, but not mutually exclusive, mechanism may involve ACE2R, which is also a glycoprotein and may express ABH glycans. It is possible that these glycans affect SARS-CoV-2 viral binding to ACE2R, the number of ACE2R proteins on a given cell surface, and/or the efficacy of internalization of the virus: receptor complex. In this case, ACE2R expressing H-antigen glycans may not be as effective at binding and internalizing SARS-CoV-2 produced by any source, irrespective of ABO type. This could also underlie COVID-19 disease severity.

It is also possible that the ABH glycans themselves could serve as (alternative) lower-affinity receptors for SARS-CoV-2 S protein or bind other viral envelope structures. Although current evidence suggests that this is unlikely, if it were relevant, then ABH glycan levels on cell surfaces, in plasma, and in secretions would be important and could affect initial infection and disease severity. For this purpose, determining the 'secretor phenotype' and Lewis blood group types would be helpful [37, 72]. Moreover, secretor status and Lewis antigen frequencies can affect host immunity [37, 72].

Because COVID-19 severity relates significantly to cardiovascular [73], thromboembolic [74, 75] and inflammatory complications, the patient's ABO type may be a surrogate for these effects and have nothing to do with blood type *per se*, the presence of naturally occurring anti-A and/or anti-B or viral-target cell interactions. For example, as described above, ABO type influences circulating VWF and factor VIII levels, which influence cardiovascular risk and hemostatic function, even in the absence of infection [76].

Although an association between ABO blood group and the risk of susceptibility to COVID-19 disease or disease severity is compelling, the practical significance is uncertain. COVID-19 convalescent plasma (i.e. plasma collected from those who recover from COVID-19) is being employed as an investigational therapy for treating COVID-19 [77, 78]. One theoretical ramification of an association with ABO type could affect such a therapy; specifically, donors with higher titres of antibodies could be recruited selectively based on blood type [79].

Table 4 Proposed mechanisms, theoretical pathways and suggested experiments for studying the association Between ABO blood groups and SARS-CoV-2 infection

Proposed mechanisms for association between ABO blood type and SARS-CoV-2 infection
Anti-A and/or anti-B antibodies serve as viral neutralizing antibodies by binding to A and/or B antigens expressed on the viral envelope, thereby preventing infection of target cells
The SARS-CoV-2 S protein is bound by human anti-A antibodies, which may block the interaction between the virus and ACE2R, thereby preventing entry into the lung epithelium
An increase in ACE-1 activity in group A individuals predisposes to cardiovascular complications, accounting for severe COVID-19
Variation of VWF and Factor VIII levels by ABO type with higher levels in group A individuals contributing to risk of thromboembolic disease and severe COVID-19
ABH glycans, if present on SARS-CoV-2 S protein, may modify the affinity of SARS-CoV-2 for ACE2R, its cellular receptor.
ABH glycans on target cells could serve as alternative, lower-affinity receptors for SARS-CoV-2 S protein or bind other viral envelope structures.
Suggested experiments to confirm the proposed associations between ABO blood groups and SARS-CoV-2 infection
In vitro production of recombinant SARS-CoV-2 S protein in cell lines that can synthesize ABH glycans to determine whether these are, indeed, on the recombinant protein.
Culturing SARS-CoV-2 in cell lines capable of synthesizing ABH glycans, isolating the virus and determining whether anti-A and anti-B prevent infection of cell lines that do not express the corresponding ABH antigen. These experiments would also allow testing whether IgM and IgG anti-A (or anti-B) antibodies were equally effective.
Producing recombinant SARS-CoV-2 S protein in identical host cell lines (e.g. by transfecting in the relevant glycosyltransferases) and then quantifying the affinity of the purified proteins for their receptor. This can determine if ABH glycans are present on SARS-CoV-2 S protein and if they modify the affinity of SARS-CoV-2 for ACE2R.
Produce SARS-CoV-2 *in vitro* in ABH-expressing cell lines; and quantify the infectivity of a given target cell line with purified virus.
Quantify IgA anti-A and/or anti-B and correlate with risk of susceptibility to infection by SARS-CoV-2 and severity COVID-19 illness.

Nonetheless, this would still require definitive proof of differential titres by ABO type. Routine ABO testing of COVID-19 patients could also guide decision-making, for example, by lowering thresholds for escalating care with higher risk blood groups. However, realistically, this is unlikely to happen given that risk, if present, is not sufficiently convincing to alter population-based care.

Conclusions

The role of ABO blood group in SARS-CoV-2 infectivity and COVID-19 disease severity requires additional study; however, accumulating evidence suggests that, at biochemical and physiological levels, there may be a contribution of ABO blood type to disease biology. It also must be recognized that host factors already identified as contributing to COVID-19 severity, play a dominant role, coupled with timely access to appropriate medical care. By contrast, the role of ABO type is likely secondary and non-modifiable.

Conflict of interest

RG serves on the medical advisory board of Rigel and reports personal consulting fees from Alexion Pharmaceuticals and TERUMO BCT outside of the submitted work. EMB reports personal fees and non-financial support from Terumo BCT, personal fees and non-financial support from Grifols Diagnostics Solutions and Abbott Laboratories, outside of the submitted work; EMB is a member of the United States Food and Drug Administration (FDA) blood Products Advisory Committee. Any views or opinions that are expressed in this manuscript are that of the author's, based on his own scientific expertise and professional judgement; they do not necessarily represent the views of either the Blood Products Advisory Committee or the formal position of FDA and also do not bind or otherwise obligate or commit either Advisory Committee or the Agency to the views expressed. DVD serves on the advisory board of Macopharma. SLS serves on the advisory board of Hemanext, Inc. is a consultant with Tioma, Inc., and is the Executive Director of the Worldwide Initiative for Rh Disease Eradication (WIRhE).

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References

1 Zhu N, Zhang D, Wang W, et al.: A Novel Coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; **382**:727–33.

2 Wiersinga WJ, Rhodes A, Cheng AC, et al.: Pathophysiology, transmission, diagnosis, and treatment of coronavirus disease 2019 (COVID-19): a review. *JAMA* 2020; **324**:782–93.

3 Barnkob MB: Reduced prevalence of SARS-CoV-2 infection in ABO blood group O. *Blood Adv* 2020; **4**(20):4990–4993.

4 Hoiland RL: The association of ABO blood group with indices of disease severity and multiorgan dysfunction in COVID-19. *Blood Adv* 2020; **4**(20):4981–4989.

5 Zhao J, Yang Y, Huang H-P, et alRelationship between the ABO Blood Group and the COVID-19 Susceptibility. *medRxiv* 2020.

6 Latz CA, DeCarlo C, Boitano L, et al.: Blood type and outcomes in patients with COVID-19. *Ann Hematol* 2020; 1–6.

7 Li J, Wang X, Chen J, et al.: Association between ABO blood groups and risk of SARS-CoV-2 pneumonia. *Br J Haematol* 2020; **190**:24–7.

8 Piccinni MP, Vultaggio A, Scaletti C, et al.: Type 1 T helper cells specific for Candida albicans antigens in peripheral blood and vaginal mucosa of women with recurrent vaginal candidiasis. *J Infect Dis* 2002; **186**:87–93.

9 Yamamoto F, Clausen H, White T, et al.: Molecular genetic basis of the histo-blood group ABO system. *Nature* 1990; **345**:229–33.

10 Blood groups and red cell antigens. Dean L., Bethesda (MD): National Center for Biotechnology Information (US). 2005.

11 Holodick NE, Rodriguez-Zhurbenko N, Hernandez AM: Defining natural antibodies. *Front Immunol* 2017; **8**:872.

12 Gérard C, Maggipinto G, Minon JM: COVID-19 & ABO blood group: another viewpoint. *Br J Haematol* 2020; **190**:e93–e94.

13 Stussi G, Huggel K, Lutz H, et al.: Isotype-specific detection of ABO blood group antibodies using a novel flow cytometric method. *Br J Haematol* 2005; **130**:954–63.

14 Daniele F: Anti-A Isohemagglutinin titers and SARS-CoV2 neutralization: implications for children and convalescent plasma selection. *Br J Haematol* 2020; **190**:e148–e150.

15 Zimring JC, Spitalnik SL: Pathobiology of transfusion reactions. *Annu Rev Pathol* 2015; **10**:83–110.

16 Branch DR: Anti-A and anti-B: what are they and where do they come from? *Transfusion* 2015; **55**(Suppl 2): S74–9.

17 Mourant AE, Kopec AC, Domaniewska-Sobczak K: *The Distribution of Human Blood Groups and Other Polymorphisms*. London, Oxford University Press, 1976.

18 Estrada-Mena B, Estrada FJ, Ulloa-Arvizu R, et al.: Blood group O alleles in Native Americans: implications in the peopling of the Americas. *Am J Phys Anthropol* 2010; **142**:85–94.

19 Clausen H, Hakomori S: ABH and related histo-blood group antigens; immunochemical differences in carrier isotypes and their distribution. *Vox Sang* 1989; **56**:1–20.

20 Dean L: *Blood Groups and Red Cell Antigens*. Bethesda (MD), National Center for Biotechnology Information (US), 2005.

21 Brockhausen I: Crossroads between bacterial and mammalian glycosyltransferases. *Front Immunol* 2014; **5**:492.

22 Zouine S, Marnissi F, Otmani N, et al.: Expression of histo-blood group antigens in tumor and adjacent normal breast tissues as prognostic markers of breast carcinoma. *J Breast Cancer* 2020; **23**:69–79.

23 Gampa A, Engen PA, Shobar R, et al.: Relationships between gastrointestinal microbiota and blood group antigens. *Physiol Genomics* 2017; **49**:473–83.

24 Sousa NC, Anicchino-Bizzacchi JM, Locatelli MF, et al.: The relationship between ABO groups and subgroups, factor VIII and von Willebrand factor. *Haematologica* 2007; **92**:236–9.

25 Liumbruno GM, Franchini M: Beyond immunohaematology: the role of the ABO blood group in human diseases. *Blood Transfus* 2013; **11**:491–9.

26 Franchini M, Capra F, Targher G, et al.: Relationship between ABO blood group and von Willebrand factor levels: from biology to clinical implications. *Thromb J* 2007; **5**:14.

27 Ibrahim-Kosta M, Bailly P, Silvy M, et al.: ABO blood group, glycosyltransferase activity and risk of venous thromboembolism. *Thromb Res* 2020; **193**:31–5.

28 Wu O, Bayoumi N, Vickers MA, et al.: ABO(H) blood groups and vascular disease: a systematic review and meta-analysis. *J Thromb Haemost* 2008; **6**:62–9.

29 Grundbacher FJ: Changes in the human a antigen of erythrocytes with the individual's age. *Nature* 1964; **204**:192–4.

30 Ravn V, Dabelsteen E: Tissue distribution of histo-blood group antigens. *Apmis* 2000; **108**:1–28.

31 Rummel SK, Ellsworth RE: The role of the histoblood ABO group in cancer. *Future Sci OA* 2016; **2**:FSO107.

32 Karakas M, Baumert J, Kleber ME, et al.: A variant in the ABO gene explains the variation in soluble E-selectin levels-results from dense genotyping in two independent populations. *PLoS One* 2012; **7**:e51441.

33 Pare G, Chasman DI, Kellogg M, et al.: Novel association of ABO histo-blood group antigen with soluble ICAM-1: results of a genome-wide association study of 6,578 women. *PLoS Genet* 2008; **4**:e1000118.

34 Franchini M, Lippi G: The intriguing relationship between the ABO blood group, cardiovascular disease, and cancer. *BMC Med* 2015; **13**:7.

35 Randi AM, Laffan MA: Von Willebrand factor and angiogenesis: basic and applied issues. *J Thromb Haemost* 2017; **15**:13–20.

36 Eder AF, Spitalnik SL: Blood Group Antigens as Receptors for Pathogens; in: Blancher A, Klein J, Socha W (eds): *Molecular Biology and Evolution of Blood Group and MHC Antigens in Primates*. Berlin, Heidelberg, Springer, Berlin Heidelberg, 1997:268–304.

37 Cooling L: Blood groups in infection and host susceptibility. *Clin Microbiol Rev* 2015; **28**:801–70.

38 Kaufmann B, Baxa U, Chipman PR, et al.: Parvovirus B19 does not bind to membrane-associated globoside in vitro. *Virology* 2005; **332**:189–98.

39 Munakata Y, Saito-Ito T, Kumura-Ishii K, et al.: Ku80 autoantigen as a cellular coreceptor for human parvovirus B19 infection. *Blood* 2005; **106**:3449–56.

40 Weigel-Kelley KA, Yoder MC, Srivastava A: Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. *Blood* 2003; **102**:3927–33.

41 Cooling LL, Koerner TA, Naides SJ: Multiple glycosphingolipids determine the tissue tropism of parvovirus B19. *J Infect Dis* 1995; **172**:1198–205.

42 Brown KE, Hibbs JR, Gallinella G, et al.: Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). *N Engl J Med* 1994; **330**:1192–6.

43 Borén T, Falk P, Roth KA, et al.: Attachment of Helicobacter pylori to human gastric epithelium mediated by blood group antigens. *Science (New York, NY)* 1993; **262**:1892–5.

44 Rossez Y, Maes E, Lefebvre Darroman T, et al.: Almost all human gastric mucin O-glycans harbor blood group A, B or H antigens and are potential binding sites for Helicobacter pylori. *Glycobiology* 2012; **22**:1193–206.

45 Aspholm-Hurtig M, Dailide G, Lahmann M, et al.: Functional adaptation of BabA, the H. pylori ABO blood group antigen binding adhesin. *Science (New York, NY)* 2004; **305**:519–22.

46 Chakrani Z, Robinson K, Taye B: Association between ABO blood groups and *Helicobacter pylori* infection: a meta-analysis. *Scientific Reports* 2018; **8**:17604.

47 Cheng Y, Cheng G, Chui CH, et al.: ABO blood group and susceptibility to severe acute respiratory syndrome. *Jama* 2005; **293**:1450–1.

48 Leaf RK, Al-Samkari H, Brenner SK, et al.: ABO phenotype and death in critically Ill patients with COVID-19. *Br J Haematol* 2020; **190**:e204–e208.

49 Göker H, Karakulak EA, Demiroğlu H, et al.: The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. *Turkish J Med Sci* 2020; **50**:679–83.

50 Abdollahi A, Mahmoudi-Aliabadi M, Mehrtash V, et al.: The novel coronavirus SARS-CoV-2 vulnerability association with ABO/Rh blood types. *Iran J Pathol* 2020; 156–60.

51 Zeng X, Fan H, Lu D, et al.: Association between ABO blood groups and clinical outcome of coronavirus disease 2019: Evidence from two cohorts. medRxiv 2020.

52 Wu Y, Feng Z, Li P, et al.: Relationship between ABO blood group distribution and clinical characteristics in patients with COVID-19. *Clin Chim Acta* 2020; **509**:220–3.

53 Patel EU, Bloch EM, Grabowski MK, et al.: Sociodemographic and behavioral characteristics associated with blood donation in the United States: a population-based study. *Transfusion* 2019; **59**:2899–907.

54 Golding J, Northstone K, Miller LL, et al.: Differences between blood donors and a population sample: implications for case-control studies. *Int J Epidemiol* 2013; **42**:1145–56.

55 Takagi H: Down the Rabbit-Hole of blood groups and COVID-19. *Br J Haematol* 2020; **190**:e268–e270.

56 Zietz M, Tatonetti NP: Testing the association between blood type and COVID-19 infection, intubation, and death. medRxiv 2020.

57 Ellinghaus D, Degenhardt F, Bujanda L, et al.: Genomewide Association Study of Severe Covid-19 with Respiratory Failure. *N Engl J Med* 2020; **383**:1522–1534.

58 Breiman A, Ruven-Clouet N, Le Pendu J: Harnessing the natural anti-glycan immune response to limit the transmission of enveloped viruses such as SARS-CoV-2. *PLoS Pathog* 2020; **16**: e1008556.

59 Li F, Li W, Farzan M, et al.: Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* 2005; **309**:1864–8.

60 Krokhin O, Li Y, Andonov A, et al.: Mass spectrometric characterization of proteins from the SARS virus: a preliminary report. *Mol Cell Proteomics* 2003; **2**:346.

61 Ying W, Hao Y, Zhang Y, et al.: Proteomic analysis on structural proteins of Severe Acute Respiratory Syndrome coronavirus. *Proteomics* 2004; **4**:492–504.

62 Watanabe Y, Allen JD, Wrapp D, et al.: Site-specific glycan analysis of the SARS-CoV-2 spike. *Science* 2020; **369**:330–3.

63 Lan J, Ge J, Yu J, et al.: Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* 2020; **581**:215–20.

64 Guillon P, Clément M, Sébille V, et al.: Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. *Glycobiology* 2008; **18**:1085–93.

65 Breiman A, Ruvën-Clouet N, Le Pendu J: Harnessing the natural anti-glycan immune response to limit the transmission of enveloped viruses such as SARS-CoV-2. *PLoS Pathogens* 2020; **16**:e1008556-e.

66 Wu S, Arthur CM, Wang J, et al.: SARS-CoV-2 Receptor Binding Domain Preferentially Recognizes Blood Group A. *Blood Adv.* 2020 (in press).

67 de França NDG, Poli MCC, Ramos PGdA, et al.: Titers of ABO antibodies in group O blood donors. *Rev Bras Hematol Hemoter* 2011; **33**:259–62.

68 Tendulkar AA, Jain PA, Velaye S: Antibody titers in Group O platelet donors. *Asian J Transfus Sci* 2017; **11**:22–7.

69 Gallian P, Pastorino B, Morel P, et al.: Lower prevalence of antibodies neutralizing SARS-CoV-2 in group O French blood donors. *Antiviral Res* 2020; **181**:104880.

70 Wrapp D, Wang N, Corbett KS, et al.: Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* 2020; **367**:1260–3.

71 Rieben R, Buchs JP, Fluckiger E, et al.: Antibodies to histo-blood group substances A and B: agglutination titers, Ig class, and IgG subclasses in healthy persons of different age categories. *Transfusion* 1991; **31**:607–15.

72 Anstee D: The relationship between blood groups and disease. *Blood* 2010; **115**:4635–43.
73 Dai X: ABO blood group predisposes to COVID-19 severity and cardiovascular diseases. *Eur J Prev Cardiol* 2020; **27**(13):1436–1437.
74 Ackermann M, Verleden SE, Kuehnel M, et al.: Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in Covid-19. *N Engl J Med* 2020; **383**:120–8.
75 Bilaloglu S, Aphinyanaphongs Y, Jones S, et al.: Thrombosis in hospitalized patients with COVID-19 in a New York City health system. *JAMA* 2020; **324**:799–801.
76 O'Donnell J, Laffan MA: The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. *Transfus Med* 2001; **11**:343–51.
77 Bloch EM, Goel R, Wendel S, et al.: Guidance for the procurement of COVID-19 convalescent plasma: differences between high- and low-middle-income countries. *Vox Sang* 2020 (in press).
78 Al-Riyami AZ, Schafer R, van den Berg K, et al.: Clinical use of Convalescent Plasma in the COVID-19 pandemic: a transfusion-focussed gap analysis with recommendations for future research priorities. *Vox Sang* 2020 (in press).
79 Bloch EM, Patel EU, Marshall C, Littlefield K, Goel R, Grossman BJ, Winters JL, Shrestha R, Burgess I, Laeyendecker O, Shoham S, Sullivan D, Gehrie EA, Redd AD, Quinn TC, Casadevall A, Pekosz A, Tobian AAR: ABO blood group and SARS-CoV-2 antibody response in a convalescent donor population. Accepted. *Vox Sang* 2020 (in press).

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ORIGINAL PAPER

Inequality averse and compassionate blood donor: implication for interventions

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Vox Sanguinis

Background and objectives Blood donors, compared to non-donors, are more likely to show a preference to help others either by sharing resources to *directly* compensate those in need or *indirectly* by punishing those who act unfairly. Knowing the dominant cooperative preference for blood donors will inform the development of targeted interventions. We test which preference dominates and an initial intervention based on these findings.

Materials and methods We report two studies. The first compares compensation and punishment preferences in blood donors and non-donors (N = 372) using a third-party-compensation-and-punishment game. Based on the results of Study 1, Study 2 (N = 151) is a feasibility experiment of an intervention based on advantageous inequality aversion ('As a healthy person, you can give blood and help those less healthy than you'.).

Results Blood donors, compared to non-donors, have a preference for compensation. Organ donors have a preference for punishment. Those exposed to the advantageous inequality aversion intervention, compared to control conditions, show a greater behavioural propensity to donate blood (this was especially the case for non-donors).

Conclusion Blood donors have a clear preference for direct helping through compensation that can be translated into a simple effective intervention to enhance blood donor recruitment and retention.

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Key words: donor motivation, donor recruitment, donors.

Introduction

Cooperation within a society can be sustained either by giving resources (e.g. time, money) to help someone in need or by *punishing* those who act unfairly [1–3]. The former offers *direct* help to an individual, signals compassion and initiates reciprocity [1–4]. The later *indirectly* helps individuals, in general, by enforcing wider societal norms of fairness and dissuading selfishness [1–3]. Both direct-cooperation and punishment are theorized to operate by reducing inequality and re-establishing fairness [5, 6]. While blood donors, compared to non-donors, are more likely to show both direct-cooperation and punishment [7–10], no study has compared donor's preferences for either direct-cooperation or punishment when both options are simultaneously available. Thus, we ask for the first time: 'Are blood donors primarily motivated to care for an individual or to ensure wider societal fairness?' Knowing donors' dominant preferences will indicate how best to target interventions to recruit donors: 'Do blood donors donate to help individuals in need or to ensure there is sufficient blood?' This paper explores, for the first time, which preference dominates for blood donors and provides some initial data on translating this into an intervention.

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Inequality aversion and preferences to compensate or punishment

A Third-Party-Punishment-and-Compensation (3PPC) game [11, 12] can be used to explore preferences for direct-cooperation and punishment simultaneously. In a 3PPC game, a third-party witnesses someone being treated unfairly by a perpetrator and can choose, to either: (1) compensate the victim, (2) punish the perpetrator or (3) both compensate and punish, all at a personal cost or can choose to do nothing and incur no cost [6, 11, 12]. It is argued that people cooperate or punish, partly because, they are motivated to *reduce inequality* between themselves and others: they are *inequality averse* [5]. There are two forms of inequality aversion. First, there is *advantageous inequality aversion* [AIA] which occurs when a person is relatively better-off than another, and *guilt* motivates them to reduce this inequality [5]. Second, there is disadvantageous inequality aversion [DIA] which occurs when one person is relatively worse off than another. Here, *envy* motivates them to find strategies to reduce inequality [5]. In this third-party context, inequality aversion suggests that the third-party would increase the victim's resources to a fair level and not over-compensate, and reduced the perpetrator's resources to a fair level and not be overly punitive [6, 13]. Indeed, being overly punitive creates an inequality that can be viewed as *spiteful* and may be counter-productive as the perpetrator may feel unjustly treated and subsequently not act fairly [13].

Philanthropic choice space

People's choice of a philanthropic act (blood donation, organ donation, volunteering or donating money) are personal, specific and differentially motivated [10, 14, 15]. For example, blood donors are motivated by feelings of warm-glow and compassion [7–10] both of which underlie c*ompensation* in a 3PPC game [4]. Therefore, it is hypothesized that compensation will be the dominant preferences for blood donors. Those who sign on the organ donor register are motivated by civic duty and solidarity to provide resources for all [16–18]. As third party punishment is linked to enforcing fairness norm [1–3], those who have signed on the organ register, compared to those who have not, should have a preference for punishment. No clear preference emerges for non-health-based helping.

Clinical trials approach

It has been argued that a clinical trials approach is needed when developing interventions to recruit blood donors [10, 19, 20]. Behavioural interventions, like pharmaceutical ones, contain active ingredients that can have side-effects as well a benefits [20]. Therefore, national campaigns (Phase IV trials) need to be developed via phase I (modelling), II (exploratory) and III (RCT) trials [19]. In phase I, information is gathered on the potential components of an intervention, and phase II provides initial evidence on an interventions effectiveness and any unforeseen consequences. Set within this approach the first study (akin to phase I) reported here explores the cooperative preference of donors and non-donors. Study 2 reports a small scale laboratory-based experiment (akin to phase II) to explore the benefits and unforeseen consequence of an intervention based on findings from Study1.

Study 1: Compensation and punishment in blood donors

Materials and methods

The sample

As women, in general, are more prosocial then men [21] a non-probability purposive convenience sampling strategy was used to ensure an equal number of male and female participants. We did not recruit participants who were specifically involved in any philanthropy to avoid bias [10]. The final sample consisted of 372 participants (52% female, mean age 22·12 years, SD 4·05 years).

Measures

Third-Party-Punishment-and-Compensation (3PPC) game: Participants played a standard one-shot 3PPC game [12]. There is evidence that exposure to repeated fairness leads to more free-riding and repeated unfairness to increased punishment and compensation in 3PPC games [11]. However, revealed altruism shows that initial allocations are more likely to reflect the person's underlying cooperative preference and as such we use a one-shot game [22, 23]. Participants read the instructions to the game and were told that the game involved three players (A, B & C) (File S1). They were informed that player-A has been *given* £10 ($13 US, 11 Euro) and told that they can share some, none or all of it with player-B. Player-B has £0. Player-B has to accept Player-A's decision. Player-C (the 3rd party) has £5 ($6·68 US, 5·57 Euro) and can choose to spend some of this to either (1) *compensate Player-B*, (2) *punish Player-A*, (3) *do a mixture of compensation and punishment* or (4) *do nothing* and keep the money. The decision was made efficient as every £1 Player-C spends to compensate increases Player-B's allocation by £2, and every £1 Player-C spends to punish reduces Player-A's allocation by £2. Thus, the participants were indicated to spend in £1 units.

The game was played in private and decisions were made anonymously. Participants were told that they

would be Player-C and faced a scenario where Player-A had given £2 of their £10 to Player-B. The participant then made a decision to either (1) punish Player-A (*punish-only*), (2) compensate Player-B (*compensate-only*), (3) both punish and compensate or (4) to do nothing. They could choose only one option. Participants were informed that the game between A and B was hypothetical but that they were playing for *real money* and the choice they made would constitute their final pay-off. Participants were told that a random number of participants would be selected and paid based on the decision they had made. Evidence shows that this payment procedure has no effects on the pattern of results compared to paying all participants [24, 25]. Thus, decisions made by the participant will affect their pay-off but not Players-A and -B. Therefore, the participant's decision signals their *intent* to punish or compensate which is an important external-signal and self-signal about their reputation [26, 27]. Furthermore, if participants were only concerned about the actual direct effects of their actions on Players-A and -B, rather than what their decisions signal, they would keep the money and do nothing.

Assessments of Philanthropy: We assessed blood donor status by asking participants if they have ever donated blood (Yes, No). This question is a reliable and accurate assessment of whether or not someone has donated blood [28] and has been widely used to assess blood donor behaviour [8–10]. We assessed other philanthropic acts as follows: (1) volunteering (have you ever volunteered? Yes or No), (2) financial helping (have you ever donate money to charity? Yes or No), (3) organ donor registration (have you registered to be an organ donor? Yes or No).

3PPC pay-offs, equality and fairness

The discussion below is represented in Fig. 1. The 'Start State' has Player-A with £10 and Player-B with £0. As the monetary allocation is unearned house-money a 50:50 share would be considered a 'Fair Offer' (=£5:£5) [29, 30]. However, Player-A makes an 'Actual Offer' that is 80:20 (=£8:£2), which is known to be perceived as unfair [4]. Player-C can act selfishly and 'Do Nothing'. However, Player-C can restore a fair outcome to the victim (Player-B) by choosing the compensate-only option and, on average, 'Compensate-B' by spending £1·5 to raises their outcome to £5 (the £2 received by player-A plus £1·5 × 2 from Player-C). This leaves Player-B with a fair outcome [4]. Player-C can restore a fair outcome by choosing the punish-only option and, on average, 'Punishing-A' by £1·5 decreasing their outcome to £5 (the £2 given away by Player-A plus £1·5 × 2 deducted). While this leaves Player-B (the victim) with a less than fair outcome it signals a wider socially orientated strategy to enforce norms of fairness. Being overly punitive to Player-A would be spiteful, and potentially counter-productive as the transgressor may feel hard-done-by and may act unfairly in future interactions [6, 13]. The most equitable and fair strategy is to choose the 'Compensate-B and Punish-A' option and spend £1·5 on each (£3 in total) so that the transgressor and victim both have £5. However, this is the most costly strategy to Player-C, thus fairness can be restored more economically by choosing to either the compensate-only or punish-only options.

Ethical approval

This study was approved following the ethical procedures of the school of psychology University of Nottingham (references codes: 534, 554 & 654).

Power Analysis

With respect to blood donor behaviour in economic games, a medium effect size (Cohen's d = 0·62; r = 0·28) is reported for generosity in dictator games [8] and punishment (Cohen's d = 0·67, r = 0·32) in an ultimatum game [10]. To attain a power of 0·80, with an alpha of 0·05 this indicates that for compensation 41 blood donors need to be compared 41 non-donor and for punishment 36 donors to 36 non-donors.

Results & discussion

Effects of sex and age on preferences

Preference choice did not vary by sex ($\chi^2_{(3)}$ 0·757, P = 0·860) or age ($F_{(3,\ 367)}$ = 0·448, P = 0·719). So there is no evidence for bias by sex or age of participants.

3PPC preferences and payments

Consistent with the literature the majority of participants chose compensation-only (n = 156, 41·9%) or kept the money (n = 136, 36·6%) with punishment-only used the least (n = 30, 8·1%) and 'compensation and punishment' chosen by 50 (13·4%) [11, 29].

Those who chose to 'compensation-only' spent on average £1·63 (SD = 1·07) giving the victim £5·26 on average. When 'compensation and punishment' was chosen participants spent on average £1·45 (SD = 0·62) to 'compensate' (on average the victim's outcome is £4·90) and £1·50 (SD = 0·73) to 'punish' (on average the transgressor's outcome is £5). Those choosing the 'punish-only' option spent £2·43 (SD = 1·04) to reduce the transgressor's outcome to £3·14. Thus, those choosing a preference to 'compensate' or both 'compensate and punish' show unfairness and inequality aversion and those choosing 'punishment-only' are more spiteful (Texts S2 and Table S1 for more details).

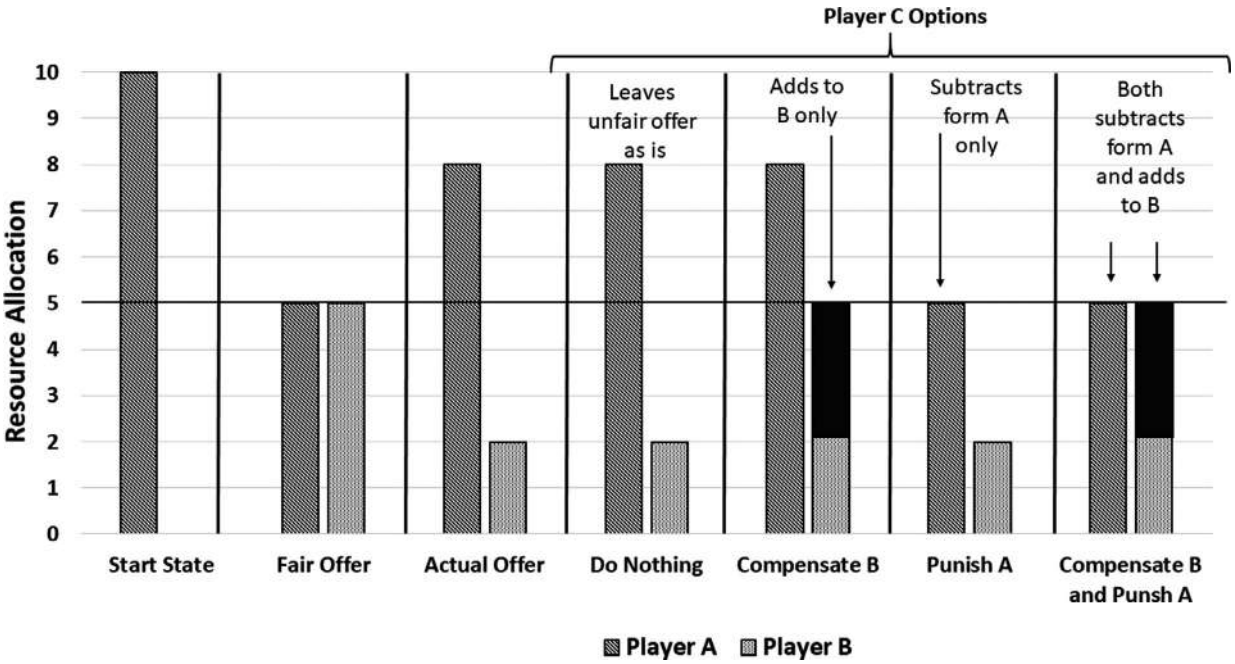

Fig. 1 Payoff patterns for the 3PPC.

Philanthropic behaviours

Of the participants 25·7% had donated blood at some time (N = 95), 42·2% had registered as an organ donor 42·3% (N = 155), 82·6% had donated money (N = 304) and 90·3% volunteered (334).

Predicting preferences to cooperate

A multinomial regression model examined if a specific preference to either compensate-only, punish-only, or do both, relative to doing nothing was observed for blood donors, registered organ donors, having donated money or volunteered. The results (Table 1: Panel A) showed that blood donors are twice as likely to choose to compensate-only compared to do nothing. Those on the organ donor register are twice as likely to choose to punish-only compared to do nothing. Thus, those who have donated blood show a preference for *direct* helping, resulting in a more equal allocation to the victim and those on the organ donor register for *indirect* punishment-only. There was no specific preference observed for donating time or money (File S3, Table S2 for robustness checks).

The options to 'compensate-only' and to both 'compensate and punish' produced average allocation patterns that resulted in more equal/fair splits of resources. Therefore, the choices to 'compensate-only' and both 'compensate and punish' were collapsed into a single category that reflected 'fairness and equality'. A second multinomial regression (Table 1: Panel B) compared 'fairness and equality' and 'punish-only', relative to doing-nothing. The results show that blood donors were significantly more likely to choose a 'fairness and equality' option and organ donor's punishment-only.

This suggests that for blood donation, interventions that emphasize *a fair direct sharing of personal resources* to minimize any difference between themselves and others in need would be effective. Study 2 reports on a feasibility study to explore this possibility.

Study 2: Inequality Aversion: Transferring the Resource of Health Through Blood Donation

The theoretical basis of the intervention strategy suggested by study 1 is inequality aversion [5]. Indeed, motivations based on *advantageous inequality aversion* (AIA) have been reported as part of blood donor motivations [31]. Here, the healthier donor is motivated to donate to help those less healthy [7, 31]. Furthermore, models of AIA suggest that guilt motivates this desire to reduce inequality [5] and indeed, guilt has been shown to be a motivator for blood donation [32]. Thus, a message based on AIA should be an effective motivator to donate blood. However, manipulating guilt may be disadvantageous if it is perceived as manipulative [33, 34]. Thus, a message that highlights inequality and enacts guilt without engendering feelings of manipulation is desirable. It has been proposed that this can be achieved using massages such as 'As a healthy person, you can give blood and help those less healthy than you'. [7, 31]. This experiment

Table 1 Multi-nominal regression predicting specific cooperative preferences

Preference	Predictor	Coef (SE)	*P* =	OR	OR 95% CI	
					Lower	Upper
	Panel A: Comparison Across all Choices					
Compensate only						
	Blood Donor	**0·667 (0·306)**	**0·029**	**1·94**	**1·07**	**3·54**
	Organ Donor	0·246 (0·263)	0·349	1·28	0·76	2·14
	Donated Money	–0·184 (0·346)	0·596	0·83	0·42	1·64
	Volunteered	0·830 (0·448)	0·073	2·23	0·83	5·67
Punish only						
	Blood Donor	0·231 (0·518)	0·655	1·26	0·46	3·47
	Organ Donor	**0·897 (0·451)**	**0·047**	**2·45**	**1·01**	**5·94**
	Donated Money	–0·401 (0·563)	0·476	0·67	0·22	2·02
	Volunteered	–0·596 (0·555)	0·283	0·55	0·18	1·63
Both 'Compensate and Punish'						
	Blood Donor	0·730 (0·410)	0·075	2·07	0·93	4·64
	Organ Donor	0·192 (0·369)	0·603	1·21	0·59	2·50
	Donated Money	–0·734 (0·435)	0·091	0·48	0·20	1·12
	Volunteered	0·678 (0·609)	0·265	1·97	0·59	6·50
R^2 (Nagelkerke)	0·062					
	Panel B: Comparison of Inequality Averse vs Punishment Choices					
'Compensate only' Plus 'compensate and punish' ('Fairness and Equality')						
	Blood Donor	**0·682 (0·291)**	**0·019**	**1·98**	**1·18**	**3·50**
	Organ Donor	0·233 (0·248)	0·349	1·26	0·77	2·05
	Donated Money	–0·340 (0·320)	0·287	0·71	0·38	1·33
	Volunteered	0·767 (0·405)	0·058	2·15	0·97	4·76
Punish only						
	Blood Donor	0·231 (0·518)	0·655	1·26	0·46	3·47
	Organ Donor	**0·897 (0·451)**	**0·047**	**2·45**	**1·01**	**5·94**
	Donated Money	–0·401 (0·563)	0·476	0·67	0·22	2·02
	Volunteered	–0·596 (0·555)	0·283	0·55	0·18	1·63
R^2 (Nagelkerke)	Blood Donor	0·231 (0·518)	0·655	1·26	0·46	3·47

Reference category is a preference to do nothing.
Significant values are highlighted in bold.

explores the effectiveness of this message against both an anticipatory guilt message and a pure control (no message) condition.

Materials and methods

Participants, design & power

A one-way between-groups design with 3-levels (anticipatory guilt [AG], advantageous inequality aversion [AIA] or pure control [PC]) was used. As there are no studies comparing AIA messages, the association between guilt and pro-sociality was used as the basis of the power calculation, as the underlying mechanism for AIA. This is a medium effect, with an *r* of 0·30 equating to a $d_{cohen's}$ of 0·629 [35]. To achieve 80% power with an α of 0·05 this requires 40 participants per condition. We oversampled to allow for some exploratory analysis with 151 participants recruited using a non-directive convenience sample (M_{age} = 20·9, SD_{age} = 2·019; 50·3% female). These were randomly allocated to one of the three conditions, with 50 participants in the AG and AIA conditions and 51 in the PC condition. Forty-nine participants described themselves as blood donors, with 28% in the AG, 32% in the AIA and 35% in the PC conditions.

Messages

All participants were provided with an image depicting a cartoon drop of blood followed by 'Donate Blood. . .Save Life'. Underneath the image, participants in the AG condition were presented with the slogan: 'If people like you do not donate blood, there will be continuing shortages in the future'. [34]. The AIA appeal was as follows: 'As a healthy person, you can give blood and help those less healthy than you' [31] (File S4).

Reactions to the messages

After reading the appeal participants rated 'to what extent the recruitment advert made them feel…' (1) 'Guilty for not donating blood' ('Guilt'), (2) 'Healthier than others' ('Healthier') and (3) 'Like you can donate blood in the future to improve the lives of others' ('Future Donation'). They also indicated the degree to which they felt manipulated: 'Did you find the recruitment advert was manipulative?' ('Manipulative'). All questions were answered on a seven-point scale (1 = not at all, 7 completely).

Behavioural proxy

To assess if any of the appeals increased the desire to donate, participants could take, at end of the study, information on how to become a donor and/or make a donation if already a donor.

Ethical approval

This study was approved following the ethical procedures of the school of psychology University of Nottingham (reference: 738R).

Results & discussion

Predicting behavioural proxy

The specific focus is on exploring any unforeseen consequences of the intervention for donors and non-donors. To do this it is necessary to compare, for donors and non-donors, separately, variation in behaviour across messages relative to the PC condition. Specifically, when donors and non-donors are considered separately is one of the messages more or less effective. A moderated logistic regression predicting the behavioural proxy was specified (Table 2). Compared to the PC, this model showed, that those in the AG and AIA conditions were significantly more likely to take the information. The nature of the significant interaction (Table 2 and Fig. 2), between conditions and blood donor status, was explored using Stata's *margins* routines (Table 3). The margins analyses (Panel A) shows that, compared to non-donors in the PC condition, non-donors in the AG and AIA conditions are more likely to take the leaflet. However, compared to donors in the PC condition, donors exposed to the AG condition were less likely to take information (Panel A). For completeness donors, compared to non-donors, were more likely to take the information under the PC condition only (Panel B). Thus, an AG intervention had potential detrimental effects on donors.

Evaluation of campaign appeals

A 3 (*Intervention*: AG vs AIA vs PC) by 2 (*donors-status*: donated vs non-donor) between-groups MANOVA was used to explore how the interventions were evaluated. The overall model showed significant main effects for intervention ($F_{(8,\ 286)\ \text{Pillia's trace}} = 10{\cdot}565$. $P = 0{\cdot}000$, $\eta p^2 = 0{\cdot}226$), donor status ($F_{(4,\ 142)\ \text{Pillia's trace}} = 4{\cdot}565$, $P = 0{\cdot}002$, $\eta p^2 = 0{\cdot}114$) and the interaction between intervention and donor status ($F_{(8,\ 284)\ \text{Pillia's trace}} = 2{\cdot}189$. $P = 0{\cdot}028$, $\eta p^2 = 0{\cdot}058$).

For the intervention, there were significant main effects for all four evaluations: (1) 'Guilt' ($F_{(2,\ 145)} = 20{\cdot}061$. $P = 0{\cdot}000$, $\eta p^2 = 0{\cdot}217$); (2) 'Healthier' ($F_{(2,\ 145)} = 14{\cdot}865$. $P = 0{\cdot}000$, $\eta p^2 = 0{\cdot}170$), (3); 'Future Donation' ($F_{(2,\ 145)} = 10{\cdot}636$. $P = 0{\cdot}000$, $\eta p^2 = 0{\cdot}128$) and (4); 'Manipulative' ($F_{(2,\ 145)} = 15{\cdot}190$. $P = 0{\cdot}000$, $\eta p^2 = 0{\cdot}173$). Such that, guilt was significantly higher in the AIA condition (mean 3·971, 95% CI 3·551, 4·392) compared to the AG (mean 2·776, 95% CI 2·332, 3·220) and PC (mean 2·101, 95% CI 1·668, 2·514) conditions. Those in the AIA condition rated themselves as healthier (mean 4·547, 95% CI 4·005, 5·039) compared to the AG (mean 3·472, 95% CI 2·953 3·991) and PC (mean 2·649, 95% CI 2·166, 3·132)

Table 2 Logistic regression models predicting blood donor behaviour proxy

			OR 95% CI	
	***P* =**	**OR**	**Lower**	**Upper**
Condition	**0·048**			
Anticipatory Guilt	**0·026**	**3·120**	**1·147**	**8·487**
Advantageous Inequality Aversion	**0·049**	**2·760**	**1·005**	**7·580**
Donor Status	**0·006**	**9·600**	**1·896**	**48·599**
Condition X Donor Status	**0·009**			
Anticipatory Guilt X Donor	**0·002**	**0·040**	**0·005**	**0·315**
Advantageous Inequality Aversion X Donor	0·075	0·147	0·018	1·209
R^2 (Nagelkerke)	0·073			

Reference category for condition is the 'pure control' and for donor status it is non-donor (N = 151).
Significant values are highlighted in bold.

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Vox Sanguinis (2021) **116**, 862–871

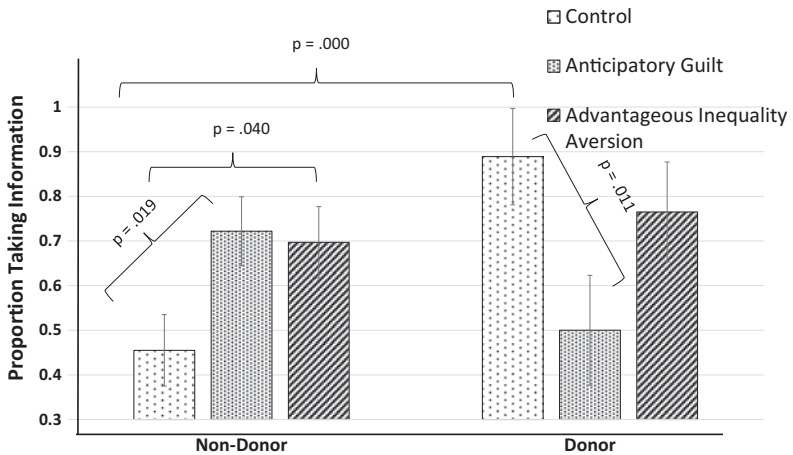

Fig. 2 Blood donor status by condition on behavioural proxy (Error bars = Standard Errors).

Table 3 Margin effects for intervention by donor-status interaction

			95% CI	
	dy/dx (SE)	***P***	**Lower**	**Upper**
Base: pure control				
Anticipatory Guilt				
Non-donor	**0·268 (0·114)**	**0·019**	**0·043**	**0·491**
Donor	**−0·389 (0·153)**	**0·011**	**−0·688**	**−0·089**
Panel A: comparison across condition				
Advantageous Inequality Aversion				
Non-donor	**0·242 (0·118)**	**0·040**	**0·011**	**0·474**
Donor	0·124 (0·127)	0·327	−0·327	0·124
Panel B: comparison across donor status				
Base: non-donor				
Pure control	**0·434 (0·114)**	**0·000**	**0·211**	**0·657**
Anticipatory Guilt	−0·222 (0·153)	0·147	−0·522	0·078
Advantageous Inequality Aversion	0·068 (0·130)	0·603	−0·188	0·323

Significant values are highlighted in bold.

conditions. Those in the AIA (mean 5·390, 95% CI 4·865, 5·915) and AG (mean 4·796, 95% CI 4·242, 5·349) conditions stated that they were significantly more likely to donate in the future than those in the PC (mean 3·699 95% CI 3·184, 4·215) condition. Those in the AG (mean 3·571, 95% CI 3·086, 4·0571) and the AIA (mean 2·710, 95% CI 2·250, 3·171) conditions, rated feeling manipulated significant more than those in the PC (mean 1·727, 95% CI 1·275, 2·179) conditions. A Bonferroni corrected post hoc comparison showed that AG was rated as more manipulative than AIA ($P = 0·036$: mean difference 0·861, 95% CI = 0·041, 1·681). Thus, the AIA was rated as less manipulative than AG, as more likely to encourage future donations and engenders a sense of health, with guilt as a motivation. Thus, the AIA condition is one of low-manipulative guilt, energizing donation intentions.

Donor status was significant for 'Healthier' only ($F_{(1, 145)} = 10·817$. $P = 0·001$, $\eta p^2 = 0·069$), with donors rating themselves as feeling healthier (mean 4·035, 95% CI 3·561, 5·509) than non-donors (mean 3·077, 95% CI 2·751, 3·404).

The intervention by donor-status interaction (Fig. 3) was significant for: (1) 'Guilt' ($F_{(2, 145)} = 3·310$. $P = 0·047$, $\eta p^2 = 0·0410$, (2) 'Healthier'(2) $F_{(2, 145)} = 4·587$. $P = 0·012$, $\eta p^2 = 0·060$ and (3) 'Future donation' ($F_{(2, 145)} = 4·809$. $P = 0·01$, $\eta p^2 = 0·062$). Examining Fig. 3 shows that for 'Healthier' and 'Future donation' donors, compared to non-donors, in the control condition, felt both healthier and were more likely to donate in the future, with this difference disappearing in both the AIA and the AG conditions. Also 'Healthier' and 'Future Donation' increase for the non-donors, compared

to the PC, for both the AIA and the AG conditions. Thus, the AIA and AG intervention encouraged non-donors to respond more like donors. For guilt, compared to the PC, both the AIA and AG conditions resulted in greater feelings of guilt for non-donors and the AIA for donors. There was not no significant difference between donors and non-donors, however, non-donors in the PC had lower guilt than donors in AIA.

General discussion

These studies show, for the first time, that inequality aversion is a potential key determinant of donors cooperative motivation and this is directed at a person in need rather than considering the wider societal need [5]. We also show, for the first time, that an intervention focusing on advantageous inequality aversion with respect to health between the donor and recipients is potentially a successful intervention to recruit non-donors. The findings and implications are discussed below.

Blood donors cooperative profile

Blood donors and organ donors (both examples of health-based philanthropy) have distinct cooperative profiles. Blood donors, compared to non-donors, have a stronger preference to *directly* help an individual in need to reduce their inequality [5]. Those registered as organ donors, however, had a preference for punishment which is consistent with their concern for societal solidarity [16–18]. However, the level of punishment seen by organ donors was overly punitive. This could be counter-productive if the person punished perceives this as unfairly draconian and reacts against this by continuing to act unfairly [13]. Finally, no specific preference was identified for non-health-based philanthropy for those who either volunteer time or donate money.

Implications for Practice: 'I'm Healthy, I can Help'

The motivational pattern for blood donors was to help a relatively disadvantaged individual rather than ensure wider societal fairness. This motivation to reduce inequality is the likely causal mechanism for this preference. This translates into interventions that focus on reducing inequality in health between the donor and the recipient, rather than focusing on ensuring sufficient supply of blood. As such, transfusion services should not just focus interventions on helping the recipient but emphasize *both* the relative difference in health between donor and recipient, as well as how the healthy donor can improve the relative health of the recipient. We show, for the first time, that campaigns with a slogan that encompasses this idea (i.e. 'As a healthy person, you can give blood and help those less healthy than you'.) are a simple technique transfusion services can consider implementing to recruit non-donors. It is certainly an intervention worthy of future

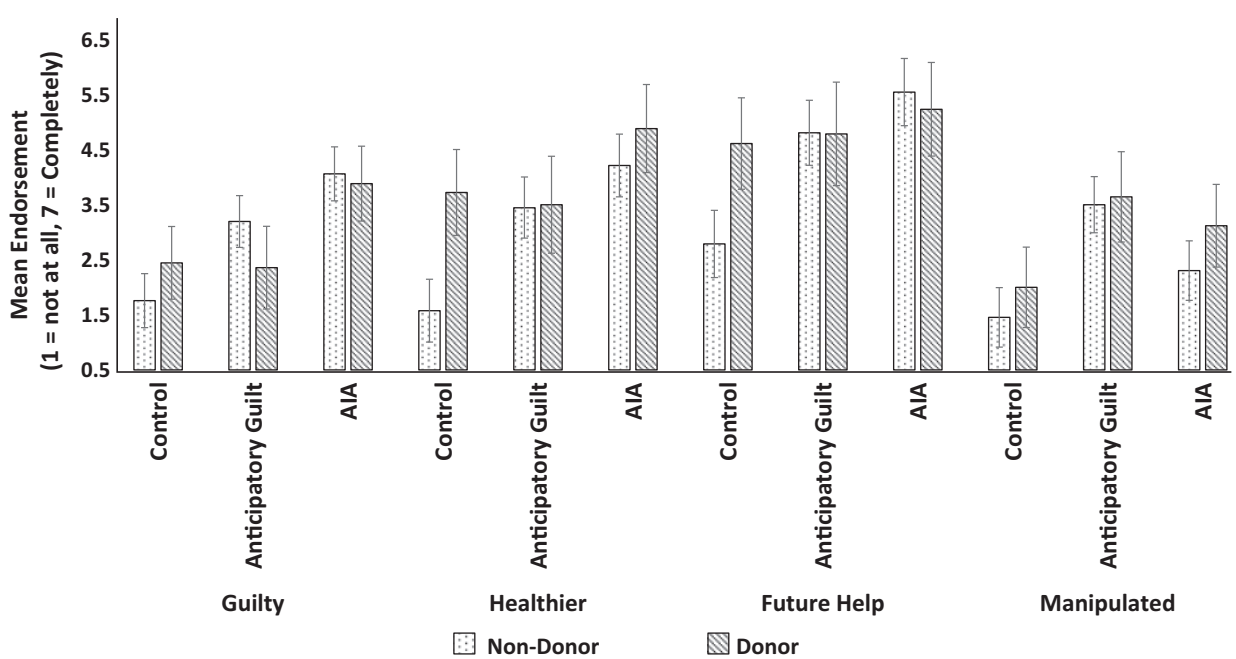

Fig. 3 Blood donor status by condition on message evaluations (AIA = Advantageous Inequality Aversion; Error bars = 95% C.I.s).

consideration and development within a clinical trials model.

Limitations

While behavioural willingness and behavioural proxies are not perfect predictors of actual behaviour they are good indicators [36], and a useful analogue within a clinical trials approach to identify any potential detrimental effects. Furthermore, it should be acknowledged that we explored preferences in a one-shot game, and future research may wish to explore any potential leaning effects. For example, do donors compared to non-donors start to express preferences for punishment with repeated exposure to unfairness in a 3PPC game, or if their underlying preference for compassion raisins unaltered [11].

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Conflict of interests

None Declared.

References

1 Fehr E, Gächter S. Altruistic punishment in humans. Nature 2002;415:137–40.
2 Nowak MA. Five rules for the evolution of cooperation. Science 2006;314:1560–3.
3 Rand DG, Nowak MA. Human cooperation. Trends Cogn Sci 2013;17:413–25.
4 Ferguson E, Quigley E, Powell G, Stewart L, Harrison F, Tallentire H. To help or punish in the face of unfairness: men and women prefer mutually-beneficial strategies over punishment in a sexual selection context. Roy Soc Open Sci 2019;6:181441.
5 Fehr E, Schmidt K. A theory of fairness, competition and cooperation. Q J Econ 1999;114:817–68.
6 Sun L, Tan P, Cheng Y, Chen J, Qu C. The effect of altruistic tendency on fairness in third-party punishment. Front Psychol 2015;6:820.
7 Ferguson E. Mechanism of altruism approach to blood donor recruitment and retention: a review and future directions. Trans Med 2015;25:211–26.
8 Ferguson E, Taylor M, Keatley D, et al. Blood Donors' helping behavior is driven by warm glow more evidence for the blood donor benevolence hypothesis. Transfusion 2012;52:2189–200.
9 Ferguson E, Lawrence C. Altruistic and warm-glow motivations: variation by blood donor career. Testing Psychomet Method Appl Psychol 2019;29:6339–651.
10 Ferguson E, Lawrence C. It's only fair: blood donors are more sensitive to violations of fairness norms than non-donors – converging psychometric and ultimatum game evidence. Vox Sang 2018;113:424–50.
11 Van Doorn J, Zeeenberg M, Breugelmans SM. An exploration of thirds parties' preferences for compensation over punishment: six experimental demonstrations. Theor Dec 2019;85:333-51.
12 Leliveld MC, Dijk E, Beest I. Punishing and compensating others at your own expense: The role of empathic concern on reactions to distributive injustice. Euro J Soc Psychol 2012;42:135–40.
13 Chavez AK, Bicchieri C. Third-party sanctioning and compensation behavior: Findings from the ultimatum game. J Economic Psychol 2013;39:268–77.
14 Ferguson E, Dorner L, France CR, et al. Blood donor behaviour, motivations and the need for a systematic cross-cultural perspective: the example of moral outrage and health-and non-health-based philanthropy across seven countries. ISBT Sci Ser 2018;13:375–83.
15 Studte S, Clement M, Soliman M, Boenigk S. Blood donors and their changing engagement in other prosocial behaviors. Transfusion 2019;59:1002–15.
16 Saunders B. Altruism or solidarity? The motives for organ donation and two proposals. Bioethics 2012;26:376–81.
17 Ferguson E, Zhao K, O'Carroll RE, Smillie LD. Costless and costly pro-sociality: correspondence among personality traits, economic preferences, and real world pro-sociality. Soc Psychol Pers Sci 2019;10:461–71.
18 Ferguson E, Shichman R, Tan JHW. Lone wolf defectors undermine the power of the opt-out default. Sci Rep 2020;10:8973.
19 Campbell M, Fitzpatrick R, Haines A, et al. Framework for design and evaluation of complex interventions to improve health. BMJ 2000;321:694–69.
20 Lorenz T, Oliver K. Adverse effects of public health interventions: a conceptual framework. J Epid Comm Health 2014;68:288–90.
21 Eagly AH, Crowley M. Gender and helping behaviour: A meta-analytic review of the social psychology literature. Psychol Bull 1986;100:283–308.
22 Cox JC, Friedman D, Sadiraj V. Revealed altruism. Econometrica 2008;76:31–69.
23 Skatova A, Spence A, Leygue C, Ferguson E. Guilty repair sustains cooperation, angry retaliation destroys it. Sci Rep 2017;7:46709.
24 Charness G, Gneezy U, Halladaya B. Experimental methods: Pay one or pay all. J Econ Behav Org 2016;131:141–50.
25 Engel C. Dictator games: A meta study. Exp Econ 2011;14:583–610.
26 Falk A, Fehr E, Fischbacher U. Testing theories of fairness—Intentions matter. Games Econ Behav 2008;62:287–30.
27 Bodner R, Prelec D. Self-signaling in a neoCalvinist model of everyday decision making. In Brocas I, Carillo J eds. Psychology of Economic Decisions. London, UK, Oxford University Press; 2003:105–26.

28 Bertalli NA, Allen KJ, McLaren CE, et al. A comparison of self-reported and record-linked blood donation history in an Australian cohort. Transfusion 2011;51:2189–98.
29 Andreoni J, Bernheim BD. Social image and the 50–50 norm: a theoretical and experimental analysis of audience effects. Econometrica 2009;77: 1607–36.
30 FeldmanHall O, Sokol-Hessner P, van Bavel JJ, Phelps EA. Fairness violations elicit greater punishment on behalf of another than for oneself. Nat Comm 2014;5:5306.
31 Ferguson E, Hill A, Lam M, et al. Typology of blood donor motivations. Transfusion 2020;60:2010–20.
32 France CR, Kawalsky JM, France JL, et al. The blood donor identity survey: A multidimensional measure of blood donor motivations. Transfusion 2014;54:2098–105.
33 O'Keefe DJ. Guilt as a Mechanism of Persuasion. In Dillard JP, Pfau M eds. The persuasion handbook: Developments in theory and practice. London, SAGE; 2002:329–40.
34 Renner S, Lindenmeier J, Tscheulin DK, et al. Guilt appeals and prosocial behaviour: an experimental analysis of the effects of anticipatory versus reactive guilt appeals on the effectiveness of blood donor appeals. J Nonprof Pub Sect Marketing 2013;25:237–55.
35 Stanulewicz NK. Guilt and the emotional underpinnings of human prosociality. PhD thesis, University of Nottingham. http://eprints.nottingham.ac.uk/50149/ 2018.
36 Wilding S, Conner M, Sandberg T, et al. The question-behaviour effect: A theoretical and methodological review and meta-analysis. Euro Rev Soc Psychol 2016;27:196–230.

Supporting Information

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

Text S1 Third-Party Punishment-Compensation Economic Game.
Text S2 Identifying Preferences that are Fair and Equality restoring.
Text S3 Sensitivity Analysis for Health and Non-Health Based Philanthropy.
Text S4 Study 2: Inequality Aversion: Transferring the Resource of Health Through Blood Donation.
Table S1 One sample *t*-test for the amount spent to compensate and punish within preferences.
Table S2 Multi-Nominal Regression Predicting Specific Cooperative Preferences.

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Lessons learned in the collection of convalescent plasma during the COVID-19 pandemic

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Background The lack of definitive treatment or preventative options for COVID-19 led many clinicians early on to consider convalescent plasma (CCP) as potentially therapeutic. Regulators, blood centres and hospitals worldwide worked quickly to get CCP to the bedside. Although response was admirable, several areas have been identified to help improve future pandemic management.

Materials and methods A multidisciplinary, multinational subgroup from the ISBT Working Group on COVID-19 was tasked with drafting a manuscript that describes the lessons learned pertaining to procurement and administration of CCP, derived from a comprehensive questionnaire within the subgroup.

Results While each country's responses and preparedness for the pandemic varied, there were shared challenges, spanning supply chain disruptions, staffing,

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impact of social distancing on the collection of regular blood and CCP products, and the availability of screening and confirmatory SARS-CoV-2 testing for donors and patients. The lack of a general framework to organize data gathering across clinical trials and the desire to provide a potentially life-saving therapeutic through compassionate use hampered the collection of much-needed safety and outcome data worldwide. Communication across all stakeholders was identified as being central to reducing confusion.

Conclusion The need for flexibility and adaptability remains paramount when dealing with a pandemic. As the world approaches the first anniversary of the COVID-19 pandemic with rising rates worldwide and over 115 million cases and 2·55 million deaths, respectively, it is important to reflect on how to better prepare for future pandemics as we continue to combat the current one.

Key words: blood collection, blood component production, blood donation testing, blood safety, plasma, transfusion medicine.

Introduction

Throughout human history, mankind has struggled with and died from many pandemics. Various organizations, including the World Health Organization (WHO), have developed guides to help inform national and international pandemic preparedness [1]. Echoes of past epidemics, including the flu pandemic of 1918, are seen today as the world struggles with this new pandemic, while waiting for a vaccine and a cure. The lack of definitive treatment or preventative options for coronavirus disease 2019 (COVID-19) led many early on to consider COVID-19 convalescent plasma (CCP) as potentially therapeutic. Convalescent plasma (CP) had been used for over a century on a smaller scale to treat a variety of infections (airborne, droplet or vectorially transmitted), including SARS-CoV and MERS-CoV. However, the COVID-19 pandemic marked the first time where CP was deployed on a massive scale. Blood centres navigated the obstacles successfully to bring CCP to patients, collecting CCP even as regulators scrambled to provide a regulatory framework for the collection, manufacturing, labelling, transfusion and reimbursement, and while scientists tried to address fundamental questions relating to the immunopathogenesis and testing for a novel virus, with the main goal of providing patients an effective and validated new therapy.

COVID-19 highlighted many areas for improvement in pandemic management from the perspective of supply and demand of both traditional blood products as well as provision of novel therapeutics. The challenges (and concomitant lessons) span from donor qualification, to supply chain maintenance, staffing and safety and to the testing and labelling of products. The current success is largely due to the flexibility and open communication between donor centres, hospitals, governmental administrators and regulators throughout the pandemic as new information required ongoing development and refinement of policies and practices. For many countries, coordination of the response has been lacking or ineffective contributing to disjointed efforts, hoarding of supplies and public confusion. In others, coordinated messaging facilitated donor education, encouraging testing and donation.

Materials and methods

The International Society of Blood Transfusion (ISBT) established a Working Group (WG) with members from all 6 WHO regions, to address existing practices and provide recommendations on the collection and use of CCP. A subgroup was assigned to draft a manuscript to document the lessons learned pertaining to CCP with a view to offer guidance for future pandemics. This manuscript was drafted as a companion to earlier publications on procurement and clinical use of CCP by the same group [2, 3].

Clinical trials, compassionate use and support from regulators

Consider convalescent plasma is still considered an investigational therapy, and in many countries, its use is restricted to research (clinical trials). While not unique to CCP, regulators actively encourage clinical trials for unproven therapies to provide strong evidence of efficacy [4–6]. By contrast, compassionate use treatment and observational studies may facilitate access, but result in slower scientific yield. The pandemic highlighted the ethical challenge of reconciling compassionate and research

use of an investigational product. Compassionate use, by diverting patients eligible for inclusion in clinical trials, impeded trials and slowed quality data gathering. The counterpoint is how to defer treatment in favour of research when an investigational product has already been shown to be safe, exhibits some signals of efficacy, and where a viable alternative to treatment is not available [7]. Understandably, the lack of treatment options for COVID-19 drove early enthusiasm for CCP and pressure for easy access. In countries where both clinical trials and compassionate use were allowed, trial enrolment was hampered by ease of acquiring CCP through compassionate use which bypassed the risk of a patient being randomized to a non-CCP arm. In future epidemics, clinicians, researchers, ethicists and policymakers will likely continue to struggle balancing access to a promising therapeutic (the need of the one) with gathering quality data through trials that restrict access (the needs of the many).

Health authorities and clinicians should work together during pandemics to accelerate authorization of clinical trials, especially for therapies like CP whose parent component, plasma, is well-known to medicine and has a well-understood safety profile. Clinical trials require a major investment in time and resources, which is especially true during a global health crisis. Infection is often waning by the time trials get underway using existing authorization processes, leaving studies underpowered to test their primary outcomes, as evidenced by the 2014-2016 West African Ebola outbreak [8] and SARS-CoV-2 pandemic [9]. Too many trials may prove to be as detrimental as too few, tying resources to studies that are unlikely to achieve targeted outcomes, while fewer protocols across many collaborating sites might otherwise have succeeded.

Failure to determine whether CP can be an effective early phase therapeutic during pandemics would be a lost opportunity, impacting future pandemics [10]. A proposal for future pandemic management is to authorize a group of internationally recognized experts, key regulators and related professional organizations to oversee an emergency pandemic clinical trial protocol. This protocol would – ideally– be developed ahead of the next global health crisis, thus enabling rapid, harmonized deployment across multiple sites and countries. Such a protocol would serve to standardize key questions that need to be addressed, along with the data collection structure to follow. This would encourage investigators to coordinate trials more effectively, allowing rapid execution of large, multi-site trials, and help standardize the collection of key data elements spanning infectivity and recovery, treatment and testing, safety, dosing and overall efficacy of potential therapeutics.

Ongoing involvement of regulatory authorities would be paramount. All key stakeholders, including biotechnology companies, blood suppliers, researchers, healthcare providers and international peers, would attend frequent joint meetings with their respective regulatory agencies to allow for quick, conclusive and prompt actions. The framework must also include the provision for allocation of national budgets to help early funding.

The pandemic has already spurred innovative approaches to data gathering. These include pairing of trials that were initiated independently of each other to accelerate enrolment goals or pooling of data where independent enrolment was suboptimal, such as the 'COMPILE' study to pool data from ongoing and discontinued RCTs pertaining to CCP [11], and analysis from several countries using Bayesian statistical methodologies to determine the effect of CCP on clinical status as the primary outcome, including the effect of various covariates. Similarly, some efforts have been made to coordinate international clinical trials, including the REMAP CAP study [12] of CCP efficacy in critically ill patients and the involvement of hospitals in the US and Brazil in the Canada-led CONCOR-1 trial [13] in moderately ill patients treated within 24 h of hospital admission. Other ongoing, multinational studies are underway. The expectation is that more uniform data gathered globally will enable better answers that will ultimately drive improved patient outcomes.

Donor centre logistical challenges

SARS-CoV-2 highlighted areas for improvement in supply chain management both of traditional blood products as well as novel therapeutics. Blood centres, already struggling with low blood inventories and declining community interest in donation, embraced the challenges to establishing a new blood product line with unique (and often dynamic) donor qualification and product labelling requirements.

CCP competes for the same limited resources as blood components and products derived from plasma fractionation, impacting the supply of all. Donor centres rely on a 'just in time' delivery model for many consumables (e.g. personal protective equipment – PPE, bags and test tubes), so are susceptible to supply chain disruptions (shipment delays); increased utilization of supplies; and increased demand for supplies not routinely used in pre-pandemic operations.

Blood centres must be considered as part of the critical infrastructure, taking active part in coordination efforts, especially at local levels. Clear and frequent communication is essential. They must be able to communicate critical changes in their supplies and availability of all blood

products including CP to governmental organizers and hear how hospitals will be adjusting their service levels that might impact blood inventory requirements.

Managing frontline staff exposure and use of personal protection equipment (PPE)

Frontline workers not only faced potential exposure to COVID-19 in their personal lives, but also increased occupational risk of exposure given interactions with each other and the public (donors). Adequate staffing was especially challenging during peak infectivity as staff become infected, are quarantined following exposure and miss work to care for affected family members or due to fear of becoming infected. Feelings of stress, fatigue, depression and helplessness are common for all staff throughout the pandemic, especially when given new tasks, extra shifts or redeployed. It is important for staff to feel heard, protected and provided frequent, active communications about any changes.

In most countries, the lack of a stable supply of PPE was an ongoing challenge even as guidance on their usage changed. The use of hand sanitizer, masks and other PPE was ultimately adopted everywhere. However, the lack of adequate supplies forced many to make their own supplies and/or repurpose single-use PPE, particularly in low- and middle-income countries (LMICs) where challenges in finding sufficient PPE to protect staff (let alone the public) were common, adding to staff and donor stress. Referenced policies should be created to provide staff with the most up-to-date information on any PPE workarounds, including how to make, clean or how long to reuse them.

Collection space limits, access to apheresis equipment and CCP donor room management

Social distancing forced the modification of the collection environment, reducing significantly collection capacity, as fewer whole blood beds and apheresis machines could be placed within a given area to guarantee at least 2 metres (6 feet) between donors. Mobile drives were severely limited as businesses closed, restricted outside access or adopted remote workforce policies. Plasma collection was especially challenging for LMICs as whole blood was the primary mechanism to collect both regular plasma and CCP and without or with limited apheresis equipment, collecting sufficient plasma and CCP from the limited donor pool was difficult.

Additional spacing was not necessary for CCP collection, although donors and staff occasionally expressed concern about having potentially infectious 'recovered patients' present. This was especially true initially when data were lacking on the kinetics of infection. In response, some centres allocated separate rooms or collected CCP outside standard working hours.

Recruiting CP donors

Donor recruitment during a pandemic is challenging. Studies on what motivates convalescent individuals to donate CP are limited. Most likely they give out of altruism, as relief or gratitude for surviving, as a directed donation for a loved one, or for knowledge about their immunity. Multiple pathways must be deployed to identify and maximally recruit CP donors. Hospitals, clinics, clinicians, testing laboratories, government officials and public health departments are valuable partners. Recovered patients should be encouraged to donate several times, especially at the beginning to help bolster the inventory. If CP donors donate more frequently or have modified suitability criteria (e.g. lower haemoglobin requirements), additional medical oversight will be needed to ensure donor safety.

In exchange for recruiting donors, partners may insist that some or all collections be dedicated to a specific hospital, physician's group, geographic location, research protocol and/or patient or patient group. While a great source of donors, this adds much complexity and stress. The overall management follows the high-touch model of a specialized donation programme rather than the lower-touch model of traditional allogeneic blood donation. To scale collections and maximize resources, blood centres need to apply as many routine blood collection policies as possible. The high-touch model, however, likely will be critical early on.

While Healthcare and public health authorities work to recruit donors, it is important to maintain safeguards to prevent unauthorized access to or improper use of protected information. Maintaining donor trust and attention to donor safety and privacy concerns is key to convincing the community to become regular CP donors. Weeks of negotiation might be needed before recruitment begins, with the development of additional questionnaires, policies, and talking points. Workflows should be created to identify which parts of the recruitment process are handled by which blood centre staff versus any potential recruiting partners. A standardized check list of pre-requisite information and approved testing is helpful. Once qualified, the recruitment team must have the infrastructure and flexibility to work around donor's schedules and ability to travel to minimize delays.

As soon as reliable patient testing is available, recruitment should focus to recovered individuals with documented presence of the infectious agent positive, such as the nasopharyngeal swab real-time polymerase chain

reaction (RT-PCR) test for COVID-19. Consistent and ongoing education and messaging about the need to donate across all media platforms is needed to drive donors to self-recruit directly with the blood centre to further increase the inventory. In the end, unique solutions may be needed to optimize local opportunities and demands.

Media partners and pressures

It is important to utilize all media platforms in recruiting donors: social media, messaging applications, billboards, national papers, news programmes and radio/TV. Each is uniquely positioned to reach different groups. National promotions with coordinated messaging can be very effective particularly with use of national leaders and prominent persons as spokespersons. A clear message however is not always a nuanced message. CCP was often touted as a 'cure' despite the lack of peer-reviewed evidence, increasing general demand for CCP even as clinical trials were struggling to enrol patients.

Donor/product qualification

COVID-19 is not considered transfusion-transmitted [14]; however, CCP must follow all the national requirements to prevent infection common for all blood products. During future pandemics, where transmission mechanisms might be unknown, pathogen reduction should be considered [15].

The definition of what constitutes clinical recovery and, consequently, CP donor acceptance will evolve as new tests and data become available. International societies/organizations (ISBT, AABB, WHO), scientists, publishers and the media are important in rapidly disseminating and releasing policies and research results. For example, over the course of the first few months of the pandemic, WHO simplified the criteria for discharge from isolation for COVID-19 [16] and acknowledged that residual cough or loss of taste/smell could linger while still waiting for results on residual live viral shedding, roughly 20 days (range 6–59) [17–19] and after 28 days [20] for SARS-CoV-2, respectively. Antigen-based rapid diagnostic tests, while faster, cheaper and easier to perform than RT-PCR, are frequently less sensitive. Saliva sampling for RT-PCR may be attractive alternatives to nasopharyngeal swabs for respiratory viruses, although are not without limitations [21].

The US FDA currently recommends a nAb titre $\geq$1:160 and $\geq$1:80 for CCP as optimal and acceptable for use, respectively, although it is still unclear which antibody specificities provide the best neutralizing potential, what minimal titre levels are optimal, how long nAbs titres in donors remain at therapeutic levels, and which surrogate assays provide the best answers to these questions. Testing with live viruses – viral neutralization tests (VNT) or plaque reduction neutralization tests (PRNT) – remains the gold standard to detect and measure nAbs. However, they require sophisticated containment facilities, highly trained personnel, are time-consuming (4–7 days), produce live pathogenic virus and not available in many parts of the world.

Another potential solution uses pseudotyped lentiviruses (lentivector plasmids) [22], which are extensively modified to reduce pathogenicity and express a luciferase reporter gene for easy detection. The method is sensitive, reproducible, faster (1–2 days) and requires only a biosafety level 2 laboratory. Several studies have shown good agreement between the various nAb methods [20, 23, 24]; however, no formal standardization exists, limiting result comparisons across platforms.

Ab titres may quickly wane, increase as the immune response matures or remain stable for months [25]. Testing each donation via a quantitative assay as a release test is the only way to provide quality assurance for CP [26] and determining optimal CP dosing. While important for dosing and outcomes analysis, large scale nAb testing as a CP release test is not possible. Other assays must be utilized, such as ELISA- and CLIA-based assays, commonly employed for blood centre testing. Several organizations (ISBT, AABB, BEST, ASCP) are currently working collaboratively to determine correlations. There is a good agreement with some but not all nAb titres and ELISA or CLIA tests [19, 27], with variation depending on antigen source (spike or nucleocapsid antigens). SARS-CoV-2-specific IgG titre >1:1000 and >1:640 has shown promise in two early trials [28, 29].

Recently, the FDA issued new updates about the emergency use authorization (EUA) to 'limit the authorization to the use of high titre COVID-19 convalescent plasma for the treatment of hospitalized patients early in the disease course and to those hospitalized with impaired humoral immunity and cannot produce an adequate antibody response' defining a number of tests to be used for CCP screening, with a minimum qualification test criteria to be followed [30]. With this new FDA guidance, units with low antibody titres should be reliably identified as soon as possible and either relabelled as frozen plasma or sent for plasma fractionation.

Triaging donors based on symptoms, recovery time and test results

NAbs have a peak detection in severe patients 10–15 days after infection, with higher titres seen in more severe than mild or asymptomatic cases [19]; therefore, recently

recovered symptomatic patients confirmed positive via a validated nasopharyngeal RT-PCR are ideal CCP donors, with higher detectable nAb estimates. Donors who are 'presumed positive' are the next best donors (had symptoms and a strong epidemiologic link). In The Netherlands and South Africa, approximately 40% and 16% of CCP collections, respectively, have no SARS-CoV-2 antibody (Vrielink and Vermeulen, personal communication). Donors who have had no symptoms and who have not had a diagnostic test are least likely to have high titre nAbs and require additional testing with different methodologies for confirmation. These donors have limited usefulness as CP donors (at great expense) unless a significant percentage of the overall community has been infected. The lack of sensitive and specific assays may result in providing products with unknown efficacy, confounding outcomes analysis. It is important to freeze samples until suitable tests are available for retrospective testing.

Qualifying donors and assessing donor suitability

CP donors are more likely to be first time donors, requiring more assurances and education. They should meet the same qualifications as regular plasma donors, including HLA and possibly HNA antibody screening in multiparous women, to ensure the greatest overall safety profile [31]. Exceptions to allogeneic criteria may be necessary especially for early or frequent donors and will require additional policies and medical oversight. Symptoms like increased post-donation fatigue, excessive vasovagal reactions, declining haemoglobin levels and increased bruising may need to be monitored to ensure donor safety.

Managing donor questions about immunity and reinfection

Specific questions about CCP donor safety centre around either the implications of donor testing regarding protection against future infection, or the adverse effect of donation on a donor's immunity. The relative roles of humoral and cellular immunity in protection from recurrence of any infection are largely unknown as is any definitive predictive statement linking antibody titre or specificity with future infection or reinfections.

Recurrence risk is related to host factors, mutations in the virus and therapies that have been received. While COVID-19 persisted in patients with B-cell deficiency-related hypogammaglobulinaemia, despite a robust anti SARS-CoV-2 T-cell response [32], and while high-frequency plasma donation is associated with depletion of a variety of plasma proteins including plasma immunoglobulins [33, 34], there are no current data suggesting an increased infectious risk or other negative health effect relative to short term (2–6 months) increased apheresis donations of any type.

Donors were also afraid donating CP would permanently reduce the level of protective antibodies in their body. Current US and European regulations require periodic determination of IgG level in high-frequency plasma donors to monitor loss, although it is unclear if such testing is needed for CP donors who might donate at higher frequency for a limited number of months. SCANDAT may eventually provide an answer relative to short- and long-term CCP donor health [35], as could haemovigilance programmes. As we understand more about COVID-19, it will help to update a common, readily accessible repository where donors can be directed to answer their questions and concerns.

Inventory management; how to provide units with short supplies?

CP is a limited resource. The blood centre and the transfusion service are ultimately guardians of the blood supply, distributing or issuing components, respectfully, following common principles of patient care, transfusion medicine best practice, distribution guidance by national regulatory agencies and/or dosing regimens by study protocols and peer-reviewed literature. One must always be consistent in 'not treating physician's anxiety but treating patient's needs'.

Some places will triage CP inventory with a limited number of transfusion specialists especially when inventory is low. Others will provide CCP only within the context of a clinical trial or after ethics committee approval to maximize the effectiveness signal and minimize demand. Others – especially where compassionate use is high – will try to ensure as many patients as possible have access to at least one unit of CCP. Each solution has its pros and cons. Concerns about restricting access to minority groups should be considered, regardless of which method is used. Good communication between expert prescribing and BTS physicians is key to developing and updating clinical guidelines and order sets.

Exclusive use of group AB CP for all patients is not possible; therefore, ABO isogroup units should be the first choice for transfusion. When ABO compatible CP is unavailable and transfusion is recommended, consider using units with low titre ABO isoagglutinins ($\leq$64–$\leq$100) or that are 'least incompatible' (i.e. B plasma to an AB patient).

Therapeutic value

The therapeutic efficacy of CP can only be established in well-controlled clinical trials with properly consented

patients using products that are well-characterized. Early reports have shown that very early administration (within 72 h after the onset of symptoms) in high-risk patients reduce significantly the frequency of disease worsening and hospitalization [36], or decrease in mortality when high titre nAb CCP was transfused [37]. Data have also shown that once patients start producing nAbs [38] or developing end organ damage [39], CCP transfusion is not beneficial, and several CCP trials in patients well into their disease course have been stopped for futility. Without widespread access to reliable and correlated testing, data collection on efficacy and dosage will be hampered.

Several clinical trials involve maintaining retention samples from the donor or CCP hoping that analyses of the samples mapped to specific patient outcomes may allow the identification of all therapeutic molecules and their optimal doses, whether it be anti-RBD (receptor binding domain) or nAb titres, cytokines or other immunomodulatory molecules. Preferred Ab classes and epitope specificities promoting antibody-dependent cellular cytotoxicity (ADCC) or other immune defence mechanisms should also be investigated to optimally characterize CP or hyperimmune globulin in the future. Although previous experience with influenza [40] suggests that hyperimmune globulin could eventually provide a more consistent and higher potency therapy compared to CP, the additional manufacturing and qualification required for hyperimmune globulin delays initial availability.

Conclusion

The pandemic is not over, and the response is still evolving. Many questions remain unanswered that must be addressed by the global scientific community, such as the real therapeutic value of convalescent plasma, the ideal dosage and timing for usage, and whether CP still has a role after hyperimmune globulins or therapeutic monoclonal blends are available. How do the vaccines, CP, or other immune-bolstering therapies alter the native immune response and how do they impact CP donor suitability? As variant strains emerge, will there be a need to characterize wild type versus variant CP?

Some of what we believe now might prove to be incorrect in future. Changes in supply chain management, investment in infrastructure, staff safety and coordination of data gathering to enhance our ability to be flexible and adapt to novel and dangerous new threats, whatever the transmission mechanism might be, are just a few lessons hopefully learned during this pandemic. Preparedness for pandemics outside of pandemic situations must be taken seriously in the future.

Conflict of interests

The authors declare no conflict of interests.

Author contributions

Silvano Wendel and Kevin Land share together the co-authorship, where both had the same dedication to the manuscript.

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References

1 WHO. Pandemic influenza risk management: a WHO guide to inform and harmonize national and international pandemic preparedness and response. 2017. https://apps.who.int/iris/handle/10665/259893
2 Bloch EM, Goel R, Wendel S, et al. Guidance for the procurement of COVID-19 convalescent plasma: differences between high- and low-middle-income countries. Vox Sang 2020. 116:18–35.
3 Al-Riyami AZ, Schafer R, van den Berg K, et al. Clinical use of Convalescent Plasma in the COVID-19 pandemic: a transfusion-focussed gap analysis with recommendations for future research priorities. Vox Sang 2021;116:88–98.
4 Furtado RHM, Berwanger O, Fonseca HA, et al. Azithromycin in addition to standard of care versus standard of care alone in the treatment of patients admitted to the hospital with severe COVID-19 in Brazil (COALITION II): a randomised clinical trial. Lancet 2020;396:959–67.
5 Cavalcanti AB, Berwanger O, Zampieri FG: Hydroxychloroquine with or without Azithromycin in Covid-19. Reply. N Engl J Med 2021;384:191.
6 Dyer O. Covid-19: Remdesivir has little or no impact on survival, WHO trial shows. BMJ 2020;371:m4057.
7 Joyner MJ, Bruno KA, Klassen SA, et al. Safety update: COVID-19 convalescent plasma in 20,000 hospitalized patients. Mayo Clin Proc 2020;95:1888–97.
8 Group PIW, Multi-National PIIST, Davey RT Jr, et al. A Randomized, Controlled Trial of ZMapp for Ebola Virus Infection. N Engl J Med 2016;375:1448-56.
9 Li L, Zhang W, Hu Y, et al. Effect of Convalescent plasma therapy on time to clinical improvement in patients

with severe and life-threatening COVID-19: A randomized clinical trial. JAMA 2020;324:460–70.
10 Bloch EM: Convalescent plasma to treat COVID-19. Blood 2020;136:654–5.
11 Petkova E, Antman EM, Troxel AB: Pooling data from individual clinical trials in the COVID-19 era. JAMA 2020;324:543–5.
12 Study RC. REMAP CAP Study. REMAP CAP Study. 2021.
13 CONCOR-1. CONCOR-1 Study. CONCOR-1. 2021(February 22, 2021).
14 Cappy PCD, Sauvage V, Lucas Q, et al. No evidence of SARS-CoV-2 transfusion transmission despite RNA detection in blood donors showing symptoms after donation. Blood 2020;136:1888–91.
15 Epstein J, Smid WM, Wendel S, et al. Use of COVID-19 convalescent plasma in low- and middle-income countries: a call for ethical principles and the assurance of quality and safety. Vox Sang 2021;116:13–4.
16 WHO. Diagnostic testing for SARS-CoV-2: interim guidance. 2020. 11 September, 2020.
17 Long QX, Tang XJ, Shi QL, et al. Clinical and immunological assessment of asymptomatic SARS-CoV-2 infections. Nat Med 2020;26:1200–04.
18 Zhou F, Yu T, Du R, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet 2020;395:1054–62.
19 Wendel S, Kutner JM, Machado R, et al. Screening for SARS-CoV-2 antibodies in convalescent plasma in Brazil: Preliminary lessons from a voluntary convalescent donor program. Transfusion 2020;60:2938–51.
20 Wang Y, Zhang L, Sang L, et al. Kinetics of viral load and antibody response in relation to COVID-19 severity. J Clin Invest 2020;130:5235–44.
21 Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva or nasopharyngeal swab specimens for detection of SARS-CoV-2. N Engl J Med 2020;383:1283–6.
22 Nie J, Li Q, Wu J, et al. Establishment and validation of a pseudovirus neutralization assay for SARS-CoV-2. Emerg Microbes Infect 2020;9:680–6.
23 Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA vaccine against SARS-CoV-2 - preliminary report. N Engl J Med 2020;383:1920–31.
24 Liu L, Wang P, Nair MS, et al. Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature 2020;584:450–6.
25 Wendel S, Fontao-Wendel R, Fachini R, et al. A longitudinal study of convalescent plasma (CCP) donors and correlation of ABO group, initial neutralizing antibodies (nAb), and body mass index (BMI) with nAb and anti-nucleocapsid (NP) SARS-CoV-2 antibody kinetics: Proposals for better quality of CCP collections. Transfusion 2021. https://doi.org/10.1111/trf.16323. [Epub ahead of print].
26 Zilla M, Wheeler BJ, Keetch C, et al. Variable performance in 6 commercial SARS-CoV-2 antibody assays may affect convalescent plasma and seroprevalence screening. Am J Clin Pathol 2021;155:343–53.
27 Luchsinger LL, Ransegnola BP, Jin DK, et al. Serological Assays Estimate Highly Variable SARS-CoV-2 Neutralizing Antibody Activity in Recovered COVID-19 Patients. J Clin Microbiol 2020;58:e02005-20.
28 Shen C, Wang Z, Zhao F, et al. Treatment of 5 critically Ill patients with COVID-19 with convalescent plasma. JAMA 2020;323:1582–9.
29 Duan K, Liu B, Li C, et al. Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci USA 2020;117:9490–6.
30 FDA In Brief: FDA Updates Emergency Use Authorization for COVID-19 Convalescent Plasma to Reflect New Data (2021). Available at https://www.fda.gov/news-events/fda-brief/fda-brief-fda-updates-emergency-use-authorization-covid-19-convalescent-plasma-reflect-new-data (last access February 23, 2021).
31 European Comission. An EU programme of COVID-19 convalescent plasma, collection and transfusion - Guidance on collection, testing, processing, storage, distribution and monitored use. Version 1.0. 2020. April, 2020.
32 Hueso T, Pouderoux C, Pere H, et al. Convalescent plasma therapy for B-cell-depleted patients with protracted COVID-19. Blood 2020;136:2290–5.
33 Laub R, Baurin S, Timmerman D, et al. Specific protein content of pools of plasma for fractionation from different sources: impact of frequency of donations. Vox Sang 2010;99:220–31.
34 Schulzki T, Seidel K, Storch H, et al. A prospective multicentre study on the safety of long-term intensive plasmapheresis in donors (SIPLA). Vox Sang 2006;91:162–73.
35 Edgren G, Hjalgrim H: Epidemiology of donors and recipients: lessons from the SCANDAT database. Transfus Med 2019;29(Suppl 1):6–12.
36 Libster R, Perez Marc G, Wappner D, et al. Early high-titer plasma therapy to prevent severe Covid-19 in older adults. N Engl J Med 2021;384:610–8.
37 Klassen SA, Senefeld JW, Johnson PW, et al. Evidence favoring the efficacy of convalescent plasma for COVID-19 therapy. medRxiv. 2020.
38 Agarwal A, Mukherjee A, Kumar G, et al. Convalescent plasma in the management of moderate covid-19 in adults in India: open label phase II multicentre randomised controlled trial (PLACID Trial). BMJ 2020;371:m3939.
39 Simonovich VA, Burgos Pratx LD, Scibona P, et al. A randomized trial of convalescent plasma in Covid-19 severe pneumonia. N Engl J Med 2020;384:619–29.
40 Hung IFN, To KKW, Lee CK, et al. Hyperimmune IV immunoglobulin treatment: a multicenter double-blind randomized controlled trial for patients with severe 2009 influenza A(H1N1) infection. Chest 2013;144:464–73.

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MATRA-A: A study on massive transfusion

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Background We use massive transfusion in various clinical conditions and it is associated with high mortality. Although some massive transfusion protocols improve patient outcomes, the clinical circumstances requiring it are not well defined.

Methods MATRA-A is a multicenter retrospective study. Six University and Training Research Hospitals in Ankara participated in the study. We collected clinical data on patients (>18 years) who received massive transfusions (≥10 units/24 h) from 2017 through 2019.

Results Overall, 167 (0·27% of transfused patients) received a massive transfusion of 2586 units of red blood cells (1·5% of total RBCs transfused). The median interquartile range values for RBCs, fresh frozen plasma (FFP) and platelets were 13 (11–176), 16 (9–33) and 4 (0–11), respectively. Surgical patients received 90% of massive transfusions. The most common clinical indications for massive transfusion were cardiovascular diseases (42·6%), trauma (20·3%) and malignancies (11%). FFP: RBC: Platelets ratio was 1·9:1:0·5. The overall and trauma-related mortality rates were 57·4% and 61·8%, respectively. The hospital mortality rates of trauma patients that received high vs. low ratio (FFP: RBCs > 1:1·5 vs. ≤1:1·5) transfusions were 47·6% and 86·6% and the difference was statistically significant ($P = 0·03$).

Conclusion Cardiovascular diseases and trauma occasion are the most common causes of massive transfusion. It is infrequent in clinical settings and is associated with high mortality rates. Additionally, in massively transfused trauma patients, a high FFP:RBCs ratio seems to be associated with increased survival. Focused prospective studies are required to define the areas that need improvement on a national scale.

Key words: indications, massive transfusion, mortality, Turkey.

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Abbreviations CATES, Central Anatolia Trauma and Emergency Surgery; FFP, fresh frozen plasma; IQR, interquartile range; LOS, length of stay; MATRA-A, massive transfusion

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Ankara; MATRA-Istanbul, massive transfusion Istanbul; MT, massive transfusion; MTP, massive transfusion protocol; RBC, red blood cells; SPSS, statistical package for the social sciences.

Introduction

Critical bleeding can kill people [1,2]. Broadly, critical bleeding is defined as high volume haemorrhage, likely to require massive transfusion (MT) [3]. Although most MT data comes from trauma studies [4–10], it is needed in various clinical settings and incurs increased mortality [9,11–15]. MT has various definitions based on RBC units (red blood cells) transfused and the duration of transfusion. The most commonly used definition is the loss of one blood volume (approximately 7% of body weight) in 24 h and transfusion of ≥10 units of RBCs in 24 hours [3,12,16].

Massive blood transfusions are not without complications: infection, organ failure and metabolic complications are all potential risks which need to be addressed [16–18]. In the last decade, MT protocols (MTP) that comprise higher Fresh Frozen Plasma (FFP)/RBCs/Platelets ratios have been reported to improve patient outcomes, particularly in trauma patients.[14]

Although there is information on the indications for MT, we know little about how often it is used in various patient groups (including paediatric). During the Central Anatolia Trauma and Emergency Surgery group meeting on 2 March 2019, we emphasized the lack of national MT and MTP data, and decided that it needed to be studied. Initially, a literature search found no retrospective or prospective studies in Turkey. Fortunately, during the conduct of this study, the Technical Assistance Project for Improving the Blood Transfusion Management System in Turkey was started on 19 March 2019 [19]. Included in the project's essential aspects is the adoption of an MTP [19] for the Turkish Health System. This has been accomplished and is currently under final review.

The current study variables resemble those of Halmin *et al.* [20] for comparative purposes. We aimed to clarify and document the epidemiology of MT in Ankara by a retrospective descriptive study to define the clinical characteristics of MT patients.

Methods

Initially, we carried out an extensive literature search on PubMed. Publications between 1990 and 2020 were filtered. Search phrases included 'massive transfusion', 'massive bleeding', 'critical bleeding', 'Epidemiology' and 'Turkey'. Publications from Turkey consisted of case reports, retrospective descriptive analyses of upper and lower gastrointestinal bleeding, military trauma, case series from various clinics and reviews written in Turkish. International literature was dominated by trauma studies, and we selected relevant articles.

The study was approved by the University of Health Sciences Gulhane Non-Invasive Investigation Ethics Committee (2020-45/11.02.2020). Study comprises only medical institutes located in Ankara. Overall, three training and research hospitals and hospitals affiliated with three different universities participated. (Table 1) Our study's time frame included data from two consecutive years between 1 January 2017 and 1 January 2018 (Period 1), and 2 January 2018 and 1 January 2019 (Period 2). We separated Tables 2 and 3 to enable future comparisons. Summed values are analysed in the results section and in Table 4.

We collected data from the laboratory information system (LIS) of hospitals and local blood bank registries, entering the formula: patients confirmed to receive ≥10 RBCs within 24 h. Data were cross-checked with the local blood banks to avoid errors. Upon identifying an MT patient's identification number, we diligently checked the clinical background as well as the data acquired from the LIS.

All patients (age > 18 years) that received MT (≥10 Units of RBCs within 24 h) during their stay at the hospital were included. General hospital data referable to the study included the total number of patient referrals, the number of patients admitted to each participating hospital, the number of patients transfused and the total number of RBC units transfused during the study period. Specific data comprised patient demographics, admitting clinic, clinical diagnosis associated with MT, length of stay (LOS), outcome (mortality) and the number of RBCs, FFP and platelet units transfused during MT. A platelet unit refers to one apheresis platelet unit or a pool of 4 to 6 whole blood-derived platelet concentrates. Mortality was defined as death from any cause that occurred any time during the hospital stay.

Peralta *et al.* [21] emphasized the lack of optimal cut-off between high and low FFP:RBC ratios and compared the outcomes of FFP: RBC ratios (≥1:1·5 vs. <1:1·5). Similarly, they divided the clinical indications and FFP:RBCs ratios into trauma/others and high/low (FFP: RBCs

Table 1 Medical institutions included in the study

- Ankara University Faculty of Medicine
  - (a) Ibni Sina and Cebeci Hospitals
- Gazi University Faculty of Medicine Hospital
- Hacettepe University Faculty of Medicine Hospital
- University of Health Sciences
  - (a) Diskapi Yildirim Bayezit Training and Research Hospital
  - (b) Gulhane Training and Research Hospital
  - (c) Kecioren Training and Research Hospital

ratio > 1:1·5 vs. ≤ 1:1·5) ratio groups. They analysed the associations between the high/low ratio and the mortality/no-mortality groups for both trauma and other causes of MT.

We analysed our data using SPSS v.22 software (Statistical Package for the Social Sciences (IBM Inc.; Armonk, NY, USA). Statistical significance was set at <0·05. Initially, normality tests were run using SPSS, and results showed that the assumption of normality was violated, and the significance value was <0·05. Therefore, we presented continuous data as the median and interquartile range (IQR) values. Categorical data were reported as frequencies. We analysed associations between continuous variables using Pearson's test. The differences between dichotomous and continuous variables that were not distributed normally were analysed using the Mann–Whitney U-test. Fischer's exact test was used to analyse the differences between the two dichotomous variables. Independent samples t-test was used to analyse the differences between the dichotomous and continuous variables. The one-way MANCOVA test determined the age-adjusted effect of surgery/no-surgery, mortality/no-mortality, and gender groups on the amount of transfused total and MT RBC units. Bonferroni corrections were used for pairwise comparisons.

Results

The medical institutions hospital software systems recorded a total of 18 512 768 patients between 2017 and 2019, and of those 603 879 (3·3%) were admitted as inpatients. Some 61·157 (10%) of the admitted patients received 173·088 units of RBCs. However, 167 (0·27%) of those transfused patients had MT and received a total of 2586 units of RBCs (1·5% of total RBCs transfused) (Tables 2 and 3). Hacettepe University, Diskapi Yildirim Bayezit and Gazi University Hospitals had 26%, 23·4% and 19·2% of cases, respectively. Cardiovascular surgery (45·5%) and general surgery clinics (25%) were most commonly involved in MT. The clinical indications for MT are given in Table 4. Between 2017 and 2019, the median number of RBCs transfused during an MT episode was 13 units. During their full hospital stay, these patients received a median of 18 units of RBCs.

There was a moderate, positive correlation between these two data ($r = 0·3$, $P < 0·001$). Patients received 16 (9–33) units of plasma and its association with RBCs transfusion was statistically significant ($r = 0·2$, $P = 0·002$). The median platelet units transfused to these MT patients were 4 (0–11), and we found no statistically significant association between RBCs and platelet transfusions. MT patients' ratio of transfused FFP: RBCs: Platelets was 2:1: 0·5.

The median age of patients who died was 61 (43–68), 73% of them male. Their LOS was 6 (2–17) days ($P = 0·003$). Like the indications for MT, the ones most commonly fatal were cardiovascular surgery (50%), trauma (21·9%), cancer surgery (6·3%) and transplantation (5·2%). Median RBC units administered in fatal and non-fatal cases were 14 (11–18) and 12 (10–15), respectively. The difference between the groups was statistically significant ($P = 0·005$). After adjusting for the age covariate, there were no statistically significant differences between the surgery/nonsurgery, and gender groups regarding the amount of transfused total and MT RBC units ($P > 0·05$).

Trauma was the second most common cause of MT. Trauma patients' median age was 31·5 (26–42) years, and 31 (91·2%) were male. These patients were administered 13 (10–15) units of RBCs, and 14 (6–2 7) FFP and 2 (0–6) platelet units. The trauma patients' FFP: RBCs: Platelets ratio was also 2:1:0·5. Massively transfused trauma patients' mortality rate was 61·8%, and there was no statistically significant difference between the mortality and no-mortality group and the amount of transfused RBCs units ($P > 0·05$). Trauma was compared with other causes of death, and there was no statistically significant difference between the variables ($P > 0·05$).

The dichotomized group (trauma and other) for MT indications was analysed individually. The mortality rates of trauma patients that received high and low ratio (FFP: RBCs ratio > 1:1·5 vs. ≤ 1:1·5) transfusions were 47·6% and 84·6%, respectively ($P = 0·03$). The other group's mortality rates between the high and low ratio groups were 55·4% and 59·4%, not statistically different ($P = 0·7$).

Hacettepe University Hospital's MT data require special attention. Six patients received 824 (62% of their total) units of FFP. Their median (IQR) FFP, RBCs and Platelets values were 105 (79–137), 41 (22–55), and 24 (12–27) units, respectively. Retrospectively, those patients' laboratory data showed that their prothrombin time and

Table 2 General statistical data of medical institutions between 1 January 2017 and 1 January 2018

	Period 1 (2017–2018)							
	Overall Hospital Statistics				MT			
Institute Name	No. patient referrals	No. patient admitted	No. patient transfused	Transfused RBC units	No. of patients	RBCs (unit)	FFP (unit)	Platelet (unit)
Ankara University İbni Sina and Cebeci Hospitals	1·244·447	78·454	6677	22·585	12	250	572	96
Gazi University Hospital	1·032·652	60·235	5895	15·484	18	223	712	60
Hacettepe University Hospital	1·184·608	56·018	4965	22·563	25	363	1323	451
Diskapi Yildirim Bayezit Training and Research Hospital	2·724·873	46·717	11·750	11·718	12	197	289	64
Gulhane Training and Research Hospital	1·258·932	31·287	3165	11·322	12	162	502	157
Kecioren Training and Research Hospital	1·509·646	20·199	1698	2911	1	10	2	0
Total	8·955·158	292·910	34·150	86·583	80	1205	3400	828

FFP, fresh frozen plasma; MT, massive transfusion; RBC, red blood cell.

Table 3 General statistical data of medical institutions between 2 January 2018 and 1 January 2019

	Period 2 (2018–2019)							
	Overall Hospital Statistics				MT			
Institute Name	No. patient referrals	No. patient admitted	No. patient transfused	Transfused RBC* units	No. of patients	RBCs (unit)	FFP (unit)	Platelet(unit)
Ankara University İbni Sina and Cebeci Hospitals	1·350·357	84·619	6299	21·880	12	145	304	128
Gazi University Hospital	1·127·559	57·836	5708	15·100	14	191	241	46
Hacettepe University Hospital	1·223·083	55·713	4643	21·619	20	313	687	169
Diskapi Yildirim Bayezit Training and Research Hospital	2·735·433	50·572	4221	12·165	27	365	1651	74
Gulhane Training and Research Hospital	1·611·542	41·568	4158	12·731	11	287	211	92
Kecioren Training and Research Hospital	1·5096·36	20·661	1978	3010	3	80	42	9
Total	9·557·610	310·969	27·007	86·505	87	1381	1650	518

FFP, fresh frozen plasma; MT, massive transfusion; RBC, red blood cell.

Table 4 Characteristics of MT patients (2017-2019)

Total number of MT patients	167
Number of MT patients per Institution, *n* (%)	
Ankara University Hospitals	24 (14·3%)
Gazi University Hospital	32 (19·2%)
Hacettepe University Hospital	45 (26·9%)
Diskapi Yildirim Bayezit TR Hospital	39 (23·4%)
Gulhane TR Hospital	23 (13·8%)
Kecioren TR Hospital	4 (2·4%)
Age, (median, IQR) years	59 (37–66)
Sex, *n* (%)	
Female	51 (30·5%)
Male	116 (69·5%)
LOS, (median, IQR) days	10 (3–20)
Setting of MT, *n* (%)	
Surgical	152 (91%)
Non-surgical	15 (9%)
Mortality, *n* (%)	
Present	96 (57·5%)
Absent	71 (42·5%)
Admission Clinic, *n* (%)	
Cardiovascular Surgery	76 (45·5%)
General Surgery	42 (25%)
Gynecology and obstetrics	8 (5%)
Orthopedics and traumatology	5 (3%)
Anesthesiology ICU	13 (8%)
Emergency Medicine	5(3%)
Cardiology	4 (2%)
Urological Surgery	3 (2%)
Other	11 (6·5%)
Indications, *n* (%)	
Cardiovascular surgery	72 (42·6%)
Trauma	34 (20·3%)
Cancer Surgery	18 (11%)
Transplantation Surgery	12 (7·2%)
Gastrointestinal Bleeding	14 (8·4%)
Obstetric care	6 (3·9%)
Hematologic disease	1 (0·6%)
Other	10 (6%)
Total RBC units transfused (median, IQR)	18 (14–26)
RBC units transfused during MT (median, IQR)	13 (11–17)
Fresh Frozen Plasma units received by MT patients (median, IQR)	16 (9–33)
Platelet units received by MT patients (median, IQR)	4 (0–11)

IQR, interquartile range; LOS, length of stay; MT, massive transfusion; RBC, red blood cell; TR, training and research.

activated partial thromboplastin time values were >1·8 times the normal levels.

Discussion

Population-based MT studies are scant in the literature. Halmin *et al.* [20], showed that the transfused patients' MT incidence was between 0·25 and 0·45%. Doughty *et al.* [22] reported that cardiac services (42·7%) and trauma (17·6%) most often led to MT and 87·1% of episodes were surgical. Our MT criterion was ≥10 units of RBC to enable comparison with the above studies. In our study, 0·27% of transfused patients experienced MT, and over 90% were surgical. Contrasting with the traditional definition stated above, Mitra *et al.* [23] proposed using ≥5 RBC units in 4 h to define MT. Green *et al.* [24] used both definitions of MT, and thus captured 701 episodes of MT within a 3-year period, and the incidence rate was 1·7 per 1000 admissions.

Massive transfusion most commonly occurs in cardiac surgery settings [15]. Koch *et al.* [25] demonstrated a dose-dependent relationship between each unit of transfused RBCs and the adjusted and unadjusted odds for postoperative mortality. The unadjusted mortality rate was >20% for those receiving ≥10 units of RBCs. Delaney *et al.* [26] also showed that high ratio FFP: RBCs transfusions were associated with less organ dysfunction and lower mortality rates.

Approximately 3%–5% of civilian and 2·4%–10% of military trauma patients are expected to require MT [27–31]. Eksert *et al.* [29] showed that torso injuries constituted 93·3% of MT in military settings and 71·4% of MT were due to thoracic and thoraco-abdominal injuries. Outcome studies of trauma patients focus on the FFP: RBCs: Platelet ratios and established MTPs. Holcomb *et al.* [7] demonstrated in their PROPPR study that more trauma patients in the 1:1:1 group achieved haemostasis and fewer experienced exsanguination-related death. Our trauma patients' FFP: RBCs: Platelets ratio is 2:1:0·5. Studies [21,32,33] that compared high vs. low ratio FFP/RBCs (≥1/1·5 vs. <1/1·5) transfusions demonstrated significantly lower mortality in trauma patients. Likewise, the trauma patients' mortality rate was approximately 1·8 times lower than the low ratio group in the current study.

The optimal FFP: RBCs: platelet ratio remains controversial [10]. Halmin *et al.* [20] reported that the plasma to RBCs ratio slightly increased from 0·36 (1996–2006) to 0·6 (2006–2010). Doughty *et al.* [22] showed a more balanced use of the haemostatic components; the reported FFP: RBC: platelet ratio was 0·79:1:0·85 during 2008–2015. The FFP:RBCs:platelet ratio was 2:1:0·5 in this study. Platelet supply is usually maintained in-near-equilibrium to the routine demand and supply [31], which may also cause the low platelet ratio reported in this study. Nevertheless, Turkey's ongoing technical assistance programme may significantly improve goal-directed transfusion practices and blood resource management.

The overall hospital mortality rate of MT patients was 57·5% in this study, which was associated with higher RBCs transfusions. Cardiovascular surgery (66·7%) and trauma (61·8%) were the most common causes. Halmin

et al. [20] showed that their 30-day mortality was 24·8%; however, they also emphasized that their 5-year mortality rate was high (54·6%). Using different definitions for MT, two other studies [12,24] reported 23% and 32% overall mortality rates. Green *et al*. [24] reported that mortality was higher for patients who required 10–14 RBCs (36%) and ≥15 RBCs (66%), compared with the patients that were transfused 5–9 units (23%).

Fresh frozen plasma (FFP) contains >70% of the normal levels of clotting factors and has been used as a source of coagulation factors worldwide [10]. MT data from Hacettepe University Hospital revealed six actively bleeding patients with severe sepsis/septic shock. Besides the RBCs and platelets, they were transfused a total of 824 units of FFP. Despite the lack of any demonstrated benefit, the transfusion of FFP has been recommended for those actively bleeding patients with severe sepsis/septic shock who have increased PT (INR), APTT [10,34–36].

Clinicians in Turkey promote the use of component therapy for all transfusion indications, including massive transfusion. Turkish Red Cross, Turkey's leading blood supplier, does not have any organization to meet demand regarding whole blood use. Our country needs to improve the new blood transfusions concepts that involve whole blood supply at least for some exceptional circumstances like massive transfusion indication.

Our study has significant limitations that are inherent to its design. We depict available occurrences, with their frequencies, associations and differences. Stages and types of cancers, injury-severity scores of trauma cases, associated morbidities of patients with cardiovascular diseases, postoperative infections and sepsis rates, transfusion-related complications and other possible causes of transfusions and mortalities are not included in the study. Our data do not include 24–48 h or 30-day mortality rates.

Conclusion

To our knowledge, this is the first large scale multicenter study that describes the clinical indications of MT in Turkey. We found that cardiovascular disease, trauma and cancer are the three most frequent, and the setting was almost invariably surgical. MT is uncommon but not negligible and leads to significant numbers of blood transfusions, and a high mortality rate. High FFP: RBCs ratio transfusion may be associated with increased survival. Now we know the most frequent factors leading to massive transfusion, but larger studies on specific patient groups are required to fill the knowledge gap.

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Conflict of interest

The authors declare no conflict of interests.

References

1 Eastridge BJ, Mabry RL, Seguin P, *et al*. Death on the battlefield (2001–2011): implications for the future of combat casualty care. J Trauma Acute Care Surg 2012;73:S431–7.
2 Ruseckaite R, McQuilten ZK, Oldroyd JC, *et al*. Descriptive characteristics and in-hospital mortality of critically bleeding patients requiring massive transfusion: results from the Australian and New Zealand Massive Transfusion Registry. Vox Sang 2017;112:240–8.
3 Patient Blood Management Guidelines: Module 1 Critical Bleeding/Massive Transfusion, 2011. https://www.blood.gov.au/pbm-module-1 [Accessed March, 2020].
4 Cantle PM, Cotton BA. Prediction of massive transfusion in trauma. Crit Care Clin 2017;33:71–84.
5 Nunez TC, Voskresensky IV, Dossett LA, *et al*. Early prediction of massive transfusion in trauma: simple as ABC (Assessment of blood consumption)? J Trauma 2009;66:346–52.
6 Kautza BC, Cohen MJ, Cuschieri J, *et al*. Changes in massive transfusion over time: an early shift in the right direction? J Trauma Acute Care Surg 2012;72:106–11.
7 Holcomb JB, Tilley BC, Baraniuk S, *et al*. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015;313:471–82.
8 Stephens CT, Gumbert S, Holcomb JB. Trauma-associated bleeding: management of massive transfusion. Curr Opin Anaesthesiol 2016;29:250–5.
9 Abuzeid AM, O'Keeffe T. Review of massive transfusion protocols in the injured, bleeding patient. Curr Opin Crit Care 2019;25:661–7.
10 Spahn DR, Bouillon B, Cerny V, *et al*. The European guideline on management of major bleeding and coagulopathy following trauma: fifth edition. Crit Care 2019;23:98.
11 Sawyer PR, Harrison CR. Massive transfusion in adults. Diagnoses, survival and blood bank support. Vox Sang 1990;58:199–203.
12 Zatta AJ, McQuilten ZK, Mitra B, *et al*. Elucidating the clinical characteristics of patients captured using different definitions of massive transfusion. Vox Sang 2014;107:60–70.
13 Johnson DJ, Scott AV, Barodka VM, *et al*. Morbidity and mortality after high-dose transfusion. Anesthesiology 2016;124:387–95.

14 Sommer N, Schnuriger B, Candinas D, *et al*. Massive transfusion protocols in nontrauma patients: a systematic review and meta-analysis. J Trauma Acute Care Surg 2019;86:493–504.
15 Dzik WS, Ziman A, Cohn C, *et al*. Survival after ultramassive transfusion: a review of 1360 cases. Transfusion 2016;56:558–63.
16 Flint AWJ, McQuilten ZK, Wood EM. Massive transfusions for critical bleeding: is everything old new again? Transfus Med 2018;28:140–49.
17 Cotton BA, Au BK, Nunez TC, *et al*. Predefined massive transfusion protocols are associated with a reduction in organ failure and postinjury complications. J Trauma 2009;66:41–8; discussion 8–9.
18 Seghatchian J, Samama MM. Massive transfusion: an overview of the main characteristics and potential risks associated with substances used for correction of a coagulopathy. Transfus Apher Sci 2012;47:235–43.
19 Technical Assistance for Improving the Blood Transfusion Management System in Turkey, 2019. https://hastakanyonetimi.saglik.gov.tr/Content/Index/?id=2 [Accessed May, 2020].
20 Halmin M, Chiesa F, Vasan SK, *et al*. Epidemiology of massive transfusion: a binational study from Sweden and Denmark. Crit Care Med 2016;44:468–77.
21 Peralta R, Vijay A, El-Menyar A, *et al*. Trauma resuscitation requiring massive transfusion: a descriptive analysis of the role of ratio and time. World J Emerg Surg 2015;10:36.
22 Doughty H, Apelseth TO, Sivertsen J, *et al*. Massive transfusion: changing practice in a single Norwegian centre 2002–2015. Transfus Med 2018;28:357–62.
23 Mitra B, Cameron PA, Gruen RL, *et al*. The definition of massive transfusion in trauma: a critical variable in examining evidence for resuscitation. Eur J Emerg Med 2011;18:137–42.
24 Green L, Tan J, Grist C, *et al*. Aetiology and outcome of massive transfusion in two large London teaching hospitals over a 3-year period (2012–2014). Transfus Med 2017;27(Suppl 5):342–7.
25 Koch CG, Li L, Duncan AI, *et al*. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. Crit Care Med 2006;34:1608–16.
26 Delaney M, Stark PC, Suh M, *et al*. Massive transfusion in cardiac surgery: the impact of blood component ratios on clinical outcomes and survival. Anesth Analg 2017;124:1777–82.
27 Morse BC, Dente CJ, Hodgman EI, *et al*. Outcomes after massive transfusion in nontrauma patients in the era of damage control resuscitation. Am Surg 2012;78:679–84.
28 Pham HP, Shaz BH. Update on massive transfusion. Br J Anaesth 2013;111(Suppl 1):i71–82.
29 Eksert S, Unlu A, Aydin FN, *et al*. Analysis of anatomical localization and severity of injury in patients with blood transfusion in Urban Terrain Hospital. Ulus Travma Acil Cerrahi Derg 2020;26:937–42.
30 Ramsey G. Blood component transfusions in mass casualty events. Vox Sang 2017;112:648–59.
31 Ramsey G. Blood transfusions in mass casualty events: recent trends. Vox Sang 2020;115:358–66.
32 Gunter OL Jr, Au BK, Isbell JM, *et al*. Optimizing outcomes in damage control resuscitation: identifying blood product ratios associated with improved survival. J Trauma 2008;65:527–34.
33 Lustenberger T, Frischknecht A, Brüesch M, *et al*. Blood component ratios in massively transfused, blunt trauma patients–a time-dependent covariate analysis. J Trauma 2011;71(5):1144–51; discussion 50–1.
34 Dellinger RP, Levy MM, Rhodes A, *et al*. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013;39:165–228.
35 Murthy T. Blood transfusion practices in sepsis. Indian J Anaesth 2014;58:643–6.
36 Tupchong KKA, Foran M. Sepsis, severe sepsis, and septic shock: A review of the literature. Afr J Emerg Med 2015;5:127–35.

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ORIGINAL PAPER

Amlodipine as adjuvant therapy to current chelating agents for reducing iron overload in thalassaemia major: a systematic review, meta-analysis and simulation of future studies

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Background and Objectives Iron overload in thalassaemia is a crucial prognostic factor and a major cause of death due to heart failure or arrhythmia. Therefore, previous research has recommended amlodipine as an auxiliary treatment to current chelating agents for reducing iron overload in thalassaemia patients.

Materials and Methods A systematic review and meta-analysis of the results of three randomized clinical trials evaluating the use of amlodipine in thalassaemia patients through 12 databases were carried out.

Results Our final cohort included 130 patients. Insignificant difference in decreasing liver iron concentrations was found between amlodipine and control groups {weighted mean difference = −0·2, [95% confidence interval = (−0·55–0·15), P = 0·26]}. As regards serum ferritin, our analysis also showed no significant difference in serum ferritin between amlodipine and control groups {weighted mean difference [95% confidence interval = −0·16 (−0·51–0·19), P = 0·36]}. Similarly, there was insignificant difference in cardiac T2* between amlodipine and control groups {weighted mean difference [95% confidence interval = 0·34 (−0·01–0·69), P = 0·06]}.

Conclusions Despite the growing evidence supporting the role of amlodipine in reducing iron overload in thalassaemia patients, our meta-analysis did not find that evidence collectively significant. The results of our simulation suggest that when more data are available, a meta-analysis with more randomized clinical trials could provide more conclusive insights.

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Introduction

Thalassaemia is caused by inherited autosomal recessive abnormalities in haemoglobin synthesis [1]. These abnormalities result in an imbalanced production of whole alpha- or beta-globin chains, leading to the formation of defective haemoglobin molecules in both alpha- and beta-thalassaemia, respectively [2]. Depending on the clinical severity and haemoglobin levels, thalassaemia patients may require regular packed red blood cell transfusions with iron chelation therapy, splenectomy or bone marrow transplantation in selected cases.

Iron overload occurs frequently along the course of treatment of transfusion-dependent chronic anaemia and contributes to mortality in thalassaemia patients [3]. For example, the heart lacks a negative feedback mechanism to protect cardiomyocytes from surges in systemic iron, rendering it more susceptible to iron deposition, serious cardiac arrhythmia and sudden death [4]. Thus, iron-chelating agents were devised to lower total body iron, minimize the production of reactive oxygen species and avoid end-organ damage in thalassaemia patients with a long history of blood transfusion, leading to lower morbidity and improved survival [5]. Yet, iron chelators demonstrate a wide array of adverse side effects. For instance, deferoxamine is associated with visual loss and ototoxicity [6], whereas, deferasirox gives rise to renal impairment [7].

Previous research has shown that iron uses high-capacity calcium channels to pass into body organs, and that it is reasonable to believe that blocking these channels may potentially aid in preventing the accumulation of tissue iron and curbing its long-term complications [8]. Recently, growing evidence from randomized clinical trials (RCTs) has recommended administering amlodipine as a calcium-channel blocker (CCB), to be utilized as an adjuvant remedy to current iron-chelating agents although these conclusions have been drawn out from small study cohorts [9–11]. Therefore, we aimed to pool the findings from all published trials to assess the clinical effectiveness of amlodipine in addition to iron- chelation therapy in reducing the iron overload in patients with thalassaemia.

Methods

Search strategy and selection criteria

Under the recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [12], we conducted our systematic review and meta-analysis (Table S1). Our protocol has been recorded within the prospective international register of systematic reviews (PROSPERO) with a registration number CRD42018085424. On 10 January 2018, we performed electronic searching through twelve databases including; PubMed, Scopus, Web of Science (ISI), POPLINE, Virtual Health Library (VHL), (System for Information on Grey Literature in Europe) SIGLE, Global Health Library (GHL), The New York Academy of Medicine (NYAM), Controlled Trials (mRCT), WHO International Clinical Trials Registry Platform (ICTRP), ClinicalTrials.com, and Google Scholar. The following integrated search terms were used to assemble all related articles: (calcium-channel blocker OR Norvasc OR amlodipine) AND thalassaemia major. No search filters were applied to language, year or study design. Identified studies were grouped into Endnote to remove duplicates. Three independent reviewers screened titles and abstracts of Endnote-imported articles for eligibility against inclusion and exclusion criteria. Inclusion criteria were; (a) RCTs reporting the use of amlodipine or any other CCBs in thalassaemia major, (b) reporting data from humans, (c) with no restriction concerning language, sex, age, and area. Exclusion criteria were; (a) study design other than RCTs, (b) non-extractable data, (c) overlapping data sets, (d) where only abstract is available, (e) animal or *in vitro* studies, (f) duplicated and irrelevant data. Finally, a manual search was conducted using references of the included studies, and looking for similar studies in PubMed and Google Scholar, and contacting authors and research groups. Conflicts regarding including or excluding articles were solved after consulting a senior study member.

Data extraction

Three independent reviewers performed the data extraction task. A template in Microsoft Excel was used to report baseline study characteristics and expected outcomes. Conflict resolution about any issues related to data extraction was finalized after reviewers' discussion and consensus with a senior author, whenever needed. All data have been re-checked by more than one reviewer. The liver-iron concentration (LIC) of one study was extracted from an included graph [11]. Also, we have contacted one author to get the raw data to include it with accuracy in the analysis [10].

Statistical analysis

All data were analysed using R statistical software version 3.4.3. First, we prepared the data by using a uniform format for the expression of liver iron loads. For that, LIC was used as the standard, and T2* data were converted

into iron concentration data (1·5T) using the appropriate conversion equations [13,14]. Second, the standardized mean difference, its variance and standard error (SE) were calculated using the pre- and post-intervention data for drug versus control groups [15,16]. After that, all data were pooled using the 'meta' package.

A fixed-effect model, according to the method of Mantel–Haenszel, was used if there were no evidence of heterogeneity between studies. Otherwise, a random-effects model using the method of Der Siomonian and Laird was employed [17]. Heterogeneity between studies was assessed using the Q statistic and I^2 test which describe the percentage of variability in the effect estimates, considering it significant with I^2 value >50% or *P*-value <0·05 [17,18]. The publication bias assessment, using Egger's regression, was not a possible test due to the small number of included studies (less than 10) [19,20].

Using Stata software (version 14.2, StataCorp, College Station, TX, USA), simulation methods were used to create graphs demonstrating the power achieved by an additional randomized trial to change the results of the meta-analysis at different sample sizes up to a maximum of 1000 patients [21,22]. This process was literally described by Crowther *et al.* as follows, [23] 'from a meta-analysis of the existing studies, a distribution for the chosen outcome measures in a new clinical trial or diagnostic test accuracy study is derived. An estimate for the outcome measure from this distribution is then sampled, representing the underlying effect in the new (simulated) study. Data representing the new study are generated stochastically according to the estimate sampled in step 1 for a specified sample size. These simulated study data are then added to the existing meta-analysis, which is then re-meta-analysed'.

Results

Literature search and patient characteristics

The electronic search yielded 95 studies from the 12 databases. After excluding duplicates through title/abstract screening, we were left with 46 relevant studies for full-text screening. Of those, only three studies fulfilled our inclusion criteria. The manual search did not result in any additional studies (Fig. 1). Our analysis included 130 participants (64 from treatment groups and 66 controls), the mean age (±standard deviation) for the treatment and control groups was 23·51 (±8·09) and 23·67 (±10·72),

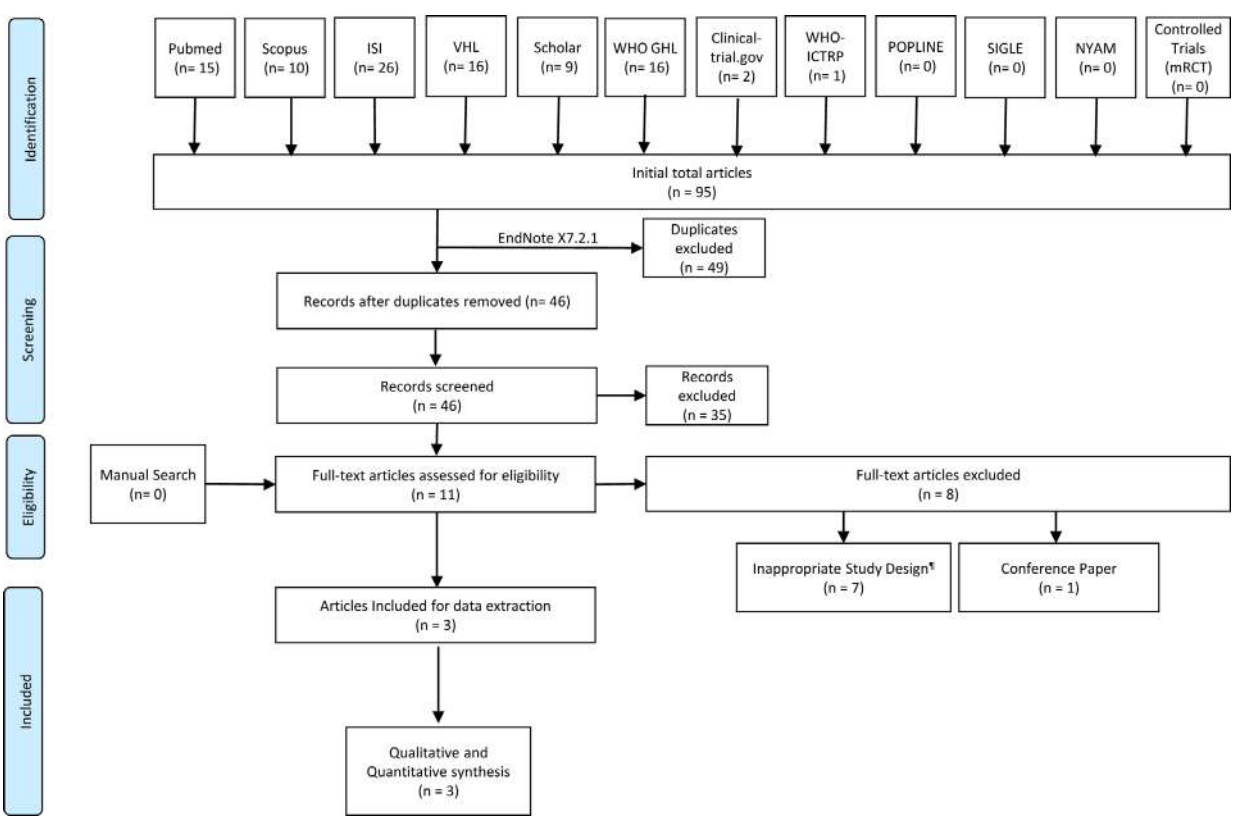

Fig. 1 PRISMA flow diagram of the study selection and screening.

respectively. 46·2% of the included participants were males. In the Fernandes studies, [10,11] 22 Hepatitis C patients were included whereas Eghbali *et al.* [9] excluded them. Dr. Fernandes was contacted to provide the raw data of his two studies, which eliminated any possibility of duplicate patients. Patients' characteristics are included in Table 1. Iron-chelating agents used included deferiprone, deferoxamine and deferasirox. Their side effects were mild and were limited to gastrointestinal tract (GIT) complaints, mild enema, skin allergies and dizziness. All administered iron-chelating medications and their associated adverse effects are reported in Table 2.

Quality assessment of included studies

To assess the risk of bias within the included RCTs, we made use of the Cochrane Collaboration's quality assessment tool [24]. Three independent reviewers carried out that assessment and resolved conflicts by discussing them with a senior author. The three included RCTs displayed a low risk of bias as regards selective reporting and attrition. Random sequence generation and allocation concealment were unclear in two studies [9,11]. Results of the quality assessment are summarized in Fig. 2.

Liver iron concentration

Three studies (130 patients) have reported changes in liver iron concentration (LIC) among the amlodipine and control groups. Pooling these data, the results showed a weighted MD of −0·20 with a null value of zero lying within the 95% CI (−0·55–0·15). This implies there is no statistically significant difference between the amlodipine and control groups ($P = 0·26$) **(Fig. 3a)**. No significant heterogeneity was found between the included studies [$I^2 = 0\%$, $P = 0·87$]. A simulation, with 100 replications in each arm, was done and yielded a power estimate of 21% with 95% CI between 13·94 and 30·29, indicating that the *P*-value was below 0·05 in 21 of the 100 iterations. Moreover, a power curve for a future meta-analysis was generated, specifying that a future trial with a sample size of 900 patients with 450 in each arm will establish a meta-analysis with an adequate power that is close to 60% **(**Fig. 3b**)**. To reach a more acceptable power of 80%, a future trial with a total sample size of 2000 subjects, 1000 for each arm will be required, which may not be totally feasible to fulfil.

Serum ferritin (SF)

As regards the serum ferritin fluctuations (pre- and post-treatment), the results showed no statistically significant difference between the amlodipine and control groups

Table 1 Baseline characteristics of included randomized clinical trials

		Sample size *N*		Age by years Mean (SD)		Male *N* (%)		Splenectomy *N* (%)		Hepatitis C *N* (%)		Ferritin ng/ml Mean (SD)		Follow-up
Author / Year	Country	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	(months)
Fernandes/2016 [10]	Brazil	30	29	23·3 (7·7)	23·5 (10·2)	13 (43)	17 (59)	5 (17)	2 (7)	9 (30)	6 (21)	2682·3	2662·6	12
Fernandes/2013 [11]	Brazil	5	10	31·2 (3·9)	26·8 (4·8)	3(60)	6 (60)	2 (40)	7 (70)	3 (60)	4 (40)	1110 (837)	1602 (1084)	12
Eghbali/2017 [9]	Iran	29	27	22·4(8·3)	22·7(12·5)	9(31)	12(44·4)	10(37)	14(48)	0	0	1544(715)	1169(672)	12

N, number; SD, standard deviation.

Table 2 Administered chelating agents and reported side effects

	Deferiprone		Deferoxamine		Deferasirox		Deferoxamine & Deferasirox		Adverse events	
Author / Year	Treatment *N* (%)	Control *N* (%)	Treatment *N* (%)	Control *N* (%)	Treatment *N* (%)	Control *N* (%)	Treatment *N* (%)	Control *N* (%)	Treatment	Control
Fernandes/2016 [10]	5 (16)	6 (21)	3 (10)	4 (14)	17 (57)	13 (45)	0	1 (3)	2 (6·6): mild ankle enema 1 (3): dizziness 1 (3): mild cutaneous allergy	0
Fernandes/2013 [11]	1 (20)	4 (40)	2 (40)	2 (20)	0	3 (30)	2 (40)	1 (10)	-	-
Eghbali/2017 [9]	4 (13·7)	2 (7·4)	2 (6·8)	1 (3·7)	22 (75·8)	20 (74)	1 (3·4)	4 (14·8)	8 (30): mild GI symptoms 2 (7): mild enema	11 (42): mild GI symptoms 5 (19): joint swelling

All data are presented as number (%).
GI, gastrointestinal.

[weighted MD (95% CI) = −0·16 (−0·51–0·19), $P = 0·36$]. (Fig. 4a). There was no significant heterogeneity between the included studies [$I^2 = 0\%$, $P = 0·62$]. A simulation, with 100 replications in each arm, was done and produced a power estimate of 45% with 95% CI between 35·03 and 55·27, indicating that the p-value was below 0·05 in 45 of 100 iterations. Moreover, a power curve for a future meta-analysis was generated, recommending that a future trial with a total sample size of 1000 patients with 500 in each arm be conducted to provide a meta-analysis with an adequate power that is close to 70% (Fig. 4b). To reach a more acceptable power of 80%, a future trial with a total sample size of 1500 subjects with 750 in each arm will be required, which may not be so feasible to conduct.

Heart T2*

Monitoring the effects of amlodipine using changes in heart T2* (pre- and post-treatment), the pooled data showed no significant difference in increasing the heart T2* between the amlodipine and control groups [weighted MD (95% CI) = 0·34 (−0·01–0·69), $P = 0·06$] (Fig. 5a). The significant heterogeneity was absent among the included studies [$I^2 = 44$ %, $P = 0·17$]. A simulation, with 100 replications in each arm, was done and gave a power estimate of 77% with 95% CI between 67·51 and 84·83, indicating that the *P*-value was below 0·05 in 77 of 100 iterations. Moreover, a power curve for a future meta-analysis was generated; specifying that a future trial with a total sample size of 500 patients (250 in each arm) and 1000 patients (500 in each arm) will ensure a meta-analysis with an adequate power that is close to 80% and 90%, respectively (Fig. 5b). These numbers are feasible with adequate recruitment since the condition is rare.

Quality of the evidence

Only three small trials were included in the review so, the overall quality of the evidence for all outcomes reported was moderate to low in quality, due to risks of bias and indirectness (not applicable to wider population). We tried to improve the quality of evidence by our access to the individual patients' data of two studies. Two of the three included trials were conducted by the same investigators with the multicentre study having a larger follow-up. To verify results, further trials with wider follow-up duration, more patients and different investigators are required.

Discussion

Worldwide, iron overload constitutes a major health problem. In the context of patients with thalassaemia, iron

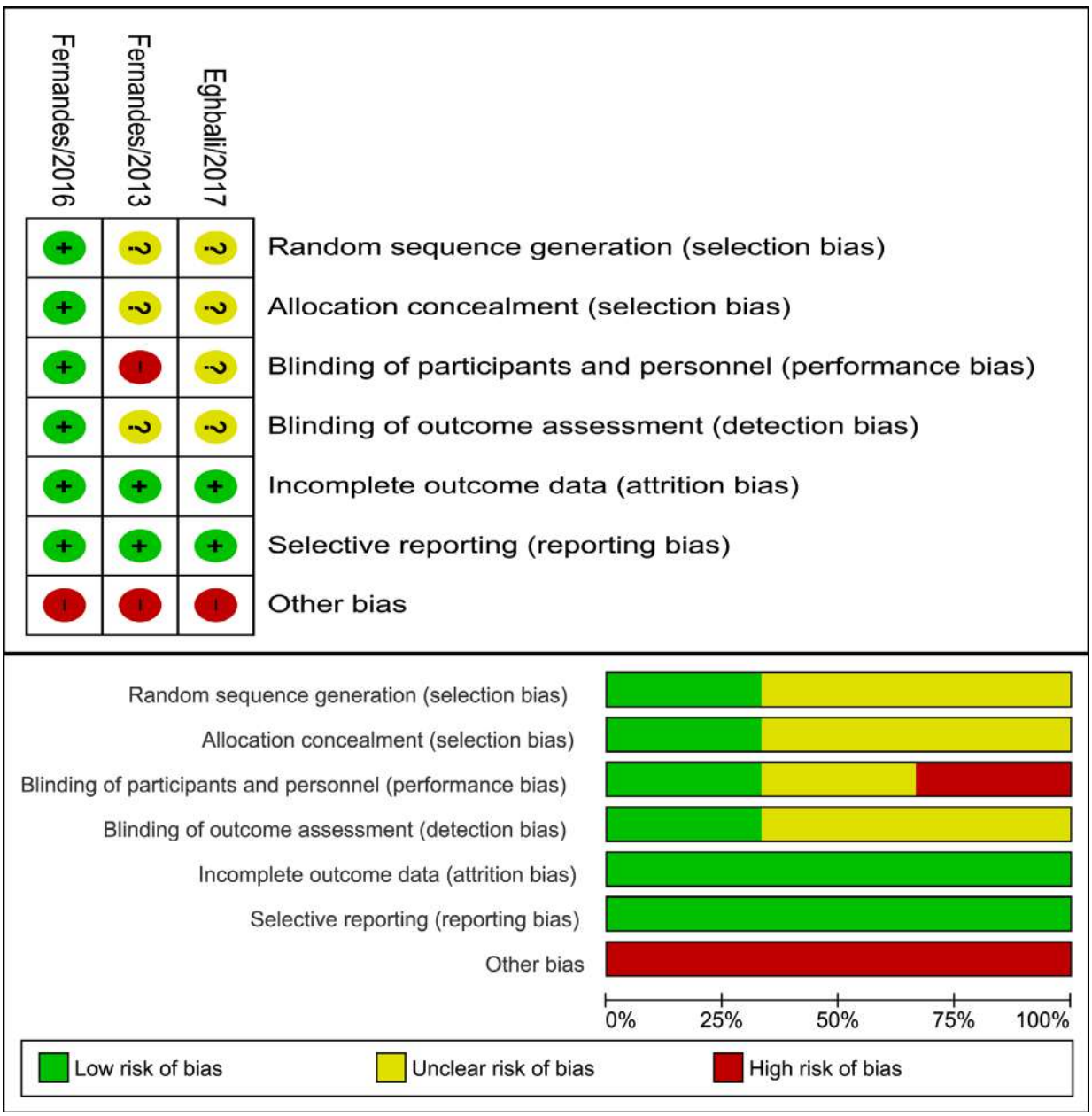

Fig. 2 Summary of quality assessment among included RCTs.

overload most commonly occur after regular blood transfusions. In this study, we shed some light on the effectiveness of amlodipine in providing an additional iron-lowering potential to commonly used iron-chelating agents through its effect on changes in LIC, SF, and heart T2* pre- and post-treatment. Although we found a beneficial effect of using amlodipine, however, due to the limited number of included patients, we were unable to draw definitive conclusions.

The initial investigation questioning the role of CCBs in reducing iron uptake by heart and liver cells started in 2003, when scientists suggested that L- and T-type calcium channels were the main portal of entry of iron into cardiomyocytes of iron-overloaded transgenic mice and that CCBs, by reducing iron entry into heart and liver cells, improved cardiac muscle function and survival in thalassaemic mice [8,25,26]. The choice of amlodipine in our included studies was explained by its safety, low cost and prolonged half-life [27,28]. There were no serious side effects exhibited in included patients, however, it is noteworthy, being a member of the dihydropyridine family of CCBs, which exert most of their anti-hypertensive

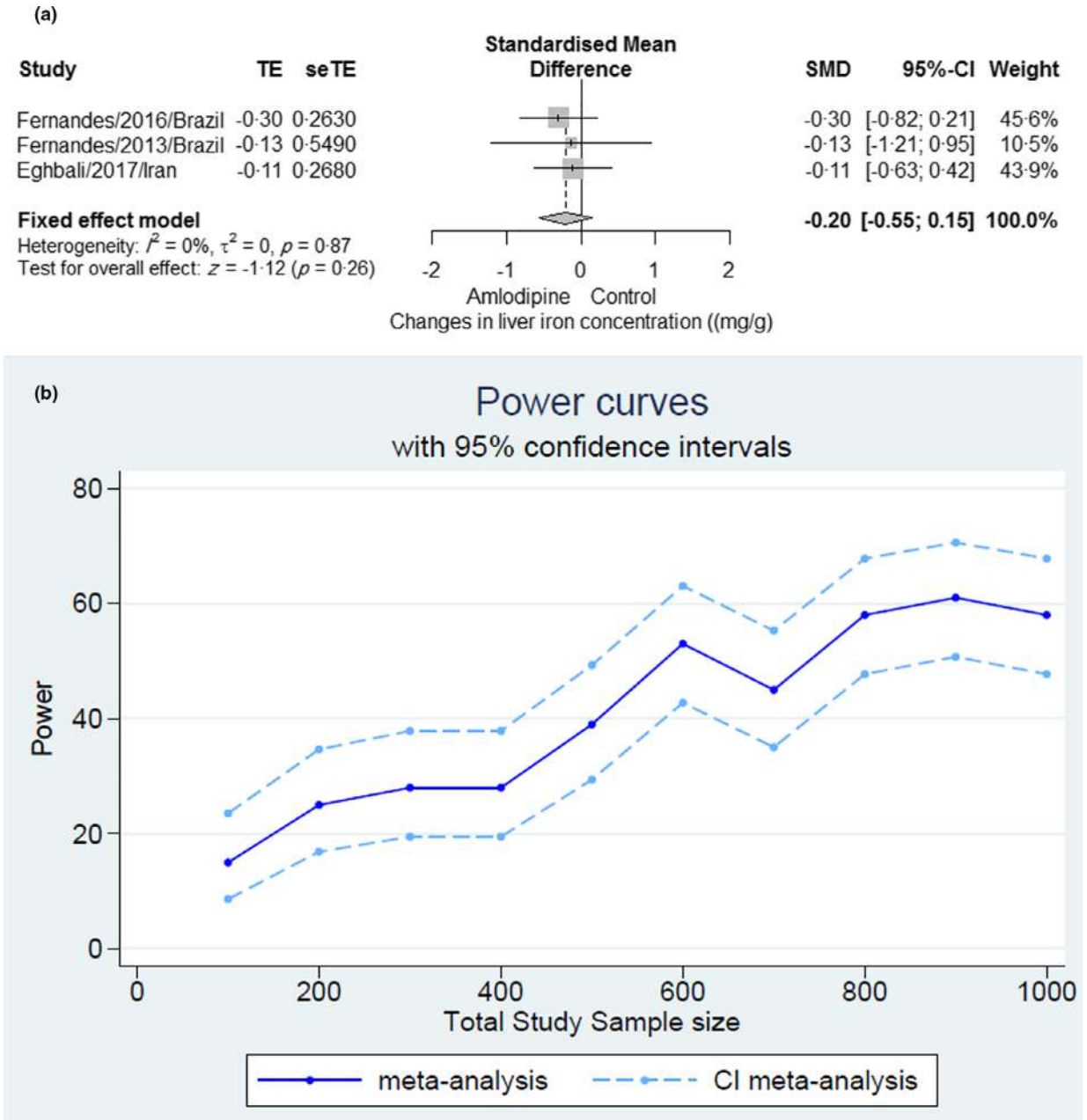

Fig. 3 (a) Forest plot meta-analysis comparing changes in LIC between amlodipine and control group. (b) Simulation of the power of the study in LIC changes for a future trial and meta-analysis using a fixed-effect model. seTE, standard error of treatment effect; TE, treatment effect.

effect through reducing peripheral vascular resistance, that amlodipine may increase the odds of experiencing hypotension and reflex tachycardia [29]. In contrast, it may be not recommended to replace it by the non-dihydropyridine group members like verapamil and diltiazem, since their long-term use might be associated with increased risk of heart failure, bradycardia, ventricular fibrillation and sudden death [27].

Our meta-analysis found no significant difference between the amlodipine and control groups in changing the cardiac T2*. However, in the 2013 pilot study by Fernandes *et al.*, a significant difference in cardiac T2* (pre- and post-treatment) was observed at 6 months, yet became non-significant at 12 months after treatment [11]. Moreover, in the 2016 trial conducted by the same author, which employed a larger sample size, it was

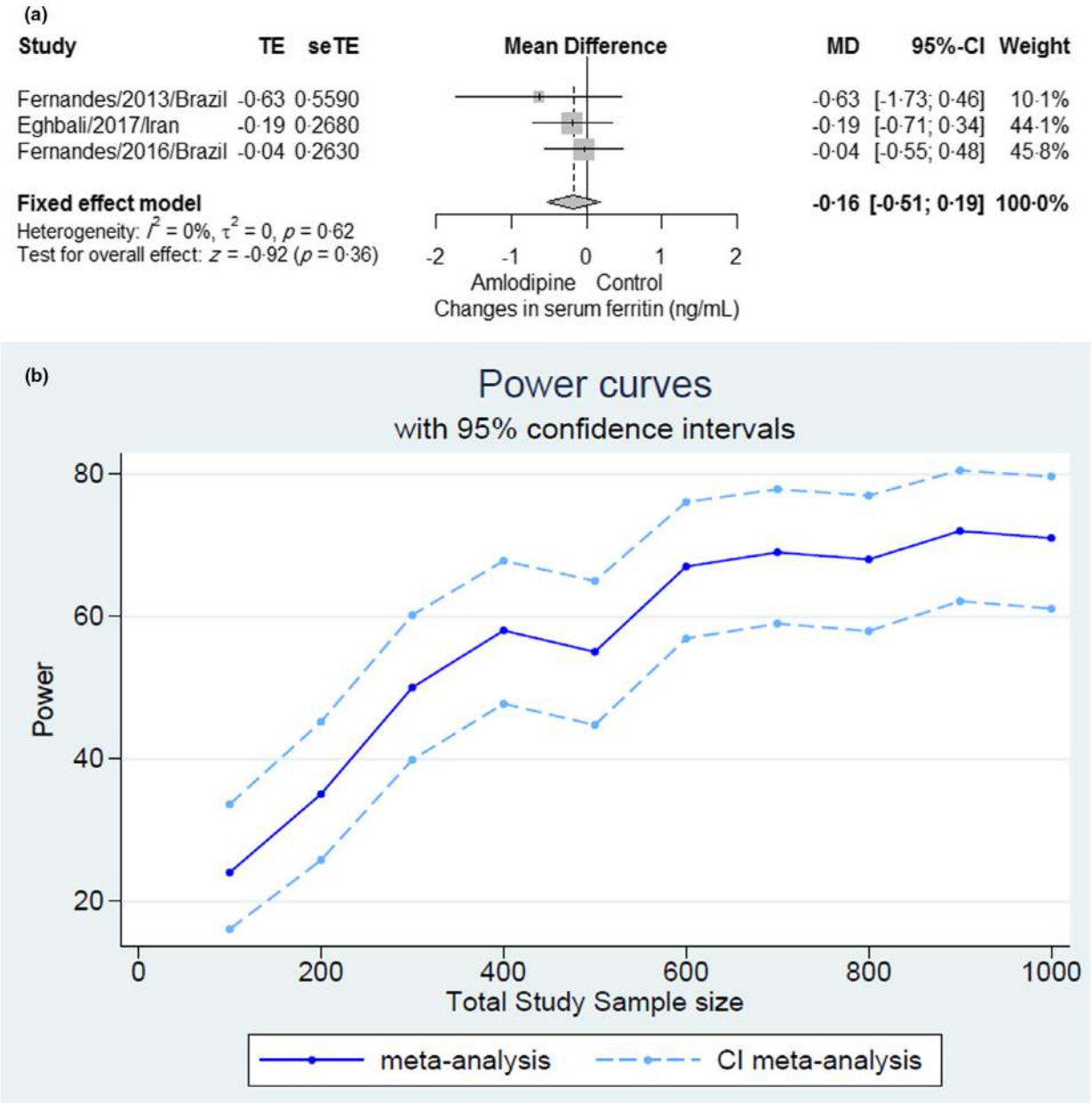

Fig. 4 (a) Forest plot meta-analysis comparing changes in SF between amlodipine and control group. (b) Simulation of the power of the study in SF for a future trial and meta-analysis using a fixed-effect model. seTE, standard error of treatment effect; TE, treatment effect.

shown that a significant decrease in Myocardial Iron Concentration (MIC) after 12 months occurred only between the amlodipine and control groups whose baseline MIC was initially above the normal mean threshold (MIC > 0·59 mg/g dry weight or T2* ≤ 35 ms) [10]. These results were similar to those of a study by Sadaf et al. in which, although non-significant changes in heart T2* were shown, other changes in MIC were significant, suggesting a possible role for baseline MIC in the response of thalassaemia patients to CCBs [30]. Although Fernandes et al. attributed the differences in cardiac T2* and MIC to the larger sample size in their 2016 study, we propose an additional hypothesis that needs to be confirmed with future studies. It has been largely established that the iron chelator deferiprone unloaded cardiac iron faster than deferoxamine [31,32]. In the Fernandes's 2013

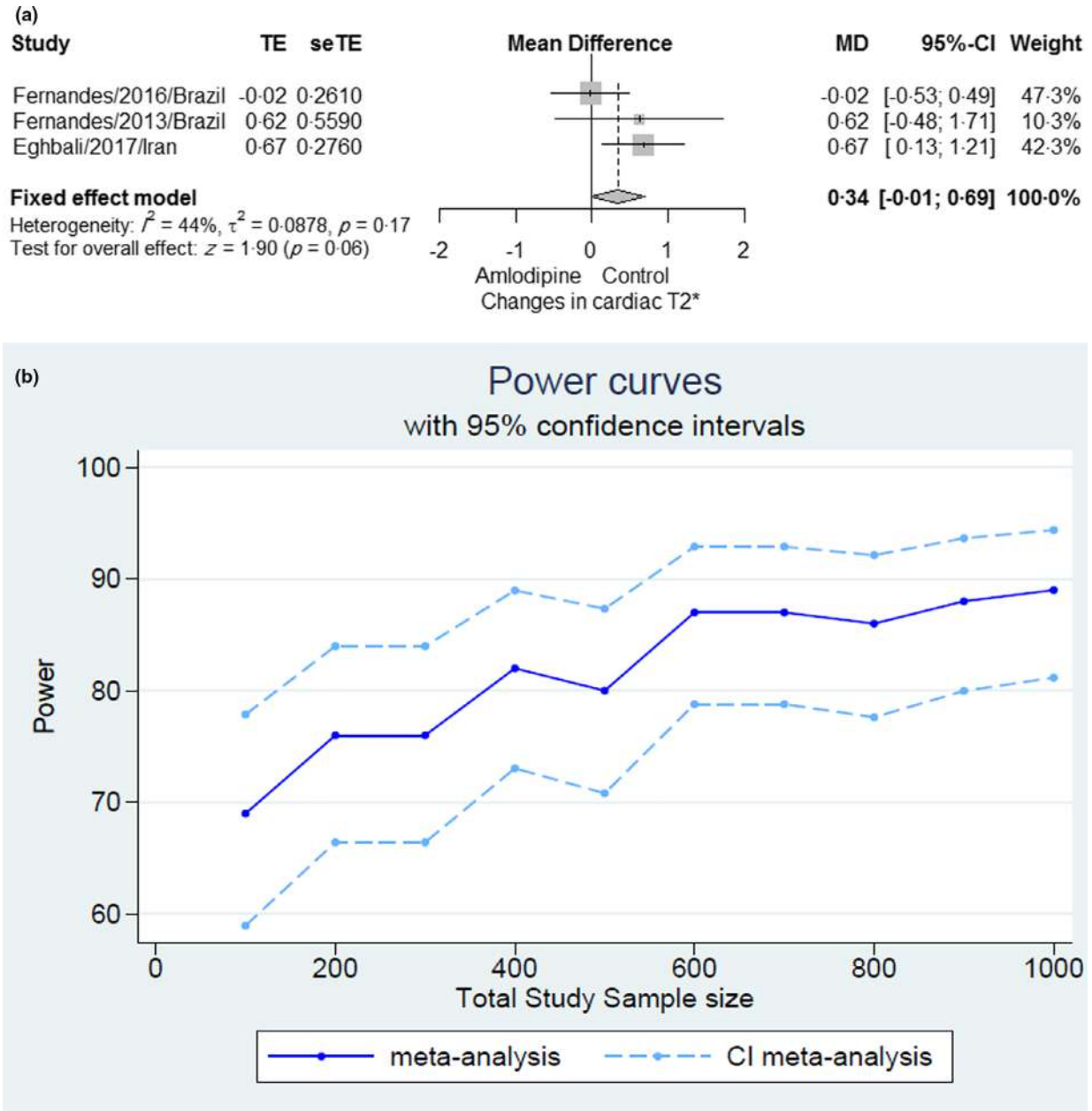

Fig. 5 (a) Forest plot meta-analysis comparing changes in heart T2* between amlodipine and control group. (b) Simulation of the power of the study in heart T2* changes for a future trial and meta-analysis using a fixed-effect model. seTE, standard error of treatment effect; TE, treatment effect.

trial, since only one (20%) of the amlodipine group, while four (40%) of the control group, was on deferiprone, this may have been responsible for the reported significant difference after 6 months of treatment between the amlodipine and control groups, which eventually waned with the continuity of treatment. Due to the differences in the unloading potentials of various iron chelators, future studies should focus on comparing treatment and control groups using only one iron-chelating agent.

In our study, none of included RCTs reported a significant decrease in LIC after treatment. Their explanation for that observation leaned on data from animal studies, where iron uptake into liver cells was less dependent on calcium channels as compared with myocardial cells [26,33]. Yet, we find it unlikely that this was the only explanation for such a phenomenon. In 2007, it was proposed by Ludwiczek *et al*. that CCBs mobilized iron from the livers of iron-overloaded mice and enhanced urinary

iron excretion through modulation of the divalent metal transporter-1 (DMT-1) [34]. However, later on, McKenzie *et al.* refuted the alleged DMT-1 mechanism, suggesting another unknown mechanism to be in effect [35]. We believe that other factors may have influenced the liver's capability to uptake iron. This has been disclosed in a later experimental study inspecting the harmful effects of obesity on the autophagic mechanisms in human hepatoma cell lines or HepG2 cells [36]. It was found that verapamil was effective in preventing chronic rising of cytosolic calcium inside those cells, leading to restoration of autophagosome and lysosome linkages. We, therefore, hypothesize that the metabolic state of the liver cells has an impact on its ability to uptake or unload iron. The LIC level was not measured as an outcome in the aforementioned experiment, however, future studies are warranted to confirm whether the metabolic state of liver cells can affect its ability to utilize iron and in which of those metabolic states can CCBs be better applied to reduce iron uptake in thalassaemia patients.

As regards the effect of amlodipine on SF, our meta-analysis showed no significant difference before and after treatment. However, Eghbali *et al.* were the only researchers to demonstrate a significant reduction in SF from baseline to 12 months after treatment in both the amlodipine and the control groups [9]. In our opinion, a possible explanation for this effect might have been the more sound selection of study subjects. Eghbali *et al.* specifically excluded subjects who were known to have inflammatory or infectious hepatic diseases, thus eliminating the possibility of an increased SF as an inflammatory marker in such subjects. Furthermore, a significant rise in SF might have been an inevitable consequence of the fact that the Eghbali study had a greater number of splenectomized patients. Previous research has shown significantly higher SF levels in splenectomized patients with β-thalassaemia than in non-splenectomized ones [37,38]. Further randomized controlled trials should consider this effect during patients' enrolment to get a better efficacy measurement. Otherwise, the effect of amlodipine on the renal excretion of iron through renal DMT-1 receptors was not consistent and therefore need more evidence [35].

A notable strength within our research was the novelty of its idea. The notion that an oral, relatively inexpensive CCB with a long half-life, such as amlodipine, could aid current iron chelators in unloading and preventing excess iron accumulation into target organs was an idea worthy of consideration. Another strong point was emphasizing the importance of selection criteria for subjects in similarly focused studies, and its possible influence on study outcomes. Nevertheless, a major limitation of our study was that we could not draw a decisive conclusion because of the small number of included patients. In addition, the lack of well-thought inclusion and exclusion criteria for similar study subjects imposed a threat to the generalizability of the results. Comparing amlodipine to control groups comprising heterogeneous iron chelators instead of making the comparison with only one type can yield misleading information since it has been shown that certain iron chelators were more efficient than others were. Future studies overcoming the above limitations are warranted.

In conclusion, our study investigating the effect of amlodipine on changes in LIC, SF and heart T2* before and after treatment of thalassaemia patients showed favourable differences that were yet statistically non-significant. Due to the small number of eligible studies within our analysis, and the presence of some confounding factors that would otherwise explain the demonstrated results, we conclude that future studies with larger and more homogenous study cohorts are warranted. Moreover, similar studies involving solely children, specific iron-chelating agents, or investigating the potential roles of baseline MIC, splenectomy and other relevant patient-specific factors are highly recommended.

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Conflict of interests

The authors declare no conflict of interests.

References

1 Hodroj MH, Bou-Fakhredin R, Nour-Eldine W, *et al.* Thalassemia and malignancy: an emerging concern? Blood Rev 2019;37:100585.
2 Mousavi Z, Yazdani Z, Moradabadi A, *et al.* Role of some members of chemokine/cytokine network in the pathogenesis of thalassemia and sickle cell hemoglobinopathies: a mini review. Exp Hematol Oncol 2019;8:21.
3 Brissot P. Optimizing the diagnosis and the treatment of iron overload diseases. Expert Rev Gastroenterol Hepatol 2016;10:359–70.
4 Paterek A, Mackiewicz U. Iron and the heart: a paradigm shift from systemic to cardiomyocyte abnormalities. J Cell Physiol 2019;234:21613–29.
5 Tanner MA, Galanello R, Dessi C, *et al.* A randomized, placebo-controlled, double-blind trial of the effect of combined therapy with deferoxamine and deferiprone on myocardial iron in thalassemia major using cardiovascular magnetic resonance. Circulation 2007;115:1876–84.

6 Al-Khabori M, Bhandari S, Al-Huneini M, *et al.* Side effects of deferasirox iron chelation in patients with beta Thalassemia major or intermedia. Oman Med J 2013;28:121–4.
7 Kontoghiorghes GJ. Introduction of higher doses of deferasirox: better efficacy but not effective iron removal from the heart and increased risks of serious toxicities. Expert Opin Drug Saf 2010;9:633–41.
8 Oudit GY, Trivieri MG, Khaper N, *et al.* Role of L-type Ca2+ channels in iron transport and iron-overload cardiomyopathy. J Mol Med (Berl) 2006;84:349–64.
9 Eghbali A, Kazemi H, Taherahmadi H, *et al.* A randomized, controlled study evaluating effects of amlodipine addition to chelators to reduce iron loading in patients with thalassemia major. Eur J Haematol 2017;99:577–81.
10 Fernandes JL, Loggetto SR, Veríssimo MP, *et al.* A randomized trial of amlodipine in addition to standard chelation therapy in patients with thalassemia major. Blood 2016;128: 1555–61.
11 Fernandes JL, Sampaio EF, Fertrin K, *et al.* Amlodipine reduces cardiac iron overload in patients with thalassemia major: a pilot trial. Am J Med 2013;126:834–7.
12 Moher D, Liberati A, Tetzlaff J, *et al.* Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
13 Garbowski MW, Carpenter J-P, Smith G, *et al.* Biopsy-based calibration of T2* magnetic resonance for estimation of liver iron concentration and comparison with R2 Ferriscan. J Cardiovasc Magn Reson 2014;16:40.
14 Hankins JS, McCarville MB, Loeffler RB, *et al.* R2* magnetic resonance imaging of the liver in patients with iron overload. Blood 2009;113:4853–5.
15 Huedo-Medina T, Johnson B. *Estimating the standardized mean difference effect size and its variance from different data sources: A spreadsheet.* Authors, 2011.
16 Hedges LV, Pustejovsky JE, Shadish WR. A standardized mean difference effect size for multiple baseline designs across individuals. Res Synth Methods 2013;4:324–41.
17 DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
18 Higgins JPT, Thompson SG, Deeks JJ, *et al.* Measuring inconsistency in meta-analyses. BMJ 2003;327:557–60.
19 Egger M, Smith GD, Schneider M, *et al.* Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629–34.
20 Peters JL, Sutton AJ, Jones DR, *et al.* Comparison of two methods to detect publication bias in meta-analysis. JAMA 2006;295:676–80.
21 Michael JC, Sally RH, Alison D, *et al.* Simulation-based sample-size calculation for designing new clinical trials and diagnostic test accuracy studies to update an existing meta-analysis. Stata Journal 2013;13:451–73.
22 Sutton AJ, Cooper NJ, Jones DR, *et al.* Evidence-based sample size calculations based upon updated meta-analysis. Stat Med 2007;26:2479–500.
23 Crowther M, Hinchliffe S, Donald A, *et al.* Simulation-based sample-size calculation for designing new clinical trials and diagnostic test accuracy studies to update an existing meta-analysis. Stata J 2013;13:451–73.
24 Higgins JP, Altman DG, Gøtzsche PC, *et al.* The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ 2011;343:d5928.
25 Kumfu S, Chattipakorn S, Chinda K, *et al.* T-type calcium channel blockade improves survival and cardiovascular function in thalassemic mice. Eur J Haematol 2012;88:535–48.
26 Oudit GY, Sun H, Trivieri MG, *et al.* L-type Ca2+ channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. Nat Med 2003;9:1187–94.
27 Abernethy DR, Schwartz JB. Calcium-antagonist drugs. N Engl J Med 1999;341:1447–57.
28 Flynn JT. Efficacy and safety of prolonged amlodipine treatment in hypertensive children. Pediatr Nephrol 2005;20:631–5.
29 Godfraind T. Discovery and development of calcium channel blockers. Front Pharmacol 2017;8:286.
30 Sadaf A, Hasan B, Das JK, *et al.* Calcium channel blockers for preventing cardiomyopathy due to iron overload in people with transfusion-dependent beta thalassaemia. Cochrane Database Syst Rev 2018;7:CD011626.
31 Borgna-Pignatti C, Cappellini MD, De Stefano P, *et al.* Cardiac morbidity and mortality in deferoxamine- or deferiprone-treated patients with thalassemia major. Blood 2006; 107:3733–7.
32 Pennell DJ, Berdoukas V, Karagiorga M, *et al.* Randomized controlled trial of deferiprone or deferoxamine in beta-thalassemia major patients with asymptomatic myocardial siderosis. Blood 2006;107:3738–44.
33 Tsushima RG, Wickenden AD, Bouchard RA, *et al.* Modulation of iron uptake in heart by L-type Ca2+ channel modifiers: possible implications in iron overload. Circ Res 1999; 84:1302–9.
34 Ludwiczek S, Theurl I, Muckenthaler MU, *et al.* Ca2+ channel blockers reverse iron overload by a new mechanism via divalent metal transporter-1. Nat Med 2007;13:448–54.
35 Mackenzie B, Shawki A, Ghio AJ, *et al.* Calcium-channel blockers do not affect iron transport mediated by divalent metal-ion transporter-1. Blood 2010;115:4148–9.
36 Park HW, Lee JH. Calcium channel blockers as potential therapeutics for obesity-associated autophagy defects and fatty liver pathologies. Autophagy 2014;10:2385–6.
37 Dedeoglu S, Bornaun H. Pulmonary hypertension in children with β Thalassemia Major, are splenectomy and high-ferritin levels related or not? J Pediatr Hematol Oncol 2017;39:259–65.
38 Pootrakul P, Vongsmasa V, La-ongpanich P, *et al.* Serum ferritin levels in thalassemias and the effect of splenectomy. Acta Haematol 1981;66:244–50.

Supporting Information

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

Table S1. PRISMA checklist.

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Frequency and timing of all-cause deaths in visits involving suspected transfusion reactions, and the significance of cardiopulmonary disturbances

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Vox Sanguinis

Abstract

Background/objectives Transfusion reactions (TRs) may cause or contribute to death. Cardiopulmonary TRs are distressing, and collectively account for most transfusion fatalities, though the degree to which they alter survival more broadly is unclear. Deaths (and their timing) after TRs may provide further insights.

Materials/Methods Adult (tri-hospital network) haemovigilance data (2013–2016) recorded referrals with conclusions ranging from unrelated to transfusion (UTR) to entities such as: septic TRs, serologic/haemolytic reactions, transfusion-associated circulatory overload (TACO), transfusion-associated dyspnoea (TAD), transfusion-related acute lung injury (TRALI), allergic transfusion reaction (ATR), and others. For (in- or out-patient) visits involving suspected TRs (VISTRs), all-cause mortalities (% [95% confidence interval]) and associated time-to-death (TTD) (median days, [interquartile range]) were compared. Diagnoses were defined inclusively (possible-to-definite) or strictly (probable-to-definite).

Results Of 1144 events, rank order VISTR mortality following (possible-to-definite) TRs, and associated TTDs, were led by: DHTR 33% [6–19], 1 death at 123d; TRALI 32% [15–54], 6 deaths: 3d [2–20]; BaCon 21% [14–31], 17 deaths: 10d [3–28]; TACO 18% [12–26], 23 deaths: 16d [6–28]; TAD 17% [11–26]: 18 deaths, 6d [3–12]. Higher-certainty TRs ranked similarly (DHTR 50% [9–91]; BaCon 29% [12–55], 4 deaths: 12d [3–22]; and TACO 25% [16–38], 15 deaths: 21d [6–28]). VISTR mortality after TACO or TRALI significantly exceeded ATR (3·3% [2·4–5·8], $P < 0·00001$) but was not different from UTR events ($P = 0·3$).

Conclusions Only half of cardiopulmonary TRs constituted high certainty diagnoses. Nevertheless, cardiopulmonary TRs and suspected BaCon marked higher

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VISTR mortality with shorter TTDs. Short (<1 week) TTDs in TAD, BaCon or TRALI imply either contributing roles in death, treatment refractoriness and/or applicable TR susceptibilities in the dying.

Key words: transfusion reactions, hemovigilance, TRALI, haemolytic transfusion reaction, blood safety, bacterial contamination.

Introduction

Transfusions, though generally safe, may be associated with adverse effects leading to death [1]. Respiratory transfusion reactions such as transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO) predominate among transfusion-related deaths and associate with in-hospital mortality (IHM) [2–6]. While preclinical models of TRALI (and its mechanisms of inflammation, endothelial leak and respiratory failure) have been shown to cause death [7,8], comparable models of fatal TACO are not yet described [9]. The degree to which the association of TACO with IHM reflects causality or a disproportionate susceptibility in those already dying is, therefore, unclear.

Explorations of transfusion reaction (TR) associations with mortality have yielded heterogeneous results [4,10–20]. Explanations for variability include differences in patient populations, reporting mechanisms and adopted definitions [10,15]. Incidences can vary significantly with age, as shown by TR incidences in paediatric, obstetric and other adult populations [21–23]. Case ascertainment may vary by orders of magnitude between passive (voluntary) to more active (mandatory or electronic) systems [24–26]. Available case information and the diagnostic criteria for a given definition may also yield different classification outputs. Diagnostic 'gating' – the inclusion of possible cases for the sake of sensitivity, versus stringent qualification (for only the most certain cases) for specificity will also shape apparent incidences [27–29]. Improvements in documentation and standardization with revised definitions [29,30] are desirable for data comparability and aggregate measures of incidence and outcomes.

In studies of TACO describing IHM, few report on the time to death (TTD) from the reaction [4,10–20], a potentially revealing indicator for a role in fatalities. High IHM with narrow TTDs may together suggest direct roles, even if not considered by clinicians witnessing and/or documenting events in real-time. Conversely, high IHM with longer TTDs may simply reflect recipient susceptibility to certain reactions, with deaths due to other causes.

This study primarily examines in- and out-patient hospital visits involving TRs, for the associated rank-order visit mortalities and TTD. Inclusive (sensitive) counts featured possible-to-definite TRs; whereas, strict (specific) counts were limited to TR diagnoses that were more certain (probable-to-definite). Secondarily, we assessed the impact that inclusive or strict coding had on TR counts and mortalities, given the particular challenge of definitively meeting criteria for cardiopulmonary TRs, when contrasted with other TRs (such as allergic or serologic) with less mistakable features. How stringency affected counts in TRALI or TACO was, therefore, also of interest.

Methods

Study setting

The Research Ethics Board approved the retrospective study of haemovigilance data for the 4 years (2013–2016) period (CAPCR-ID 17-5235·0 [108180·0]) [31]. From the recorded dates of registration, reaction, post-reaction discharge and status (living/deceased) at discharge, mortalities and intervals (TTD) were calculated.

The tri-hospital setting of the University Health Network (UHN) consists of Princess Margaret Cancer Centre, Toronto General Hospital and Toronto Western Hospital in Toronto, Canada. Each hospital blood transfusion laboratory (BTL) dispenses blood *products*, defined either as *components* (red-blood-cell units [RBC], adult platelet dose concentrates [PLT: pools from four whole blood-derived buffy coats, or single donor apheresis platelets), frozen plasma [FP], cryoprecipitates [CPP]); or *derivatives* (purified protein products such as albumin, IVIG, prothrombin complex concentrates and fibrinogen concentrates). Reaction reporting rates surpass other hospitals across the province [31,32]. All suspected TRs are referred to the BTL for investigation by the transfusion safety officer (TSO [AE, FT]). TRs are reviewed by on-service trainees and transfusion medicine physicians (TM MD [JP, LL, YL, JC, CC]), and log in a BTL database with final digital medical record signout [CC]. Internally, the Hospital Transfusion Committee receives TR summary reports, and externally, applicable TRs are reported in accordance with the tools, contracts and regulations of the haemovigilance system (the Transfusion Transmitted Injuries Surveillance System [TTISS] and the Public Health Agency of Canada/Canada Vigilance Programme) [33].

Event counts

Referrals to evaluate for suspected TRs reflected ≥ 1 (clinical, radiologic and/or laboratory) disturbance(s) associated with ≥1 blood product administrations, as experienced on a given encounter date. Acute reportable disturbances in policy include fevers, mucocutaneous changes (eg urticarial rash), cardiopulmonary events (eg hypoxic desaturation and/or dyspnoea) and/or other concerns (pain and changes in level of consciousness). Implicated products in a referral may encompass ≥ 1 products on an encounter-date (eg 1 PLT and 2 u RBC). The referred event may, therefore, follow several implicated products and manifest with ≥1 disturbance, thereby generating a number of potential TR conclusions, each with a respective degree of certainty and product imputability.

Visits involving suspected transfusion reactions (VISTRs)

Registrations consisted of all length of stay (LOS) options, ie- either outpatient visits (LOS 0) or LOS ≥ 1 (inpatient admissions, and ambulatory or emergency visits escalating to admission) for the given TR referral.

Transfusion reactions and comparators

TR categories included: allergic – all varieties (ATR), allergic-bronchospasm (ABSTR as a respiratory subset of ATR), acute haemolytic transfusion reaction (AHTR), septic transfusion reactions/bacterial contamination (BaCon), delayed haemolytic transfusion reaction (DHTR), delayed serologic transfusion reaction (DSTR), febrile non-haemolytic transfusion reaction (FNHTR), intravenous immunoglobulin (IVIG)-related headache (IVIG headache), passive haemolytic transfusion reaction (PHTR), passive serologic transfusion reaction (PSTR), transfusion-associated circulatory overload (TACO), transfusion-associated dyspnoea (TAD) and transfusion-related acute lung injury (TRALI). The significance of a category was explored against ATRs and 'reaction' unrelated to transfusion (UTR) events as a pragmatic, multi-severity control. In UTRs, the transfusion was deemed less imputable than the patient's underlying condition (Table 1) [34].

Stringency of TR definitions

The reviewer's certainty on any given TR diagnosis had been classified at the time of reporting (Table 2, with examples of suspected TACO). Probable-to-definite certainties reflected TRs with high confidence (and valued for their specificity), while the inclusion of possible cases was to gain sensitivity for events that may have been undercounted due to limits in information. (Suspected TRALI was important to report to the producer [ie-Canadian Blood Services for components, or to Health Canada and derivative manufacturers], while suspected TACO held value as a risk or quality indicator if clustering in specific care areas.)

VISTR mortality

TR-associated mortality reflected status at discharge among VISTRs (or among inpatients), expressed as proportions with 95% confidence intervals (95% CI) (http://vassarstats.net/prop1.html), while TR-specific TTDs were expressed as medians with interquartile ranges (IQR).

Results

General information and demographics

University Health Network transfused 250 978 components over 4 years, with 1144 referrals (0·5%) to investigate suspected TRs (in a cohort of 902 unique reactors, 55% male: 45% female [$P < 0{\cdot}0001$]), expanding into 2425 possible-to-definite TRs of interest, and a smaller (56%) subset of TRs with greater confidence/certainty in the conclusion (1358 probable-to-definite) (Fig. 1). For TRs occurring among inpatients (62% of possible-to-definite TRs [or 53% of probable-to-definite TRs]), discharge vital status was recorded in 100%.

Frequency of reported TRs

The commonest possible-to-definite conclusion was UTR (Fig. 1B), reflecting the ubiquity of attributable comorbidities. The commonest possible-to-definite TR was FNHTR, while ATR led among the more certain (probable-to-definite) diagnoses. TACO placed 3rd (whether inclusively or strictly defined), with TAD 4th-5th. There were 43% (to 53%) fewer TACO cases after eliminating merely possible events; whereas, uncertainty-related losses were rare in TRs with definitive features (IVIG headache, ATR and/or laboratory-defined DSTR/DHTR).

VISTR mortality

In this 4-year period, 113 of 902 individuals investigated for TR died (13% [11–15]). Though no reactions were judged as directly fatal, two DHTRs (of 9 hyperhaemolysis cases) and 3 cardiopulmonary TRs were thought to have contributed to later deaths. In TR-associated all-cause deaths, mortalities (Figure 2) ranged as widely as 0–33%, with confidence intervals widest on infrequently reported and more serious TRs. Among the most commonly

Table 1 Definitions.

TR	Definition
ABSTR	Acute-onset mucocutaneous signs/symptoms of allergy (pruritis, angioedema, urticaria, erythema) with respiratory involvement, ie- upper airway (hoarseness, stridor) and/or lower bronchopulmonary (dyspnea, cough, wheezing/bronchospasm, hypoxemia).
AHTR	Hemolytic transfusion reaction manifesting within 24 h of transfusion (host vs. product).
ATR	Skin reaction (transient urticaria or other skin rash with pruritus associated with transfusion ± angioedema without respiratory dysfunction or distress); anaphylactic reactions with hypotension ± loss of consciousness, circulatory collapse requiring vasopressors.
BaCon	Suspected bacterial contamination event, with culture-positive findings after applicable febrile/hemodynamic disturbances; deemed *definite* if the same organism is seen in both product and patient; *probable* if the product is culture positive with a non-contaminant (and the patient is either untested or construed as falsely negative); *possible* if the implicated product is not retrieved for culture or construed as falsely negative, whereas patient is culture-positive for an organism that is likeliest product-attributable after clinical review.
DHTR	Hemolytic transfusion reaction >24 h and <1 month after transfusion.
DSTR	Emergence of new alloantibodies < 28 days following transfusion.
FNHTR	Either fever $\geq$ 38°C by $\Delta \geq$ 1°C from baseline, or chills/rigors, not attributable to other drivers.
I/PTR	Inflammatory/pain reaction.
IVIG Headache	Headache during or shortly after IVIG administration.
PHTR	Hemolysis attributed to passively infused antibodies (product vs. host, eg- IVIG isoagglutinin-mediated anemia).
PSTR	Serologic change attributed to passive antibodies (eg- minor mismatched platelets with eluted isoagglutinin).
TACO	Within 12 h of transfusion: respiratory distress (tachypnea, dyspnea, cyanosis, hypoxia) and/or pulmonary edema (crackles, cardiac wheeze, cough, S3, transudative sputum, congestive changes on radiography), supported by cardiovascular features (tachycardia, JVP rise, BP change, peripheral edema), fluid status (positive balance, diuretic response), or natriuretic peptide findings.
TAD	Respiratory distress within 24 h of transfusion not meeting criteria for TRALI, TACO, ABSTR, or underlying condition.
TRALI	New acute onset ALI (during or within 6 h of transfusion), hypoxemia ($PaO_2/FiO_2 \leq 300$ or oxygen saturation < 90% on room air), bilateral lung infiltrates on chest radiography, and absence (or non-accountable stability) of left atrial hypertension; *definite (type 1)* if lacking alternative ARDS risk factors; or *possible (type 2)* if underlying (direct or indirect) lung injuries existed just prior (but were stable in the pre-transfusion period).
UTR	"Transfusion reaction" was most likely unrelated to the product, but rather due to underlying condition(s) and temporally-coincidental progression.

Definitions adapted from Canada Public Health Agency Transfusion Transmitted Injuries Surveillance System (TTISS) User's Manual Version 3.0 (2007).

reported possible-to-definite TRs (all registrations [2A]), ATRs associated with the lowest mortality (3·8% [2·5–5·9]), followed by FNHTR (14% [11–17]) and UTR (18% [15–21]). VISTR mortalities appeared highest in DHTR, TRALI and BaCon [2A,2C], or DHTR, BaCon and higher-certainty TACO [2B,2D]. VISTR mortalities after cardiopulmonary reactions (TRALI or TACO) were not different from UTR ($P = 0{\cdot}3$) but were significantly higher than ATR ($P < 0{\cdot}00001$).

Time to death (TTD)

Time to death [IQR] varied among the TRs and with diagnostic confidence (Fig. 3). The TTD (in days) was shorter in possible-to-definite cardiopulmonary TRs such as TRALI (3 [2–30]) and TAD (6 [3–12]), followed by the cluster of TACO, ABSTR and FNHTR (16 [6–28], [3–24], [10–59]) and ATR (24 [7–57]). TRs in the ≥15% VISTR mortality range with a median TTD of ≤10 days were limited to TRALI, TAD and BaCon, whereas, those with <20% mortality and TTD > 15 days included TACO, FNHTR and ATR/ABSTR (3C).

Discussion

In this TR cohort, the proportions (and certainties) of specific TRs varied widely, as did their ensuing (all-cause) mortalities and TTD. Common minor disturbances (allergic TRs and FNHTRs) were more confidently concluded, and presented in visits exhibiting low mortality and long TTD. Serologic TRs (DSTR/DHTR) occurred in higher mortality admissions but had long TTDs. By contrast, suspected cardiopulmonary or bacterial contamination events were more often uncertain, and occurred in higher mortality visits with the shortest TTDs. These hypothesis-generating data suggest that cardiopulmonary (and contamination) events are either contributing to death and/or are effects in those whose morbidities are priming or already terminal.

Comparisons of these results with other studies are challenged by differences in patient populations and the haemovigilance system (from definitions used, to mandates that may either favour sensitivity [inclusivity to prevent underestimates], or specificity [exclusivity to reduce overestimates and unnecessary donor deferrals]) [10,21,22,24–

Table 2 Certainty in TR.

	Definite	Likely Most likely	Probable	Possible – can neither rule in nor rule out	Doubtful, but cannot fully rule out	Ruled out
Rubric:	Most or all relevant examinations were conducted and documented to objectively satisfy required criteria in the formal diagnosis	Criteria are met, but evidence in a given criterion may be equivocal, or reasonably imputed (if absent)	Most (but not all) criteria are met, but the TR remains likelier than the underlying condition(s) The "missing link" criterion is either missing or may be explained by the underlying condition.	Many features are consistent with the TR, but the TR and underlying condition(s) are equally likely Criteria are not satisfied because of information gaps or non-specificity	Documentation of relevant features may have been limited; while few features concur, alternatives are more likely	Most or all features establish the alternative diagnosis, while any features unique/specific to the TR (if they exist) are technically absent
Sample query TACO cases: Reaction after 1 u RBC given over 2 h:	New dyspnea/hypoxia, crackles + S3, radiologic overload, hypertension, charted weight gains, BNP elevation	New dyspnea/hypoxia, crackles, mild CXR edema without cardiac silhouette enlargement, tachycardia, pedal edema (pre = post)	Underlying pneumonia, but worsened dyspnea with new effusions, worsened hypertension, +diuretic response (no BNP testing)	New dyspnea/hypoxia, noting post-op atelectasis, tachycardia (with background panic disorder), and normoxia after independent euvolemia	Worsening dyspnea with risk factors, but all features are pre-existing (O_2 saturation, vital signs, CXR CHF); BNP is elevated but without comparators	Worsening dyspnea but CXR shows aspiration pneumonitis with unchanged cardiovascular/volume indicators (including similarly elevated pre/post BNP)

27,35]. We examined the effects of definitional stringency on TRs to discern shifts in representation and outcomes. These TRs occurred in an adult population evaluated within a single haemovigilance system marked by robust reporting, diverse settings, experienced BTL staff and uniform TTISS definitions [31,32]. By including possible cases, the number of diagnoses exceeded the number of referrals. While we often concluded that referrals were UTR, these assignments were uncertain one-third of the time. Gaps in information and consequential uncertainty were most pronounced in complex (multi-source evidence-dependent) cardiopulmonary TRs, which were reported often, with TACO and TAD following FNHTR and allergic TR frequencies. By this unorthodox approach (given that 'possible' cardiopulmonary TRs other than TRALI are not formally recognized), many cases were merely 'possible' (with TACO, TAD and TRALI being uncertain 50, 80 and 100% of the time, respectively). This flexibility was as much to offset known reporting underestimates as to be sensitive to potential safety (or documentation quality) issues. Indeed, suspected cases of TRALI, BaCon and TAD were among the hardest to verify, with near-elimination by rigid inquiry. Others have also exposed the tensions of strict definitions, which may capture homogeneous states for study, but at the expense of diminishing measures [26,36].

A wide range of IHM has been reported for different TRs, with cardiopulmonary TRs among the deadliest [2–6]. In suspected TRALI, nearly 1/3rd of affected visits ended in death, though there were no evaluable high-probability cases, reflecting its vanishingly low incidence with mitigation and strict definitions for definite (type 1) cases. TACO, conversely, had its 1-in-5 deaths/visit (among inclusive cases) rise to 1-in-4 when strictly defined. Mortalities in possible-to-definite TAD resembled possible-to-definite TACO, though like ABSTR, TAD had lower mortalities (akin to ATR) when defined strictly. TACO outcomes in this cohort align with the uppermost frequencies of death (up to 27%) across other studies (Table 3) [4,9–17,19,20].

Non-respiratory TRs with comparably high mortalities were BaCon and DHTR (in the range of 1 in 3 to 1 in 2). However, when re-sorting TRs by TTD, DSTR/DHTR exhibited long durations in a small (underpowered) sample, arguing against causality and instead for ascertainment bias, as discoveries are more likely in those with admissions long enough to involve multiple transfusions and tests. Suspected and more definitive BaCon cases exhibited indistinguishably high mortality rates and short TTDs, suggesting that suspected cases were either legitimate or as serious.

Though *definite* TRALI was not captured, nearly 20 *possible* cases were seen, and these showed the highest mortality and shortest TTD, transcending either BaCon or UTR. UTR is an important category, as underlying illness

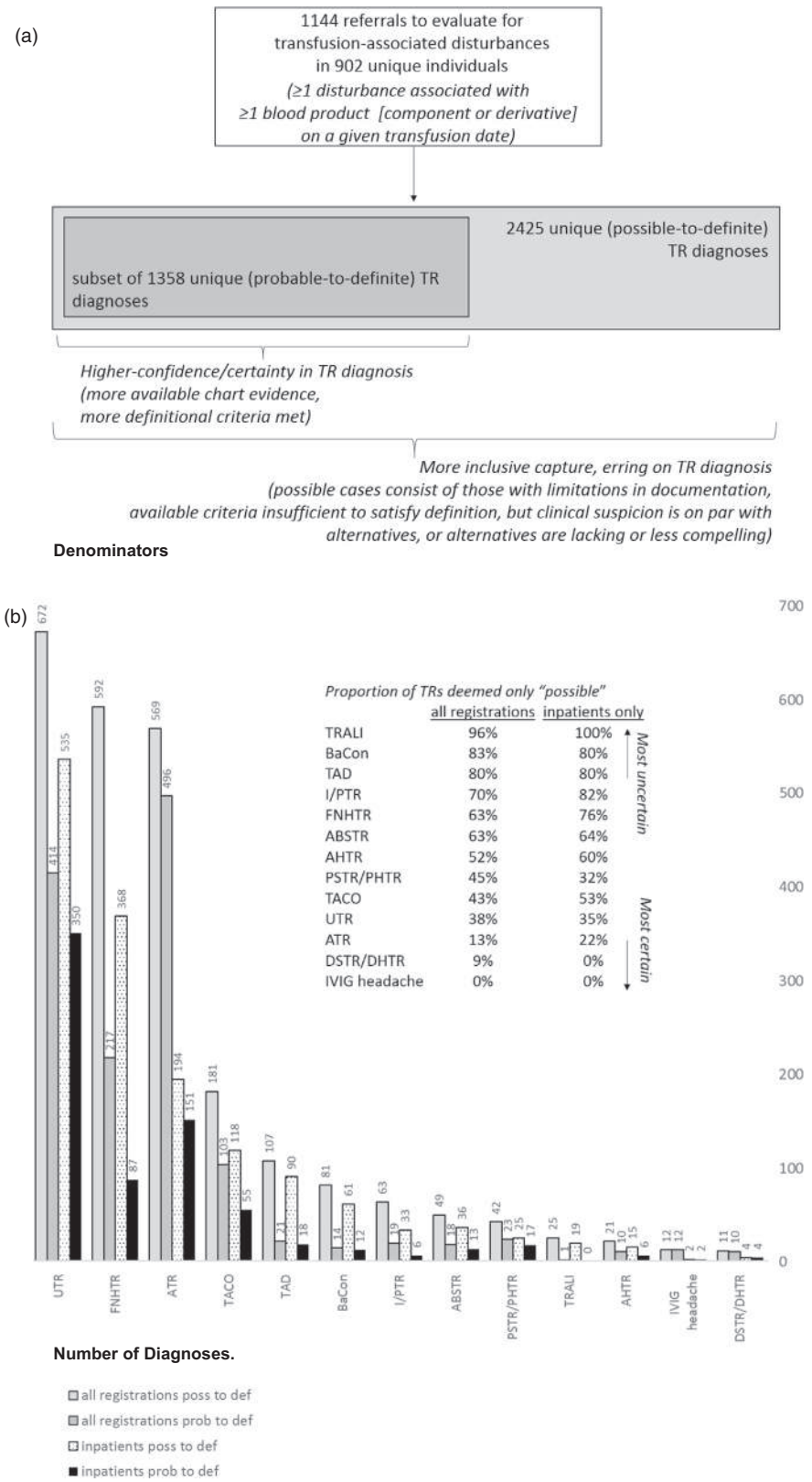
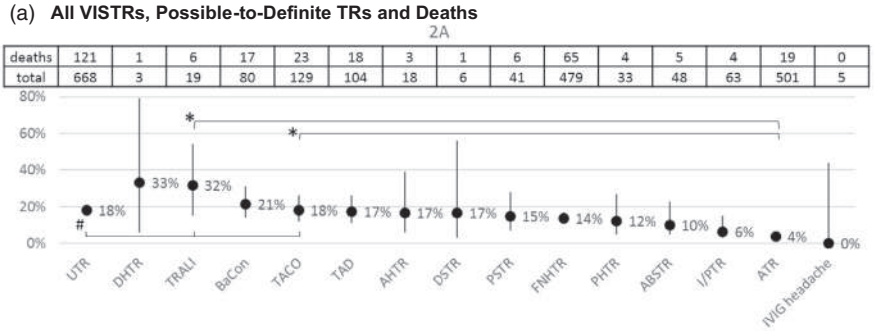


Fig. 1 Referral volumes, reaction counts, and certainty. (A) Denominators. (B) Number of diagnoses.

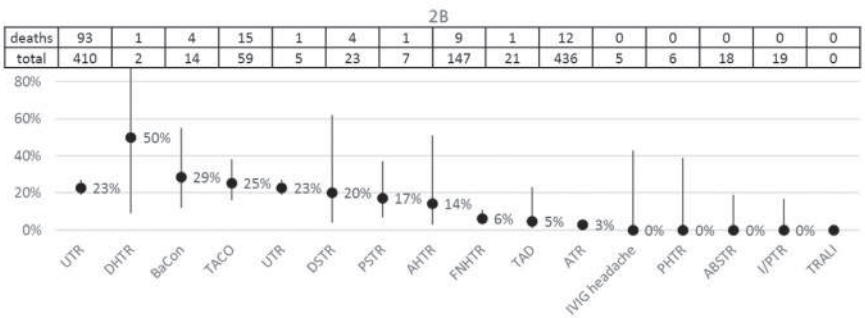
(a) **All VISTRs, Possible-to-Definite TRs and Deaths**



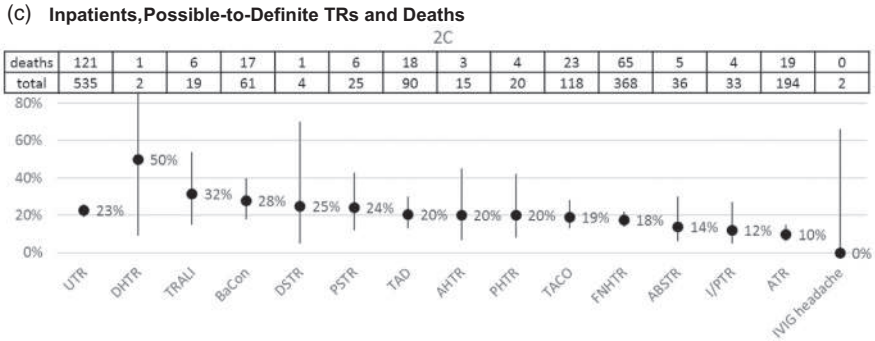
#: Non-significance for 3-way comparison of UTR, TRALI, TACO;

*: P<0.00001 for TRALI or TACO vs ATR

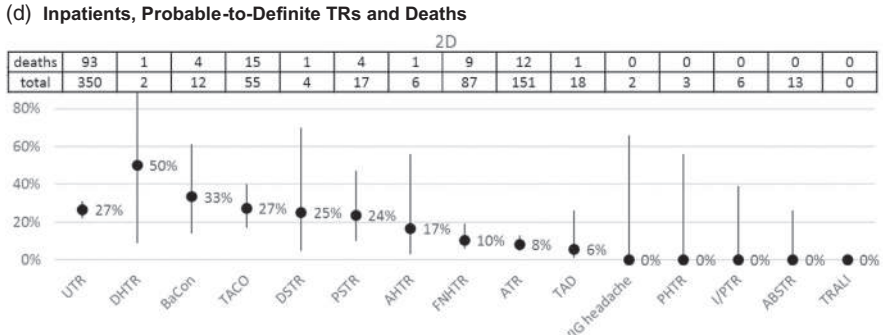
(b) **All VISTRs, Probable-to-Definite TRs and Deaths**



(c) **Inpatients,Possible-to-Definite TRs and Deaths**



(d) **Inpatients, Probable-to-Definite TRs and Deaths**



Variation: 95% CI.

Fig. 2 Post-reaction mortality. (A) All VISTRs, possible-to-definite TRs and deaths. #Non-significance for 3-way comparison of UTR, TRALI, TACO; *$P < 0{\cdot}00001$ for TRALI or TACO vs. ATR. (B) All VISTRs, probable-to-definite TRs and deaths. (C) Inpatients, possible-to-definite TRs and deaths. (D) Inpatients, probable-to-definite TRs and deaths. Variation: 95% CI.

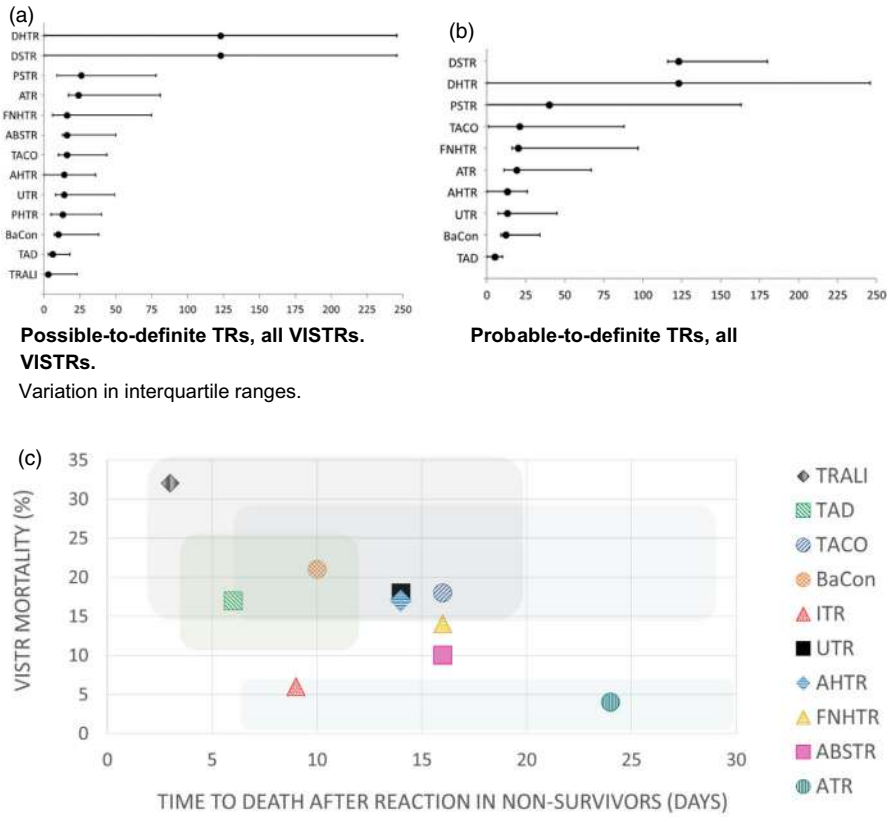


Fig. 3 TTD (in days) in non-survivors with TR. (A) Possible-to-definite TRs, all VISTRs. (B) Probable-to-definite TRs, all VISTRs. Variation in interquartile ranges. (C) Mortality and TTD in possible-to-definite TRs (all VISTRs). Variances shown for TRALI, TAD, TACO, and ATR.

is most often blamed when 'transfused ARDS' or possible [type 2] TRALI are considered. However, the pattern of death in possible TRALI did not exactly overlap with UTR. AHTR, on the other hand, did so in the 2 × 2 mortality/TTD construct.

TRALI and TACO share in preying upon at-risk or 'primed' patients in a 2-hit process [37]. TACO had a similarly high IHM but a longer TTD, challenging the notion that its deadliness was on par with TRALI or BaCon. As such, TACO may select for moribund patients but not necessarily hasten death, the rate of which can already exceed 30% in ICU patients, irrespective of transfusion status [38]. Alternatively, diuretics (which are less helpful to TRALI) may temporarily delay but not prevent death in TACO, which may, therefore, be less reversible than assumed. More research must establish the extent to which TACO incurs or merely associates with death. Establishing causation heightens the mandate for (and may grant insights on) prevention and treatment. At a minimum, these data caution on transfusion harms in patients already contending with advanced diseases.

Applying Bradford Hill criteria for causation [39] to the association of death with TACO yields mixed results (Table 4) [4,6,9–20]. Physical examination and laboratory markers of congestion [40] are shared between TACO and congestive heart failure (CHF), the latter of which may be terminal [5,18,41]. The one reported rat model of TACO did not characterize experimental lethality [9]. Dose responsiveness (biological gradient) is also unpredictable in TACO. Whereas, some report volume dependency; [12,14,42] others describe severe TACO after a single unit; most often, RBCs are invoked [43]. In previous studies, intervals between TACO and death are either unavailable [11,14,16,18,19], or reported as occurring within-admission [15,20] or as 1 [4,12], 2 [17] or 3 [10,12,13] month mortalities.

While a strength of this study was its inclusion of various TRs and their probabilities, with minor reactions (or the UTR) as comparators, the analysis nevertheless lacked a cohort of matched untransfused or disturbance-free transfused controls by which to best infer TR contributions to death. Subgroup analyses by service, underlying

Table 3 TACO Mortality in Literature [4,10–17,19,20,51].

Number of TACO cases (% of total)	Mortality in TACO (%)	Mortality in Control (%)	TACO Reporting – Definition	Refs
Inpatient adult academic hospital Possible 48 (1%) Probable-to-definite 39 (0·8%)	90 day: 0	Transfused, no TACO: 90 day: 14	Systemic active surveillance Expert adjudication, Definitions: CDC, ISBT	[10]
Adult ICU 51 (6%)	n/a	Age, sex, ICU diagnosis matched and transfused: n/a	Hydrostatic pulmonary edema < 6 h post transfusion	[11]
Adult ICU 66 (6%)	28 day: 22·7 90 day: 27·3	Transfused, no TACO: 28 day: 18·8 Pulmonary edema, not transfused: 28 day: 24·2	Drop in P/F ratio to <300 < 6 h post transfusion and clinical CHF not induced by other causes	[12]
Academic hospital (multisite) 83 (0·2%)	90 day: 16·8	Transfused, no TACO: 90 day: 4	Active electronic surveillance of transfused patients with ABG Expert panel (CDC definition), pulmonary edema < 6 h, with at least 3 TACO symptoms	[13]
Academic hospital (multisite) 100 (0·05%)	2	n/a	PHAC TACO definition; dyspnea, cyanosis, orthopnea, hypertension, or CHF < 6 h of transfusion	[14]
Adult non-cardiac surgery 176 (4%)	Inpatient: 8·5	Transfused, no TACO: 2·4	Electronic TACO screening algorithm Hypoxia < 6 h from transfusion correlated with CXR then expert screening with NHSN > 3 TACO criteria	[15]
Adult/pediatric 600 hospitals 4138 (3%)	n/a	n/a	ICD-9 billing codes (pulmonary edema) and transfusion	[16]
Inpatient adult academic hospital (multisite) 200 (1%)	50 day: 21	Transfused, no TACO: 50 day: 11	Active electronic surveillance with CXR < 12 h from transfusion, research nurse verified pulmonary edema; standardized forms (reviewed by expert panel), NHSN pulmonary edema < 6 h and associated criteria	[17]
Pediatric non-cardiac surgery 14 (3%)	30 day: 0	Transfused, no TACO: 30 day: 1·8	Electronic screening algorithm P/F < 300, CXR, expert screening with NHSN > 3 TACO criteria	[4]
Adult outpatients and inpatients: 26 (6%), 79 (5%)	Outpatient: 0 Inpatient: 0	Outpatient: 0·5 Inpatient: 0·7	Transfusion reaction reports (eg TACO) were extracted from the national hemovigilance database system of the French National Agency for Medicine and Health Products Safety	[19]
Adult multisite 221 (21%)	2	n/a	Dyspnea, orthopnea, cyanosis, tachycardia, hypertension, pulmonary edema, CXR, in proximity to transfusion	[49]
Adult multisite 1340 (0·06%)	Inpatient: 5·7	Transfused non-TACO inpatients: 6·8	ICD-9-CM billing code (TACO, transfusion)	[20]

ABG, arterial blood gas analysis; CDC, Centre of Disease Control; CXR, chest radiograph; ICD-9 (CM), International classification of diagnosis; ISBT, International Society of Blood Transfusion; NHSN, National Healthcare Safety Network; P/F, partial pressure arterial oxygen/fraction inspired oxygen; PHAC, Public Health Agency of Canada.

Table 4 Bradford-Hill criteria in TACO and death [4,6,9–20].

Criteria	Evidence	Supportive
Effect size	Changes in IHM with TACO [6,14]	YES
Consistency	Effects variable across studies [4,10–20]	NO
Specificity	"Sick selection" vs. causality [15]	NO
Temporality	Reactions are acute, but deaths are delayed (TTD > 16 days)	NO
Gradient	In contributing cases, severity/death is proportional to the volume transfused [13,15,20]	YES
Plausibility	Acute cardiopulmonary edema, CFR > 30%	YES
Coherence	Laboratory correlates with mortal outcomes are unknown [18]	NYD
Experiment	Not ethical in humans; the only existing animal model does not assess TACO-induced death (beyond examination after sacrifice) [9]	NO
Analogy	Non-cardiogenic pulmonary edema (TRALI) has CFR of 5–24%	YES
Reversibility	Diuretic and/or volume-conserving measures	NYD

CFR, case fatality rate; IHM, in-hospital mortality; NYD, not yet determined.

diagnosis or severities were not performed. Conclusion multiplicities and uncertainties were enriched among cardiopulmonary cases, reflecting classification challenges. Despite how high the highest mortalities were, true mortality rates were likely still underestimated, as they reflected the given visit's outcome, rather than ongoing outcomes in a longer predefined surveillance period.

This database is also used prior TACO and TRALI definitions rather than the latest revisions [29,30], though it is unlikely that these revised criteria would have significantly changed the assessments of the cases reported here. The inclusion of 'possible' TACO led to a rough doubling in counts, which may either have over-estimated, 'caught up to', or still underestimated true frequencies. This cohort also predated the COVID-19 pandemic, the impacts of which remain unknown in relation to TR susceptibility or severity. By analogy with COVID-19, true TR incidences are undercounted, and while roles in individual deaths may not always be clear-cut, exposure-specific changes in population death rates infer impacts by degrees [44,45].

The relationships observed in this study underscore the difficulty (or technical impossibility) of disentangling causes and effects, despite some evidence on independent effects of transfusion on mortality [46–49]. Each of our observations can be explained by well-accepted interdependencies: Patients with cardiomyopathy are likelier to die, and to experience TACO, which may hasten demise. Patients with sepsis may be primed for TRALI or progress coincidentally with transfusions. Patients with immunodeficiency may be more susceptible to (or suffer more severely from) a range of septic TRs.

Alongside information gaps is the challenge of underlying morbidities and the possibility of multiple (or multi-feature) TRs occurring (eg FNHTR and TACO or 'hot TACO') [50]. In an active prospective study applying a more rigorous and agnostic investigative approach to cardiopulmonary and/or febrile TRs aims include improving capacity to reach definitive conclusions and measuring real-life case complexity (TADPOL, NCT04267029).

In summary, the occurrence of a TR in a hospital visit reflects a range of odds that the visit will not be survived. In cardiopulmonary or septic TRs, this may be owing to directly injurious effects, whereas, serologic events may be surrogates of serious and protracted comorbidities. While global haemovigilance data underscore the predominance of cardiopulmonary TRs among transfusion-related deaths, these infrequent calamities remain a call to improve practice. However, the extent to which TRs intersect with the 'expected' rates of death in hospitals (by selection or contribution) is unknown. Despite not having a large sample of transfusion-induced deaths in this dataset, the distribution of mortalities and TTD reinforced minor categories as benign and laid out a rough hierarchy (for higher mortality, shorter TTD) in TRs known among leading causes of transfusion-related death.

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All authors: Contibuted to the review. MMcV and CC: Wrote the manuscript. CC: Conceptualized the study. AE and FT: Collected the data. JP, LL, YL, JC and CC: Performed case assessments. CC, MMcV, VA and RC: Performed analysis/graphics. MMcV and CC: Contributed to literature review.

Conflicts of interest

No conflicts of interest.

References

1 Harvey AR, Basavaraju SV, Chung KW, *et al*. Transfusion-related adverse reactions reported to the National Healthcare Safety Network Hemovigilance Module, United States, 2010 to 2012. Transfusion 2015;55:709–18.

2 Otrock ZK, Liu C, Grossman BJ. Transfusion-related acute lung injury risk mitigation: an update. Vox Sang 2017;112:694–703.

3 Roubinian NH, Triulzi DJ. Transfusion-associated circulatory overload and transfusion-related acute lung injury: etiology and prevention. Hematol Oncol Clin North Am 2019;33:767–79.

4 Thalji L, Thum D, Weister TJ, *et al*. Incidence and epidemiology of perioperative transfusion-related pulmonary complications in pediatric noncardiac surgical patients: a single-center, 5-year experience. Anesth Analg 2018;127:1180–8.

5 Bosboom JJ, Klanderman RB, Migdady Y, *et al*. Transfusion-associated circulatory overload: a clinical perspective. Transfus Med Rev 2019;33:69–77.

6 Bolton-Maggs PHB. Serious hazards of transfusion - conference report: celebration of 20 years of UK haemovigilance. Transfus Med 2017;27:393–400.

7 McKenzie CG, Kim M, Singh TK, *et al*. Peripheral blood monocyte-derived chemokine blockade prevents murine transfusion-related acute lung injury (TRALI). Blood 2014;123:3496–503.

8 McVey MJ, Kapur R, Cserti-Gazdewich C, *et al*. Transfusion-related acute lung injury in the perioperative patient. Anesthesiology 2019;131:693–715.

9 Klanderman RB, Bosboom JJ, Maas AAW, *et al*. Volume incompliance and transfusion are essential for transfusion-associated circulatory overload: a novel animal model. Transfusion 2019;59:3617–27.

10 Hendrickson JE, Roubinian NH, Chowdhury D, *et al*. Incidence of transfusion reactions: a multicenter study utilizing systematic active surveillance and expert adjudication. Transfusion 2016;56:2587–96.

11 Li G, Rachmale S, Kojicic M, *et al*. Incidence and transfusion risk factors for transfusion-associated circulatory overload among medical intensive care unit patients. Transfusion 2011;51:338–43.

12 Bosboom JJ, Klanderman RB, Zijp M, *et al*. Incidence, risk factors, and outcome of transfusion-associated circulatory overload in a mixed intensive care unit population: a nested case-control study. Transfusion 2018;58:498–506.

13 Murphy EL, Kwaan N, Looney MR, *et al*. Risk factors and outcomes in transfusion-associated circulatory overload. Am J Med 2013;126:357.e29-38.

14 Lieberman L, Maskens C, Cserti-Gazdewich C, *et al*. A retrospective review of patient factors, transfusion practices, and outcomes in patients with transfusion-associated circulatory overload. Transfus Med Rev 2013;27:206–12.

15 Clifford L, Jia Q, Yadav H, *et al*. Characterizing the epidemiology of perioperative transfusion-associated circulatory overload. Anesthesiology 2015;122:21–8.

16 Magee G, Zbrozek A. Fluid overload is associated with increases in length of stay and hospital costs: pooled analysis of data from more than 600 US hospitals. Clinicoecon Outcomes Res 2013;5:289–96.

17 Roubinian NH, Hendrickson JE, Triulzi DJ, *et al*. Contemporary risk factors and outcomes of transfusion-associated circulatory overload. Crit Care Med 2018;46:577–85.

18 Klanderman RB, Bosboom JJ, Migdady Y, *et al*. Transfusion-associated circulatory overload-a systematic review of diagnostic biomarkers. Transfusion 2019;59:795–805.

19 Moncharmont P, Barday G, Odent-Malaure H, *et al*. correspondants d'hemovigilance et de securite transfusionnelle de la region Auvergne Rhone A. Adverse transfusion reactions in recipients transfused in out-of-hospital. Transfus Clin Biol 2018;25:105–8.

20 Menis M, Anderson SA, Forshee RA, *et al*. Transfusion-associated circulatory overload (TACO) and potential risk factors among the inpatient US elderly as recorded in Medicare administrative databases during 2011. Vox Sang 2014;106:144–52.

21 Oakley FD, Woods M, Arnold S, *et al*. Transfusion reactions in pediatric compared with adult patients: a look at rate, reaction type, and associated products. Transfusion 2015;55:563–70.

22 De Cloedt L, Emeriaud G, Lefebvre E, *et al*. Transfusion-associated circulatory overload in a pediatric intensive care unit: different incidences with different diagnostic criteria. Transfusion 2018;58:1037–44.

23 Thurn L, Wikman A, Westgren M, *et al*. Incidence and risk factors of transfusion reactions in postpartum blood transfusions. Blood Adv 2019;3:2298–306.

24 Sahu A, Bajpai M. Determining the true incidence of acute transfusion reactions: Active surveillance at a specialized liver center. Hematol Transfus Cell Ther 2019;42(4):326–32.

25 Fujihara H, Yamada C, Furumaki H, *et al*. Evaluation of the in-hospital hemovigilance by introduction of the information technology-based system. Transfusion 2015;55:2898–904.

26 Raval JS, Mazepa MA, Russell SL, *et al*. Passive reporting greatly underestimates the rate of transfusion-associated circulatory overload after platelet transfusion. Vox Sang 2015;108:387–92.

27 Vande Vusse LK, Caldwell E, Tran E, *et al*. The epidemiology of transfusion-related acute lung injury varies according to the applied definition of lung injury onset time. Ann Am Thorac Soc 2015;12:1328–35.

28 Toy P, Gajic O, Bacchetti P, *et al*. Transfusion-related acute lung injury: incidence and risk factors. Blood 2012;119(7):1757–67.

29 Vlaar APJ, Toy P, Fung M, *et al*. A consensus redefinition of transfusion-related acute lung injury. Transfusion 2019;59:2465–76.

30 Wiersum-Osselton JC, Whitaker B, Grey S, *et al*. Revised international surveillance case definition of transfusion-associated circulatory overload: a classification agreement validation study. Lancet Haematol 2019;6: e350–e8.

31 Cohen R, Lima A, Escorcia A, *et al.* Serologic assessments in acute transfusion reactions: practices and yields. Vox Sang 2019;114:749–61.

32 St Bernard R, Yan M, Ning S, *et al.* Sustained and significant increase in reporting of transfusion reactions with the implementation of an electronic reporting system. Transfusion 2016;56:1247–8.

33 TTISS-On. Ontario Guide for Reporting Transfusion Reactions [monograph on the internet]. 2018. Available from: https://mctr.mcmaster.ca/surveys/?s=ELF8R8JPYE

34 Public Health Agency of Canada: Transfusion Transmitted Injuries Surveillance System, User's Manual, Version 3.0. 2007. https://ttiss.mcmaster.ca/wp-content/uploads/2016/10/Current-V3-CTAERF-Manual-E-2008-04-15.pdf

35 Clifford L, Jia Q, Subramanian A, *et al.* Characterizing the epidemiology of postoperative transfusion-related acute lung injury. Anesthesiology 2015;122:12–20.

36 Gosmann F, Norgaard A, Rasmussen MB, *et al.* Transfusion-associated circulatory overload in adult, medical emergency patients with perspectives on early warning practice: a single-centre, clinical study. Blood Transfus 2018;16:137–44.

37 Vlaar AP, Juffermans NP. Transfusion-related acute lung injury: a clinical review. Lancet 2013;382:984–94.

38 Shehata N, Forster AJ, Lawrence N, *et al.* Transfusion patterns in all patients admitted to the intensive care unit and in those who die in hospital: a descriptive analysis. PLoS One 2015;10:e0138427.

39 Hill AB. The environment and disease: association or causation? Proc R Soc Med 1965;58:295–300.

40 Callum JL, Cohen R, Cressman AM, *et al.* Cardiac stress biomarkers after red blood cell transfusion in patients at risk for transfusion-associated circulatory overload: a prospective observational study. Transfusion 2018;58:2139–48.

41 Tobian AA, Sokoll LJ, Tisch DJ, *et al.* N-terminal pro-brain natriuretic peptide is a useful diagnostic marker for transfusion-associated circulatory overload. Transfusion 2008;48:1143–50.

42 Marshall AL, Levine M, Howell ML, *et al.* Dose-associated pulmonary complication rates after fresh frozen plasma administration for warfarin reversal. J Thromb Haemost 2016;14:324–30.

43 Popovsky MA, Moore SB. Diagnostic and pathogenetic considerations in transfusion-related acute lung injury. Transfusion 1985;25:573–7.

44 Rosenbaum L. The untold toll - the pandemic's effects on patients without Covid-19. N Engl J Med 2020;382:2368–71.

45 Woolf SH, Chapman DA, Sabo RT, *et al.* Excess deaths from COVID-19 and other causes, March-July 2020. JAMA 2020;324:1562–4.

46 Hopewell S, Omar O, Hyde C, *et al.* A systematic review of the effect of red blood cell transfusion on mortality: evidence from large-scale observational studies published between 2006 and 2010. BMJ Open 2013;3(5):e002154.

47 Hebert PC, Wells G, Blajchman MA, *et al.* A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion requirements in critical care investigators, canadian critical care trials group. N Engl J Med 1999;340:409–17.

48 Schmidt AE, Henrichs KF, Kirkley SA, *et al.* Prophylactic preprocedure platelet transfusion is associated with increased risk of thrombosis and mortality. Am J Clin Pathol 2017;149:87–94.

49 Warner MA, Jia Q, Clifford L, *et al.* Preoperative platelet transfusions and perioperative red blood cell requirements in patients with thrombocytopenia undergoing noncardiac surgery. Transfusion 2016;56:682–90.

50 Parmar N, Pendergrast J, Lieberman L, *et al.* The association of fever with transfusion-associated circulatory overload. Vox Sang 2017;112:70–8.

51 Piccin A, Cronin M, Brady R, *et al.* Transfusion-associated circulatory overload in Ireland: a review of cases reported to the National Haemovigilance Office 2000 to 2010. Transfusion 2015;55:1223–30.

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Association of the ABO blood group with SARS-CoV-2 infection in a community with low infection rate

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Abstract

Background and objectives Reports on the association of the ABO phenotypes with infection by the SARS-CoV-2 virus have mostly come from countries with high infection rates. This study examined the possible association between SARS-CoV-2 infection and the ABO phenotype in Black Africa.

Materials and methods This report is from a single centre where both asymptomatic and symptomatic patients were quarantined. At the time of this report, Oyo State, Nigeria had carried out 15 733 tests of which 3119 were positive for the virus with 1952 recoveries and 37 deaths. The ABO distribution of patients was compared with that of a blood donor population.

Results Of the 302 participants, 297 (98%) had their blood group determined, asymptomatic and symptomatic individuals were 123 (40·7%) and 179 (59·3%) respectively. Blood group O was significantly less represented among the patients ($P < 0{\cdot}01$) while blood groups B and AB were significantly more represented ($P < 0{\cdot}01$, $P = 0{\cdot}03$ respectively). Patients with anti-B (groups A and O) were significantly less represented than those without anti-B (B and/or AB): B and AB ($P < 0{\cdot}001$), B ($P = 0{\cdot}002$), AB ($P = 0{\cdot}01$). There was no difference in the blood group distribution of symptomatic and asymptomatic patients (χ^2 (3, N = 302) = 2·29; $P = 0{\cdot}51$), but symptomatic patients with anti-A (groups B and O) were more represented than asymptomatic patients with anti-A (χ^2 4·89; $P = 0{\cdot}03$).

Conclusion The higher prevalence of blood group O and more potent beta haemolysins (anti-B antibodies) are likely reasons for the lower infectivity by the SARS-CoV-2 virus and severity of COVID-19 disease in the community.

Key words: antibodies, blood group, COVID-19, haemolysin, isoagglutinins, serology.

Introduction

The ABO blood group antigens are present on cellular elements in the blood and body secretions. This could explain why it has been associated with some diseases especially those of the respiratory and gastrointestinal tracts [1]. COVID-19, a disease resulting from SARS-CoV-2 viral infection, reportedly affects most systems of the body; it is also linked to the ABO blood group phenotypes [2]. Most studies have reported protection from blood group O but susceptibility of group A [2–5]. GWAS confirmed these findings and an association signal which

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coincides with the ABO blood group locus. [6] There is evidence to suggest that the anti-A antibodies present in the serum rather than the blood group itself could be responsible for the association [7, 8]. Infection rates and fatality from COVID-19 have been low in most African countries [9] where blood group O is more prevalent than other ABO blood group phenotypes in the population [10–12]. We therefore explore the likelihood that the low infection rates may be deduced from this.

Methods

This study was conducted at an Infectious Diseases Centre situated in Ibadan, the capital of Oyo state, Nigeria. The estimated population of Ibadan, the capital of Oyo State, is about six million. At the time of compilation of this report, Oyo State in South Western Nigeria had carried out 15 733 tests and of this number, 3119 were confirmed positive for the SARS-CoV-2 virus with 1952 recoveries and 37 deaths. Asymptomatic individuals were identified contacts of confirmed positive cases who also tested positive to the virus. In accordance to the guidelines of the Nigeria Centre for Disease Control (NCDC), asymptomatic individuals who tested positive for SARS-CoV2 and considered unable to appropriately self-isolate because of poor living conditions were quarantined and observed in the isolation centres until negative. This is to prevent the spread of the disease. This prospective data were therefore obtained from both asymptomatic and symptomatic individuals, and the study was carried out between 27th April 2020 and 30th August 2020, and the positivity rate was 19·8%.

COVID-19 testing was performed by Real-Time Polymerase Chain Reaction on nasopharyngeal and oropharyngeal swabs collected into Viral Transport Media. Briefly, within 2 h of collection, viral RNA was extracted from the nasopharyngeal and throat swabs using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. A commercial real-time RT-PCR kit (BGI, Europe) which detects the open reading frame 1ab (ORF1ab) was used for the real-time RT-PCR assay. The total reaction volume was 30μL, and the reaction was set up according to manufacturer's protocol. The reaction procedure was 50°C for 20 min, 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. Threshold cycle (Ct value) greater than 38 indicated a positive sample. Each run cycle included known positive and negative control as well as the internal control.

Information obtained from all patients managed at the centre included demographics, presenting symptoms and premorbid ailments. The blood group was obtained as part of the laboratory work up for all patients. The ABO phenotype was carried out by tile grouping, the method used during emergency for ABO blood group determination in our setting. The tile grouping done during emergency situations are thereafter confirmed by tube grouping in the hospital setting. Confirmation by tube grouping was not done in this instance because the blood group determination was for record purpose and not for blood transfusion. Blood donors of a teaching hospital in a neighbouring state in South Western Nigeria, a city about 140km from the study centre, served as controls [13].

Statistical analysis

The ABO frequencies of the patients were compared with that of the blood donors. The presenting symptoms were coded as respiratory, gastrointestinal and various combinations including respiratory and gastrointestinal. In addition, the participants were categorized into symptomatic and asymptomatic cases. A cross tabulation of the coded symptoms was carried out with the four ABO phenotypes and with the blood group coded as 'O' and 'non-O' blood group. The coded symptoms were also compared with the blood group coded as presence of anti-A antibody in the serum (blood groups O and B) and absence of these antibodies (blood groups A and AB). Chi-squared test was used to compare the association of the frequencies of the ABO of the participants with that of the blood donors and also to test for association between the coded blood groups and symptoms. Logistic regression was used in a model to test if age, gender or the different coded blood groups could predict the likelihood of an infected person developing symptoms. The level of significance for all the tests was set at 5%. Data were entered and analysed using STATA version 13 (StataCorp, College Station, Texas, USA).

Results

A total of 302 participants were analysed, of which 179 (59·3%) were symptomatic and 123 (40·7%) were asymptomatic. There were 202 (66·9%) males and 100 (33·1%) females with a mean age of 38·8 ± 16·1 years. Five of the participants did not have their blood group recorded and were excluded from the analyses. Respiratory symptoms were experienced by 70 (39·1%) participants, gastrointestinal symptoms by 21 (11·7%) participants while 6 (3·4%) experienced both. Blood group O was significantly less represented among the patients ($P < 0·01$) compared with donor controls while blood groups B and AB were significantly more represented ($P < 0·01$, $P = 0·03$ respectively). There was no significant difference between the A phenotype among the COVID-19 patients and the donor population ($P = 0·28$). (Table 1). A comparison of those

Table 1 ABO Phenotypes of COVID-19 patients in an Isolation Centre in Nigeria compared with a Blood Donor Population Distribution

ABO blood group	COVID-19 patients (N = 302)	Donor population (N = 9138)	χ^2	*P*
A	58 (19·1%)	2098 (23%)	1·19	0·28
AB	17 (5·6%)	307 (3·3%)	4·85	0·03
B	77 (25·3%)	1771 (19·4%)	19·72	<0·01
0	145 (47·7%)	4962 (54·3%)	23·61	<0·01

Five COVID-19 patients did not have their blood groups recorded.

with or without corresponding antibodies (A or B) in their sera showed that, patients with anti-B (groups A and O) were significantly less represented than those without anti-B (B and/or AB): A and AB ($P < 0·001$), B ($P = 0·002$), AB ($P = 0·01$). However, patients with anti-A showed no significant difference compared with those without anti-A except for the AB population which showed more infection rate ($P = 0·04$) (Table 2). The ABO distribution of symptomatic and asymptomatic patients is shown in the Figure 1. There was no significant difference between the blood group distribution between symptomatic and asymptomatic patients (χ^2 (3, N = 302) = 2·29; $P = 0·51$) but symptomatic patients with anti-A (groups B and O) were more represented than asymptomatic patients with anti-A (χ^2 4·89; $P = 0·03$). There was no difference between symptomatic and asymptomatic patients with anti-B (Table 3). Blood group was not associated with whether the patients had respiratory (χ^2 (1, N = 302) = 1·03; $P = 0·31$) or gastrointestinal symptoms (χ^2 (1, N = 302) = 1·6; $P = 0·2$). Age modestly predicted if a patient infected with SARS-CoV-2 would become symptomatic (OR 0·98 (95%CI 0·96, 0·99; $P = 0·01$). Gender, blood group or blood group recoded as 'O' and 'non-O' showed no association in the model ($P = 0·98$; $P = 0·96$; $P = 0·61$ respectively).

Table 2 Comparisons of the presence/absence of antibodies (anti-A, anti-B) in the serum of patients and donors

	ABO Blood Group	Donor Control, *n* (%)	COVID-19 Patients, *n* (%)	χ^2	*P*
With anti-B	O and A	7060 (77·3)	203 (68·4)		
	B and AB	2078 (22·7)	94 (31·6)	12·88	<0·001
Without anti-B	B	1771 (19·4)	77 (25·9)	9·3	0·002
	AB	307 (3·4)	17 (5·7)	6·6	0·01
With anti-A	O and B	6733 (73·7%)	222 (74·7)		
	A and AB	2405 (26·3)	75 (25·3)	0·17	0·68
Without anti-A	A	2098 (23)	58 (19·5)	1·39	0·24
	AB	307 (3·4)	17 (5·7)	4·11	0·04

Discussion

The findings of this study showed that blood group O is protective against COVID-19 infection while blood groups B and AB are risk factors. Expectedly, we also noted that patients with anti-B (Blood groups O and A) in their serum were less likely to be infected by the virus and that patients with anti-A (blood groups O and B) were more likely to become symptomatic from the infection. Unlike other studies, we did not find susceptibility of group A to the infection but rather an underrepresentation suggesting a possible protection, though this did not reach a significant level. The male gender was twice more susceptible to infection by the virus than the female gender, similar to the finding of male susceptibility by other studies [14, 15]. Contrary to other studies, the mean age for our patients was 39 years, which is at least a decade younger than found in most studies [14, 15].

The protection conferred by the group O phenotype was reported by studies which compared ABO blood group frequencies in COVID-19 patients with the community blood donor population, and these studies at the same time observed susceptibility to the infection by blood group A [2, 3]. A study which compared ABO frequencies between COVID-19 patients and other hospitalized patients found no significant difference between both groups [16]. The findings of our study differed from other studies by the observation of susceptibility in both groups B and AB rather than in group A only. This observed difference is more likely to be region specific and therefore could be genetic or influenced by environmental factors. We opined that these findings might be similar in other African or Black populations where blood group O is in the majority [10–12]. The findings of no difference between the observed and expected ABO distribution in Blacks and Hispanics (with high blood group O prevalence) compared with Whites where the observed frequency was significantly different from the expected [17] supports this hypothesis. This shows that infection by the virus differed between Blacks and Hispanics with high frequencies of the O phenotype compared with Whites with low O phenotype.

Analysis of the association between ABO distribution and COVID-19 infection by looking at the presence or absence of the corresponding antibodies confirms the difference between our studies and previous published data. Our study found the prevalence in those with anti-B to be significantly different from those without which again is at variance with previous studies which found such a difference between those with and without anti-A [7]. The protection conferred by blood group O has been attributed to circulating anti-A antibodies of the IgG type which could interfere with the virus-cell adhesion process [18]. Similarly, anti-B from group O is often IgG in contrast to

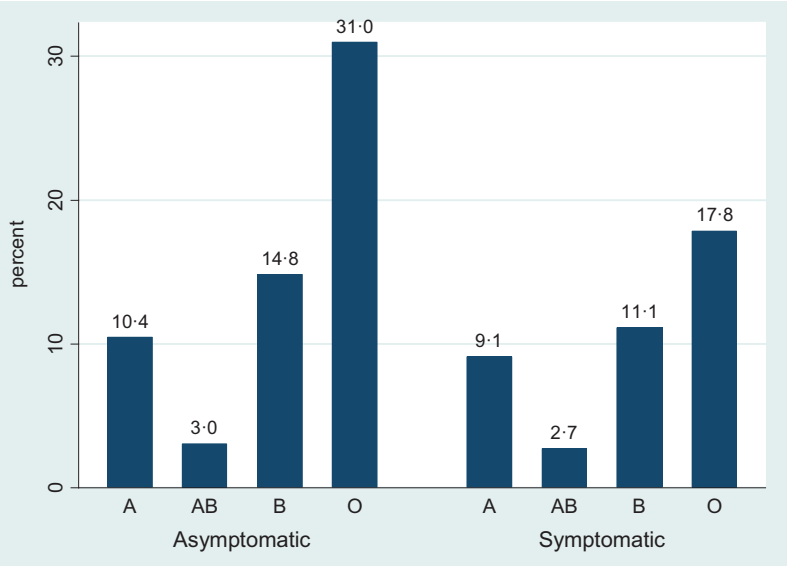

Fig. 1 A comparison of ABO phenotypes between symptomatic and asymptomatic patients with SARS-CoV-2.

Table 3 Comparisons of the presence/absence of antibodies (anti-A, anti-B) in the serum of symptomatic and asymptomatic patients

	ABO Blood Group	Symptomatic Patients, *n* (%)	Asymptomatic Patients, *n* (%)	χ^2	*P*
With anti-A	O and B	136 (77·3%)	86 (71·1)		
	A and AB	40 (22·7)	35 (28·9)	4·89	0·03
Without anti-A	A	31 (17·6)	27 (22·3)	1·17	0·28
	AB	9 (5·1)	8 (6·6)	0·46	0·5
With anti-B	O and A	123 (69·9)	80 (66·1)		
	B and AB	53 (30·1)	41 (33·9)	0·47	0·49
Without anti-B	B	44 (25)	33 (27·3)	0·28	0·60
	AB	9 (5·1)	8 (6·6)	0·38	0·54

antibodies from group A or B which are mostly IgM. It is thus likely that as suggested for anti-A, the anti-B from blood group O is more potent against the virus than anti-B from blood group A [7, 19]. Hence, the protection accorded to patients with blood group O would differ from that from blood group A since the anti-B is mostly of the IgM type. This could be an explanation for the non-significant level of protection attributable to patients with blood group A, though it could also be argued that the reason for the non-significant difference is that the study was not sufficiently powered to detect a difference.

Another peculiarity of our study is that it clearly shows that blood group AB is a risk factor for COVID-19 infection, and this difference compared with the blood donor population is statistically significant ($P = 0·03$). It is not surprising that the AB phenotype is susceptible to infection by the virus since individuals who have the AB blood group lack either anti-A and anti-B. This is also supported by the difference in the level of significance shown to blood group AB by those with anti-A ($P = 0·04$) and those with anti-B ($P = 0·01$) (Table 2). Though other studies showed increased risk of blood group AB to infection and severity of the disease, but these did not reach a statistically significant level [5, 19]. In the review of 28-day mortality which included the different races in the USA, Leaf RK et al reported similar mortality rate across the races but the graph showed a clear increased mortality in Blacks with the AB phenotype [17]. This would further support our hypothesis of the ABO phenotype showing a different trend in the response of Blacks to COVID-19 infection. A response which is likely linked to the higher prevalence of the O blood group and possibly more potent anti-B (haemolysin).

The strength of this study is that 98% of the participants had their blood group documented as well as the availability of information for individuals who were infected by the virus but were asymptomatic. The limitation of the study is the relatively smaller study size, and that it is a study from a single centre in comparison with other related studies. Though the choice of the donor population for this study was from a study carried out in another city which could be a limitation, the city is however in the same geographical location and of the same tribe as our community. The results also are similar to that of another donor population in the eastern part of the country [20] and that of a well-baby clinic from our institution [21] suggesting that the ABO prevalence is

more representative of that of the general population. Furthermore, the selective choice of blood group O as donors, done elsewhere, [16] is not practiced in Nigeria. It is therefore unlikely that the donor population was enriched by group O blood donors.

Conclusion

This study confirmed the association between ABO phenotypes and COVID-19 infection and the severity of infection. It also confirms the protection of blood group O from infection. However, contrary to other studies, we found that individuals with blood groups B and AB were more susceptible to the disease. The higher prevalence of blood group O and the presence of beta haemolysins which are very potent are possible reasons for a lower infectivity by the SARS-CoV-2 virus and severity of the COVID-19 disease in communities like ours.

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Conflict of interest

The authors have no conflict of interest.

Author contributions

TOA, AF conceptualized the study; TRK, TOA designed the study; TRK, OIF, ASA analysed and interpreted the data. TRK prepared the first draft, and all authors approved the final draft.

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References

1 Uwaezuoke SN, Eze JN, Ayuk AC, *et al.*: ABO histo-blood group and risk of atopy in children: a review of published evidence. *Paediatric Health Med Ther* 2018; **9**:73–79

2 Zhao J, Huang H, Li D, *et al.*: Relationship between the ABO blood group and the covid-19 susceptibility. 2020. https://doi.org/10.1093/cid/ciaa1150.

3 Li J, Wang X, Chen J, *et al.*: Association between ABO blood groups and risk of SARS-CoV-2 pneumonia. *Brit J Haematol* 2020). **190**(1), 24–27. https://doi.org/10.1111/bjh.16797

4 Gallian P, Pastorino B, Morel P, *et al.*: de-Lamballerie X: Lower prevalence of antibodies neutralizing SARS-CoV-2 in group O French blood donors. *Antiviral Res* 2020). **181**, https://doi.org/10.1016/j.antiviral.2020.104880

5 Goker H, Aladag-Karakulak E, Demiroglu H, *et al.*: The effects of blood group types on the risk of COVID-19 infection and its clinical outcome. *Turk J Med Sci* 2020; **50**:679–683

6 Ellinghaus D, Degenhardt F, Bujanda L, *et al.*: Genome wide association study of severe Covid-19 with respiratory failure. *N Eng J Med* 2020). **383** (16), 1522–1534. https://doi.org/10.1056/NEJMoa2020283

7 Gerad C, Maggipinto G, Minon J: COVID-19 and ABO blood group: another viewpoint. *Brit J Haematol* 2020; **190**(2), e93–e94. https://doi.org/10.1111/bjh.16884

8 Focosi D: Anti-A isohaemagglutinin titres and SARS-CoV-2 neutralization: implications for children and convalescent plasma selection. *Brit J Haematol* 2020). **190**(3), e148–e150. https://doi.org/10.1111/bjh.16932

9 Njenga MK, Dawa J, Nanyingi M, *et al.*: Why is there low morbidity and mortality of COVID-19 in Africa? *Am J Trop Med Hyg* 2020; **103**(2):564–569

10 Oyedeji OA, Adeyemo TA, Ogbenna AA, *et al.*: Prevalence of anti-A and anti-B hemolysis among blood group O donors in Lagos. *Nig J clin Pract* 2015; **18**(3):328–332

11 Smith S, Okai I, Abaidoo CS, *et al.*: Association of ABO blood group and body mass index: A cross-sectional study from a Ghanaian population. *J Nutr Metab.* 2018; **2018**:1–6. https://doi.org/10.1155/2018/8050152

12 Jahanpour O, Pyuza JJ, Ntiyakunze EO, *et al*: ABO and Rhesus blood group distribution and frequency among blood donors at Kilimanjaro Christian medical center, , Moshi, Tanzania. *BMC Res Notes* 2017; **10**:738. https://doi.org/10.1186/s13104-017-3037-3

13 Apecu RO, Mulogo EM, Bagenda F, *et al.*: ABO and Rhesus (D) blood group distribution among blood donors in rural south western Uganda: a retrospective study. *BMC Res Notes* 2016; **9**:513. https://doi.org/10.1186/s13104-016-2299-5

14 Li L, Huang T, Wang Y, *et al.*: COVID-19 patients' clinical characteristics, discharge rate and fatality rate of meta-analysis. *J Med Virol* 2020; **92** (6):577–583

15 Ambrosino I, Barbagelata I, Ortona E, *et al.*: Gender differences in patients with COVID-19: a narrative review. *Monaldi Arch Chest Dis* 2020; **90** (2):318–324

16 Dzik S, Eliason K, Morris EB, *et al.*: COVID-19 and ABO blood groups. *Transfusion* 2020). **60**(8), 1883–1884. https://doi.org/10.1111/trf.15946

17 Leaf RK, Al-Samkari H, Brenner SK, *et al.*: ABO phenotype and death in critically ill patients with COVID-19. *Brit J Haematol* 2020; **190**(4), e204–e208. https://doi.org/10.1111/bjh.16984

18 Guillon P, Clement M, Sebille V, *et al.*: Inhibition of the interaction between SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. *Glycobiology* 2008; **18**:1085–1093

19 Wu BB, Gu DZ, Yu JN, *et al.*: Association between ABO blood groups and COVID-19 infection, severity and demise: A systemic review and meta-analysis. *Infect Genet Evol* 2020). **84**, https://doi.org/10.1016/j.meegid.2020.104485

20 Okeke TC, Ocheni S, Nwagha UI, *et al.*: The prevalence of Rhesus negativity among pregnant women in Enugu, Southeast Nigeria. *Nig J Clin Pract* 2012; **15**(4):400–402

21 Omotade OO, Adeyemo AA, Kayode CM, *et al.* Gene frequencies of ABO and Rh (D) blood group alleles in a healthy infant population in Ibadan, Nigeria. *West Afr J Med* 1999; **18**(4):294–297

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ORIGINAL PAPER

Prevention of hypokalaemia and hypomagnesaemia following peripheral stem cell collection – a prospective cohort study

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Background and objectives Citrate-based anticoagulation reduces plasma potassium and free magnesium in patients undergoing peripheral stem cell collections. Whether the effects may be mitigated by pre-procedure oral electrolyte supplements has not been previously assessed.

Materials and methods Results from a historic cohort (2010–2013) guided a systematic prospective intervention in subjects deemed at risk for clinically meaningful hypokalaemia and hypomagnesaemia. From 2015 to 2019, 136 patients were enrolled in the study. Pre- and post-apheresis electrolyte levels were measured, and oral potassium and magnesium supplements were systematically administered based on the pre- electrolyte levels.

Results We saw a 37% absolute reduction in severe hypokalaemia and 39% absolute reduction in hypomagnesaemia in the prospective intervention cohort when compared to the historic cohort. Multivariate analyses indicated that part of the effect was due to the electrolyte intervention, while part of the effect likely stemmed from other procedure-related changes implemented during the study period.

Conclusion Oral potassium and magnesium prophylaxis appear to reduce hypokalaemia and hypomagnesaemia following peripheral stem cell collection. Whether the effect size is sufficient to motivate the intervention warrants further investigation, preferably in a prospective randomized trial setting.

Key words: apheresis, haematopoietic stem cell, haemovigilance.

Introduction

Transplantation of haematopoietic stem cells is an essential part of treatment in several haematological malignancies. Autologous haematopoietic stem cells are collected to ensure haematopoietic recovery following myeloablative regimens, and are preferentially acquired by peripheral stem cell collection (PSCC). PSCC is the method of choice in >99% [1] of collections, partly due to convenience, but also given the fact that this leads to higher stem cell yields enabling quicker transplant engraftment. In PSCC, the stem cells are mobilized to peripheral blood by granulocyte colony-stimulating factor (G-CSF) and collected by centrifuge based apheresis.

In general, PSCC is regarded as a safe procedure and non-transient complications are rare [2, 3]. However, the safety is dependent on proper preparation and surveillance as large volumes of blood are processed and continuous anticoagulation (AC) is administered during the apheresis procedure.

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Citrate-based solutions are a common option to prevent extracorporeal clotting and act by lowering free calcium, thereby inhibiting the coagulation cascade. Citrate effects are transient, but can cause symptoms, not only from Ca^{2+} reduction, but also from concomitant effects on potassium and magnesium.

Symptoms of hypocalcaemia are common, and apheresis centres seek to reduce these by administering oral or intravenous calcium, or by reducing citrate load. Adverse effects of hypomagnesaemia or hypokalaemia are less evident, and science-based strategies to mitigate these are lacking. Thus, we here firstly conducted a retrospective analysis of our clinic's latest PSCCs. Post-PSCC hypokalaemia and hypomagnesaemia were found to be common, and that paved way for a subsequent oral potassium and magnesium intervention. We here present the findings from the retrospective data set and from the prophylactic intervention cohort.

Methods

Background of the intervention

Patients eligible for autologous bone marrow transplantation in northern Sweden are all admitted to the Hematology Department, University Hospital of Umeå, Sweden, and PSCC are performed at the Transfusion Medicine Department at the same hospital. As part of our quality and safety assurance programme, we, in 2014, conducted a look back at electronic health records of 103 individuals who had undergone PSCC from 2010 to 2013.

Among other blood tests, pre- and post-venous electrolyte levels were assessed, revealing a high prevalence of post-PSCC hypokalemia and hypomagnesaemia (results). Given the lower prevalence in patients having received oral potassium and/or magnesium pre-PSCC (results), a systematic intervention guided by the pre-PSCC figures was established. A pre-PSCC concentration of <4·0 mmol/l and <0·70 mmol/l of potassium and magnesium, respectively, triggered oral supplementation of the equivalent electrolyte. Potassium chloride (1500 mg, slow release 6–8 h) and/or magnesium hydroxide (500 mg) was given orally approximately 12 and 2 h prior to PSCC initiation. A potassium of <3·1 mmol/l elicited continuous 3-lead ECG monitoring during the PSCC.

Albumin-corrected calcium was elevated in most subjects (results) potentially causing clinicians to overestimate the clinically relevant ionized calcium concentration. Ionized calcium measurements were therefore added to the prospective cohort and analysed in relation to the calcium infusion speed.

The prospective evaluation cohort

From 2015 to 2019, a total of 136 patients underwent PSCC and were part of the new potassium and magnesium supplementation routine. Identically to the 2010–2013 cohort, most patients were treated with a chemotherapy-based priming regimens and all patients received subcutaneous administration of a granulocyte colony-stimulating factor (G-CSF) 5 μg/kg/day for 4–7 consecutive days prior to PSCC. The patients arrived at the hospital the day before their scheduled PSCC, and pre-PSCC venous samples were drawn upon arrival.

Electrolyte analyses and anthropometric measurements

Venous blood was collected in heparinized BD Vacutainer® plasma tubes and was treated as clinical routine samples. The samples were analysed at Clinical Chemistry Laboratory, University Hospital of Umeå, Sweden, within one hour. Apart from ionized calcium, all samples were analysed on the Cobas® 8000 (Roche, Mannheim, Germany). The ISE, c701 and c502 modules were used for analyses of potassium, calcium and magnesium as well as albumin, respectively. Ionized calcium was assessed using the 9180 Electrolyte Analyzer (Roche, Mannheim, Germany). Albumin correction of calcium was calculated by adding (39-albumin (g/l)) ×0·01 to the original calcium value.

The lab-specific reference for potassium was 3·5–4·4 mmol/l, magnesium 0·70–0·95 mmol/l, albumin-corrected calcium 2·15–2·50 mmol/l and ionized calcium 1·08–1·29 mmol/l.

Body weight and length were measured at the patients' regular hospital facility at the start of the priming regimen.

Apheresis

All PSCCs were initiated in the morning, commonly between 8 and 9 am, and the patients were instructed to eat a calcium-rich breakfast before the procedure. A peripheral venous access was used in the majority of patients. Until 2014, a Cobe Spectra (Terumo BCT, Japan) was used for cell separation, and citrate–phosphate–dextrose 50 (CPD50, 4·4% citrate) for AC. The AC to whole blood ratio was increased in a stepwise manner during the procedure: 1:18 for the first 1·5 blood volume, 1:20 for the following 0·5 blood volume and from thereon 1:22. For most patients, the processing target was 4 blood volumes. From 2014 and onwards, a Spectra Optia

(Terumo BCT, Japan) was used for cell separation and the AC was switched to anticoagulant–citrate–dextrose solution A (ACD-A, 3·0% citrate). From 2014, the AC ratio for the first blood volume was 1:13, for the following 0·5 blood volume 1:15 and thereafter 1:17. From 2015, the processing goal was lowered to 3·2 blood volumes and some runs were terminated earlier based on calculations of sufficient CD34+ yields. During the entire study period, the processing speed was governed by the calculated safety limit of the citrate infusion speed. All patients received a continuous calcium infusion (calcium gluconate 10%) through the return line at the infusion speed prescribed by the apheresis medical doctor. The speed of infusion was occasionally adjusted based on reports of symptoms deemed attributable to hypocalcaemia. Venous samples for post-PSCC electrolyte assessment were drawn immediately after completion of the apheresis. The patients were generally discharged on the same day.

Statistics

Descriptive data are presented as means ± standard deviations (SD). Approximate normal distribution of continuous variables was verified visually on Q-Q plots, supported by skewness and kurtosis estimations. In repeated measures (i.e. pre- and post-PSCC electrolytes), mean differences were assessed using paired sample t-test. The Students' *t*-test for independent samples was used for evaluation of mean group differences. Chi-squared test served to evaluate differences in distribution between categorical variables and linear regression for assessing the post-PSCC electrolyte levels. The linear regression models included the following covariates: age, sex, blood volume (estimated from Nadler's formula), amount of citrate infused, cohort (where applicable) and the pre-PSCC electrolyte level. The reported β-values refer to the unstandardized measure and correspond to change per relevant unit. The effect of potassium and magnesium substitution in the prospective cohort was assessed on a per protocol basis. Calculations were performed using SPSS version 27 (IBM, United States). All statistical test were 2-sided, and a *P*-value of <0·05 was considered statistically significant.

Ethics

The study was considered part of the clinics' safety and quality process and was therefore not reviewed by an external ethics review board. The patients in the two cohorts received treatment according to the normal clinical practice at the time of participation. The patients signed informed consent for sharing anonymized data for quality improvements and research purposes.

Results

Historic cohort (2010–2013)

Baseline characteristics of the historic cohort (*n = 103*) are shown in Table 1.

Potassium

Potassium declined significantly (Δ –0·76 mmol/l, $P < 0·001$) during the PSCC and the greatest absolute decline was in patients with high pre-PSCC potassium (Fig. 1). In the multivariate linear regression model, male patients declined slightly more (β = 0·16, $P = 0·04$), whereas age, infused citrate or the patients' blood volume was not independently associated with the potassium decline ($P > 0·05$ for all). Patients having received oral potassium prophylaxis ($n = 8$) declined by 0·33 mmol/l compared to 0·80 mmol/L in non-prophylaxis recipients ($n = 95$), but the effect was not significant in the multivariate model (β = 0·18, $P = 0·07$).

There was a significant correlation between potassium and magnesium change (partial correlation 0·37, $P < 0·001$), but magnesium prophylaxis was not associated with potassium change ($P = 0·7$) in the covariate adjusted model. The same was true for PSCC calcium infusion speed ($P = 0·19$).

93% of subjects had a post-PSCC potassium lower than the reference boundary of 3·5 mmol/L, and 38% were below 3·0 mmol/L (Table 2). One patient was accidently discharged with potassium at 2·1 mmol/L, but luckily recovered without reported adverse effects.

Based upon expert advice, it was decided to aim for a post-PSCC-potassium level of ≥3·0 mmol/L. The pre-PSCC potassium was the strongest determinant of post-PSCC potassium ≥3 mmol/L (area under curve (AUC) 0·8, $P < 0·001$). Based on the receiver operating characteristic (ROC) curve, it was determined that a pre-PSCC potassium of <4·0 mmol/L would be an appropriate cut-off for oral prophylactic potassium administration. This cut-off resulted in sensitivity of 78%, specificity 53%, positive predictive value 50% and a negative predictive value of 79% for post-PSCC potassium <3·0 mmol/l.

The cut-off was subsequently used to guide potassium administration in the prospective cohort.

Magnesium

Plasma magnesium remained static in prophylaxis recipients ($n = 7$, Δ 0·00 mmol/l, $P = 0·98$) and declined significantly in subjects not receiving magnesium ($n = 96$, Δ –0·13 mmol/l, $P < 0·001$). The apparent prophylaxis effect was significant also in the multivariate regression model (β = 0·09, $P = 0·03$). The decline was more

Table 1 Baseline characteristics of the study cohorts

	Historic cohort (2010–2013)	Prospective cohort (2015–2019)	*P* for difference between groups
Demographics			
n	103	136	
Age (years)	58 ± 10	59 ± 10	0·37
Men/women (%)	64/36	61/39	0·63
Height (cm)	174 ± 10	172 ± 10	0·27
Weight (kg)	83 ± 15	80 ± 16	0·12
Diagnosis			0·89
Plasma cell disorder (%)	63	63	
Lymphoma (%)	33	32	
Other disease (%)	4	5	
Electrolyte levels			
Potassium (mmol/l)	3·8 ± 0·4	3·8 ± 0·3	0·70
Magnesium (mmol/l)	0·73 ± 0·10	0·76 ± 0·09	<0·05
Calcium, albumin corrected (mmol/l)	2·32 ± 0·09	2·31 ± 0·08	0·36
Calcium, ionized (mmol/l)	Not measured	1·22 ± 0·04	-
Prophylaxis			
Potassium	8%	65%	<0·001
Magnesium	7%	27%	<0·001
Apheresis data			
Peripheral access/femoral access (%)	77/23	78/22	0·82
Blood volume (l)	5·0 ± 0·9	4·8 ± 0·9	0·16
Apheresis duration, h:mm	4:33 ± 0:30	4:20 ± 0:52	<0·05
Processed volume (l)	20·3 ± 4·6	13·8 ± 4·0	<0·001
Citrate infused (g)	48 ± 10	30 ± 8	<0·001
Calcium gluconate (10%) infused (ml)	71 ± 17	40 ± 11	<0·001

pronounced in patients with higher pre-PSCC magnesium concentration ($\beta = 0{\cdot}22$, $P = 0{\cdot}03$), whereas the other covariates were non-predictive of magnesium decline.

The pre-PSCC magnesium was a strong predictor of post-PSCC magnesium ≥0·7 mmol/l (AUC 0·82, $P < 0{\cdot}001$). A cut-off of pre-PSCC magnesium of <0·7 mmol/l was set to guide prophylactic magnesium administration in the prospective cohort.

Albumin-corrected calcium

Calcium gluconate 10% (94 mg/ml) was prescribed at a rate of 15·6 ± 3·4 (range 7–25) ml/h resulting in a total dose of 70·9 ± 16·7 ml. As to be expected, there was a significant positive correlation between both the speed (Pearson correlation 0·28, $P < 0{\cdot}01$) and total volume (Pearson correlation 0·38, $P < 0{\cdot}001$) of infused calcium gluconate in relation to the change in albumin-corrected calcium. 96% of patients had a post-PSCC albumin-corrected calcium above the reference of 2·5 mmol/l.

Prospective cohort (2015-2019)

As shown in Table 1, apart from a slightly higher baseline magnesium, there were no major differences in the underlying characteristics of the prospective and the historic cohort. With regard to the apheresis procedure, the changes made during the study period (see method) resulted in less average citrate and calcium gluconate load, and less blood was processed ($P < 0{\cdot}001$ for all) in the prospective cohort. There was also a small difference in the average apheresis duration ($P < 0{\cdot}05$).

Potassium

90% (81/90) of patients at a pre-PSCC potassium <4·0 mmol/l received potassium prophylaxis as stipulated by the study protocol. In these patients, the medium potassium decline was significantly smaller than in the non-recipients (Δ-0·1 versus Δ-0·5 mmol/l, $P < 0{\cdot}01$). In the total cohort, 99% of the patients displayed a post-PSCC potassium above the predefined target (≥3·0 mmol/l) as compared to 60% in the historic cohort ($P < 0{\cdot}001$). The lowest observed post-PSCC potassium in the prospective cohort was 2·8 mmol/l as compared to 2·1 mmol/l in the historic cohort.

In a multivariate regression model, including both the historic cohort and the prospective cohort, there was a significant positive effect of potassium prophylaxis ($\beta = 0{\cdot}13$, $P < 0{\cdot}01$). There was also a cohort-specific

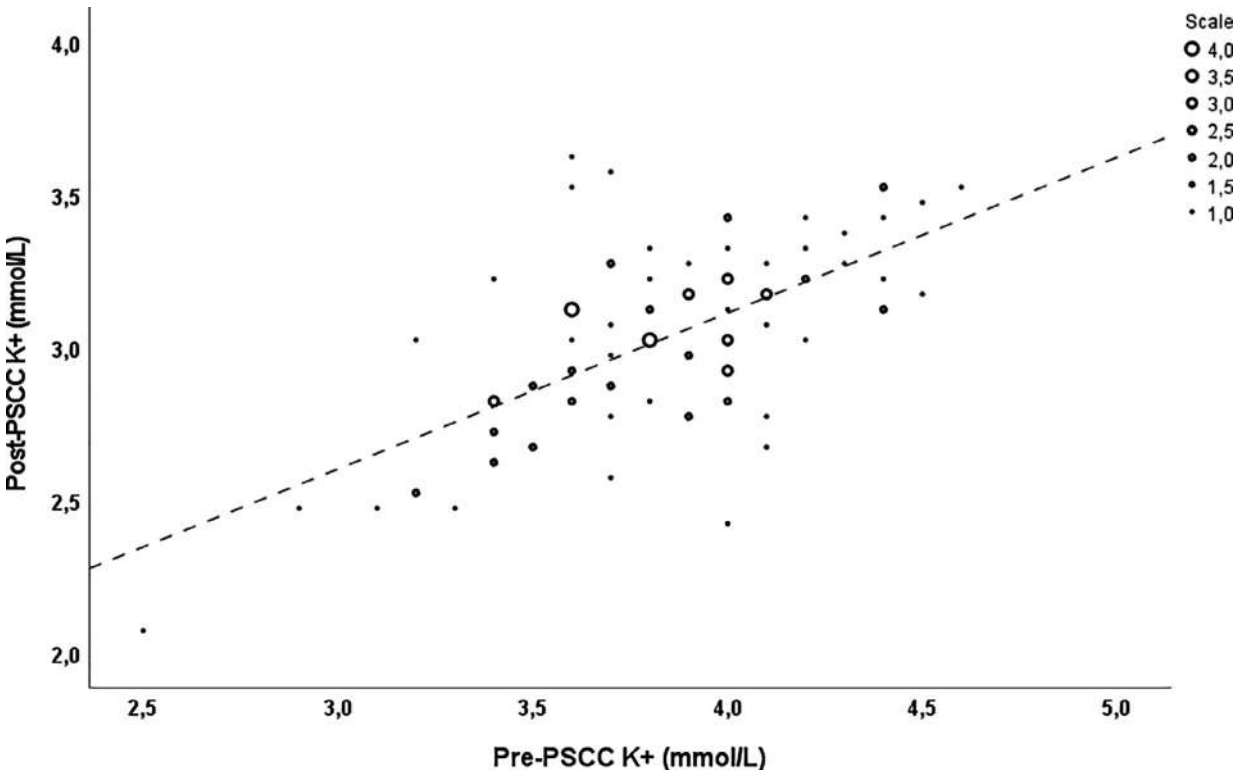

Fig. 1 Relation between pre- and post-PSCC plasma potassium concentration in the historic cohort. PSCC, peripheral stem cell collection.

effect (β = 0·34, $P < 0·001$), potentially indicating that changes in the apheresis procedure, not fully captured by the citrate adjustment, may have contributed to the reduction in potassium decline.

Magnesium

93% (26/28) of patients at a pre-PSCC magnesium <0·7 mmol/l received magnesium prophylaxis as stipulated by the study protocol. These patients had an increase in plasma magnesium (Δ0·02 mmol/l) whereas non-magnesium recipients instead declined ($n = 99$, Δ-0·05).

In a multivariate regression model, including both the historic cohort and the prospective cohort, there was borderline significant effect of magnesium prophylaxis (β = 0·03, $P = 0·05$). As with potassium, the reduced decline in magnesium was also partly explained by a cohort-specific effect (β = 0·05, $P < 0·001$).

Calcium

Although the calcium infusion speed tended to be lower in the prospective cohort (Table 1), the prevalence of albumin-corrected hypercalcaemia remained high (87%). In addition, ionized calcium was measured, and there was a moderate correlation between albumin-corrected and ionized calcium both pre- and post-PSCC (Pearson correlation 0·65, respectively, 0·52, $P < 0·001$ for both). Unlike the post-PSCC albumin-corrected figures, ionized calcium remained within the reference range in the majority of patients (90%) and only 9% had plasma levels below 1·08 mmol/l.

There was no significant relationship between calcium infusion speed (ml/h) and change in ionized calcium (β = 0·007, $P = 0·08$) in the multivariate model.

3-lead ECG monitoring

In total, two patients were monitored by continuous 3-lead ECG due to pre-PSCC-potassium of <3·1 mmol/l. Neither of these patients experienced cardiac-related symptoms or ECG alterations during the PSCC.

Discussion

In the present study, we consistently saw declines in plasma potassium and magnesium following PSCC and many patients ended up at levels known to cause symptoms and increase arrhythmia risk. After adjusting for potential confounders, the PSCC-associated declines appeared to be moderately mitigated by oral prophylaxis.

The results are in line with Schlenke et. al. reporting from 200 PSCC procedures [4]. They not only noted a significant potassium decline, but also reported a need for

Table 2 Pre- and post-PSCC plasma concentration within the two cohorts

	Historic cohort[a]	Prospective cohort[b]
Mean pre-PSCC K+ (mmol/l)	3·8 ± 0·4	3·8 ± 0·3
Mean post-PSCC K+ (mmol/l)	3·0 ± 0·3	3·5 ± 0·3
Δ K+ (mmol/l)	0·8 ± 0·3	0·3 ± 0·4
Distribution of post-PSCC K+		
<2·5 mmol/l	2%	0%
2·5–2·9 mmol/l	36%	1%
3·0–3·4 mmol/l	55%	39%
≥3·5 mmol/l	7%	60%
Mean pre-PSCC Mg (mmol/l)	0·78 ± 0·05	0·76 ± 0·09
Mean post-PSCC Mg (mmol/l)	0·65 ± 0·8	0·72 ± 0·09
Δ Mg (mmol/l)	−0·13 ± 0·07	−0·04 ± 0·07
Distribution of post-PSCC Mg		
<0·7 mmol/l	76%	37%
≥0·7 mmol/l	24%	63%
Mean pre-PSCC Ca^{2+} (mmol/l)	-	1·22 ± 0·04
Mean post-PSCC Ca^{2+} (mmol/l)	-	1·16 ± 0·07
Δ Ca^{2+} (mmol/l)	-	−0·07 ± 0·07
Distribution of post-PSCC Ca^{2+}		
<1·08 mmol/l	-	9%
≥1·08 mmol/l	-	91%

Abbreviation: PSCC, peripheral stem cell collection.

[a] Subjects receiving prophylaxis excluded.

[b] Subjects in violation of the study protocol excluded.

potassium replacement in 52% of their procedures. Declines in plasma potassium have also been noted in platelet- [5, 6] as well as allogenic peripheral stem cell donation [7], supporting a citrate effect that was procedure and patient/donor independent.

Also for magnesium, a decline across both procedure types and patient groups has been reported. The ionized magnesium dropped by 30–56% [5, 8–12], whereas the effect on total magnesium was significantly smaller (<12%) [5, 8, 9, 12]. In the present study, only plasma total magnesium was measured, and an approximate 16% decline was seen in subjects not receiving magnesium prophylaxis. Magnesium prophylaxis appeared to have a significant, but rather modest, mitigative effect. Hypothetically, the magnesium prophylaxis may have had a larger impact on ionized magnesium, but we were regrettably unable to assess this in the present trial.

Citrate-based anticoagulant solutions not only exert direct effects through the binding of divalent cations, but also have metabolic consequences upon conversion to bicarbonate. Given that the overwhelming majority of both magnesium and potassium is located intracellularly [13], small absolute shifts can have a profound impact on plasma concentration.

It is believed that citrate decreases plasma potassium concentration in three ways, all sharing the common path of increased intracellular Na+ resulting in enhanced Na+ K–ATPase activity [14]. The pathways are as follows: Na+ shifted intracellularly by HCO_3- induced co-transport, alkalosis induced H+ efflux and Na+ influx through the Na+ H+ antiporter, and lastly insulin activation of the same antiporter. The net result, increase in Na+ K—ATPase activity, causes intracellular potassium uptake and thus a corresponding decrease in plasma potassium [14–16].

Unlike for potassium, the citrate primarily exerts its effect on magnesium by chelation of the free (ionized) portion [15]. As such, the relatively small reduction in total magnesium, seen in this study, and others [5, 8, 9, 12] do not fully reflect the impact of the biologically active form.

Hypokalaemia and hypomagnesaemia have clinical implications as they may adversely impact both neuromuscular, cardiovascular and metabolic function. Symptoms may range from mild to life threatening. The perhaps most significant impact is the effect on the conduction system on the heart, increasing arrhythmia risk. Pronounced hypokalaemia may manifest as U waves, T-wave flattening, prolongation of QT interval and ST-segment depression [17]. The hypokalaemia induced hyperexcitability is exhibited by an increase in supraventricular and ventricular ectopy, resulting from intracellular Ca^{2+} overload [18]. Hypokalaemia is well known to increase the risk of ventricular fibrillation in acute myocardial infarction [19], and similar effects have been shown also in normal canine heart [20].

Hypomagnesaemia may aggravate hypokalaemia effects trough blockage of calcium channels, thereby increasing arrhythmia risk. Magnesium administration may prevent triggered action potentials and is an efficient treatment in torsades de pointes [18]. Further, hypomagnesaemia may aggravate hypocalcaemia by inhibiting PTH secretion [13, 21], thus causing common symptoms as tingling, paraesthesia, headache and tetany [15]. The same symptoms may also be caused by hypomagnesaemia itself, potentially leading to unnecessary calcium administration in the absence of laboratory data support [15].

Despite citrate induced electrolyte shifts, severe adverse events in PSCC are fortunately rare. Reports from the World Apheresis Registry estimate that approximately 0·1% of autologous PSCC has had to be terminated prematurely due to complications [2]. Given the rarity of severe arrhythmia, it is inherently difficult to assess the effects of electrolyte surveillance and prophylaxis using hard end-points. There is however some indirect support of risk mitigation. Laspina et al. noted consistent citrate induced QTc time prolongation during platelet donation [22], corresponding to a well-described increase in arrhythmia risk [23, 24]. Further, hypokalaemia associates with arrhythmia in a dose–response manner [19, 24]. Both arrhythmias and cardiac

arrest have been reported in PSCC donors [3, 20, 25], whereas reports in PSCC patients are lacking.

In the present study, we saw significant but rather modest effects of oral potassium and magnesium prophylaxis. Although there were no reports of adverse prophylaxis effects, the number needed to treat to prevent one serious event is likely to be very large, and risk–benefit ratios are uncertain. There may however be other positive effects such as decreased need for interruptions and reduced apheresis speeds and a reduction of more commonly reported symptoms such as fatigue, muscle weakness and tingling. Also, a reduction in significant hypokalaemia may lessen the need for post-PSCC medical surveillance. We suggest that the effect of prophylaxis on PSCC-associated symptoms and other medical aspects should be assessed in a blinded prospective trial.

With regard to calcium, we saw consistent elevation of albumin-adjusted figures, whereas ionized calcium remained within normal reference range in most patients. This is a natural reflection of the continuous calcium infusion in conjunction with citrate induced chelation. The rather weak correlation between the total and the ionized fraction suggests that the total calcium would serve as a poor guide for calcium administration. Interestingly, there was no significant association between calcium infusion speed and the post-PSCC ionized plasma calcium. Speculatively, the long apheresis duration provides sufficient time for PTH to mobilize endogenous calcium. If confirmed, clinicians may consider reducing the calcium infusion speed throughout the procedure.

The present study comprised two well-defined cohorts, both representative of real-world conditions in haematological and apheresis clinics. Data from the historic cohort guided a prospective systematic intervention, serving to validate the historic cohort results. Blood analyses were performed in a consistent way, and the overwhelming majority of patients received intervention as stipulated by the study protocol. The underlying characteristics of the cohorts were similar, but potentially important alterations of parameters (equipment, AC and AC ratio, calcium infusion speed and processed volumes) took place during the study period that was beyond the control of the study. In multivariate analysis, these changes appeared to reduce the potassium and magnesium decline, resulting in an overestimation of the prophylaxis effect in unadjusted models. After adjustments, the prophylaxis effect appeared modest and we acknowledge that assessments of effect size are quite sensitive to model assumptions.

Conclusion

In conclusion, changes in the apheresis procedure throughout the study appeared to have a material impact on electrolyte change, limiting the ability to draw firm conclusions on the intervention itself. The data were indicative of a moderate positive effect of potassium and magnesium prophylaxis, but whether the effect sizes were large enough to motivate the intervention remains unclear. Future randomized trials are needed to reliably quantify the laboratory and clinical effects of oral potassium and magnesium prophylaxis.

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Author contributions

Lars Skagerlind and Fredrik Toss jointly worked on study design, data acquisition, data analyses and the interpretation of data; contributed to the writing process and approved the final version of the manuscript; and acknowledge accountability for all aspects of the work.

Conflict of interest

The authors declare no conflicts of interest.

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References

1 Passweg JR, Baldomero H, Chabannon C, *et al.*: The EBMT activity survey on hematopoietic-cell transplantation and cellular therapy 2018: CAR-T's come into focus. *Bone Marrow Transplant* 2020; **55**:1604–13

2 Mortzell Henriksson M, Newman E, Witt V, *et al.*: Adverse events in apheresis: An update of the WAA registry data. *Transfus Apher Sci* 2016; **54**:2–15

3 Halter J, Kodera Y, Ispizua AU, *et al.*: Severe events in donors after allogeneic hematopoietic stem cell donation. *Haematologica* 2009; **94**:94–101

4 Schlenke P, Frohn C, Steinhardt MM, *et al.*: Clinically relevant hypokalaemia, hypocalcaemia, and loss of hemoglobin

and platelets during stem cell apheresis. *J Clin Apher* 2000; **15**:230–5
5 Bolan CD, Greer SE, Cecco SA, *et al.*: Comprehensive analysis of citrate effects during plateletpheresis in normal donors. *Transfusion* 2001; **41**:1165–71
6 Bolan CD, Cecco SA, Yau YY, *et al.*: Randomized placebo-controlled study of oral calcium carbonate supplementation in plateletpheresis: II. Metabolic effects. *Transfusion* 2003; **43**:1414–22
7 Bolan CD, Leitman SF: Management of anticoagulation-associated toxicity during large-volume leukapheresis of peripheral blood stem cell donors. *Blood* 2002; **99**:1878
8 Bolan CD, Cecco SA, Wesley RA, *et al.*: Controlled study of citrate effects and response to i.v. calcium administration during allogeneic peripheral blood progenitor cell donation. *Transfusion* 2002; **42**:935–46
9 Mercan D, Bastin G, Lambermont M, *et al.*: Importance of ionized magnesium measurement for monitoring of citrate-anticoagulated plateletpheresis. *Transfusion* 1997; **37**:418–22
10 Patidar GK, Sharma RR, Marwaha N: Frequency of adverse events in plateletpheresis donors in regional transfusion centre in North India. *Transfus Apher Sci* 2013; **49**:244–8
11 Mielcarek-Siedziuk M, Gajek K, Musial J, *et al.*: Safety and efficacy of autologous mononuclear cell and stem cell apheresis in very low-weight children-Experience at a single center. *J Clin Apher* 2019; **34**:563–70
12 Das SS, Chaudhary R, Khetan D, *et al.*: Calcium and magnesium levels during automated plateletpheresis in normal donors. *Transfus Med* 2005; **15**:233–6
13 Weisinger JR, Bellorin-Font E: Magnesium and phosphorus. *Lancet* 1998; **352**:391–6
14 Aronson PS, Giebisch G: Effects of pH on potassium: new explanations for old observations. *J Am Soc Nephrol* 2011; **22**:1981–9
15 Lee G, Arepally GM: Anticoagulation techniques in apheresis: from heparin to citrate and beyond. *J Clin Apher* 2012; **27**:117–25
16 Halperin ML, Kamel KS: Potassium. *Lancet* 1998; **352**:135–40
17 Alfonzo AV, Isles C, Geddes C, *et al.*: Potassium disorders–clinical spectrum and emergency management. *Resuscitation* 2006; **70**:10–25
18 El-Sherif N, Turitto G: Electrolyte disorders and arrhythmogenesis. *Cardiol J* 2011; **18**:233–45
19 Nordrehaug JE, Johannessen KA, von der Lippe G: Serum potassium concentration as a risk factor of ventricular arrhythmias early in acute myocardial infarction. *Circulation* 1985; **71**:645–9
20 Hohnloser SH, Verrier RL, Lown B, *et al.*: Effect of hypokalemia on susceptibility to ventricular fibrillation in the normal and ischemic canine heart. *Am Heart J* 1986; **112**:32–5
21 Vetter T, Lohse MJ: Magnesium and the parathyroid. *Curr Opin Nephrol Hypertens* 2002; **11**:403–10
22 Laspina SJ, Browne MA, McSweeney EN, *et al.*: QTc prolongation in apheresis platelet donors. *Transfusion* 2002; **42**:899–903
23 Moss AJ: Measurement of the QT interval and the risk associated with QTc interval prolongation: a review. *Am J Cardiol* 1993; **72**:23B–5B
24 Ravens U, Cerbai E: Role of potassium currents in cardiac arrhythmias. *Europace* 2008; **10**:1133–7
25 Loseto G, Canaris D, Mele G, *et al.*: Paroxysmal atrial fibrillation caused by hypokalemia in a healthy donor who underwent PBSC apheresis. *Bone Marrow Transplant* 2013; **48**:155–6

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INTERNATIONAL FORUM

International Forum on Walking Blood Bank Programmes: Summary

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Introduction

Blood products remain the pivotal non-surgical tool in the management of massive haemorrhage, which results in the majority of preventable death following traumatic injury [1]. The management of massive haemorrhage in trauma has evolved significantly over the last two decades, resulting in new standards of care. First, blood products should be transfused as soon as possible following injury [2]. Second, plasma transfusion is superior to crystalloid [3]. Third, platelet transfusion should not be delayed [4]. Finally, the ratio between red blood cells, plasma, and platelets should be close to 1:1:1 [5].

Blood supply is a challenge wherever you are in the world. Aiming to follow the latest evidence, blood products are required as early as possible in the patient pathway. Logistical and regulatory constraints make implementing massive transfusion safely extremely complex, especially in austere or military settings, where the majority of casualties die. This is one reason why walking blood bank (WBB) programmes have been implemented. The term WBB describes a setting where fresh whole blood is drawn from a pre-tested 'walking' donor pool for immediate use in bleeding casualties. A WBB programme allows for the availability of blood products even in very degraded situations such as mass casualty events, war zones, or following compromise of the blood supply chain. First practiced during World War I, fresh whole blood represents a promising solution [6].

Given the increasing interest in whole blood transfusion, the time is propitious to conduct a Vox Sanguinis International Forum on current WBB programme practices. The main aim of this International Forum is to assess the variability and the similarity in current WBB programmes around the world. The insight gained from this survey can be used as a basis to implement guidelines, developed from the best available evidence, and for benchmarking between comparable countries.

The participants in the survey were selected from diverse parts of the world to study a wide variety of practices. From January to September 2020, an invitation was sent by email to blood bankers around the world, selected according to their membership in recognized organizations such as the NATO Blood Panel, ISBT, African Blood Association or the Asian Blood Association. A total of 28 invitations were transmitted. The questionnaire was composed of 8 sections, embracing the whole chain: from the WBB programme structure to haemovigilance and training, including a focus on pre-screening and blood collection. We received 15 (54%) completed questionnaires. A summary of findings is reported:

Structure of WBB programme

Questions: *Does a WBB programme exist in your country? Is the WBB programme Military or civilian? How many personnel are required to run the WBB programme? When did the WBB programme commence? What is the legal framework under which the WBB programme is regulated/governed?*

Ten countries report an existing WBB programme; one country is currently planning to develop such a programme (Belgium), whereas the remaining four countries have neither an existing nor a planned programme (Brazil, Cameroon, Luxembourg and Malaysia). Eight countries report that their WBB programme is a military programme, while one country reports a civilian programme (Mali) and another reports both military and civilian WBB programmes (Norway). Even though WBBs are not a new concept, the oldest programme only started in the late 1980s, with seven (70%) respondents with active WBB programmes reporting establishment the programme in the last 15 years (Fig. 1). With respect to the legal framework under which the programme is regulated, the majority (70%) of programmes follow civilian regulations for their country, where applicable. Those specifications that fall outside of this regulatory framework are governed by military regulations.

Personnel/command and control

Questions: *What are the situations in which you expect to activate the walking blood bank programme? Who will be responsible for activation of the WBB? Who will organize/co-ordinate donor collection? What are the qualifications of individuals performing donor selection, whole blood collection, donor testing, recipient testing, confirming compatibility between donor and recipient and transfusion of whole blood to recipient?*

One of the principal questions regarding WBB programme relates to the circumstances leading to activation of the WBB. In military settings, WBB activation is based on the need of a blood product for an emergency associated with a shortage of (re) supply. This included mass casualty situations, or when platelets are required and not carried by the deployed blood bank. Six (60%) respondents have developed WBB programmes to support deployed troops with no blood supply including special operations forces or troops working in remote locations with a low occurrence of haemorrhage where a full-time blood bank would lead to a high rate of blood product wastage. The activation of the programme is under the responsibility of a chief/senior medical officer for all countries except one, which authorizes paramedics to activate it during special operations. Regarding the organization of the blood collection, phlebotomy is performed by appropriately trained personnel, rather than requiring a specific level of education (e.g. doctor). Donor selection remains a doctor's prerogative in three (30%) countries.

Pre-screening of potential donors

Questions: *Are potential WBB blood donors pre-screened? What are the tests performed? Which tests are mandated? Where and by whom are the tests performed? How are the test results reported and who is responsible for dissemination of results and donor care? How long is the TTI pre-screening valid?*

All countries base their strategy on pre-screening donors for the WBB. This pre-screening fulfils local blood donation control criteria and is performed in a blood bank laboratory, often co-ordinated at a single-centralized site. Results are forwarded to the physician by various methods and recorded in the donor's medical file in two (20%) countries. The validity of pre-screening ranges from 30 days to one year (Table 1).

Pre-transfusion point of care testing

Questions: *What are the rules used to determine compatibility of blood transfusion? Is a point of care (POC) test used to confirm compatibility of donated whole blood and recipient prior to the transfusion? What is the name of the device? Are additional POC tests (e.g. transfusion-transmitted infection (TTI) screen) performed on the recipient pre-transfusion? Which POC test(s) and device (s) are used? Are repeat TTI tests performed on the donor prior to transfusion? What tests are performed? Which device is used? Are donor/recipient samples also taken for testing at a later date? What tests are performed? Who/where performs the testing? Who is responsible for these tests?*

The key points of safety during the activation of a WBB programme are ensuring immune-haematologic (IH) ABO blood group compatibility and minimizing the risk of transfusion-transmitted infection. Regarding IH compatibility, four (40%) countries collect only blood from low-titre O donors, preventing the risk of an ABO mismatch. Despite this, eight (80%) countries still require

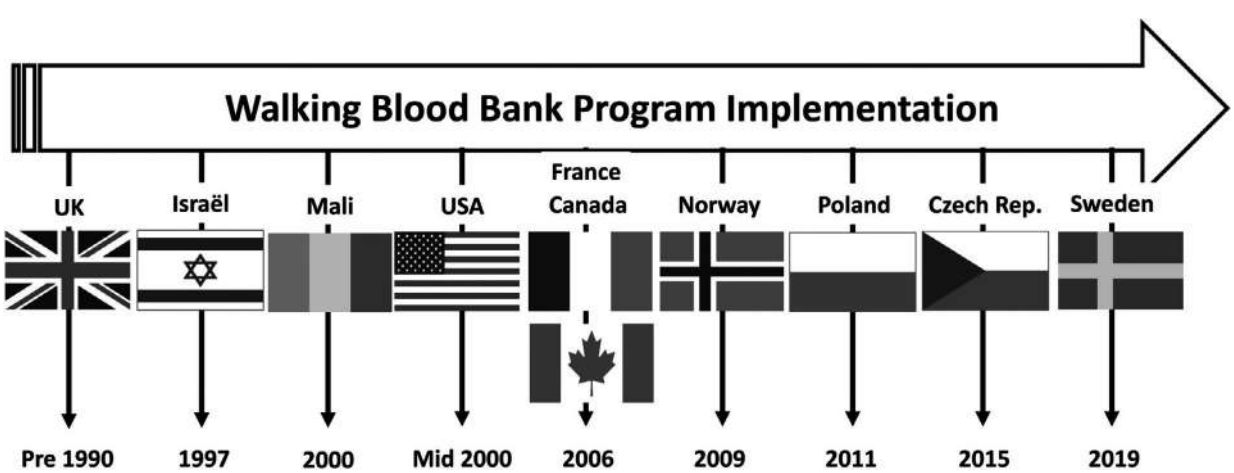

Fig. 1 International Forum responding Countries with Walking Blood Bank Programs and their date of implementation.

Table 1 Pre-screening analysis of WBB donors

	Canada	Czech Republic	France	Israël	Norway	Poland	Sweden	UK	USA
ABO typing	X	X	X	X	X	X	X	X	X
Rh typing	X	X	X	X	X	X	X	X	X
Antibody against HIV, HBV, HCV	X	X	X	X	X	X	X	X	X
Syphilis testing	X	X	X				X	X	
Antibody against HTLV	X		X	X				X	X
NAT HIV, HBV, HVC			X	X		X	X		X
Anti-RBC antibodies		X	X	X	X	X		X	X
Haemolysins	X	X	X	X	X	X	X	X	X
Additional testing	WNV** Chagas* Malaria*		Chagas* Malaria*	WNV**		WNV** Malaria*		Ab against HEV	Zika*** Babesia*** WNV** Chagas
Pre-screening validity (months)	3	One deployment	12	1	Tested each donation	3 to 12	6	7	12

Mali did not report specifics about donor pre-screening.

NAT: nucleic acid testing.

*if required by past medical history.

**West Nile Virus NAT, seasonal.

***all three by NAT.

rapid ABO testing of donor and recipient prior the transfusion. Tests used for ABO grouping are either the EldonCard (Eldon Biologicals A/S) or the ABTest Card (Diagast). Regarding testing of donors for donor for infectious disease, rapid blood born virus (HIV, HBV (HBs Ag) and HCV) testing is required by five (50%) countries, with the UK also performing syphilis (VDRL) assay and malaria testing when required. Finally, eight (80%) countries obtain samples from donors at the time of donation and send to a reference laboratory to perform deferred infectious screening.

WB specifications

Questions: *What type of bag is the whole blood collected into (manufacturer and model)? Does the whole blood undergo leukodepletion? Which device is used? Is a pathogen inactivation technology used? Which device is used? What type of administration set is used? Does the administration set incorporate a filter? What type of filter is used?*

Whole blood is collected in single bag without leukoreduction in all but one country. The latter uses a platelet sparing leucodepletion developed to filter whole blood for civilian uses, in these situations, while leukoreduction is feasible when the WBB programme is activated in non-emergency situations. In terms of storage solutions, CPD and CPDA1 are equally used, with some using both. There are differences regarding the use of the whole blood: some devote the collected whole blood to a specific recipient and no blood banking is allowed, whereas some collect the whole blood and can store the units in a blood bank for up to 28–35 days, depending on the storage solution. For the first hours post collection, whole blood is maintained at room temperature (up to 24 h) before storage at 4°C (Table 2).

Haemovigilance

Questions: *Is there a haemovigilance reporting system implemented for the WBB programme? Describe the organization/structure/process of reporting? How are incidents reported? Who is responsible for reporting? Have there been any incidents/near misses reported? What is the nature of these incidents? What is the process for managing incidents/near misses if they have occurred? Where incidents/near misses have been reported has corrective action been taken? Where incidents/near misses have been reported, have changes occurred to prevent future incidents?*

Haemovigilance is the process by which transfusion related adverse effects and near misses are reported. Reporting is mandatory, from blood collection to

Table 2 Characteristics of WB collection device and instructions of use

	Canada	Czech Republic	France	Israël	Mali	Norway	Poland	Sweden	UK	USA
Whole blood collection bag	Fenwal 450 ml	CompoFlex®	CompoFlex®	Chinook Medical	Dual bags	Terumo ± Imuflex	Terumo	Terumo	CompoFlex®	Fenwal
Additive solution	CPD	CPD	CPDA1	CPD	CPDA1	CPD CPDA1	CPDA1	CPDA1	CPDA1	CPD CPDA1
Leukodepletion	No	No	No	No	No	No/Yes	No	No	No	No
Blood banking allowed?		X			X	X	X	X	X	X
Maximal storage (days)	<1	Not defined (14–21)	1–2	0	30	35	35	35	28	35 (CPDA-1) 21 (CPD)
Storage temperature	4°C	2–6°C	2–6°C After 6 h at RT	Not specified	Not specified	2–6°C After 8 h at RT	2–6°C	2–6°C After 6 h at RT	4°C After 24 h at RT	1–6°C

CPD : citrate phosphate dextrose; CPDA: citrate phosphate dextrose adenine; RT: room temperature.

post-transfusion, and includes blood processing steps. Haemovigilance helps ensure the highest level of blood transfusion safety. The set-up of a haemovigilance system for a WBB programme is highly desirable but faces difficulties in the austere, remote and battlefield settings in which the WBB programme is activated. Half of the countries report using a haemovigilance system merged with the civilian system in their country. The responsible physician in the field is in charge of reporting adverse events. Only one country declared the availability of an online reporting system, which may be more efficient. No adverse events were reported. Data regarding overall WBB activity is shared/available from a few countries and highlights the fact that the activation of the WBB is an exceptional event.

Training

Questions: *How is training delivered to implement and maintain the WBB programme? Who is responsible for overseeing this training? Who performs the training? Who is trained? How long does the training take? How long is the training valid?*

As highlighted previously, the activation of the WBB is a rare event, occurring mainly in austere settings in response to an emergency. Therefore, comprehensive training is required for all personnel involved in the activation of the WBB programme. All countries have well-developed training programmes under the coordination of their national authority. Some have developed specific courses on the topic. One country reports remote training, close to the place of WBB programme activation under the supervision of the blood programme officer. Personnel who are trained include nurses, laboratory technologists, paramedics and physicians. The duration of the training and frequency is variable from one country to another (Table 3).

Summary

WBB programmes were developed to address blood supply shortages and ensure optimal care of bleeding patients by providing blood transfusion as early as possible. However, safety requirements in transfusion medicine are some of the most stringent in medical care. The implementation of a WBB programme has to address these constraints, ensuring the highest safety in the most austere and emergency situations. This challenge was reported by all 10 responders in this forum. Interestingly, all protocols for WBB programmes include methods for pre-screening and activating donors but the use of collected blood and the monitoring for adverse reactions reveal several different approaches. The approach

Table 3 WBB programme training duration and period of validity

	Canada	Czech Republic	France	Israël	Mali	Norway	Sweden	UK	USA
Training duration	2 days	5 days	2 days	A few hours	5 days	3–5 days	1 day	5 days	Defined locally
Period of validity	1–3 years	Not defined	2 years	As long as they are in position	Not defined	Not defined	Not defined (1 year)	2 years	Defined locally

to mitigating transfusion-transmitted infections at the point of collection differs between countries and no consensual guidelines exist regarding the fate of non-transfused blood products. This forum also highlights the infrequent activation of WBB programmes, making training and skill maintenance a key priority. Currently, civilian WBB programmes must plan for mass casualty events where the local blood supply could become exhausted or (re) supply disrupted due to terror attacks or natural disaster. These reflections around WBB programmes are highly interesting. Such programmes may even have relevance for space exploration [7]!

References

1 Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. J Trauma 2006;60 (6 Suppl):S3–11.
2 Shackelford SA, Del Junco DJ, Powell-Dunford N, Mazuchowski EL, Howard JT, Kotwal RS, et al. Association of prehospital blood product transfusion during medical evacuation of combat casualties in afghanistan with acute and 30-day survival. JAMA 2017;318:1581–91.
3 Sperry JL, Guyette FX, Brown JB, Yazer MH, Triulzi DJ, Early-Young BJ, et al. Prehospital plasma during air medical transport in trauma patients at risk for hemorrhagic shock. N Engl J Med 2018;26:315–26.
4 Cardenas JC, Zhang X, Fox EE, Cotton BA, Hess JR, Schreiber MA, et al. Platelet transfusions improve hemostasis and survival in a substudy of the prospective, randomized PROPPR trial. Blood Adv 2018;2:1696–704.
5 Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015;313:471.
6 Spinella PC, Cap AP. Whole blood: back to the future. Curr Opin Hematol 2016;23:536–42.
7 Nowak ES, Reyes DP, Bryant BJ, Cap AP, Kerstman EL, Antonsen EL. Blood transfusion for deep space exploration. Transfusion 2019;59:3077–83.

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INTERNATIONAL FORUM

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International Forum on Walking Blood Bank Programmes: Responses

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United Kingdom

Section A – Structure of WBB programme

Question 1

Not within the UK but there is a Military equivalent WBB programme for use overseas deployments.

The UK has a well-established National Blood Service which can provide blood and blood components at very short notice to fulfil a requirement.

1.1 Military.
1.2 Each deployment will have a minimum of 2 people to manage the WBB. Pre-screening and administration within the UK require an additional 4 people.
1.3 Pre 1990.
1.4 Pre-screening of volunteer donors is regulated by the UK Medicines and Healthcare Products Regulatory Agency (MHRA) following EU Directives. Donation in the field follows as closely as possible to the above regulations an urgent requirement and environmental considerations may be waived in emergencies.

Section B – Personnel/Command and Control

Question 2

In order to satisfy clinical need for whole blood or platelets. In the event of components being unavailable due to logistical difficulties.

Question 3

Senior Clinician in the area.

Question 4

Biomedical Scientist (BMS) / suitably trained healthcare professional.

Question 5

Information on Qualifications.

5.1 UK DMS delivered Blood Donation Storage and Supply Course (BDSS).
5.2 UK DMS delivered Blood Donation Storage and Supply Course (BDSS).
5.3 Health and Care Professions Council (HCPC) Registration as Biomedical Scientist.
5.4 Health and Care Professions Council (HCPC) Registration as Biomedical Scientist.
5.5 Health and Care Professions Council (HCPC) Registration as Biomedical Scientist.
5.6 Registered medical professional (Drs, Nurse, Operating Department Practitioner (ODP)).

Section C - Pre-screening of potential donors

Question 6

Yes, in the UK.

6.1 Blood Group × 2, Allo-antibody Screen, HIV 1 + 2, HCV, HBV, HeV, HTLV 1 + 2, Syphilis, High Titre Anti A and B.
6.2 All above.
6.3 National Health Service Blood and Transplant (NHSBT) Filton.
6.4 Results are electronically transferred by NHSBT to UK DMS. Donor care is part of the NHSBT service.
6.5 7 Months.

Section D – Pre-transfusion point of care (POC) testing

Question 7

BMS managed WBB = Compatibility testing, Blood group, POCT prior to transfusion.

Question 8

Yes, if BMS managing WBB, No, if not:

8.1. Diamed Gel ID system.

Question 9
No.
9.1. NA.

Question 10
Yes.
10.1. Abbott Determine HIV 1 + 2, Abbott Determine HbsAg, Abbott Determine Syphilis, SD Bioline HCV, (Binax Now Malaria).
10.2 RDT.

Question 11
Yes.
11.1 HIV, HIV, HCV, Syphilis.
11.2 NHSBT Filton.
11.3 Defence Medical Services (Centre of Defence Pathology).

Section E – Whole blood specifications

Question 12
Fresenius Kabi Compo Flex Single System.

Question 13
No.
13.1. NA.

Question 14
No.
14.1. NA.

Question 15
NA.
15.1. Yes.
15.2. In-line leukodepletion filter.

Section F – Fate of whole blood unit

Question 16
No.
16.1 NA.
16.2 24 h at RT + 48 h @ 4°C, or 28 days @ 4°C.

Question 17
Yes.
17.1 In Blood Bank.
17.2 Validated cool box.
17.3 Up to 28 days @ 4°C.

Section G – Current WBB programme activity

Question 18
Data for WWB Program.
18.1 552.
18.2 No comment.
18.3 No comment.
18.4 No comment.
18.5 No comment.
18.6 No comment.
18.7 No comment.
18.8 No comment.

Section H – Haemovigilance

Question 19
Yes, UK National SABRE/SHOT programs.
19.1 Online reporting to MHRA.
19.2 Via web page.
19.3 Responsible Person (Blood) via Centre of Defence Pathology.

Question 20
No.
20.1 NA.

Question 21
Notification made to Defence Medical Service Blood Supply Organisation (Centre of Defence Pathology), who then progress to clinical and/or regulatory authorities as required.

Question 22
NA.

Question 23
NA.

Section I – Training

Question 24
Information on training to implement and maintain the WBB programme:
24.1 The UK Defence Academy.
24.2 HCPC Registered BMS, Registered Nurses.
24.3 Healthcare Professionals required to undertake WBB.
24.4 5 days.
24.5 2 years.

Question 25
None [1].

References

1 Guidelines for the Blood Transfusion Sciences https://www.transfusionguidelines.org/red-book.

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Mali

Section A – Structure of WBB programme

Question 1

Yes, a WBB program exists in my country. The program is included in the activities of the Centre National de Transfusion Sanguine (CNTS) of Bamako (National Blood Transfusion Center).

1.1 The WBB program is civilian.
1.2 Fifty persons are required to run the WBB program.
1.3 The WBB program started in 2000.
1.4 The CNTS is a Public Scientific and Technological Establishment created by Ordinance No. 00-041/P-RM of September 20, 2000, ratified by Law No. 01-027 of June 01, 2001. It is headed by a Director General, under the responsibility of the Ministry of Health.

Section B – Personnel/Command and Control

Question 2

The situations in which we expect to activate the WBB program: situations of wounded wars in the context of insecurity in our country, traffic accidents resulting in several wounded, other disasters necessitating transfusions, in Bamako or outside, and during the rainy season (June to October) when malaria endemic peaks.

Question 3

The Director General of CNTS will be responsible for activation of the WBB.

Question 4

The head of the department of promotion and blood collection will organize/co-ordinate donor collection.

Question 5

The qualifications of individuals performing the following are:

5.1 Doctors, medical assistants, transfusion-trained medical interns.
5.2 Nurses, health technicians.
5.3 Laboratory technicians, biologists.
5.4 Laboratory technicians, biologists.
5.5 Laboratory technicians, biologists.
5.6 Doctors, nurses.

Section C – Pre-screening of potential donors

Question 6

Yes, potential WBB blood donors are pre-screened.

6.1 The tests performed are haemoglobin pre-donation.
6.2 Hemoglobin pre-donation is the only test mandated.
6.3 The tests are performed by laboratory technicians or nurses.
6.4 The results are communicated confidentially by the doctor in charge of the medical selection or the doctor in charge of the collections.
6.5 The TTI pre-screening is valid until next blood donation.

Section D – Pre-transfusion point of care (POC) testing

Question 7

Generally the compatibility of blood transfusion is used for sickle cell patients, women of childbearing age, and pediatric patients.

Question 8

The compatibility is made in indirect test with antiglobulin gel filtration technique.

8.1 The device used is a gel card centrifuge of the brand Diamed.

Question 9

No other test is performed on the recipient pre-transfusion.

Question 10

Yes, TTI tests are performed on the donor prior to transfusion.

10.1 Tests performed are: HIV antibodies, HBs antigen, HCV antibody, syphilis.
10.2 The device used is ARCHITECT ABBOTT.

Question 11
Yes, donor/recipient samples are also taken for testing at a later date.

11.1 Tests performed are: blood group antigens.
11.2 The tests are done at the CNTS by the technicians and the biologists.
11.3 The responsible for these tests is responsible for the laboratory.

Section E – Whole blood specifications

Question 12
The bags used are dual bags containing CPDA-1 anticoagulant.

Question 13
No, the whole blood does not undergo leukodepletion.

Question 14
No, a pathogen inactivation technology is not used.

Question 15
The type of administration set is not used.

15.1 No, the administration set does not incorporate a filter.

Section F – Fate of whole blood unit

Question 16
No, the unit can be kept.

Question 17
Yes, the whole blood can be stored.

17.1 It can be stored in a refrigerated container.
17.2 The logistical chain from donor to storage is transport in coolers.
17.3 They can be stored at the right temperature (2–6°) for a month.

Section G – Current WBB programme activity

Question 18
Data are available in the CNTS activity report (reference: Rapport d'activités, 30ème session du Conseil d'Administration du CNTS 2018).

18.1 The number of donations collected is 55 935, of which 27% volunteers and 73% from the compensation.
18.2 The number of deferrals for infectious disease was 8960, of which 1·9% HIV, 10·17% hepatitis B, 3·9% hepatitis C and 0·03% syphilis.
18.3 The number of deferrals for immuno-haematological markers was 32 for grouping errors and 180 for cases of transfusion incompatibilities.
18.4 All donations were collected in total blood.
18.5 Only 12% of donations have been in a WBB.
18.6 The number of patients is not known for whom the WBB was activated.
18.7 The number of whole blood units collected was 6473.
18.8 The number of blood units transfused is not known.

Section H – Haemovigilance

Question 19
There is no haemovigilance reporting system implemented for the WBB programme.

Question 20
No incidents/near misses have been reported.

Question 21
There is no process for managing incidents/near misses if they occur.

Question 22
Not applicable.

Question 23
Not applicable.

Section I – Training

Question 24
The training delivered to implement and maintain the WBB programme, is provided in the CNTS compound.

24.1 The Director and head of training are responsible for overseeing this training.
24.2 Doctors and pharmacists specialized in blood transfusion perform the training.
24.3 Nurses and technicians are trained.
24.4 The training lasts a week with different modules.
24.5 The training validity is not determined.

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Sweden

Section A – Structure of WBB programme

Question 1

In the Swedish Armed Forces (SAF), a WBB program is recently implemented. The program is currently introduced for designated deployed forces.

1.1 The WBB is a military program, initially aimed for deployed forces but the plan is to have also a national program in crisis and catastrophe preparedness. The pre-screening and approval of WBB donors are done by a civilian blood establishment, according to the regulations for civilian blood donors.

1.2 In the planning and start-up phase only a few personnel have been involved; a specialist in Transfusion Medicine, two anesthesiologists in the SAF, the education dept.in SAF. The organisation is not finally set yet.

1.3 The program is implemented in the autumn 2019, for forces deployed to Mali.

1.4 The program follows the national regulations, as close as possible. Standard operation procedures (SOPs) regulating exceptions, necessary in austere environment, are approved by medical and organisational authorities in SAF.

Section B – Personnel/Command and Control

Question 2

The WBB program is primarily aimed for deployed forces where we have challenges with transportation of blood components. The program will be activated in urgent and critical situations when blood component therapy is not available in time to save lives.

Question 3

The Surgeon General in the SAF is over-all responsible for the program. At site activation will be done by the senior medical officer (SMO) or by a medical person in lead.

Question 4

The senior medical officer (SMO) is responsible to activate the program locally. Blood donors are pre-screened and approved before deployment and results are documented in a blood donor register at site. The medical staff have a one-day education in collection, testing and transfusion of whole blood.

Question 5

The staff qualified are licensed doctors and nurses who are trained to perform collection and testing. The competence will be documented and annual retraining will be required.

5.1 Donor selection, whole blood (WB) collection and testing are done by licensed doctors and nurses qualified for the assignment

5.2 See 5.1.

5.3 See 5.1.

5.4 If recipient testing is required it is done by licensed doctors and nurses qualified for the assignment.

5.5 Confirming compatibility between donor and recipient is done by licensed doctors and nurses qualified for this.

5.6 Transfusion of WB is done by licensed doctors and nurses qualified for this.

Section C – Pre-screening of potential donors

Question 6

Yes potential WBB blood donors are pre-screened and health questionnaires approved according to the same regulations as for civilian blood donors.

6.1 The routine tests for donor approval in Sweden are blood type, erythrocyte antibody screening and virus tests (HBsAg, anti-HIV, -HCV,-HTLV I/II, Lues), In addition NAT (HBV, HCV, HIV) and titer anti-A/B in blood type 0 blood donors are performed in the WBB donors.

6.2 See above.

6.3 The tests are performed at the Blood Bank at Karolinska University Hospital.

6.4 The test results are reported electronically in the blood bank system at Karolinska. The individual donors are approved by a Transfusion Medicine specialist and the results are sent to the appointed responsible medical person in the SAF.

6.5 The TTI pre-screening (HBsAg, anti-HCV,-HIV) is repeated before deployment if the results are more than 3 months old and valid for 6 months.

Section D – Pre-transfusion point of care (POC) testing

Question 7

In urgent situations, priority is to use low-titer anti-A/B blood group 0 donors. If the recipient is a known blood group 0, low-titer is not necessary. If the recipient is known RhD negative 0 RhD negative donors are prioritized, but not mandatory.

Question 8
In a situation when it is decided to use ABO identical blood confirmatory blood typing is required.
7.1 The device used is "One-man-kit Eldon card".

Question 9
We plan to test available blood donors at site once a month.
9.1 The tests performed are HBsAg (Vikia) anti-HIV, HCV, HBc (Medmira). The Medmira kit is not CE approved and will be changed.

Question 10
See above.

Question 11
Tests are taken if the donors have not been tested within the last month. Tests will be done at site with the available device. At the moment virus tests are not sent to Sweden for confirmatory tests but this may be added.
11.1 The tests performed are HBsAg (Vikia) anti-HIV, HCV, HBc (Medmira).
11.2 Trained nurses and doctors do the testing.
11.3 The senior medical officer (SMO) is the main responsible person.

Section E – Whole blood specifications

Question 12
The whole blood is collected in CDPA-1 Blood Bag (Terumo).

Question 13
The whole blood does not undergo leukodepletion.

Question 14
Pathogen inactivation technology is not used.

Question 15
The transfusion is administered in the collection bag.
15.1 A transfusion filter for blood products is used.

Section F – Fate of whole blood unit

Question 16
The unit is immediately transfused or put in a blood fridge.
16.1 The unit must be transfused in two hours or put in a blood fridge, temperature +2–6°C.
16.2 See above.

Question 17
Yes.
17.1 The whole blood unit should be stored in a temperature-controlled blood fridge.
17.2 The donated unit is either transfused or put in a temperature-controlled blood fridge.
17.3 The whole blood unit can be stored in a temperature-controlled blood fridge up to 35 days.

Section G – Current WBB programme activity

Question 18
A WBB program was implemented in autumn 2019.
18.1 Until now approximately 200 donors are prescreened and approved.
18.2 None of the prescreened donors are deferred for infectious disease.
18.3 One donor is deferred because of detected erythrocyte antibodies.
18.4 Until now, no whole blood collection procedures have been performed (November 2019).
18.5 A WBB has not been activated, until November 2019.
18.6 No patients have been transfused with a unit from a WBB until November 2019.
18.7 No whole blood units have been collected until November 2019.
18.8 No whole blood units have been transfused until November 2019.

Section H – Haemovigilance

Question 19
Yes.
19.1 All units and all transfusions will be documented and reported.
19.2 In the medical records and to the responsible transfusion specialist.
19.3 SMO or a medical person in lead is responsible for reporting.

Question 20
We have not really started and have not experience yet. Guidelines will be in place how to manage and report incidents and make corrective actions.
20.1 All incidents/near misses will be documented and reported.

Question 21
See above.

Question 22
See above.

Question 23
See above.

Section I – Training

Question 24
The information is provided below:

24.1 The transfusion specialist in collaboration with the Military medical training department in SAF has done the education. Retraining will be done by appointed medical persons. The details are not implemented.
24.2 The transfusion specialist in collaboration with the Military medical training department in SAF has performed the training.
24.3 Until now doctors and nurses for deployed forces have been trained.
24.4 The initial training is one day. The retraining is not designed in detail.
24.5 Details are not in place but at least annual retraining will be required.

Question 25
The Senior medical officer will have a one day exercise in blood tactical training and there is a blood tactical memo for guidance and planning.

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Section A – Structure of WBB programme

Question 1
Yes.

1.1 Military only.
1.2 Depends on situation:
  a. In ROLE 1 or 2 (types of field hospital/facility) 1 physician + min. 2 nurses + 2 next personnel.
  b. In special task force combat operation 1 medic + 1 CLS (Combat Life Supporter).
1.3 In special forces 2015, it will expand to other armies from 2020.
1.4 The legal framework is in process as military prescription, for military operation only (not issued yet).

Section B – Personnel / Command and Control

Question 2
1/ in ROLE.
Chief doctor.
Based on information about casualties and transport options to a higher level determines:

- number of donors
- donor selection

2/ in special task force combat operation.
Chief medic (SOCM 18D etc.) or commander SOST (after assessment of the injury) and determine appropriate donors and expected amount of needed blood.

Question 3
See above.

Question 4
See above.

Question 5
Qualifications of individuals:

5.1 See above.
5.2 Combat medic, CLS, nurses.
5.3 Combat medic, nurses, physicians.
5.4 Combat medic, nurses, physicians.
5.5 Combat medic, nurses, physicians.
5.6 Combat medic, nurses, physicians.

Section C – Pre-screening of potential donors

Question 6
Potential WBB blood donors pre-screened before deployment.

6.1 Blood type (check), blood-borne infections (VHB, VHC, HIV, syphilis), anti-erythrocytes irregular antibodies, titre anti-A/B (IgM).
6.2 See above.
6.3 Military University Hospital Prague.

6.4 Reported to commander or head of medical support.
6.5 For one deployment.

Section D – Pre-transfusion point of care (POC) testing

Question 7
1/ Check dog tag, 2/ check personal card 3/ perform anti-A / anti-B bed-side test.

Question 8
Yes.
8.1 Rapid ABO test (ABTest Card®, Diagast).

Question 9
Additional tests are optional.
9.1 Not yet determined.

Question 10
Repeat TTI tests are optional.
10.1 Optional - VHB, VHC, HIV.
10.2 The device is not yet determined.

Question 11
Yes.
11.1 Blood type ABO Rh, Anti-erythrocytes irregular antibodies, TTI – VHB, VHC, HIV.
11.2 Military University Hospital Prague.
11.3 Commander for transportation, Military University Hospital Prague for tests.

Section E – Whole blood specifications

Question 12
The Blood collection single bag with CPD (CompoFlex® Single System, Fresenius Kabi).

Question 13
No.

Question 14
No.

Question 15
Transfusion set (SANGOFIX® B, B.BRAUN).
15.1 Yes.
15.2 Mesh.

Section F – Fate of whole blood unit

Question 16
Yes.
16.1 As soon as possible / immediately.
16.2 No.

Question 17
Yes.
17.1 In active cooling boxes, in ROLE only.
17.2 If not transfused, blood is stored.
17.3 Not defined yet, probably 2–3 weeks.

Section G – Current WBB programme activity

Question 18
The data is provided below:
18.1 60 (in 2019).
18.2 None.
18.3 None.
18.4 There is no information.
18.5 There is no information.
18.6 There is no information.
18.7 There is no information.
18.8 There is no information.

Section H – Haemovigilance

Question 19
There is no hemovigilance reporting system implemented for the WBB programme as yet:

Question 20
There is no reporting system in place.

Question 21, 22, 23
Not applicable as there is no reporting system in place.

Section I – Training

Question 24
The detailed training program is in preparation, so far only operatively.
24.1 Not defined yet.
24.2 Department of Hematology and Blood Transfusion, Military University Hospital Prague.
24.3 Selected combat medics/nurses/CLS.
24.4 5 days.
24.5 Not defined yet.

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Canada

Section A – Structure of WBB programme

Question 1

Yes, a WBB program does exist in Canada.

1.1 The program is Military.

1.2 There is one full time program coordinator (Critical Care or General Duty Nursing Officer) in Canada and 3 additional staff to run the program overseas (2 nurses and 1 laboratory technologists). A medical specialist physician or surgeon acts as senior advisor to the program.

1.3 The current version of the program has been in operation since early 2015, but other versions have been in operation for some time. For example, a WBB program was in place to support Canadian Forces Health Services (CFHS) Operations when Canada had responsibility for the hospital on the Kandahar base (2006–2009). During this period, the WBB screening and testing program was done by CBS under a memorandum of understanding. Since 2006, the WBB program in support of the Canadian Forces has been operated jointly between Canadian Blood Services and the Canadian Forces.

1.4 The relevant federal regulatory authority, Health Canada, provides Blood Regulations which include specific requirements for pre-assessed donor programs (i.e. walking donor programs), notably in sections 86-91. Section 4(4) states that an establishment cannot transfuse allogeneic blood that is collected from a pre-assessed donor unless the establishment has complied with requirements outlined in those sections. These include ensuring that the program is carried out under the supervision of a medical director; and it is used only when no other alternative source of blood appropriate for the recipient is available, and the recipient's physician requests the blood for use in the emergency treatment of their patient. Donors in pre-assessed donor programs must have a donor identification code at the time of acceptance into the program and have their suitability assessed every three months against the establishment's authorized criteria including assessment of medical and social history to determine infectious disease risk (questionnaire and testing), and provision of information to donors concerning donation risk, obtaining the ABO/Rh type and testing for any clinically significant antibodies, and a mechanism to acquire post-donation information. In addition, blood components from a pre-assessed donor program shall not be placed in the regular inventory or used in settings other than emergency use.

Section B – Personnel / Command and Control

Question 2

The WBB program is activated by CFHS in deployed settings when blood stocks are depleted, platelets are required, or in settings where blood is unavailable. The program should not be triggered unless a physician has requested blood for a recipient. The mission must be designated for WBB activation by CFHS Director of Health Services Operations (DHSO).

Question 3

The Director of Health Services Operations of the Canadian Armed Forces is responsible for the existence, regulation and monitoring of the program. In the field hospital setting, a senior medical authority is responsible for activation of the WBB when there is an exhausted supply of component product and/or when platelets are required in the deployed setting.

Question 4

The senior medical authority has responsibility for organization and coordination of donor collection. Once the WBB has been activated, nurses to the screening, and technologists perform any point of care testing that is required. See Annex A.

Question 5

The qualifications of individuals are provided below.

5.1 The recruitment of donors is the responsibility of the walking donor program in the Canadian Forces. Once identified donors, are screened and tested by Canadian Blood Services staff.

5.2 Whole blood is collected by individuals with appropriate training, either phlebotomists, nurses, physicians, or special operations medical technicians.

5.3 Donor testing prior to deployment is conducted by Canadian Blood Services in their consolidated testing laboratories. Staff in those laboratories are full time testing staff, either laboratory assistants or registered medical laboratory technologists. Results are reviewed by a certified medical laboratory technologist.

5.4 Testing of recipients is controlled by the clinical care team and may be performed by registered medical technologists or other trained laboratory staff. Members who have received Fresh Whole Blood are screened for HIV, hepatitis B and hepatitis C at 3, 6

and 12 months after transfusion to monitor for seroconversion.

5.5 To confirm compatibility between donor and recipient, a crossmatch is performed by a laboratory technologist with appropriate training. In austere environments, an EldonCard may be utilized.

5.6 In deployment settings, the transfusion of whole blood to recipients may be performed by a medic who is generally a family practice or specialist physician or surgeon.

Section C – Pre-screening of potential donors

Question 6

Yes, WBB blood donors are pre-screened.

6.1 Potential donors undergo standard blood donor screening used for all donors at Canadian Blood Services. This includes a blood pressure check prior to donation. All donations are tested for blood group, syphilis, hepatitis B, hepatitis C, HIV, HTLV-1/2, and West Nile virus. Depending on the answers to the screening questions, a donor may also be tested for Chagas disease. Anti-A and anti-B titers are also determined in ABO group O donors.

6.2 All tests performed are mandated.

6.3 Tests are performed by Canadian Blood Services donor testing laboratory staff in the consolidated testing laboratories of Canadian Blood Services located in either Calgary, AB, or Brampton, ON.

6.4 Test results are reported back to the Canadian Armed Forced blood program coordinator indicating which donors are suitable for the program as well as any deferrals along with the reason and the duration of the deferral.

6.5 Screening is valid for a period of 3 months; however, a possible extension to 12 months is under consideration.

Section D – Pre-transfusion point of care (POC) testing

Question 7

The donor's blood type and any titres are determined by Canadian Blood Services at the time of donation. The recipient is blood typed by a technologist who also performs a crossmatch.

Question 8

Yes, a point of care test is used to confirm compatibility of donated whole blood and recipient prior to the transfusion.

8.1 Compatibility is confirmed using a gel card or Eldon Card.

Question 9

Yes, additional POC tests are performed on the recipient pre-transfusion.

9.1 Additional testing is performed for hepatitis B, HIV, hepatitis C, syphilis and malaria.

Question 10

Yes, repeat TTI tests are performed on the donor prior to transfusion.

10.1 Tests are performed for hepatitis B, hepatitis C, HIV, syphilis and malaria.

10.2 See Annex B for information on what device is used.

Question 11

Protocols dictate that samples should be taken for testing at a later date, however, protocol violations are not uncommon.

11.1 Tests for hepatitis B, hepatitis C, syphilis, HIV and HTLV-1/2 are performed.

11.2 Testing is performed in public sector laboratories in Canada.

11.3 The staff of the Canadian Armed Forces blood program are responsible for ensuring these tests are performed and results managed.

Section E – Whole blood specifications

Question 12

Blood is collected into a Fenwal CPD bag; product code is: 4R0012MC.

Question 13

No, the blood is not leukodepleted.

13.1 N/A.

Question 14

No, pathogen inactivation technology is not used.

14.1 N/A.

Question 15

See Annex B.

15.1 See Annex B.

15.2 See Annex B.

Section F – Fate of whole blood unit

Question 16

Yes, immediate transfusion is required.

16.1 Blood must be transfused within 24 h of collection and can only be used to support the specific patient for whom it was collected.

16.2 Blood from WBB donors must be maintained at 4°C.

Question 17

Yes, blood may be stored for a brief period of time.

17.1 Whole blood may be stored in a dedicated blood refrigerator that is under the control of the laboratory.

17.2 Blood goes from collection to laboratory to donor, or from collection to laboratory to storage.

17.3 Storage cannot exceed 24 h.

Section G – Current WBB programme activity

Question 18

Data (per annum) is provided below.

18.1 The number of pre-screened donors has varied greatly depending on the activities of the Canadian military. During the period of Canadian involvement in the Afghanistan conflict, between 360 and 845 donors were screened each year. Since the program was re-established in 2016, annual screening numbers have ranged from 176 to 315.

18.2 There have been 0 deferrals for infectious disease.

18.3 There have been 2 deferrals for immuno-haematological markers.

18.4 Less than 30 whole blood collection procedures have been performed.

18.5 A WBB has been activated 6 times.

18.6 Unknown.

18.7 25 whole blood units were collected.

18.8 All 25 units that were collected were transfused.

Section H – Haemovigilance

Question 19

No, a hemovigilance reporting system has not been implemented for the WBB programme.

Question 20

Yes, there have been incidents/near misses reported.

20.1 Protocol violations have been reported that would be considered incidents or near misses.

Question 21

A review is undertaken by the Health Services Operations of the Canadian Armed Forces.

Question 22

Yes, corrective actions were identified and applied.

Question 23

Yes, administrative and organizational, and operating procedural improvements have taken place.

Section I – Training

Question 24

Training information is provided below.

2.1 Training of Canadian Blood Services personnel involved in donor screening and donor testing is the responsibility of Canadian Blood Services. The responsibility for training of Canadian Forces personnel resides with the Director of Health Services Operations (DHSO).

24.2 For Canadian Blood Services staff, training occurs through formal staff training programs in an environment that is regulated by Health Canada. Canadian Blood Services also trains crown personnel (Canadian military personnel) in phlebotomy and donor screening. Other training of Canadian Forces personnel is the responsibility of the Blood Program officer under supervision of DHSO.

24.3 Trainees include registered nurses, laboratory technologists, special forces medics, family physicians, specialist physicians and surgeons in the Canadian Forces. At Canadian Blood Services, trainees include all screening and laboratory staff.

24.4 Training takes two days at the Canadian Forces. Training of Canadian Blood Services staff takes variable lengths of time depending on the exact type of training involved.

24.5 Some aspects of Canadian Blood Services training require annual renewal, other assessments are made by supervisors on a regular basis and retraining offered to any staff who require it. For Canadian Forces personnel, training is good for 3 years. Additionally, this group receives deployment training and mission-specific training.

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Cameroon

Section A – Structure of WBB programme

Question 1

No, a WBB program does not exist in Cameroon.

1.1 No plans are in place and there are no discussions about the issue to the best of my knowledge.
1.2 N/A.
1.3 N/A.
1.4 N/A.

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Belgium

Section A – Structure of WBB programme

Question 1

The Belgian Defence has not yet established a WBB program, but has developed a research program for the proper implementation of this concept, in order to meet Belgian needs and to allow collaboration with our NATO allies.

We plan to use the WBB in two different cases: In the first case, it will be used for small teams isolated from medical support. The second case is that of a supply chain disruption or overload.

The WBB concept when implemented cannot meet all the requirements of both the Belgian legal framework and the European directives.

1.1 Belgian Defence is currently considering developing a WBB program for military implementation only. There are certainly possible civilian applications but further discussion between both medical worlds are needed. The concept has to be first fully developed beforehand.
1.2 At this time, the research team has not assessed that part of the concept.
1.3 There is no precise planning for the implementation of the WBB, but a research project is in progress in order to take advantage of the WBB in a near future.
1.4 In Belgium, there is no specific legal framework for the WBB. Therefore, the national legal framework and the European directives for blood establishments and transfusion apply.

Section B – Personnel/Command and Control

Question 2

We plan to activate the WBB for small isolated teams deployed without nearby medical support or when the usual supply chain is disrupted or overloaded.

Question 3

The research team has not yet addressed this point, but the activation of the WBB will always be a medical responsibility.

Question 4

The organisation of blood collection will be under the responsibility of the physician in charge of the medical support on the field. This task can be delegated but the final responsibility will remain with the physician.

Question 5

The qualifications are planned as follows:

5.1 The selection of the donors is a medical responsibility but can be delegated to a properly trained medic.
5.2 The Whole Blood collection is a medical responsibility but can be delegated to a properly trained medic.
5.3 A properly trained and educated medic can perform donor testing.
5.4 A properly trained and educated medic can perform recipient testing.
5.5 The confirmation of the compatibility between donor and recipient is a medical responsibility but can be delegated to a properly trained medic.
5.6 The transfusion process is a medical responsibility but can be delegated to a properly trained medic in remote conditions. Assistance of a physician must be offered IOT advise the medic.

Section C – Pre-screening of potential donors

Question 6

Yes, we plan to pre-screen the blood donors.

6.1 The following tests will be performed: serological screening tests (HIV, HBV, HCV and syphilis) and viral genome detection tests (HIV, HBV, HCV) in accordance with the Belgian legal framework and the European directives.
6.2 The tests that we plan to perform are the mandatory tests.

6.3 The Military Medical Laboratory Capacity, the clinical biology laboratory of the Belgian Military Hospital, will perform these tests. These tests will be added to the mandatory pre-deployment tests.
6.4 The field detachment physician will receive the results so that he can manage a WBB if necessary. However, the unit physician will also receive the information so that he can ensure the dissemination of results and the medical follow-up of the troops under his responsibility.
6.5 For one year and/or before each deployment (in accordance to the NATO Blood Panel).

Section D – Pre-transfusion point of care (POC) testing

Question 7
A blood-grouping test will be performed on both the donor and the recipient to verify compatibility.

Question 8
Yes, we are planning for a point of care test to confirm compatibility of donated WB and recipient prior to the transfusion.
8.1 We plan to use the ELDON CARD as a POC test to confirm compatibility.

Question 9
At the current stage of the study, we do not plan to perform any further TTI tests on the recipient.

Question 10
Yes we plan to repeat TTI tests prior to transfusion.
10.1 We plan to carry out rapid tests for the serological screening of HIV, HBV and HCV.
10.2 The choice of the device remains to be determined in our study.

Question 11
We plan to organize a systematic return of samples taken from both donor and recipient, but we anticipate that they will not be systematically usable due to poor preservation.
11.1 This step is not yet implemented. We plan to perform serological screening tests for HIV, HBV, HCV and syphilis and viral genome detection tests for HIV, HBV, HCV in accordance with the applicable Belgian legal framework and the European directives.
11.2 The Military Medical Laboratory Capacity, the clinical biology laboratory of the Belgian Military Hospital, will perform these tests.
11.3 The Military Medical Laboratory Capacity, the clinical biology laboratory of the Belgian Military Hospital, will be responsible for carrying out the tests.

Section E – Whole blood specifications

Question 12
We plan to evaluate blood collection bags distributed by Fresenius Kabi and Terumo BCT with anticoagulant CPDA-1 type.

Question 13
Still to be determined but will probably not be carried. Indeed, in case of an emergency transfusion, the transfusion needs to be performed rapidly. The choice is based on the rapidity of transfusion in case of emergency collection. However, filtration time would delay the transfusion and decrease the chance of survival. Thus, filtration in the case of an emergency protocol does not seem to be of enough benefit [1].

Question 14
Still to be determined. The pathogen inactivation will probably not be carried out [2].

Question 15
The type is still to be determined.
15.1 The choice of the set will be discussed with anaesthesiologists.

Section F – Fate of whole blood unit

Question 16
The primary goal of Belgian Defence is to activate the WBB only when a transfusion is needed. However, we do plan to collect blood and store it in case there is an interruption or an overload of the resupply chain.
16.1 In this case, immediately means that the blood will be collected the moment the need arises.
16.2 These aspects have not yet been evaluated and will be addressed through the ongoing study [1, 3].

Question 17
We plan to store whole blood.
17.1 These aspects have not yet been evaluated and will be addressed through the ongoing study.
17.2 These aspects have not yet been evaluated and will be addressed through the ongoing study.
17.3 The shelf life of whole blood will depend on the duration of effectiveness of the platelets and according to the literature [2, 4].

Section G – Current WBB programme activity

Question 18
The Belgian WBB program is under development.

Section H – Haemovigilance

Question 19
Haemovigilance is an aspect that has not yet been studied. A feasibility study should enable us to highlight the key stages of a field haemovigilance process. This is essential to guarantee the safety of both the donor and the recipient.

19.1 To be determined.
19.2 To be determined.
19.3 A properly trained medic under the final responsibility of a physician could execute this task.

Question 20
N/A, as our program is under development.

Question 21
N/A, as our program is under development.

Question 22
N/A, as our program is under development.

Question 23
N/A, as our program is under development.

Section I – Training

Question 24
We would like to offer a training approach tailored to the staff as well as to the missions. As this is an exceptional programme and not a routine one, we insist on the importance of regular reminders and practical trainings.

24.1 The study team in close coordination with the team of the operational part of the Military Medical Laboratory Capacity will be responsible of the education and training of the key personnel.
24.2 The team of the operational part of the Military Medical Laboratory Capacity will be responsible for the training. Collaborations with key partners are to be expected.
24.3 Personnel who may not always have access to medical support and personnel responsible for transfusion processes in the field.
24.4 Still to be determined.
24.5 Depending on the level of qualification of the target personnel, the duration and frequency of the training will be adapted but regular reminders are needed for the reasons mentioned above.

References

Strandenes G, De Pasquale M, Cap AP, Hervig TA, Kristoffersen EK, Hickey M, et al. Emergency whole-blood use in the field: a simplified protocol for collection and transfusion. Shock 2014;41(Suppl 1):76–83. https://doi.org/10.1097/SHK.0000000000000114. PMID: 24365879

Pidcoke H, McFaul S, Ramasubramanian A, Parida B, Mora A, Fedyk C, et al. Primary haemostatic capacity of whole blood: a comprehensive analysis of pathogen reduction and refrigeration affects over time. Transfusion 2013;53(Suppl 1):137S–S149.

Jobes D, Wolfe Y, O'Neill D, Calder J, Jones L, Sesok-Pizzini D, et al. Toward a definition of «fresh» whole blood: an in vitro characterization of coagulation properties in refrigerated whole blood for transfusion. Transfusion 2011;51:43–51.

Davies R. Should whole blood replace the shock pack. J R Army Med Corps. 2015;1–3.

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France

Section A – Structure of WBB programme

Question 1
In France, the WBB program is under the responsibility of the French Armed Forces Health Service, under the leadership of the French Military Blood Institute (Centre de Transfusion Sanguine des Armées) [1]. Up to now, there is no civilian WBB program. The program is led by the Department of Clinical Operations, the director of the CTSA is responsible for all the process in the institute, included the WBB program. The program is embedded in the routine, no specific people is strictly devoted to it. The program started in 2006. The legal framework is a Ministerial Direction [2].

Section B – Personnel / Command and Control

Question 2, 3, 4, 5

The activation of the WBB is limited to overseas operations or high-risk mission of Armed Forces included counter-terrorist actions. The indications are restricted to the management of life-threatening emergencies due to haemorrhage: (1) in case of massive transfusion situation after the first 3 red-blood cells and lyophilized plasma transfusions in order to provide platelets to the patients, as no platelets are available for French Armed Forces physicians in austere settings and that it was showed that early transfusions of platelets are associated with survival benefit for trauma-related massive haemorrhage [3], (2) when haemostasis procedures failed to stop the bleeding or when platelets are required as assessed by laboratory testing, (3) in case of mass-casualties when the resources of the blood bank will be obviously overwhelmed or in case of shortage of the blood bank, whatever the reasons are. The activation of the WBB is under the responsibility of the intensivist (role 2) or, in remote situation (role 1), of the physician (general practitioner). The organization of the blood collection follows a well-described process under the responsibility of a physician and involved nurses and auxiliaries. Blood donor selection is preferentially done by a physician and could be delegated to a trained nurse. Whole blood collection is performed by a nurse or a physician. Donor testing is performed by a nurse, a lab technician or a physician. Recipient testing is performed by the nurse under the responsibility of the physician in charge of the management of the patient.

Section C – Pre-screening of potential donors

Question 6

Blood donors are preferentially selected among French servicemen who underwent a blood screening prior the deployment. Volunteers are informed during the pre-deployment medical consultation about the WBB program. If they agree, they undergo a medical selection for blood donors, according to the French regulation [4]. The purpose of this interview is to detect permanent deferral. If the volunteer fits the criteria then he will be tested as every blood donor [5]. All the analysis will be performed at the French Military Blood Institute (blood donation control laboratory): blood-typing, haemolysins (threshold 1/64), anti-erythrocytes irregular antibodies, antibodies against HIV1+2, HCV, HBc, HTLV1+2, syphilis, HBs antigen and nucleic acid testing for HIV1+2, HBV, HCV and HEV. Antibodies against malaria and Chagas disease are performed according to past medical history. Results are reported to the military physician who did the interview and are responsible to register this information in the medical form. This screening is valid up to 12 months.

Section D – Pre-transfusion point of care (POC) testing

Question 7, 8, 9, 10

Warm whole blood (wWB) is iso-group transfused when the recipient is blood typed according to the French regulation [6]. If the recipient is not blood-typed according to this regulation, then the wWB will be collected from an O-type donor without haemolysins. If such a donor is unavailable, the blood-type of the recipient will be assessed by rapid testing and transfused with a iso-group wWB. In any case, the blood type of the recipient and the donor will be checked by rapid testing (ABTest Card®, Diagast, France). Prior to transfusion blood samples are retrieved from the recipients to test infectious markers, as previously described, as well from the donor and tested according to the regulation in our blood donation lab. All the samples are forwarded to the CTSA for analysis. On the field, infectious rapid tests are performed on blood donors: HIV1+2 (INSTI™, Nephrotek), Ag HBs (VIKIA® HBsAg, BioMérieux, France until June 2020, replaced by Determine HBsAg2, Abbott), HCV (OraQuick® HCV, OraSure Technologies).

Section E – Whole blood specifications

Question 12, 13, 14, 15

Whole blood bags are purchased from Fresenius (COMPOFLEX, 600 ml, CPD1-1 63 ml). Neither leukodepletion, nor pathogen inactivation is performed. The wWB is transfused with a transfusion set (filter between 170 and 260 μm).

Section F – Fate of whole blood unit

Question 16, 17

Units have to be transfused within the first 6 h of collection and store at room temperature. If required, for medical evacuation for instance, units can be stored 24–48 h between 2 and 6°C. It must be specified that units collected are strictly devoted to a patient. It is not allowed to transfuse wWB unit to anticipate the blood collection if the recipient is unknown as well to transfuse a collected unit to another recipient. Each blood collection is initiated for a unique patient.

Section G – Current WBB programme activity

Question 18

The data regarding blood screening are not yet available. Around 20–50 procedures are performed each year, for the same number of recipients with an average of 4 units collected per process. From 2006 to 2019, no infectious markers (HIV, HCV or HBsAg) were identified in a procedure.

Section H – Haemovigilance

Question 19, 20, 21, 22, 23

The WBB program is integrated in the regular hemovigilance reporting system. On the field, the Chief Medical Officer is responsible for hemovigilance. He is responsible for the reporting of any adverse effects occurring during the whole procedure: from the selection of the donors to the safety of the transfusion to the patient. Every critical stages of the procedure are recorded on files that are forwarded to the CTSA: blood donation initiation (indications, circumstances, recipient), medical interview of the donors, rapid testing of the units and identity of the recipient. At our institute, those data are recorded into our transfusion software under the responsibility of the chief of the blood donation control lab. From 2006 to 2019, no infectious markers (HIV, HCV or HBsAg) were identified in any procedure.

Section I – Training

Question 24

Training is organized by the Val-de-Grâce Military Academy and all the training are delivered by the CTSA. The Chief of Clinical Operations is responsible for the design of the training in collaboration with Blood Donation Department and Blood donation control laboratory. Physician and nurses that are potentially deployed or are about to be deployed must follow the training. The training lasts 8 h on two consecutive half-days: a lecture is provided to teach the WBB program and practical exercises are done. Attendees do a simulation of a WBB donation under the supervision of a trained physician of the CTSA. A debrief is subsequently done. A written test evaluating every steps of the program ends the training. This training is valid for 2 years.

References

Sailliol A, Ausset S, Peytel E. Blood transfusion in emergency settings: French military health service experience. Transfus Clin Biol J Soc Francaise Transfus Sang 2010;17:279–83.

INSTRUCTION N° 509396/DEF/DCSSA/PC/BMA relative à la transfusion sanguine en situation d'exception. Du 7 août 2015 [Internet]. Available from: http://circulaires.legifrance.gouv.fr/pdf/2015/09/cir_40027.pdf.

Cardenas JC, Zhang XU, Fox EE, Cotton BA, Hess JR, Schreiber MA, et al. Platelet transfusions improve hemostasis and survival in a substudy of the prospective, randomized PROPPR trial. Blood Adv 2018;2(14):1696–704.

Arrêté du 17 décembre 2019 fixant les critères de sélection des donneurs de sang.

Code de la santé publique - Article D1221–6. Code de la santé publique.

Bulletin Officiel n°2002-18 [Internet]. [cited 2020 Apr 12]. Available from: https://solidarites-sante.gouv.fr/fichiers/bo/2002/02-18/a0181843.htm.

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Norway

Section A – Structure of WBB programme

Question 1

Yes, Norway has a WBB program.

1.1 Both Military (Norwegian Armed Forces Medical Services) and Civilian (on Civilian oil installations, and in the Department of Immunology and Transfusion Medicine (Blood Bank) at Haukeland University Hospital, Bergen, Norway).

1.2 To activate the program, only 1 person is required like in Military Far Forward operations and for the nurses at our oil installations. At the Blood Bank the physician on call will activate the WBB in collaboration with the Head of Department. To establish a WBB program, you need a collaborative effort between an adequately trained Blood Banker and the clinical and military personnel involved. We have training courses both for military and civilian WBBs run by the Blood Far Forward Group.

1.3 2009 (military special forces), 2015 (first civilian oil installation), 2017 the Blood Bank at Haukeland University Hospital.
1.4 Health Authorities and Norwegian Armed Forces.

Section B – Personnel/Command and Control

Question 2

Ongoing bleeding, and (1) no blood available, or (2) platelet containing blood product not available so that balanced blood transfusion cannot be given, or (3) blood component therapy do not satisfyingly provide hemostatic control.

Question 3

Military: Head of surgical team, if prehospital/far forward: trained medic. On civilian oil installations: Responsible nurse in collaboration with physician. In Blood Bank: Physician on Call and/or Head of the Department for the Blood Bank.

Question 4

Trained personnel (military and civilian oil installations); for military services: can be performed by specially trained non-authorised health personnel (medics); Trained personnel at the Donor Collection Centre (the Blood Bank).

Question 5

It is mandatory to have proper training and education to be allowed to performed WBB actions and procedures. For military services: can be performed by specially trained non-authorised health personnel (medics);

5.1 Trained personnel (military and civilian oil installations); Trained personnel at the Donor Collection Centre (the Blood Bank).
5.2 Trained personnel (military and civilian oil installations); Trained personnel at the Donor Collection Centre (the Blood Bank).
5.3 Trained personnel (military and civilian oil installations); Trained personnel at the Immunohematology section (the Blood Bank).
5.4 Trained personnel (military and civilian oil installations). Trained personnel at the Immunohematology section (the Blood Bank).
5.5 We use only low titre group O whole blood for our WBBs.
5.6 Trained personnel (military and civilian oil installations), Trained personnel at the Immunohematology section (the Blood Bank).

Section C – Pre-screening of potential donors

Question 6

Yes, potential WBB blood donors are pre-screened.

6.1 ABO type, titre anti-A and anti-B, Virus (HIV and hepatitis) testing. NAT testing for viral infections is not mandatory in Norway.
6.2 All above.
6.3 As close to the operations as possible and during mission (i.e. ABO-typing) for military and oil installations. Before donation and at the donation in the Blood Bank.
6.4 Tests taken before operations is performed and reported by approved laboratories and by the civilian Blood Bank with which the Norwegian Armed Forces and the oil company has established collaboration. Overall responsibility for the Military operations is Senior Medical officer. During missions, personnel with appropriate training (military and civilian oil installations) are responsible for the testing, reporting of results and donor care. In the Blood Bank the Head of the Department has the overall responsibility for all activity.
6.5 New testing at the time of donation.

Section D – Pre-transfusion point of care (POC) testing

Question 7

Only low titre group O blood is used.

Question 8

If time. But presently, only pretested and approved blood donors are used.

8.1 Eldon card.

Question 9

No additional POC tests are performed on the recipient pre-transfusion.

9.1 Not applicable.

Question 10

No repeat TTI tests are performed on the donor prior to transfusion.

10.1 Not applicable.
10.2 Not applicable.

Question 11

Yes. Samples are taken from donor at time of donation and 3 months afterwards.

11.1 Sent to laboratory by with which the Norwegian Armed Forces and the oil company has established collaboration.
11.2 See above.
11.3 See above.

Section E – Whole blood specifications

Question 12
In military operations and for civilian oil installations: Terumo CPDA-1 blood bags. For WBB at the Blood Bag, also Terumo CPD with Imuflex leukoreduction filter is used.

Question 13
Partly.
13.1 Terumo CPD with Imuflex leukoreduction.

Question 14
No, pathogen inactivation technology is not used.
14.1 Not applicable.

Question 15
Single spiked 200 micro.
15.1 Yes.
15.2 200 micro.

Section F – Fate of whole blood unit

Question 16
No.

Question 17
Yes, the whole blood can be stored.
17.1 Approved temperature monitored blood fridge.
17.2 Must be moved to cold storage (2-6 degrees Celsius) within 8 hours.
17.3 CPDA 35 days if stored under continuous temperature control during storage and transport.

Section G – Current WBB programme activity

Question 18
Apologize, we cannot provide data for this section.

Section H – Haemovigilance

Question 19
Yes.
19.1 Norwegian Hemovigilance Network, Norwegian Health Directorate. For military operations outside Norway, the Armed Forces Medical Services is responsible for hemovigilance reporting.
19.2 To the responsible Blood Bank which forward report electronically to the Norwegian Health Directorate.
19.3 Health personnel responsible for the medical treatment of the patient and performing the blood transfusion.

Question 20
No.

Question 21
Local action.

Question 22
Not applicable.

Question 23
Not applicable.

Section I – Training

Question 24
Please see below:
24.1 Norwegian Armed Forces Medical Services and in the Department of Immunology and Transfusion Medicine (Blood Bank) at Haukeland University Hospital, Bergen, Norway.
24.2 Norwegian Armed Forces Medical Services and in the Department of Immunology and Transfusion Medicine (Blood Bank) at Haukeland University Hospital, Bergen, Norway.
24.3 Personnel expected to perform WBB procedures.
24.4 Military and oil installations: 3–5 days education courses with follow-up training according to written requirements for the Military Medical Services or the Oil Company. Blood Bank personnel is trained according to Norwegian Health Authorities requirements and local procedures.
24.5 As for today, no time limit defined.

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Brazil

Section A – Structure of WBB programme

Question 1
No, a WBB program does not exist. Brazil is a very peaceful country, whose participation in conflicts or previous war is very uncommon. Although it has a great surface (actually, is the fifth largest country in the world), with military personnel scattered all over the country, no WBB program has been planned at this moment, as far as we are aware.

Mandatory tests are carried out for each donation covering the following agents/markers: Syphilis, Chagas Disease, Hepatitis B (HBsAg, anti-HBc and HBV-DNA), Hepatitis C (anti-HCV and HCV-RNA), Acquired Immunodeficiency Syndrome (AIDS – anti HIV1/2 and subtype O; HIV-RNA) and T-Lymphocyte Human Virus (HTLV) type I and II. These tests must be performed on a sample taken at the time of donation, with diagnostic kits licensed by the Brazilian Ministry of Health. The whole blood and its components cannot be transfused before the final non-reactive results are obtained.

1.1 Not applicable.
1.2 Not applicable.
1.3 Not applicable.
1.4 If planned one day in our country, it certainly shall be regulated by the Brazilian Ministry of Health Agency (ANVISA) norms and guidelines.

Section B – Personnel/Command and Control

Question 2
We think that natural disasters (although there are no hurricanes, tornadoes or earthquakes reported in the country), or a potential military conflict (either internal or external) might be a reason for establishing a provisional/permanent WBB in the future.

Question 3
Probably governmental authorities.

Question 4
This is unknown at the moment, but most likely the military forces will take an important role in this action.

Question 5
According to the current Brazilian Guidelines [1], all pertaining activities must be carried out under medical supervision, even though one can employ the working capacity of nurses, medical technologists, etc. Thus, there is no reason to consider a different procedure from the civil counterpart if a WBB is organized in Brazil.

Section C – Pre-screening of potential donors

Question 6
No, and at this moment it is not legally accepted in the country to have blood units transfused without a non-reactive result from a blood sample derived from the same donation.

Section D – Pre-transfusion point of care (POC) testing

Question 7, 8, 9, 10, 11
POC testing is not legally accepted in Brazil. Therefore, all questions from this section are not applicable in our country.

Section E – Whole blood specifications

Question 12, 13, 14, 15
There are several whole blood bags licensed in the country, although none is intended for WBB, at the moment. We consider that all questions below shall not be applicable, even though leukodepletion and pathogen inactivation (amotosalen) are currently licensed and utilized by several civilian blood services, where blood components are normally screened and stored.

Section F – Fate of WB unit

Question 16, 17
All questions are not applicable for Brazil.

Section G – Current WBB programme activity

Question 18
Not applicable.

Section H – Haemovigilance

Question 19, 20, 21, 22, 23
Not applicable, although there is a national hemovigilance program for civilian purposes.

Section I – Training

Question 24
Not applicable.

Question 25
Unless a major military conflict (highly unexpected for Brazil) affects our peaceful life, we consider that the possibility of WBB in our country is quite remote.

References
Brazilian Ministry of Health - PORTARIA DE CONSOLIDAÇÃO N° 5, DE 28 DE SETEMBRO DE 2017. Available at https://portalarquivos2.saude.gov.br/images/pdf/2018/marco/29/PRC-5-Portaria-de-Consolida----o-n---5--de-28-de-setembro-de-2017.pdf (last access February 27, 2020).

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Poland

Section A – Structure of WBB programme

Question 1
Yes, but only during abroad military missions (the first time in Afghanistan in 2011) but one year ago the Minister of Defense issued a regulation ordering the training of a walking blood bank as well as regulation ordering and authorizing paramedics (not only doctors and nurses as before) to collect and transfuse blood and blood components during crisis situations and to support military units (national and NATO units) - (Act: Dz.U. 2019, poz. 564 - published 26 Mar 2019).

1.1 At this moment only military.
1.2 Data are collected but I think we need about 100 medical persons and minimum 10 000 soldiers included to WBBP (dedicated for about 1 500 000 soldiers).
1.3 See above (2011 Afghanistan – ISAF).
1.4 At present only special forces use WBB procedure and training, we are still in the phase of preparation to introduce the pledge to WBB for use, a detailed program and procedures. We have legal bases for the army (see above) but not in civil applications.

Section B – Personnel / Command and Control

Question 2
Especially MASCAL, special operations e.g. SOST, abroad missions, regular military operations – level 1–2.

Question 3
Medical officer only (doctor) in regular operations, doctor or paramedic - in special operations and in level 1 during regular operations.

Question 4
Nurses, paramedics, sometimes doctors.

Question 5
Information on qualifications:

5.1 Doctors (in special operations paramedics under doctor supervision – e.g. mobile).
5.2 Nurses, paramedics, only in need doctors.
5.3 Lab technicians (before deploy and in level 2–3), nurses, paramedics (level 1 and in SOF).
5.4 None.
5.5 Only blood group checking (in level 2 and 3 possible retrospective or in time cross matching).
5.6 Paramedics, nurses, doctors (medics in SOF in some situations).

Section C – Pre-screening of potential donors

Question 6
Yes, potential WBB blood donors are pre-screened.

6.1 HCV, HIV, HBV (elisa and molecular biology TMA method – single donation), in addition TP/Syphilis (elisa) – at beginning and before deploy, every 3 months after deploy and probably will be minimum every one year in WBBP in country. Additional tests according area of operation (eg. Malaria, WNV).
6.2 HCV, HIV, HBV (elisa and molecular biology TMA method – single donation).
6.3 All WBBP members.
6.4 All results and archive probes must be sent to our facility and keep and store 30 years.
6.5 3 month for predeploy and 12 months will be or shorter for WBBP members in Poland.

Section D – Pre-transfusion point of care (POC) testing

Question 7, 8, 9, 10, 11
Not applicable.

Section E – Whole blood specifications

Question 12
Terumo blood bags (single with CPD-A).

Question 13
No, but it is possible.

Question 14
Only for blood components (Mirasol, Methylene Blue).

Question 15
We use also special device e.g. Belmont 2000.
15.1 Yes.
15.2 Different.

Section F – Fate of whole blood unit

Question 16
No, the unit is not required to be transfused immediately.
16.1 As soon as possible, but if no 72 h from collection – we will change it to longer time.
16.2 Yes, temperature 2–6°C.

Question 17
Yes, the WB can be stored.
17.1 In a dedicated medical cooler (2–6°C).
17.2 Collection in dedicated place (e.g. Container, Tent) or in the field then must be store in hand bag and transferred immediately to trauma room or stored in fridge (72 h 2–6°C).
17.3 See above.

Section G – Current WBB programme activity

Question 18
We collected about 100 units of WB and transfused about 70 units in Afghanistan only. Present we will started.

Section H – Haemovigilance

Question 19, *20, 21, 22, 23*
All blood reports must be sent to us (Warsaw – Military Blood Center) and must be stored in a special database (dedicated system called Military Blood Bank System – present providing by ASSECO Company but we will preparing a new system).

Section I – Training

Question 24
See above, we will be responsible in near future.

Question 25
See above.

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Section A – Structure of WBB programme

Question 1
No, a WBB program does not exist in our country.

The need for a civilian WBB is currently not publicly established/evaluated. Considerations are made for a military WBB in a deployed setting, but no short-term implementation is planned. The developments and evolutions of WBB programs in our main partner nations are closely followed.

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Elon Glassberg & Eilat Shinar

Israel

Section A – Structure of WBB programme

Question 1
Partially. Our WBB program is only implemented in extreme and rare cases, when a military unit will need blood but may be completely disconnected for any supply. To achieve that all combatants in that unit are tested in Magen David Adom National Blood Services (MDANBS) laboratories for TTD and blood type, to identify those with O blood group. Suitable blood bags are supplied and the paramedics and/or a military surgeon are trained to collect blood according to SOPs.
1.1 Military.
1.2 Two: one in the military and one in the national Blood Services. None of them as FTE.
1.3 1997, in the described format.
1.4 MDANBS and Medical Corps regulations.

Section B – Personnel/Command and Control

Question 2
Specific military scenarios (e.g., remote and isolated locations.

Question 3
Military surgeons.

Question 4
Military surgeons.

Question 5
Information on qualifications:

5.1 Donor selection pre-screened and selected by MDANBS phlebotomists and the IDF military surgeons.
5.2 Military surgeons and paramedics.
5.3 Donor testing is performed only as pretesting by MDANBS qualified laboratory technicians. No testing is performed at the time of donation.
5.4 We do not do recipient testing.
5.5 None.
5.6 Military surgeons.

Section C – Pre-screening of potential donors

Question 6
Yes.

6.1 ABO, Rh typing. In the coming year we plan that all type O male donors will be titered for anti A & anti B titers in order to identic compatible donors for LTOWB.
6.2 ABO, Rh, RBC Antibody screening and TTD detection (HIV, HBV HCV and HTLV using Chlia, and ID-NAT for HIV, HBV, HCV and seasonal WNV).
6.3 By MDANBS qualified laboratory technicians.
6.4 The responsible medical doctor in MDABS reports to the military surgeon who are the O type suitable donors.
6.5 For 30 days, as per the national SOPS.

Section D – Pre-transfusion point of care (POC) testing

Question 7
Pre-identification of the O type donors, as described.

Question 8
No.

Question 9
No.

Question 10
No.

Question 11
No. The recipient blood type will be determined in the Hospital Blood Bank, to which he is evacuated and data regarding the transfusion from O type WBB reported.

Section E – Whole blood specifications

Question 12
Field Blood Transfusion Kit (TMM-FBTK) by Chinook Medical Gear, Inc.

Question 13
No.

Question 14
No.

Question 15
Not applicable.

15.1 No. only filter for RBC aggregates, which is a part of the transfusion set.

Section F – Fate of whole blood unit

Question 16
Yes, units are meant only to be collected and used at times of urgent need.

16.1 As soon as the completion of the donation.

Question 17
No. MDANBS SOPs allow storing whole blood at RT for up to 8 hours. However, as the temperature in the austere conditions is not monitored, whole blood should be used immediately after the collection from the WBB.

Section G – Current WBB programme activity

Question 18
Data provided for 2019.

18.1 70, 32 donors were O type (46%), 12 of them were defined as Low Titer (<50).
18.2 None.
18.3 None.
18.4 None.

18.5 None.
18.6 None.
18.7 None.
18.8 None.

Section H – Haemovigilance

Question 19
No.

Question 20
As applying WBB in the battlefield is extremely rare, we have no data of such incidents.

Question 21
As applying WBB in the battlefield is extremely rare we have no data on incidents/near misses.

Question 22
As applying WBB in the battlefield is extremely rare we have no data on incidents/near misses.

Question 23
As applying WBB in the battlefield is extremely rare we have no data on incidents/near misses.

Section I – Training

Question 24
Information on training:
24.1 The military in cooperation with the medical doctor from the MDANBS.
24.2 Senior phlebotomists from the MDANBS.
24.3 Military Paramedics and Surgeons.
24.4 Few hours.
24.5 As long as they are in position.

Question 25
In general WBB is not encouraged in Israel. Both military and civilian paramedics are authorized to use TXA and FDP in cases of massively bleeding patients during ground transportation to the Medical facilities. In case of air-evacuation the military helicopters carry cold stored LTOWB, and the team is qualified to transfuse them during the flight. We believe that only in rare incidents, where Special Forces, need to transfuse a massively bleeding patient in a remote or austere location, and have no cold stored LTOWB or blood components (FDP), the WBB program can be exercised. This can be done on previously screened volunteer blood donors, by trained paramedics and physicians.

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United States

Section A – Structure of WBB programme

2 Does a WBB program exist in your country? No. There is not an official WBB program in the US. There is currently a WBB program for the Military that we are currently working to formalize into an official program.

If there is not a WBB program established but is planned, what is the expected structure? If no WBB program is in place or planned, please outline reasons for this.

2.1 Is the WBB program Military or civilian? Military
2.2 How many personnel are required to run the WBB program? Size depends on medical unit deployed and capabilities. Can be done with as few as 1–2 staff or much larger at R3 or R2 with up to 8–12 staff members.
2.3 When did the WBB program commence? WBB has been used by the US Military since WWI. Current concept that is practiced today was initiated during OIF/OEF in early to mid-2000's. Clinical Practice Guideline provided by the Joint Trauma System (JTS) provides an SOP for deploying units to use for WBB setup and execution.
2.4 What is the legal framework under which the WBB program is regulated/governed? US DoD and Health Affairs policies acknowledge deployed forces may need to execute a WBB for use of non-FDA approved blood products. JTC CPG provides guidance.

Section B – personnel/command and control

3 What are the situations in which you expect to activate the walking blood bank program? The goal is to always provide FDA Licensed Blood Products (to include Low Titer Group O Whole Blood) to support patients as well as to provide those products as far

forward and as close to the Point-Of-Injury as possible. Based on the current inventory levels and available re-supply, a decision will be made to activate the WBB.

4 Who will be responsible for activation of the WBB? Senior Medical Officer overseeing patient care.

5 Who will organize/co-ordinate donor collection? Medical Team/Unit Leadership to include Medical Officer and NCOs.

6 What are the qualifications of individuals performing the following? Individuals may be any medical series provider properly trained on WBB tasks. Individuals do not have to be credentialed providers for collection of WB. The order to transfuse a unit of collected WB is under the oversight of a credentialed medical provider.

6.1 Donor selection

6.2 Whole Blood (WB) collection

6.3 Donor testing

6.4 Recipient testing

Confirming compatibility between donor and recipient - same as noted above, can be a properly trained medical staff member.

6.5 Transfusion of WB to recipient - transfusion must be ordered under the oversight of a credentialed medical provider but may be performed by medic or credentialed provider.

Section C – Pre-screening of potential donors

7 Are potential WBB blood donors pre-screened? If yes:

7.1 What are the tests performed? FDA required tests: ABO/Rh; ABS; all required infectious disease screening tests (HIV-1 NAT, HBV NAT, HCV NAT, anti-HIV ½, anti-HBc, HBsAg, anti-HCV, Zika NAT, Babesia NAT, anti-HTLV I/II, anti-T. cruzi, Babesia NAT, syphilis; anti-A/anti-B for type O donors

7.2 Which tests are mandated? Same as above.

7.3 Where and by whom are the tests performed? Performed at ASBP blood donor centers prior to military unit deployment or within a theatre after unit has deployed.

7.4 How are the test results reported and who is responsible for dissemination of results and donor care? Results recorded in Theater Medical Data Store/TMDS and communicated as needed to unit senior Medical Officer for donor care.

7.5 How long is the TTI pre-screening valid? 1 Year.

Section D – Pre-transfusion point of care (POC) testing

8 What are the rules used to determine compatibility of blood transfusion? Low titer type O donors are universal / type specific for all other

9 Is a point of care test used to confirm compatibility of donated WB and recipient prior to the transfusion? If yes:

9.1 What is the name of the device? Eldon Card or saline tube testing (R3 only)

10 Are additional POC tests (e.g. TTI screen) performed on the recipient pre-transfusion? If yes:

10.1 Which POC test(s) and device(s) are used? OraQuick Advance HIV ½ antibody & OraQuick HCV antibody and CTK Test Onsite HBsAg Rapid Test

11 Are repeat TTI tests performed on the donor prior to transfusion? If yes:

11.1 What tests are performed?

11.2 Which device is used?

12 Are donor/recipient samples also taken for testing at a later date? If yes:

12.1 What tests are performed? Sample are drawn, plasma/serum frozen and then shipped back to US for testing at military donor testing laboratory. All FDA required testing performed minus ABO/Rh

12.2 Who/where performs the testing? Lackland AFB Donor Testing Lab

12.3 Who is responsible for these tests? Donor Testing Lab emails test results to COCOM Joint Blood Program Officer for entry into TMDS and notification of donors as needed.

Section E – Whole blood specifications

13 What type of bag is the whole blood collected into (manufacturer and model)? Typically CPD or CPDA1 bags manufactured by Fenwal/it depends on type of supply system used by the deploying medical unit.

14 Does the whole blood undergo leukodepletion? If yes:

14.1 Which device is used?

15 Is a pathogen inactivation technology used? If yes:

15.1 Which device is used?

16 What type of administration set is used? Standard infusion/transfusion set.

16.1 Does the administration set incorporate a filter? If yes: Standard blood administration set with 170–260 micron filter.
16.2 What type of filter is used? 170–260 micron filter.

Section F – Fate of whole blood unit

17 Must the unit be transfused immediately? If yes:
17.1 How immediately define?
17.2 Are there any other limits set (i.e. Temperature).
18 Can the wholeblood be stored? If yes:
18.1 Where can it be stored? Units must be stored at 1–6°C in an approved storage container
18.2 What is the logistical chain from donor to storage?
18.3 How long can it be stored? CPDA-1 units have a 35 day expiration and CPD units have a 21 day expiration. Units must be stored at 1–6°C.

Section G – Current WBB programme activity

19 If a WBB program is currently active, please provide the following data (per annum).
19.1 Number of pre-screened donors: Will require additional research.
19.2 Number of deferrals for infectious disease: Will require additional research.
19.3 Number of deferrals for immuno-haematological markers: Will require additional research.
19.4 Number of whole blood collection procedures performed: Will require additional research.
19.5 Number of times a WBB has been activated: Will require additional research.
19.6 If known the number of patients for whom the WBB was activated: Will require additional research.
19.7 The number of WB units collected: Over 10 000 units of WB have been collected as of 31 August 2020.
19.8 Number of blood units transfused: Over 10 000 units of WB have been collected as of 31 August 2020.

Section H – Haemovigilance

NOTE: Information in current version of the Clinical Practice Guideline is provided

Guidelines for the Walking Blood Bank (WBB) for Fresh Whole Blood (FWB) are found in the Clinical Practice Guidelines (JTS CPG). This Whole Blood Transfusion (CPG ID:21) provides the most current rationale and guidelines for WB transfusion, including but not limited to product definitions, indications, collection, storage, testing, transfusion, and documentation. The first associated CPG was published on 1 October 2006 and then updated on 24 October 2012. This most recent version of the CPG was published on 15 May 2018.

The decision to use FWB is a medical decision that must be made by a physician who has full knowledge of both the clinical situation and the availability of compatible blood products. A WBB Program should be established based on a risk assessment and the potential for casualties.

In general, the use of FWB should be limited to casualties who are anticipated to require a transfusion when the physician determines that SWB or optimal component therapy is unavailable or in limited supply, or in patients that are not responding to SWB or component therapy. The decision to initiate a FWB drive should be made in consultation with the appropriate treatment facility medical authority and Laboratory/Blood Bank Officer-In-Charge. At smaller facilities, the lead surgeons and/or facility Officer-In-Charge should be consulted on the decision to initiate the WBB.

Pre-screened donors in the WBB Program determined to be suitable should be utilized, to the greatest extent possible, before using personnel who: (1) have been pre-screened or donated in the past but do not have current (within 90 days) screening and infectious disease testing; (2) have no pre-screen or donation history. All donors must be rescreened at the time of donation.

All donors should have their ABO/Rh verified (i.e. Eldon card or laboratory testing) at the time of donation. Before any FWB is transfused, rapid infectious disease testing (i.e. HIV, HBV, HCV) of donor specimens shall be performed, to the greatest extent possible. Retrospective samples must be sent to a licensed laboratory for FDA-approved testing, regardless of whether the rapid infectious disease testing is performed pre- or post-transfusion, as these tests are not licensed for donor testing.

Upon the notification of confirmed positive infectious disease results, a medical provider or preventive medicine personnel will be notified by the Area Joint Blood Program Officer to ensure that the donor is notified and counseled. Donors and unit commanders must understand the importance of donor tracing.

If a patient receives a confirmed positive infectious disease unit, the Area Joint Blood Program Officer will notify the Armed Services Blood Program Division immediately to initiate patient notification and an evaluation of both the donor and patient.

In accordance with Health Affairs Policy on the Use of Non-U.S. Food and Drug Administration, recipients of FWB shall receive follow-up advice and infectious disease

testing as soon as possible, and at 3-, 6-, and 12-months post-transfusion.

The Theater Medical Data Stores (TMDS) is currently utilized to record FWB donations and infectious disease testing results.

While there is currently no defined hemovigilance program established, there are reporting requirements and frequency for reporting established. Data sources include the patient's record, DoD Trauma Registry and associated blood collection and transfusion databases (currently TMDS).

20 Is there a hemovigilance reporting system implemented for the WBB programme? If yes:
   20.1 Describe the organisation/structure/process of reporting? The AJBPO and Joint Blood Program Officer (JBPO) are responsible for all reporting associated with the WBB program (overall), the collection process, processing of the donor units, rapid testing, infectious disease testing and required lookback procedures. Medical personnel are required for all patient reporting.
   20.2 How are incidents reported? If the incident occurs during the donor screening and collection process, it should be reported by personnel involved in that process and documented on the donor screening form. If the incident occurs during the unit processing and testing, it should be reported by those personnel and documented on the appropriate form. If the incident occurs during the blood administration process it should be reported by those personnel and documented appropriated.
   20.3 Who is responsible for reporting? Personnel who are directly involved in the incident or discover the error/incident are responsible for documenting and reporting. The JBPO has the overall responsibility to follow-up on any incidents to ensure that any related policy or training issues are addressed.

21 Have there been any incidents/near misses reported? If yes:
   21.1 What is the nature of these incidents? While the ASBP Division would be made aware, there is currently no formalized process to track this incidents at this level.

22 What is the process for managing incidents/near misses if they have occurred? Process would be defined locally at the Medical Treatment Facility and involve the JBPO.

23 Where incidents/near misses have been reported has corrective action been taken? Would require additional research.

24 Where incidents/near misses have been reported, have changes occurred to prevent future incidents? Would require additional research.

Section I – Training

NOTE: Information in current version of the Clinical Practice Guideline is provided

In practice, all forward-deployed Medical Treatment Facilities should establish a WBB. Coordination with the Area Joint Blood Program Officer (AJBPO) is required to establish a WBB Program. Training involves the following SOPs: Blood Donor Screening SOP and the Emergency Whole Blood Drive SOP. Both are contained in the CPG.

Since the need for FWB cannot be predicted, a robust contingency operational plan should be developed by the Medical Treatment Facility staff to include the Laboratory/Blood Bank and surgical and anesthesia providers in coordination with the Area Joint Blood Program Officer. The plan should be reviewed and rehearsed regularly. Equipment and consumables should be inspected with due attention paid to storage conditions and expiration dates.

A contingency plan should be developed for activating the WBB, collecting, storing, and transfusing FWB in MASCAL situations or when it may be deemed that the current blood inventory will be exhausted prior to re-supply.

The physical donation site will vary by location and should be organized in such a way as to maintain the integrity of the screening and donation process, and to minimize the possibility of clerical errors. This is especially important in emergency situations involving more than one casualty.

Every effort should be made to adhere to the same screening, drawing, labeling, and issuing standards required for U.S. FDA-approved blood products.

25 How is training delivered to implement and maintain the WBB programme?
   25.1 Who is responsible for overseeing this training? The AJBPO is area/location.
   25.2 Who performs the training? Training will be performed by designated personnel and may vary based on location. Training personnel can include the Area Joint Blood Program Officer, laboratory personnel or other assigned medical personnel.
   25.3 Who is trained? Designated assigned personnel will be trained on the WBB program. These personnel should be identified ahead of time and participate in all required training events. It

may be necessary to use other specialty assigned personnel to assist with the WBB so that laboratory personnel can complete the required labelling, rapid testing and associated paperwork.

25.4 How long does the training take? This is defined locally by the AJBPO with oversight by the Joint Blood Program Officer.

25.5 How long is the training valid for? This is also defined locally by the AJBPO with oversight by the Joint Blood Program Officer. The length of deployment will also be considered when establishing a local program.

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Malaysia

Section A – Structure of WBB programme

Question 1

No, a WBB program does not exist in Cameroon.

1.1 No plans are in place and there are no discussions about the issue to the best of my knowledge.

1.2 N/A.

1.3 N/A.

1.4 N/A.

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Supporting Information

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

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LETTER TO THE EDITOR

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Vox Sanguinis

High-titre anti-SARS-CoV-2 convalescent plasma donation after donors' vaccination

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Dear Editor:

Early treatment of COVID-19 patients with convalescent plasma (CP) with high anti-SARS-CoV-2 IgG antibody levels is associated with a significantly reduced mortality [1–3]. The FDA established that units of CP with a signal-to-cut-off ratio of ≥12 in the Ortho VITROS SARS-CoV-2 IgG test could be qualified as high titre, but cut-off ratio has been recently lowered to ≥ 9.5 [4]. Finding donors with adequate titre of antibodies can be difficult, especially among younger donors with mild disease. We tried to explore whether the arrival of vaccines against COVID could help find optimal CP donors.

Sixteen CP donors were tested for anti-SARS-CoV-2 IgG after COVID-19 vaccination. Women comprised 68.8% of the patients, with a mean age of 41.1 years (SD: 10.46). Fourteen had received the Pfizer® vaccine (4 a single dose and 10 two doses) and two one dose of Moderna® vaccine. Importantly, most of them had previously been excluded as donors because of low antibody levels. Donor serum antibodies were assessed with the Ortho VITROS IgG assay (Ortho Clinical Diagnostics, NJ, USA), according to manufacturer's instructions. Signal-to-cut-off ratios for anti-SARS-CoV-2 IgG antibody levels were categorized as low (<9.5) or high (≥9.5).

The last measurement of antibody levels before the administration of the first dose of the vaccine was taken as a baseline determination (mean months: 3.3, SD: 1.98): 4 donors (25%) showed high levels and 12 (75%) showed low levels (median ratio: 3.0, IQR: 6.8). Post-vaccination anti-SARS-CoV-2 IgG antibody test was performed 7 to 29 days after the first dose (mean: 20.8, SD: 1.93). All donors presented high levels of antibodies (median ratio: 19.9, IQR: 1.9) significantly superior from their baseline antibody levels (Mann–Whitney test: z = −3.92, p-value: 0.000). There were no differences between donors who had received two doses or a single dose (median ratio: 20.1 vs. 19.3 respectively, p-value: 0.945). (Table 1 and Fig. 1)

The use of CP can still play an important role in the treatment of patients suffering from COVID-19 infection, before vaccines become widely available or in immunosuppressed patients. FDA has recently clarified that the authorization for administration of CP under EUA is limited to high-titre plasma units [4] and that individuals who received a vaccine after diagnosis of COVID-19 are allowed as CP donors [5]. Our study shows that convalescent donors have a rapid and significant increase in anti-SARS-CoV-2 antibody levels within a few weeks of vaccination. Additional studies are needed to confirm antibody response with different kinds of vaccines, clinical efficacy

Table 1 Ortho VITROS IgG assay ratio Signal-to-Cut-off (S/C) of SARS-CoV-2 antibodies in plasma donor pre- and post-vaccination

	Baseline ratio S/C	Post-vaccination ratio S/C	*P*-value
Mean (standard deviation)	6.7 (7.79)	19.5 (1.37)	0.000
Median (interquartile range)	3.0 (2.0–8.8)	19.9 (18.8–20.6)	
Antibody titres			
High level (%)	4 (25%)	16 (100%)	0.000
Low level (%)	12 (75%)	0 (0%)	

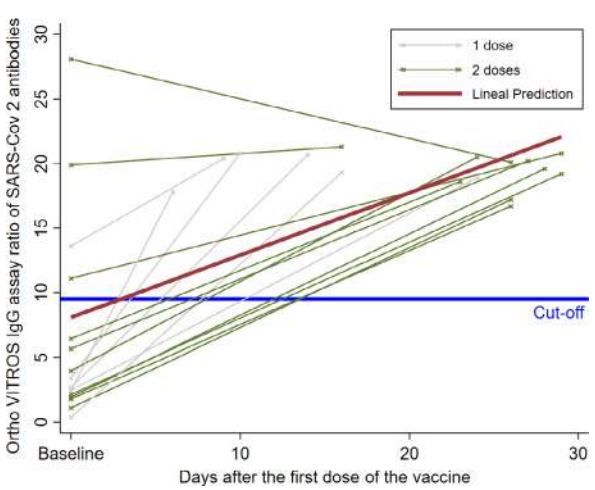

Fig. 1 Evolution of SARS-CoV-2 IgG antibodies. Cut-off is set at 9.5 S/C.

of this CP, and follow-up to discern whether long-term responses are achieved.

Conflict of interest

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References

1 Salazar E, Christensen PA, Graviss EA, et al. Treatment of Coronavirus disease 2019 patients with convalescent plasma reveals a signal of significantly decreased mortality. Am J Pathol 2020;190:2290–303.
2 Joyner MJ, Carter RE, Senefeld JW, et al. Convalescent plasma antibody levels and the risk of death from Covid-19. N Engl J Med 2021. https://doi.org/10.1056/NEJMoa2031893. (published online ahead of print on 13 January 2021)
3 Libster R, Pérez Marc G, Wappner D, et al. Early high-titer plasma therapy to prevent severe Covid-19 in older adults (published online ahead of print on 6 January 2021) N Engl J Med 2021;384:610–8. https://doi.org/10.1056/NEJMoa2033700
4 Bratcher-Bowman N. Letter of Authorization, Reissuance of Convalescent Plasma EUA. 2021. https://www.govinfo.gov/content/pkg/FR-2020-04-01/pdf/2020-06905.pdf (Last accessed February 15 2021).
5 FDA. Investigational COVID-19 Convalescent Plasma. Guidance for Industry. Food Drug Adm. 2021. https://www.fda.gov/vaccines-blood-biologics/investigational-new-drug-ind-or-device-exemption-ide-process-cber/recommendations-investigational-covid-19-convalescent-plasma (Last accessed February 15 2021).

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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