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## In this issue



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REVIEW


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# Pharmacogenomics with red cells: a model to study protein variants of drug transporter genes

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The PharmacoScan pharmacogenomics platform screens for variation in genes that affect drug absorption, distribution, metabolism, elimination, immune adverse reactions and targets. Among the 1,191 genes tested on the platform, 12 genes are expressed in the red cell membrane: *ABCC1*, *ABCC4*, *ABCC5*, *ABCG2*, *CFTR*, *SLC16A1*, *SLC19A1*, *SLC29A1*, *ATP7A*, *CYP4F3*, *EPHX1* and *FLOT1*. These genes represent 5 ATP-binding cassette proteins, 3 solute carrier proteins, 1 ATP transport protein and 3 genes associated with drug metabolism and adverse drug reactions. Only *ABCG2* and *SLC29A1* encode blood group systems, JR and AUG, respectively. We propose red cells as an *ex vivo* model system to study the effect of heritable variants in genes encoding the transport proteins on the pharmacokinetics of drugs. Altered pharmacodynamics in red cells could also cause adverse reactions, such as haemolysis, hitherto unexplained by other mechanisms.

**Key words:** pharmacogenomics, pharmacogenetics, drug transporters, erythrocytes, DMET, PharmacoScan.

## Background

Many proteins of the red cell membrane have been recognized as blood groups. The currently established 36 blood group systems are encoded by 41 genes [1]. They are involved in various cellular functions: transport of substrates (*ABCG2, ABCG6, SLC29A1, AQP1, AQP3, SLC14A1, SLC4A1 and XK*); cellular adhesion (*ACKR1, BCAM, BSG, CD151, CD44, ERMAP, ICAM4, MIC2 and SEMA7A*); enzymatic activity (*ABO, ACHE, ART4, GBGT1, GCNT2, KEL, FUT1 and FUT3*); red cell stability (*GYPC, RHAG, RHCE, RHD, SLC4A1 and SMIM1*); viral and bacterial attachment (*A4GALT, B3GALNT1, FUT3, GYPA, GYPB and GYPE*); complement interaction (*C4A, C4B, CD55, CD59 and CR1*); and unknown function (*XG*) [1–4]. Several of these membrane proteins serve as transporters that contribute to the absorption, tissue distribution and elimination of various drugs [1, 2]. Moreover, drug transporters often influence homeostatic expression of a variety of genes that regulate drug metabolism and disposition [5, 6]. The potential for these membrane proteins to influence pharmacology has been poorly studied.

Transporters are classified into 2 superfamilies: ATP-binding cassette (ABC) proteins and solute carrier (SLC) proteins. ABC transporters are involved in the translocation of a wide variety of substrates including amino acids, sugars, vitamins, inorganic ions, peptides, hormones, large polypeptides (>100 kD) and therapeutics [7, 8]. In eukaryotes, ABC proteins contribute only to the ATP-dependent efflux of substrates from cells against a concentration gradient [9, 10]. SLC proteins mediate the cellular uptake of drugs through facilitated diffusion or secondary active transport [11].

Similar to ABC and SLC transporters, the ion pumps (ATPases) [12] and ion channels [13] transport ions, such

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as $Na^+$, $K^+$, $H^+$, $Cl^-$ and $Ca^{2+}$, across the cell membrane, utilizing energy from ATP hydrolysis or electrochemical gradients, respectively. Aquaporins are a special class of bidirectional channel proteins that are involved in the transfer of water across the membrane driven by the osmotic gradient [14].

Inter-individual variation in the human genome due to single-nucleotide variations (SNVs), small-scale insertions and deletions (InDels) and copy number variations (CNVs) may result in altered pharmacokinetic and pharmacodynamic characteristics of drugs leading to a lack of therapeutic efficacy or a risk for drug-induced toxicity [15, 16]. Variations in genes encoding drug transporters have been documented to affect responsiveness to chemotherapeutic agents [15, 17]. Rarely, sensitivity of red cells to the direct toxicity of the drugs can lead to drug-induced haemolytic anaemia [18–21]. Some medications bind to the RBC cell surface or alter RBC surface antigens resulting in immune attack [22]. Drug-induced immune complexes can bind to RBCs [22], and alloantibody therapies that react with RBC antigens also cause haemolysis [23]. Lastly, oxidative injury to RBCs results from peroxide formation and subsequent haemolysis, particularly in populations who harbour deleterious variants in G6PD or haemoglobin H [24]. Drug metabolism can alter drug-induced haemolytic anaemia [25, 26]. And drugs bound to red cell proteins, including blood group proteins, can bind drug-dependent antibodies [22, 27]. Such antibodies can cause drug-induced immune haemolytic anaemia [28]. Therefore, the potential for inter-individual variation in drug binding and transport resulting from novel genetic variations should be explored and eventually considered to guide indications and dose recommendations.

The DMET Plus array, launched in 2012, scans 1936 variations (1931 SNVs and 5 CNVs) in 231 absorption, distribution, metabolism and elimination (ADME)-related genes [29, 30]. The PharmacoScan Solution array, an updated version of DMET Plus launched in 2016, scans 4627 variations in 1191 genes of known or suspected pharmacogenomic consequences. PharmacoScan incorporated all 231 genes from DMET Plus and nearly all of its variations but scans many additional variations and genes not present on DMET Plus.

The NIH Clinical Center has implemented a clinical decision support (CDS) for patients who are on medications where SNVs may assist with optimal dose or prediction of adverse events [31, 32]. In this pharmacogenomics approach, we have been screening HLA antigens by nucleotide sequencing to avoid exposure of patient with distinct HLA alleles to drugs associated with severe allergic reactions (e.g. allopurinol and carbamazepine) [31]. Nucleotide variations affecting proteins with transporter and metabolic functions have been determined by the DMET Plus microarray platform to adjust drug dose in patients with variants of high, intermediate or low activity [32].

We review the involvement of blood group proteins and other red cell membrane proteins and their potential applications to provide mechanistic insights in pharmacogenomics. As red cells are easily accessible, we propose an approach of using human red cells with variants of drug transport proteins, naturally occurring among blood donors and other healthy individuals. They can serve as an *ex vivo* model systems to study the kinetics of drug transport, as it may be affected by the protein variants.

## Data search criterion and Methods

The exact number of genes expressed in the red cell membrane with drug transport function is unknown. We examined the blood group genes with drug transport function in red cells and represented on a commercial genotyping platform: the PharmacoScan array (ThermoFisher Scientific). The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an international consortium that provides genotype-based drug guidelines to optimize drug therapy [33]. The CPIC drug–gene pairs table includes a total of 363 drug–gene interactions (DGIs), representing 214 unique drugs and 127 unique genes [33]. Among the 1,191 genes present on the 2 arrays, only 113 gene–drug pairs are covered by the CPIC guidelines. One CPIC gene *FCGR3A* is present on red cell membrane (with low confidence [34]) but not present on any of the arrays. The remaining 13 genes in CPIC (*ABL2*, *ASL*, *HPRT1*, *NAGS*, *SERPINC1*, *CYP2A7P1*, *CYB5R1*, *CYB5R2*, *CYB5R4, MT-RNR1, PROS1, TMEM43 and YEATS4*) are not present on red cell membrane and thus irrelevant for the current approach [34]. By searching the published literature and public databases [34], we retrieved the genes that are present on the PharmacoScan array and also expressed on red cell membranes.

## Red cell membrane genes among the PharmacoScan and CPIC drug–gene pairs

We found 12 red cell membrane genes that met our search criterion (Table 1). Apart from the ABC (*ABCC1, ABCC4, ABCC5, ABCG2 and CFTR*), SLC (*SLC16A1, SLC19A1 and SLC29A1*) and ATP transporters (*ATP7A*), 3 additional genes associated with drug metabolism (*CYP4F3* and *EPHX1*) and adverse drug reactions (ADRs; rs3909184 in *FLOT1*) were identified. Hegedus et al. [34] associated each gene with a confidence level to evaluate the potential validity of its protein's presence in the red

**Table 1** Genes present in the red cell membrane and routinely tested in pharmacogenomics.

| Gene | Red cell membrane confidence threshold* | Pharmacogenomics platform | |
|---|---|---|---|
| | | DMET | PharmacoScan |
| *ABCC1* | High | Yes | Yes |
| *ABCC4* | High | Yes | Yes |
| *ABCC5* | High | Yes | Yes |
| *ABCG2* | High | Yes | Yes |
| *SLC16A1* | High | Yes | Yes |
| *SLC19A1* | Medium | Yes | Yes |
| *SLC29A1* | High | Yes | Yes |
| *CYP4F3* | Medium | Yes | Yes |
| *CFTR* | High | No | Yes |
| *FLOT1* | High | No | Yes |
| *ATP7A* | High | Yes | Yes |
| *EPHX1* | High | Yes | Yes |

*High = identified in at least 2 mass spectrometry-based studies, an established blood group, or a CD marker for red cells; Medium = identified in only 1 mass spectrometry-based study [34].

cell membrane: high level if the protein was present in at least two mass spectrometry studies or was an established blood group or CD marker; medium level if the protein was present in at least 1 mass spectrometry study; and low level if the protein was identified only semi-automatically from reviews [34]. We summarized the clinical interpretation of drug-gene pairs, based on the PharmGKB Clinical Annotations tables.

## Only 2 of the 12 genes define blood group systems

Variations in the proteins of the red cell membrane are the hallmark and requirement for defining blood group systems. However, only 2 of the 12 genes from the present search are defined as blood group systems. The *ABCG2* gene encodes the JR (ISBT 032) [35, 36], and the *SLC29A1* gene encodes the AUG blood group system (ISBT 036; Table 2) [37].

### JR blood group system

The high prevalence $Jr^a$ antigen was first reported in 1970. JR was defined as a blood group system in 2012 [38]. The dbSNP database lists 341 non-synonymous or frame shift variants in the *ABCG2* gene. Until today, however, all individuals who developed anti-$Jr^a$ lack the whole JR protein from their red cell membranes. The antibody can cause haemolytic transfusion reactions and severe haemolytic disease of the foetus and newborn (HDFN) [35, 36, 39].

### AUG blood group system

The high prevalence $At^a$ antigen was first identified in 1967. AUG was defined as a blood group system in 2015 [37]. The dbSNP database lists 351 non-synonymous or frame shift variants in the *SLC29A1* gene. Only 3 variants encoding 4 antigens in the AUG system are known. Individuals carrying these variants developed alloantibodies, which can cause haemolytic transfusion reactions and mild HDFN [40, 41].

### Other blood group systems

In addition to *ABCG2* and *SLC29A1*, the 4 blood group system genes *ABO* (ABO; ISBT 001), *BCAM* (LU; ISBT 005), *ACKR1* (FY; ISBT 008) and *CR1* (KN; ISBT 022) are also represented on the PharmacoScan array. Some resources consider them having impact in pharmacogenomics [42]. We do not review these 4 blood groups because CPIC did not identify a drug-gene pair for them.

## Protein structural feature of the 12 genes

As expected for membrane transporters, 9 proteins are multi-pass transmembrane proteins (Table 3). Another 2 proteins, EPHX1 and LTB4H, are single-pass transmembrane proteins. Only 1 protein, FLOT1, is inserted in the inner leaflet of the plasma membrane of the red cell but does not traverse it. None of the 12 proteins identified were GPI-anchored [43–45]. The 12 proteins are involved in the transport of a wide variety of drugs in humans (Table 4).

## Disease association of the 12 genes

Gene variants (alleles) of any of the 12 genes have been associated with various diseases. Variations can occur at the genetic level, involve changes of the mRNA and protein expression, and affect the localization of the proteins in cellular compartments. The number of such variants is growing, and their tabulation is basic for pharmacogenomics (Table S1).

### ABCC1

*ABCC1* is the first identified member of the ABCC subgroup and is ubiquitously expressed in almost all human tissues [46]. Increased MRP1 protein or mRNA concentrations or both were found in many haematologic and solid malignancies as predictor of poor chemotherapy response

**Table 2** Genomic characteristics of the 12 genes[‡].

| Gene | Chromosome location | Genomic size | GenBank number | Exons | Length of cDNA (nucleotides) | Length of coding sequence (CDS) (nucleotides) |
|---|---|---|---|---|---|---|
| *ABCC1* | 16p13.11 | 200498 bp | NG_028268.1 | 31 | 6504 | 4596 |
| *ABCC4* | 13q32.1 | 288618 bp | NG_050651.1 | 31 | 5871 | 3978 |
| *ABCC5* | 3q27.1 | 98027 bp | NG_047115.1 | 30 | 5790 | 4314 |
| *ABCG2** | 4q22.1 | 68596 bp | NG_032067.2 | 16 | 4206 | 1968 |
| *SLC16A1* | 1p13.2 | 44507 bp | NG_015880.2 | 5 | 3927 | 1503 |
| *SLC19A1* | 21q22.3 | 29905 bp | NG_028278.2 | 6 | 4982 | 1776 |
| *SLC29A1*† | 6p21.1 | 10648 bp | NG_042893.1 | 14 | 2201 | 1497 |
| *CYP4F3* | 19p13.12 | 19864 bp | NG_007964.1 | 13 | 5053 | 1563 |
| *CFTR* | 7q31.2 | 188703 bp | NG_016465.4 | 27 | 6132 | 4443 |
| *FLOT1* | 6p21.33 | 15143 bp | NC_000006.12 | 13 | 1866 | 1284 |
| *ATP7A* | Xq21.1 | 139740 bp | NG_013224.2 | 23 | 8492 | 4503 |
| *EPHX1* | 1q42.12 | 35489 bp | NG_009776.1 | 9 | 1847 | 1368 |

**ABCG2* - Junior blood group system (JR; ISBT 032) [35, 36].

†*SLC29A1* - Augustine blood group system (AUG; ISBT 036) [37].

‡For a list of variants in the 12 genes and associated clinical outcomes, see Table S1).

[47]. A number of variations in *ABCC1* were associated with therapeutic response, cancer prognosis, drug toxicity and disease susceptibility [48, 49].

## ABCC4

Increased MRP4 membrane localization and retention were associated with drug resistance in acute myeloid leukaemia [50]. Expression changes caused by an intronic CNV in *ABCC4* correlated with an increased risk for oesophageal squamous cell carcinoma in the Chinese Han population [51]. A large number of SNVs in *ABCC4* altered the affinity for the protein's substrate drugs [49, 52, 53].

## ABCC5

*ABCC5* variants were associated with tumour response to gemcitabine-based chemoradiotherapy and survival in patients with pancreatic cancer [54]. Increased *ABCC5* mRNA concentrations were reported in lung, colon, pancreatic and breast cancer [49].

**Table 3** Protein characteristics of the 12 genes.

| Gene | Protein | Length (amino acids) | Topology* | | Transmembrane segments |
|---|---|---|---|---|---|
| | | | Amino-terminal | Carboxy-terminal | |
| *ABCC1* | Multidrug resistance-associated protein 1 (MRP1) | 1531 | Extracellular | Cytoplasm | 17 |
| *ABCC4* | Multidrug resistance-associated protein 4 (MRP4) | 1325 | Cytoplasm | Cytoplasm | 12 |
| *ABCC5* | Multidrug resistance-associated protein 5 (MRP5) | 1437 | Cytoplasm | Cytoplasm | 12 |
| *ABCG2* | ATP-binding cassette sub-family G member 2 (ABCG2) | 655 | Cytoplasm | Cytoplasm | 6 |
| *SLC16A1* | Monocarboxylate transporter 1 (MCT1) | 500 | Cytoplasm | Cytoplasm | 12 |
| *SLC19A1* | Folate transporter 1 (RFC1) | 591 | Cytoplasm | Cytoplasm | 12 |
| *SLC29A1* | Equilibrative nucleoside transporter 1 (ENT1) | 498 | Cytoplasm | Extracellular | 11 |
| *CYP4F3* | Docosahexaenoic acid omega-hydroxylase (LTB4H) | 520 | Extracellular | Cytoplasm | 1 |
| *CFTR* | Cystic fibrosis transmembrane conductance regulator (CFTR) | 1480 | Cytoplasm | Cytoplasm | 12 |
| *FLOT1* | Flotillin-1 (FLOT1) | 427 | Cytoplasm | Cytoplasm | 0 |
| *ATP7A* | Copper-transporting ATPase 1 (MNK) | 1500 | Cytoplasm | Cytoplasm | 8 |
| *EPHX1* | Epoxide hydrolase 1 (EPHX1) | 455 | Cytoplasm | Extracellular | 1 |

*Predicted or experimentally proven location of the amino- or carboxy-terminal protein ends at the cytoplasmic or extracellular side of the plasma membrane.

### ABCG2

Increased ABCG2 protein concentrations were associated with poor outcome in large B-cell lymphoma [55] and acute myeloid leukaemia [56]. Increased ABCG2 protein expression correlated with reduced survival of patients with small cell and non-small cell lung cancers [57]. A genome-wide association study (GWAS)-associated *ABCG2* alleles with hyperuricaemia and gout [58–60]. *ABCG2* variations were associated with various malignancies including colorectal cancer, lymphoma and leukaemia [61]. The *ABCG2* variant (rs2231142, Gln141Ly) causes reduction of transport activity [62] and increased drug concentrations leading to drug-induced toxicity [63]. Alloimmunizations occurred, complicated transfusions and caused HDFN disease (see JR blood group).

### SLC16A1

MCT1 protein was overexpressed in cancer cells and involved in pH regulation [64]. The *SLC16A1* variant (rs1049434, Asp490Glu) correlated with survival rates in patients with non-small cell lung [65] and colorectal cancers [66]. *SLC16A1* promoter mutations were implicated in hereditary exercise-induced hyperinsulinism and hypoglycaemia [67] and ketoacidosis [68].

### SLC19A1

*SLC19A1* variants affected methotrexate toxicity and outcome in leukaemia [69]. A recent meta-analysis suggested a role of *SLC19A1* rs1051266 variant in haematopoietic malignancies [70].

### SLC29A1

Decreased ENT1 protein expression correlated with recurrence and poor outcome in patients with hepatocellular carcinoma after surgery [71]. Expression of *SLC29A1* mRNA and ENT1 protein in tumour tissues was a predictive marker of outcome in cancer patients receiving gemcitabine [72]. *SLC29A1* promoter region variants altered gene expression and gemcitabine chemosensitivity [73]. The *SLC29A1* variant (rs45573936, Ile216Thr) may increase the risk for seizures during alcohol withdrawal [74]. Alloimmunizations occurred, complicated transfusions and caused HDFN disease (see AUG blood group).

### CYP4F3

*CYP4F3* variants were associated with the risk of ulcerative colitis [75] and lung cancer [76].

### CFTR

Absence, reduced concentration, or malfunction of the CFTR protein resulted in cystic fibrosis [77, 78] and cystic fibrosis-associated diseases, including bronchiectasis [79], chronic pancreatitis [80] and congenital bilateral absence of the vas deferens [81].

### FLOT1

The *FLOT1* gene is located 620 kb upstream of the HLA-B gene on the short arm of chromosome 6. A *FLOT1* variant (rs3909184) was identified as a tagging SNV for the *HLA-B*15:02* allele, associated with carbamazepine-induced Stevens–Jonson syndrome and toxic epidermal necrolysis in the Asian population [31, 82, 83]. A recent study identified *FLOT1* variants affecting *FLOT1* mRNA expression as susceptibility risk factor for major depressive disorder [84]. Upregulation of *FLOT1* mRNA or FLOT1 protein expression may promote oesophageal squamous cell [85], colorectal [86], breast [87] and hepatocellular cancer [88].

### ATP7A

*ATP7A* variants caused various copper transport disorders, such as Menkes disease [89], occipital horn syndrome [90] and the ATP7A-related distal motor neuropathy [91].

### EPHX1

The low-activity genotype of the *EPHX1* exon 3 variant (rs1051740, Tyr113His) was associated with a decreased risk for lung cancer in Caucasians [92]. Functional variants were also associated with susceptibility to various cancers, such as lung [93], upper aerodigestive tract [94–96], colorectal [97], bladder [98] and breast cancer [99].

## Advantages of red cells in pharmacologic studies

Previous studies, using site-directed mutagenesis, have been applied in cell cultures, such as human embryonic kidney-293 [100] and Madin–Darby canine kidney cells [101] or oocytes from *Xenopus laevis* [102]. However, these methods and cellular assays can be artificial, expensive, laborious and time-consuming. Proteomic analysis of the red cells, the most abundant cells in human body [103], has identified multiple transporter proteins in their membrane. Several of these proteins are known to be involved in the influx or efflux of clinically important drugs [34].

**Table 4** Common substrate drugs of the 12 genes.

| Gene | Substrate drugs |
|---|---|
| *ABCC1* | Doxorubicin, methotrexate |
| *ABCC4* | Antivirals: acyclovir, ritonavir, adefovir, tenofovir;<br>Diuretics: furosemide, hydrochlorothiazide;<br>Cephalosporins: ceftizoxime, cefazolin;<br>Cytotoxic drugs: methotrexate, 6-mercaptopurine, 6-thioguanine, topotecan; olmesartan, para-methoxy-N-ethylamphetamine. renal excretion of a wide variety of antiviral, cytostatic, antibiotic and cardiovascular drugs |
| *ABCC5* | Methotrexate, 6-thioguanine (anticancer drug), PMEA (anti-HIV drug), 5-fluorouracil, rosuvastatin, atorvastatin, kainic acid, domoic acid, ZJ43 |
| *ABCG2* | Anthracyclines, daunorubicin, doxorubicin, topotecan, SN-38, irinotecan, methotrexate, imatinib, irinotecan, Mitoxantrone, nucleoside analogs, prazosin, pantoprazole, statins, topotecan, rosuvastatin, teriflunomide, chlorothiazide |
| *SLC16A1* | 3-bromopyruvate (3-BrPA), a cancer drug candidate that inhibits glycolysis |
| *SLC19A1* | 5-methyl-tetrahydrofolate, methotrexate |
| *SLC29A1* | Cladribine, cytarabine (for AML, ALL etc), fludarabine, gemcitabine capecitabine fialuridine, ribavirin |
| *CYP4F3* | Metabolize numerous drug substrates |
| *CFTR* | Ivacaftor |
| *FLOT1* | Carbamazepine |
| *ATP7A* | Cisplatin, oxaliplatin, carboplatin (overexpression causes sequestration of the drugs into intracellular vesicles) |
| *EPHX1* | Carbamazepine |

The membrane structure of the red cell is arguably the best studied of all human cell types [104], which enables us to draw worthwhile conclusions [105]. Red cells can be haemolysed and later resealed to regain limited permeability [106]. This technical feature is rather unique for red cells. No wonder that several studies utilized resealed human erythrocyte membranes, dubbed ghosts, as model system for drug transport studies [107, 108]. Use of ghosts circumvented the interference from proteins and enzymes present in the erythrocyte cytoplasm [109].

## Study topics for pharmacogenomics with red cells

### Clinical syndromes: haemolysis

The *SLC28A3*, a drug transporter gene not expressed on the red cell membrane, is tested on both the DMET and PharmacoScan arrays. A *SLC28A3* variant (rs10838138) was associated with a lower incidence of severe haemolytic anaemia in patients with chronic hepatitis C receiving pegylated interferon and ribavirin [110]. Haemolytic events may however remain undetected until the haemolysis becomes rather severe. Haemolysis by drugs can be caused by 2 mechanisms: (1) non-immune mediated, and (2) immune mediated.

### Haemolysis, non-immune mediated

Non-immune–mediated drug-induced haemolytic anaemia is due to direct toxicity through irreversible damage of red cells [18–21, 25, 26, 28]. Various other factors such as red cell enzymopathy, infections, uraemia, diabetic ketoacidosis, deficient of vitamin E and low levels of glucose can increase the haemolytic effect of a drug [28]. Drugs, such as phenylhydrazine [111] cause haemolysis in all subjects in relatively low concentrations; while primaquine, acetanilid, nitrofurantoin, p-aminosalicylic acid, naphthalene, phenylsemicarbazide, sulphonamides and sulphones cause haemolysis in normal subjects only in high concentrations [28, 112, 113]. Genetic variants in drug transport or drug metabolism genes may determine the intracellular concentration of the drug and its impact on haemolysis.

### Haemolysis, immune mediated

Although underdiagnosed, an incidence of approximately 1 per million per year [114, 115] has been proposed for drug-induced immune haemolytic anaemia, a rare but severe hypersensitivity reaction to drug administration [116, 117]. It is caused by warm autoantibodies against red cells induced by many antibiotic, anti-inflammatory and chemotherapy drugs [118, 119]. A large and growing list of drugs have been associated with drug-induced immune haemolytic anaemia, and the most common are piperacillin, cefotetan and ceftriaxone [118]. Platinum-based chemotherapeutic agents such as oxaliplatin, cisplatin and carboplatin are also known to induce drug-induced immune haemolytic anaemia in rare cases [25, 118, 120, 121]. While drug-induced immune haemolytic anaemia is often diagnosed by excluding alternative causes

rather than by direct evidence, genetic variants of red cell membrane proteins, other than blood group proteins, are not routinely considered.

We wonder how many clinical haemolytic events are not properly attributed to be caused by variants of membrane proteins? Each protein variant is rare, but a large fraction of patients may carry one of the host of such variants.

### Reservoir or sink for a drug

Red cells may function as a reservoir or sink. Their effectiveness can vary if protein variants are involved. Drug transporter proteins can bind drugs to the red cell surface or transport the drug into the red cell cytoplasm. Either way, the drug's plasma concentration may be reduced, delaying or preventing efficient delivery of therapeutics to target tissues. The role of red cell membrane proteins has been studied extensively in drug transport or drug binding [122]. The effect of these proteins' variants has not been systematically evaluated so far.

### Drug delivery

Resealed red cells have been manufactured for in vivo drug delivery [123]. They have a long life span, excellent biocompatibility, complete biodegradability and low immunogenicity [124]. Protein variants may be a lesser concern when allogeneic red cells are manufactured. In an autologous setting, the variant of a red cell membrane protein in the patient would matter.

Drugs can be targeted to red cells in two ways, such as encapsulation and conjugation. The drugs are encapsulated inside the ghosts, which reduces the possibility of an immune reaction and protects the drug from inactivation [125]. Molecular variants of transport proteins may alter the entrapment and eventual release of the drug. By chemical or genetic means, drugs can be physically conjugated to lectins and other ligands that bind to distinct red cell membrane proteins [126]. For example, single-chain variable region fragment (scFv) of TER-119, a monoclonal antibody to the mouse analogue of human glycophorin A (GPA), was genetically attached to complement-regulating proteins including decay-accelerating factor (DAF) which protected the mouse red cells against lysis by complement [127]. Of course, molecular variants of red cell surface proteins can alter the binding affinity of the drug-ligand conjugates and affect the bioavailability of the drug.

### Limitations

Red cells recapitulate the *in vivo* condition where the expression of a transporter protein and presence of multiple transporters for same drug are accounted for. Studying the kinetics of drug transport using red cells harbouring naturally occurring variants of drug transport proteins may allow direct insight in pharmacokinetics for red cells. Such results may be carefully extrapolated to other cell types that express any of the 12 genes in their cell membranes. However, using ghosts as model systems has its limitations: the protein isoforms and the amount of protein expressed may differ between red cells and other tissues; also, the membrane lipid composition, cytoskeleton proteins and interacting proteins differ among cell types.

## Transplant and iatrogenic chimeras

Peripheral blood, routinely used for pharmacogenetic analysis, would reflect the genotype of the donor after a hematopoietic stem cell transplantation. Chronic transfused patients and patients with solid organ transplants are known to accept donor granulocytes and lymphocytes even with leucoreduced donor blood [128, 129]. Being an emerging field, there is a dearth of information on the relevance of donor or recipient genotype to pharmacologic outcome, and both the donor and recipient genetic backgrounds and their discrepancies should be taken into account.

## Therapeutics with potentially important RBC pharmacogenomics relationships

### Methotrexate

Methotrexate polyglutamates accumulate within erythrocytes in a dose-dependent fashion, significantly influencing long-term methotrexate plasma concentrations [130]. One study evaluated the relationship between *ABCC1* variants and methotrexate concentrations in erythrocytes, finding that rs35592 was associated with lower methotrexate polyglutamate concentrations and rs3784862 was associated with higher concentrations [131]. Other studies have identified genetic variants in the folate transporter (*SLC19A1*, *FOLT* and *RFC1*) that are associated with erythrocyte folate concentrations [132,133]. Although controversial [134], RBC methotrexate polyglutamate concentrations are associated with genetic variants in *SLC19A1* [135]. *SLC19A1* loss results in reduced methotrexate uptake and methotrexate resistance in erythroleukaemia cells [136]. Variants in RBC transporters have also been associated with methotrexate plasma concentrations [137], and RBC folate concentrations have been associated with methotrexate outcomes [138]. Although methotrexate likely targets white cells, methotrexate polyglutamates in circulating RBCs may be associated with clinical efficacy of methotrexate, determining both dose and therapeutic selection [139]. Such

relationships may underlie the association between variants in *ABCC1*, *SLC19A1* and other polymorphisms with methotrexate efficacy. Thus, understanding how allelic variants in RBC transporters influence this relationship may increase the likelihood of developing precision use of methotrexate. This field remains in its infancy.

### Mercaptopurines

Located in a variety of tissues, including erythrocytes, thiopurine methyl transferase (TPMT) is the major metabolic detoxification route for mercaptopurines. Red blood cells may act as a reservoir for mercaptopurine metabolites, and low erythrocyte TPMT activity is a marker for mercaptopurine toxicity [140] and lower risk of relapse [141]. Both MRP4 and MRP5 transport mercaptopurine out of red blood cells, whereas ENT1 is a mercaptopurine uptake transporter associated with mercaptopurine sensitivity [142–144]. The rs3765534 polymorphism in *ABCC4* impairs membrane localization and is associated with significant mercaptopurine sensitivity [145, 146]. One study determined that variants in *SLC29A1* were associated with erythrocyte concentrations of thiopurines in patients receiving azathioprine for neuromyelitis optica spectrum disorders [147]; however, the genetic influences of erythrocyte transport and its implications on the pharmacology of mercaptopurines are rather poorly studied.

### Antiretrovirals

Low erythrocyte inosine triphosphatase (ITPA) activity is associated with the development of adverse events during antiretroviral therapy [148, 149] and metabolizes purine analogues used in HIV treatment [149]. Since ITPA activity is decreased in individuals infected with HIV [150], factors influencing ITPA metabolism in erythrocytes may be of significant importance. Several studies have identified variants in transporters that are associated with the pharmacokinetics or clinical outcome of antiretrovirals [151–158]. However, to our knowledge, no study has yet determined whether these variants are associated with intra-erythrocyte concentration of these medications, and therefore, the availability of antiretroviral substrates to erythrocyte ITPA.

### Nucleoside analogues

*SLC29A1* (encoding ENT1) is involved in the pharmacology of many nucleoside analogues (e.g. cytarabine, gemcitabine, 5FU, pentostatin, zidovudine, ribavirin, dipyridamole and draflazine) [159]. Interestingly, we did not find a single study that has evaluated whether RBC ENT1 uptake effects the pharmacology of these medications. Since ribavirin is known to cause dose-limiting haemolytic anaemia [160], variants in this transporter should be studied to determine whether a population of individuals is at particular risk of haemolytic anaemia during ribavirin therapy.

### ABCG2 substrates

ABCG2 transports a very wide variety of medications from different classes, and genetic variants in *ABCG2* have been associated with the pharmacokinetics and outcomes of numerous therapeutics (Table S1). The implications of erythrocyte ABCG2 expression remain poorly characterized. Yet, changes in the expression of ABCG2 resulting from genetic variation are reflected in the red cell membrane [161]. One study discovered a novel ABCG2 variant (ABCG2-M71V; rs148475733) after noting that certain patients had very low (50% of average) ABCG2 erythrocyte membrane expression levels [162]. Thus, RBC transporter expression can be used to identify potentially important variants affecting the expression or function of transporters. Further study is warranted on ABCG2 expression in red cell membranes and the implications of such expression in pharmacology.

### CFTR potentiators

Erythrocytes are representative of the CFTR status of patients [163]. Membrane preparations from erythrocytes are already used to study CFTR structure, function and density [164–166]. Numerous genetic variants are associated with CFTR potentiators (Table S1). Thus, erythrocyte membrane preparations may be useful for non-invasive diagnostic purposes, developing novel CFTR potentiators, or understanding unusual clinical outcomes [167, 168]. Such approaches do not appear to be prevalent in the literature.

## Summary

Red cells are easily accessible for pharmacologic studies. The DMET and more recently the PharmacoScan arrays are increasingly used worldwide for clinical pharmacogenetic decision-making. A thorough search of literature identified 12 genes that are scanned by the arrays and also expressed in the red cell membrane. We propose red cells as an *ex vivo* model system to study the effect of variants of these 12 membrane proteins on the pharmacokinetics of drugs.

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## Conflict of interest

The authors declared having no competing financial interest relevant to this article.

## Statement of disclaimer

The views expressed do not necessarily represent the view of the National Institutes of Health, the Department of Health and Human Services, or the U.S. Federal Government.

## Author contribution

WAF asked the relevance of red cells for pharmacogenomics studies; KS and TMS screened the literature and compiled the tables; all authors researched and discussed the data. WAF, KS and TMS edited the drafts and wrote the manuscript.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:
Table S1: Pharmacogenomic variants and associated clinical outcomes.

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REVIEW ARTICLE

# Severe acute respiratory syndrome coronavirus-2: implications for blood safety and sufficiency

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Vox Sanguinis

**Background and Objective** Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a novel coronavirus, first identified in China at the end of 2019 and has now caused a worldwide pandemic. In this review, we provide an overview of the implications of SARS-CoV-2 for blood safety and sufficiency.

**Material and Method** We searched the PubMed database, the preprint sites bioRxiv and medRxiv, the websites of the World Health Organization, European Centre for Disease Prevention and Control, the US Communicable Diseases Center and monitored ProMed updates.

**Results** An estimated 15%–46% of SARS-CoV-2 infections are asymptomatic. The reported mean incubation period is 3 to 7 days with a range of 1–14 days. The blood phase of SARS-CoV-2 appears to be brief and low level, with RNAaemia detectable in only a small proportion of patients, typically associated with more severe disease and not demonstrated to be infectious virus. An asymptomatic blood phase has not been demonstrated. Given these characteristics of SARS-CoV-2 infection and the absence of reported transfusion transmission (TT), the TT risk is currently theoretical. To mitigate any potential TT risk, but more importantly to prevent respiratory transmission in donor centres, blood centres can implement donor deferral policies based on travel, disease status or potential risk of exposure.

**Conclusion** The TT risk of SARS-CoV-2 appears to be low. The biggest risk to blood services in the current COVID-19 pandemic is to maintain the sufficiency of the blood supply while minimizing respiratory transmission of SARS-CoV-19 to donors and staff while donating blood.

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## Introduction

On 31 December 2019, China notified WHO of a cluster of pneumonia cases with unknown aetiology in the city of Wuhan, Hubei Province [1]. By 7 January 2020, Chinese scientists had identified the pathogen as a novel coronavirus [2,3]. Initially referred to as 2019 novel coronavirus (2019-nCoV), the virus has now been designated severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), classified within the *Severe acute respiratory syndrome-related coronavirus* species, *Sarbecovirus* subgenus, *Betacoronavirus* genus and *Coronaviridae* family [4–6]. Sequence analysis has indicated that SARS-CoV-2 is closely related to SARS-CoV (approximately 80% sequence homology) [5,7,8]. The disease associated with SARS-CoV-2 has been designated as coronavirus virus disease 2019 (COVID-19) [9].

On 30 January, the WHO Emergency Committee declared the COVID-19 outbreak a Public Health Emergency of International Concern (PHEIC) [10] and on 11 March, declared it a pandemic [11]. As at 17 September 2020, WHO had reported over 29.4 million confirmed

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COVID-19 cases globally [12]. Initially, the highest number of confirmed COVID-19 cases was reported in China. However, by mid-March, the highest number of new confirmed cases was being reported in the European Region (particularly Spain, Italy, France, Germany, the UK and, subsequently, the Russian Federation); since mid-May, the highest number of new cases has been reported in the Region of the Americas, primarily due to the US and Brazil, and the South-East Asian Region, primarily due to India [12].

In this review, we summarize what is currently known about SARS-CoV-2 and the associated disease, COVID-19, particularly those characteristics of the virus that are relevant to assessing the potential risk to blood safety. We then discuss whether the virus is potentially transfusion-transmissible and consider the impact of risk mitigation strategies that can be employed by blood centres. Additional supporting references are included in the supplementary material file.

## Epidemiology of SARS-CoV-2

The origin of SARS-CoV-2 and mode of transmission to humans has not been definitively established [13–15]. Sequence homology studies indicate that SARS-CoV-2 may have originated from a bat coronavirus and transmitted to humans via an intermediate host [7,14,16,17].

Many of the earliest, although not all, reported cases of SARS-CoV-2 (prior to 1 January 2020) were directly or indirectly associated with a seafood/animal market in Wuhan, which now appears to have been due to human-to-human transmission [18,19]. Subsequently, the rapid geographical spread and increase in case numbers of SARS-CoV-2 in China and beyond has demonstrated that sustained person-to-person transmission is now the primary mode of transmission [3,20]. COVID-19 cases have been reported in clusters typified by people coming into close contact in confined spaces, often with the identification of superspreaders [21–23]. These include households, public gatherings, conferences, healthcare facilities, religious gatherings and cruise ships [3,24–26]. For example, the Diamond Princess cruise ship off Japan resulted in 712 confirmed cases [12] and there were 600 confirmed cases on the Ruby Princess in Sydney, Australia [27].

Evidence indicates that the predominant mode of human-to-human SARS-CoV-2 transmission is via airborne droplets. SARS-CoV-2 has been demonstrated to infect cells of the upper respiratory tract and isolated from a variety of human respiratory fluids including saliva, bronchoalveolar lavage fluid, nasopharyngeal and throat swabs [28–30]. Transmission by aerosol particles is not a major mode of transmission [31–33]. *Under laboratory conditions*, it has been demonstrated that infectious virus is stable for a limited time on surfaces (fomites) and in generated aerosols contaminated with cultured virus. [34,35]. Studies of isolated COVID-19 patients and hospital wards have reported natural SARS-CoV-2 RNA contamination of commonly used items, surfaces, outdoor environment and air samples, suggesting the contamination of surfaces by airborne droplets [33,36–38]. However, these studies either did not detect or did not test for infectious virus and therefore the importance of fomites in the transmission of SARS-CoV-2 is not clear.

There is evidence that SARS-CoV-2 is transmissible by infected asymptomatic and pre-symptomatic individuals [39,40]. A number of transmission clusters with evidence of possible transmission from pre-symptomatic individuals in close contact have been reported [41–44]. Several studies have reported serial intervals (time from symptom onset in a primary case to symptom onset in a secondary case) shorter than the incubation period, suggesting asymptomatic and pre-symptomatic transmission [45,46]. In addition, SARS-CoV-2 RNA has been detected in respiratory swabs and faeces from asymptomatic individuals [47,48].

There is currently no evidence for intrauterine transmission of SARS-CoV-2 [49,50] or vertical transmission to newborns [51]. A small number of cases of SARS-CoV-2 RNA detection in the breast milk of infected nursing mothers have been reported [52–54]. However, the detection of infectious virus in breastmilk or transmission by breastfeeding has not been reported, consistent with MERS-CoV and SARS-CoV. There is no evidence of sexual transmission of SARS-CoV-2 [51,55]. One study has reported evidence that SARS-CoV-2 may be transmissible by the ocular conjunctival route under some circumstances, however this has been questioned [56–58]. SARS-CoV-2 has been shown to infect cells in the ileum and colon, and infectious virus has been isolated from rectal swabs and stool, indicating that the digestive system may also be a route of infection and faecal transmission may be possible [59–62].

## COVID-19: disease characteristics

Directly estimating the proportion of asymptomatic SARS-CoV-2 infections in the general population is not currently possible as the total number of infections is unknown, and awaits the publication of reliable seroprevalence studies. In addition, reported estimates of the proportion of asymptomatic infections vary due to the differences in methodology and the epidemiology of the study population. Three studies have reported estimates based on specific study groups in which all individual

were tested for SARS-CoV-2 RNA (but not serologically) [63–65]. The estimated percentage of asymptomatic infections varied between a mean of 30·8% (95% CI: 7·7–53·8%) and a median of 34·6% (95% credible interval: 29·4%–39·8%). Subsequently, there have been a number of additional studies and meta-analyses, with estimates varying from 15% (95% CI: 12–18%) to 46% (95% CI: 18–73%) [66–69].

The incubation period for SARS-CoV-2 infection has been modelled by several studies, most showing close agreement with the estimated means/medians ranging from 3·0 days (IQR: 2·0–6·0) to 7·5 days (95% CI: 4·1–10·9) [70–72]. There was some variation between studies for the estimated ranges of the incubation period but most were within the range of 2 to 11 days and almost all infections developed symptoms by day 14. These estimates have been supported by several subsequent meta-analyses which estimated mean values between 4·24 days (95% CI: 3·03–5·44) and 6·93 days (95% CI: 6·11–7·75) [68,73–75].

Studies from several countries have demonstrated that the majority of reported confirmed COVID-19 cases in the general population are mild/moderate [76–79]. For example, a large study of reported confirmed cases in China ($n$ = 44 672) reported that 81% of cases were mild infections, 14% were severe, 5% were critical and 2·3% of cases died [3,20]. The median age of COVID-19 patients varies between countries due to differences in epidemiology and the stage of the pandemic. In initial reports, based primarily on Chinese studies, the median age of patients varied between 47 and 56 years and a majority were males (53·4–73%) [18,80]. Subsequently, studies from several countries have reported the mean age of COVID-19 patients, varying from 39 years (Brazil) to 72 years (US) [77,81–84]. For most countries, the average age of patients was >60 years. Although there is some variation between studies, typically the most common symptoms were fever (83–98%), cough (59–81%), myalgia/fatigue (44–70%) and breathing difficulties (31–55%). Less common symptoms included confusion, headache, sore throat, rhinorrhoea, congestion, expectoration, cutaneous symptoms (including chilblain-like lesions), cardiovascular complications, gastrointestinal symptoms (diarrhoea, anorexia, nausea and vomiting) and neurological symptoms. People of older age, male gender, smokers or those with underlying disease, particularly cardiovascular disease, chronic respiratory disease, hypertension, diabetes, chronic kidney disease and Down's syndrome, are at a higher risk of developing severe symptoms [3,20,77,83–88]. Patients with severe disease may also have neurologic symptoms including acute cerebrovascular diseases, impaired consciousness, seizures, meningoencephalitis, Guillain–Barré syndrome and skeletal muscle injury [89,90]. More recently, acute temporary loss or impaired taste, olfactory and chemesthesis function have been recognized as common (>60% in some studies) and specific early symptoms of SARS-CoV-2 infection [91–93]. Compared to adults, it appears children have a higher proportion of asymptomatic infections, milder symptomatic infections, a lower fatality rate and possibly a longer incubation period [94–96]. A syndrome, which has Kawasaki disease-like symptoms and referred to as multisystem inflammatory syndrome in children (MIS-C) or paediatric inflammatory multisystem syndrome – temporally associated with SARS-CoV-1 (PIMS-TS), has been reported in children with COVID-19. The syndrome has a wide range of presenting symptoms, from fever, inflammation and gastrointestinal symptoms to myocardial injury, shock and development of coronary artery aneurysms [97–100].

While the fatality rate among reported confirmed cases varies substantially between regions, the risk factors for death are consistent, namely older age, male gender and comorbidities [101–103]. The Chinese study noted above reported no fatalities in patients under 10 and 0·2% fatality rate in those between 20 and 40, but increasing to 8·0% for those 60–69 and 14·8% for those 80 or over [20,102], and similar findings have been reported by other studies [102–107]. The same Chinese study found that male patients were overall approximately 1·6 times as likely to die than female patients (2·8% vs. 1·7%), a finding also reported by other studies [102,105]. Compared to all reported COVID-19 cases, patients with a fatal outcome have higher rates of comorbidities including hypertension, diabetes, chronic vascular disease and chronic lung disease [102,104,105]. It is currently not possible to accurately estimate the total number of SARS-CoV-2 infections due to asymptomatic infections which would typically not be reported nor diagnosed, underreporting of symptomatic cases and lack of attribution of COVID-19 as cause of death [108–110]. As a consequence, it is not possible at present to estimate the infection fatality rate (IFR) for all infections (reported and unreported). However, a number of studies and meta-analyses have modelled the IFR, taking into account the proportion of unreported infections. While estimates of the mean overall IFR vary from 0% to approximately 5%, most studies reported values between 0·2% and 2% [68,111–114].

Data on SARS-CoV-2 RNA detection in blood (RNAaemia) are limited, and the blood phase of SARS-CoV-2 infection has not been well defined. A number of studies have shown that only a small proportion of COVID-19 patients had detectable RNAaemia, although most had detectable viral RNA in respiratory swabs [18,59,115–120]. The RNAaemia period appears to be brief, low level and typically associated with more severe disease

symptoms. There has been one report demonstrating that SARS-CoV-2 RNA detected in the blood of patients was not associated with infectious virus [121]. A single case study of a patient with an extended period (approximately 40 days post-symptom onset) of RNAaemia has been reported [122]. However, the RNA levels were low, anti-SARS-CoV-2 IgG was detectable and the presence of infectious virus was not demonstrated. There have also been reports of SARS-CoV-2 detection in peripheral blood mononuclear cells (PBMCs) [123] and platelets [124]. However, this appears to be rare, the levels of RNA in these cases were low and the presence of infectious virus was not demonstrated. In summary, RNAaemia is not detectable in most COVID-19 patients, is low level, brief and may not represent infectious virus.

A number of studies have reported SARS-CoV-2 antibody seroconversion times relative to time of symptom onset with mean/median times varying from 5–11 days for total antibody, 8–14 days for IgM and 10–14 days for IgG [125–130]. Neutralizing antibodies become detectable within 10–15 days of symptom onset and correlate with total antibody levels [129]. Severe COVID-19 is associated with higher levels of antibody compared to mild cases [130,131]. Long-term serological studies are not yet possible, but initial studies have indicated that IgM declines from about 2 weeks post-symptom onset. One study reported the loss of detectable IgG within 2 months [132], but most reports indicate that while IgG levels decline after approximately 2 months, levels remain relatively high for several months [131,133,134]. Assuming that detectable RNAaemia represented infectious virus, it would be expected that blood would no longer be infectious once rising titres of IgG or total antibody become detectable and viral RNA levels declined. This is indicated by a study of COVID-19 patients who were plasma RT-PCR-positive. Using a fitted curve, the plasma RT-PCR-positive rate in samples from the patients was> 90% for samples taken 1–3 days post-symptom onset but declined to <50% by 14 days [127].

## Implications for safety and sufficiency of the blood supply

Broadly, emerging infectious disease (EID) pathogens can be classified into two categories. Firstly, those that are vector-borne, with limited or no human-to-human transmission. Secondly, those that are spread predominately human-to-human, such as respiratory viruses. Both categories of pathogen may impact blood safety due to the potential transfusion-transmission risk and the sufficiency of the blood supply due to infected donors/staff being unwell and unable to donate/attend work, or the loss of donors due to deferrals or social disruption. Pathogens that are predominately transmitted human-to-human may also impact sufficiency of supply due to donors being reluctant to attend donor centres out of fear of being infected. In this section, we will assess the likelihood that SARS-CoV-2 can be transmitted by transfusion and then summarize some of the strategies that blood centres can use to mitigate any potential risk to blood safety and supply.

### Transfusion-transmissibility

The following criteria have been used to assess if an EID pathogen is a potential risk to blood safety: (1) able to establish infection in humans and spread within populations, (2) infection includes an asymptomatic blood phase, (3) able to survive during blood processing and storage, (4) transmissible by the intravenous route and (5) associated with a clinically apparent disease in at least a proportion of recipients [135].

As noted in the first part of this review, it is now clear that SARS-CoV-2 can establish infection in humans and cause disease (COVID-19), which may result in severe symptoms and death, and also spread efficiently from human-to-human within populations. Although SARS-CoV-2 RNA has been detected in respiratory swabs of asymptomatic patients [64,136], it has not been determined if SARS-CoV-2 infection includes an asymptomatic blood phase, either the pre-symptomatic period for infections that become symptomatic or during the course of infection in cases that do not develop symptoms. However, the absence of reported cases of SARS-CoV-2 RNA detection in the blood of asymptomatically infected individuals may be due to infrequent testing of blood as respiratory swabs are primarily used for laboratory diagnosis, most cases referred for laboratory testing are symptomatic and the potential viraemic period is probably brief and low level. The relative viral loads in the different constituents of blood, and whether viable SARS-CoV-2 is able to survive during blood processing and storage (for fresh products) has also not been determined. Similar to other human coronaviruses (including SARS-CoV and MERS-CoV), transfusion transmission of SARS-CoV-2 has not been reported [137–140], suggesting that transfusion transmission of coronaviruses is rare, if it occurs at all. However, it is acknowledged that SARS-CoV-2 has only recently been identified and therefore future reporting of transfusion-transmitted cases cannot be excluded.

Several studies have reported results of SARS-CoV-2 RNA testing of blood donors. A study of seven Korean donors, identified as COVID-19 cases post-donation, failed to detect SARS-CoV-2 RNA in repository samples from all donors [141]. In addition, platelets and red cells from

some of these donors were transfused, but no recipients had developed COVID-19 symptoms between 19–29 days post-transfusion. A study of blood donor screening/retrospective testing at the Wuhan Blood Center reported detectable RNAaemia in four donors [142]. However, these results should be interpreted with some caution. The RT-PCR results showed weak signals, indicating low levels of RNA and the possibility of false-positive results or assay contamination cannot be excluded. A case of a patient with very severe aplastic anaemia who received an apheresis platelet transfusion from a donor diagnosed with COVID-19 three days after donation has been reported [143]. There was no evidence of transfusion transmission as the recipient tested negative on follow-up testing and did develop symptoms. A report of SARS-CoV-2 RNA blood donor screening and retrospective testing in Wuhan on donor samples collected during January found 4 of 7425 donors were RNA-positive. In all cases, RNA was present at low levels and infectious virus was not confirmed [144]. A subsequent report of SARS-CoV-2 RNA screening of 94 342 blood donations in Hubei Province between 9 February and 30 April 2020 found no RNA-positive donations [145]. However, it was noted that this testing period was immediately after the height of the COVID-19 outbreak in Hubei. A Chinese study has estimated the number of donors who may have donated while in the COVID-19 incubation period for the period through to the 17 March [146]. Although the number of potentially infected donors in the incubation period was low (4·05 for the whole of China), it should also be noted that only a small proportion of window period cases would likely have detectable viral RNA and, as noted, it has not been established that infectious virus circulates in the blood.

## Risk mitigation strategies

As noted, a majority of SARS-CoV-2 infections probably result in symptomatic infections with a relatively short incubation period. Donors with symptomatic infection, if presenting to donate, would be deferred from donating. In addition, blood donors should be encouraged to notify the blood centre if they develop symptoms post-donation, such as fever in the two days post-donation or sudden taste or smell dysfunction, a strategy that would partly mitigate any theoretical transfusion-transmission risk associated with donors in the incubation period but more importantly, allows contact tracing to occur if required.

For countries that have either not reported SARS-CoV-2 cases or have small clusters of human-to-human transmission (i.e. no sustained human-to-human transmission), the potential SARS-CoV-2 transfusion-transmission risk can be reduced by travel-related donor deferrals, especially in the initial phase of the epidemic when most cases are imported. Blood centres in these countries can implement a deferral, either for donors returning from countries assessed as high risk for SARS-CoV-2 infection or, given that most countries are now affected by SARS-CoV-2, all donors returning from overseas. As the epidemic progresses in a particular geographical region with sustained widespread local transmission, travel-related deferrals will be less effective in mitigating transfusion-transmission risk, especially if government closes the borders to overseas travellers and imposes a period of isolation for returning citizens [147]. A deferral for donors infected with or potentially exposed to SARS-CoV-2 can be implemented to further reduce any potential transfusion-transmission risk. For example, the WHO, US FDA and Asia Pacific Blood Network (ABPN) guidelines recommend a deferral period of 28 days for donors after possible exposure and the deferral of recovering confirmed cases of SARS-CoV-2 for at least 28 days after symptom resolution [148–150]. For convalescent plasma donors, the US FDA has recommended that a period of at least 14 days after resolution before the donation [151].

Other potential risk mitigation strategies that can be used to reduce the transfusion-transmission risk of emerging infectious diseases include pathogen reduction technologies (PRTs), donor laboratory screening and quarantine of blood components with delayed release if there is no subsequent illness reported by the donor [152–154]. Commercial PRTs are effective for MERS-CoV and SARS-CoV and at least one is effective for SARS-CoV-2 [155–157]. However, for countries that have not already implemented PRTs, it is unlikely to be a cost-effective strategy, particularly as transfusion transmission of SARS-CoV-2 has not been reported [139,158]. For each country, the implementation of blood donor screening for SARS-CoV-2 would require a validated assay approved by that country's regulator and, at present, this is not an option for most countries. In addition, given the low risk, if any, of transmitting SARS-CoV-2 by transfusion, implementing a donor screening assay would not be cost-effective. Quarantining of components would be difficult to implement operationally and, particularly if there is widespread transmission of SARS-CoV-2, could potentially impact the sufficiency of supply. In addition, quarantining platelets would not be feasible due to the short shelf life.

## Sufficiency of supply and proportionate response

The response by blood centres to outbreaks and epidemics should be proportionate to the level of risk to both recipients and sufficiency of supply [150,159]. Decisions about implementing donor travel deferrals need to balance the safety and sufficiency of the blood supply. For example,

the deferral of donors will result in the loss of product in the short term and, potentially in the longer term, donors. The deferral of blood donors can have adverse psychological impacts on donors and negatively impact future donation intention [160,161]. In addition, it is important that both blood centres and government health departments carefully manage their response to infectious disease outbreaks, taking care not to create undue concern among donors and the general population as donors may be reluctant to attend donor centres due to a fear of being infected and/or reluctance to travel due to restrictions [162–165]. Therefore, it is important for blood centres to take appropriate measures to mitigate the risk of SARS-CoV-2 transmission in donor centres, as this will reassure donors and minimize the risk of transmission to staff. A potential measure to maintain donor numbers is to relax existing donor deferrals, where it is demonstrably safe to do so. For example, the US FDA has recently recommended a relaxation of donor deferrals relating to sexual activity [166]. Attracting and selecting suitable donors is an important challenge, given that convalescent plasma [167–169], intravenous immunoglobulin (IVIG) and hyperimmune globulin [170,171] are being investigated as potential treatment options for COVID-19.

## Conclusions

For countries without a substantial number of reported cases or where most cases are imported, the potential transfusion-transmission risk associated with SARS-CoV-2 could be reduced by the implementation of deferral policies relating to potential geographical exposure, a history of SARS-CoV-2 infection or potential local exposure to SARS-CoV-2 cases. For countries with widespread and sustained local transmission, in addition to the deferral of confirmed cases and those potentially exposed, PRT may be an option to reduce the transfusion-transmission risk, but each country would need to perform its own risk assessment to determine the cost-effectiveness. However, based on current knowledge of SARS-CoV-2 infection and the absence of reported transfusion transmission of coronaviruses, the risk of transmitting SARS-CoV-2 by transfusion appears to be low or may not occur at all. If it does occur, the risk is certainly substantially lower than the respiratory route. Accordingly, the biggest risk to blood services in the current COVID-19 pandemic is to maintain the sufficiency of the blood supply, including adequate provision of plasma, while minimizing respiratory transmission of SARS-CoV-19 to donors and staff while donating blood.

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## Conflict of interests

The authors declare no conflict of interests.

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## Supporting Information

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

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REVIEW

# Re-introducing whole blood for transfusion: considerations for blood providers

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Vox Sanguinis

Whole blood is the original blood preparation but disappeared from the blood bank inventories in the 1980s following the advent of component therapy. In the early 2000s, both military and civilian practice called for changes in the transfusion support for massive haemorrhage. The 'clear fluid' policy was abandoned and replaced by early balanced transfusion of platelets, plasma and red cells. Whole blood is an attractive alternative to multi-component therapy, which offers reduced hemodilution, lower donor exposure and simplified logistics. However, the potential for wider re-introduction of whole blood requires re-evaluation of haemolysins, storage conditions and shelf-life, the need for leucocyte depletion/ pathogen reduction and inventory management for blood providers. This review addresses these questions and calls for research to define the optimal whole blood product and the indications for its use.

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## Background

There is a resurgence of interest amongst clinicians for the use of whole blood (WB) in the treatment of haemorrhagic shock, initially primarily for the treatment of traumatic haemorrhage especially in the pre-hospital setting and remote locations where the availability of blood components may be limited [1, 2]. However, major haemorrhage is a clinical emergency associated with a wide variety of practice including obstetrics, gastrointestinal bleeding and major surgery. The perceived benefits of re-introducing whole blood include the delivery of optimized resuscitation together with logistic simplicity and reduced donor exposure. The re-introduction of whole blood into systems optimized for component product may be challenging. However, the feasibility of re-introducing whole blood programmes has been demonstrated by early adopters [3, 4]. The purpose of this review from members of the Biomedical for Excellence for Safer Transfusion (BEST) Collaborative is to describe the practical issues and considerations related to the 're-introduction' of WB for blood providers. For civilian blood providers, cold-stored whole blood (CSWB) rather than fresh whole blood (FWB) is the more practical option, as this can be

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refrigerated for longer periods of up to several weeks instead of the 48–72 h for FWB. Therefore, this review predominantly focuses on CSWB, information on FWB can be found elsewhere [5–7].

## Brief historical perspective of WB transfusion

The discovery in the early 1900s that citrate was an effective anticoagulant heralded the ability to collect anticoagulated WB, which could be kept for a few days. The addition of glucose to the anticoagulant (i.e. acid citrate dextrose, ACD) helped preserve the red blood cells (RBCs) for several weeks [8]. CSWB in glass bottles was the original blood component and was pioneered in World War I (1914–1918) and used widely since [9]. WB was the only blood product available for transfusion in the military and civilian settings through to the late-1960s. By this time, the need for cellular concentrates, especially platelets, and plasma derivatives enabled by the availability of plastic blood bag collection and storage systems led to the development of processing procedures to prepare separated blood components from each WB donation, that is, RBCs, platelets and plasma. CPDA-1 (citrate, phosphate, dextrose, adenine solution-1), the last WB preservative solution released into the market [10], was largely redundant by the time it was licensed in 1979 being superseded by SAG (saline, adenine, glucose), the first RBC additive solution, developed in Sweden in 1975 [11]. These developments together with leucocyte depletion, optimal component use and self-sufficiency in fractionated products contributed to the rapid disappearance of WB usage in the late 1980s. However, WB remained within military protocols, continues to be the main source of blood for transfusion in sub-Saharan Africa and many low and middle-income countries (LMIC) and has also been used in some centres for paediatric transfusion [12].

## Massive haemorrhage and transfusion support

From the 1950's, massive haemorrhage was increasingly managed with fluid replacement and blood components. During military conflicts in the 1990's and later, exsanguination was recognized as a potentially preventable cause of battlefield mortality and the treatment of haemorrhage became the focus for trauma care innovation [13]. The new understanding of the coagulopathy associated with severe trauma [14] led to development of transfusion support within the paradigm of Damage Control Resuscitation [15].

Transfusion support provides RBCs to maintain oxygen transport together with haemostatic components. In addition, the volume associated with transfusion treatment supports intravascular volume replacement and microvascular perfusion. Observational studies seeking to identify optimum trauma transfusion protocols have been conflicting and difficult to interpret [16, 17]. Despite the lack of a universally accepted transfusion protocol for the treatment of haemorrhagic shock, early transfusion of plasma and platelets has been associated with improved outcomes [18–20]. Half of critically injured patients who will die, do so before they reach the hospital. A significant percentage, estimated to be as high as 8–19% of such deaths, are caused by uncontrolled haemorrhage. Many of those deaths are potentially preventable with early haemorrhage control manoeuvres, haemostatic resuscitation and rapid delivery to temporizing or definitive surgical care. Blood products for use in such an environment needs to support blood volume, oxygen delivery and haemostasis, while remaining simple to use. Whole blood is attractive because it meets these criteria and ensures balanced resuscitation. In addition, it is efficient in terms of therapeutic impact for delivered weight, because there is only anticoagulant and no additive solution in whole blood.

The renewed use of whole blood in the civilian setting is still under debate, partly because of lack of data demonstrating a clear clinical benefit compared with component therapy, and partly due to logistical and operational considerations in supplying it. Some of the perceived barriers include ensuring appropriate haemolysin testing, a lack of choice of platelet-sparing leucocyte depletion filters, wastage of the product and impact on the supply of blood components. In the past, WB was only allowed in some countries as ABO identical transfusions. However, the move towards low titre group O whole blood as the initial resuscitative strategy, rather than separate blood components [21], has already led to a review and revision of the standards for emergency whole blood by AABB. The latest version of AABB Standards approves the use of type specific or low titre group O whole blood (LTOWB).

## Re-introduction of WB transfusion in the civilian setting

Whole blood is being re-introduced into the civilian blood bank inventory. In addition, some civilian blood banks provide whole blood for pre-hospital and military programmes. The increasing studies and case reports demonstrate that introduction is feasible in a small scale, but whole blood programmes/provision for many large national blood providers is in its infancy. In Norway, whole blood was introduced in two air ambulance services, following the successful introduction of pre-hospital use of red cell concentrates and lyophilized plasma

[22]. In USA, The University of Pittsburgh Medical Center has been using low titre group O CSWB for several years for all male and females who are >50 years old and who are hypotensive from traumatic haemorrhage. The clinical safety and feasibility of this approach have been well documented [1, 23, 24]. At the Mayo Clinic, whole blood has been introduced in the pre-hospital setting through its availability in the air ambulances. The Mayo Clinic serves a large area with scarce population and long pre-hospital transport times [2]. Later, whole blood has also been introduced at several ambulance services and hospitals in USA [3]. The Royal Caribbean Cruise Lines has developed a programme for remote whole blood transfusions; during a 36-month period, 37 severely bleeding individuals were transfused with a mortality of 13% [2]. In New Zealand, the Auckland Rescue Helicopter and land-based rapid response vehicle carry one or two units of leukoreduced blood group O negative CSWB for pre-hospital resuscitation. This programme is managed in conjunction with the New Zealand Blood Service. Other blood providers are considering the use of whole blood in the context of clinical trials to assess its efficacy/safety compared to component therapy.

## WB product basics and variables

WB preparations are diverse and data on one product type might not extrapolate to another. Apart from donor-related variables such as sex and blood group, variables in the collection, processing and storage of WB can also influence the biological composition, quality and safety of the final preparation. Examples include the type of anticoagulant, storage conditions, length of storage (shelf-life), whether WB is filtered to deplete platelets as well as leucocytes, and whether WB is pathogen inactivated. There is no consensus on the terminology used to describe or label WB products in terms of these variables, especially in relation to the storage temperature and platelet content.

Whole blood is initially collected into a citrate-based anticoagulant at a volume ratio of 1:7 anticoagulant to WB. In this sense, WB is already a diluted blood product from the outset, but not as dilute as transfusing its component parts where these may be stored in additive solution (approximately 100 ml each RBC unit, and 200 ml in each pooled platelet product if stored in additive solution with 35% plasma). CPD is the anticoagulant most used for the collection of WB units in developed countries. CPD CSWB has a 21-day licensed shelf-life when stored at 4°C, defined by the United States Food and Drug Administration's (FDA) requirement for >75% of transfused RBCs to be circulating *in vivo* 24-h after transfusion [25], although may be stored for longer according to EU guidelines. CPDA-1 is supplemented with adenine and 25% more dextrose compared to CPD, which enhances the RBC preservation properties and allows extended storage to 35 days [10].

The FDA's WB shelf-life limits were derived based on the viability of red cells and were conditional on storage of the WB units at 1–6°C. The effect on platelet and plasma constituents of the WB was not a key determinant in setting shelf-life limits. The optimal shelf-life for CSWB that is to be used for resuscitation may need re-evaluation to take into consideration the effects on the non-RBC constituents of WB, including consideration as to the optimal anticoagulant; although preferential, specific collection of units into CPDA1 for the purpose of CSWB may not always be practical. For these reasons, some have opted to restrict the shelf-life of CPD CSWB to 14 days [3, 26].

Standard blood bank storage of CSWB is without agitation. However, agitation is a potential consideration for optimal maintenance of platelet viability. It is well accepted that platelet concentrates stored at ambient temperature need agitation in gas permeable containers to remain viable [27], while platelets stored at 4°C may be kept at rest due to their decreased rate of metabolism [28]. Recent studies suggest that WB units do not require agitation but data on this aspect are sparse [24, 29, 30].

## Cold storage and impact on constituents

The cold storage of WB units is a balance between maintaining sufficient quality of the RBCs, platelets and plasma while minimizing risks associated with progressive storage lesions and potential microbial growth. Separated blood components, that is, RBCs, plasma and platelets, are stored at different optimized temperatures, that is, refrigerated at 1–6°C, frozen at −18°C or colder and room temperature 20–24°C respectively. While refrigerated storage of WB may be optimal for RBCs, the combined effect of cold temperature and length of storage on the platelet and plasma constituents in the WB unit have not been extensively investigated.

An important but under-explored consideration is the interaction between RBCs, platelets, leucocytes and plasma when stored together. Leucocytes and their biologically active factors can impact on red cells and platelets [31], and thus pre-storage leucocyte reduction of WB units is desirable. Plasma and red cells may improve buffering, which may improve platelet metabolism. Additionally red cells provide oxygen, consume nitric oxide and consume activated complement fragments in a way that probably protects platelets [32, 33]. This appears to be reflected in better recovery and survival in vivo of platelets in stored in CSWB compared with cold-stored

apheresis platelets (Stolla 2018; Slichter 2019) [29, 34]. Because of these possible interactions it is inappropriate to extrapolate outcomes of storage time and temperature on separated blood components, even when refrigerated, to that of WB units.

It has long been known that cold temperature is suboptimal for the storage of platelets in the context of *in vivo* platelet survival after transfusion for prophylactic purposes [35]. However, cold-stored platelets have increased *in vitro* aggregation and superior correction of the bleeding-time compared with platelets stored at 20–24°C [36–38]. Therefore, in scenarios, such as haemorrhagic shock, where activated platelets are required for immediate therapeutic function to stop bleeding rather than for prophylaxis, the reduced *in vivo* survival of cold-stored platelets may not be as important. Laboratory studies examining aspects of platelet function in platelet concentrates or whole blood stored refrigerated have been summarized elsewhere [1, 39]. The effect of storage times beyond seven days on the *in vivo* functionality of platelets in CSWB has not been well documented and is the subject of ongoing clinical trials. Further, there is little international consensus on which aspects of platelet function should be assessed and how to use this information to determine shelf-life.

Studies of cold-stored liquid plasma have been well described and have demonstrated the need to carefully consider its shelf-life [40]. When separated plasma is stored at 1–6°C, there is a gradual decrease of coagulation factor activity over time, most noticeably FV, FVIII and protein S, but not fibrinogen, which is very stable. In addition, contact factor activation that occurs more frequently with increasing duration of cold storage of plasma and donor-dependent variables is well described, although the clinical significance is unclear. This has led to several European countries limiting the shelf-life of liquid stored plasma to 7–14 days [41, 42]. Recent studies suggest that decline in coagulation factor activity in WB is not the same as liquid plasma, most notably a more pronounced decrease in Factor V in the presence of platelets [26, 43]. Additionally, it is now recognized that plasma appears to modulate the endotheliopathy associated with haemorrhagic shock and may reduce capillary leakage and that perhaps this may be as, if not more, important than the coagulation content of plasma [44]. A revised understanding of the role of plasma will have an impact on the future evaluation of plasma, whole blood and cold platelets stored in plasma.

Current WB collection-storage bags are made from polyvinyl chloride (PVC) plasticized with diethylhexyl phthalate (DEHP), or less commonly with other plasticizers. PVC-DEHP is advantageous for the preservation of RBCs as it has low gas permeability, which lessens oxidative-induced damage of red cells and DEHP is beneficial in stabilizing RBC membranes and reducing haemolysis [45]. The potentially negative effect of low gas permeability of PVC-DEHP for platelets is counteracted by the ability of the RBCs to release oxygen to support platelet respiration and to consume the expired carbon dioxide. As there is an international effort to reduce exposure to DEHP where appropriate, consideration will need to be given to the storage of WB as well as components in alternative plastics/plasticisers as they become available.

## Blood safety

Blood safety starts with donor selection and serological screening together with quality management systems and appropriate clinical use. Further enhancements are provided by NAT, leucodepletion and pathogen inactivation.

### Donor selection

The risk of transfusion transmissible infectious diseases (TTID) is no greater for WB than for separate blood components, provided policies for standard screening and leukocyte depletion are applied. Indeed, because a WB unit is from a single donor, whereas separate components are from multiple donors, the TTID risk of a WB unit is likely to be less.

To limit the risk of transfusion-related acute lung injury (TRALI) due to inadvertent transfusion of donor-derived anti-histocompatibility antibodies (i.e. HLA, platelet and or neutrophil antibodies), and in accordance with current AABB Standards, the selection of male donors, women who have never been pregnant, or women who have tested negative to histocompatibility antibodies since their most recent pregnancy would be necessary [46].

In the setting of WB transfusion for emergency resuscitation purposes, the selection of blood group O donors who have low titre anti-A and anti-B antibodies is advocated as a strategy to mitigate the risk of a serious HTR where group O WB containing plasma may be transfused to non-O recipients [3]. However, there is no consensus of test method to determine titre, or a definition of a 'safe' titre in WB. The US military experience defined a titre threshold of less than 1:256; however, in the civilian setting, a tighter limit of less than 1:50 has been applied by some [24].

### Leucodepletion

Leucocyte depletion reduces the risk of transmission of intra-leucocyte pathogens such as HTLV, CMV and prions

as well as conferring other benefits. For many providers, LD is now the standard of care. Most filters that remove WBC also remove platelets. At least one type of 'platelet-sparing' WB LD-filter is available and has been reported to have relatively little impact on platelet function; however, there is an approximate 10–20% loss of platelets [47, 48]. Lately, several papers have demonstrated that a platelet-sparing filter provides good platelet recovery and does not significantly impair the product quality [49–51].

### Pathogen inactivation

As for LD, PI is another strategy to mitigate the risk of transfusion-transmitted infections. Most PI methods are based on physicochemical or photochemical disruption of structural or nuclear elements to prevent replication of infectious agents. Blood elements, including cellular and protein, are also affected to varying degrees by these treatments; the challenge has been to find the balance to obtain maximal efficacy, but avoid damage to RBCs, platelets and plasma constituents. PI technology based on riboflavin and UV light is now available for WB and has recently received CE-marking for a 14-day shelf-life [52]. Other pathogen reduction technologies for whole blood are under development, but so-far the different blood components must be inactivated separately. The focus of activity for PI and WB is application in countries where access to resources/equipment for component production may be limited, and where there is a high prevalence of infectious agents such as HIV or malaria in the donor population.

## Where to from here?

### Inventory management

Cold-stored whole blood is a currently licensed blood product in most jurisdictions and could be made available as a safe transfusion product by most blood providers. As for all other blood products, CSWB should be subject to full regulatory requirements including hemovigilance.

The re-introduction of whole blood would lead to a mixed inventory of both components and whole blood. The financial implications for each producer will vary but should include considerations such the potential loss of income from whole blood-derived platelets/plasma and challenges of supplying enough quantities of group O high titre negative blood. Some have reported that the provision of whole blood has little impact on the supply of O RhD negative red cells for other patient groups [53], this will need to be established for different jurisdictions where the practicalities of how blood is supplied may differ. Like platelets, it is assumed that CSWB would have a shorter shelf-life than red cells to ensure maximal clinical efficacy as a resuscitative therapy for haemorrhagic shock, although there is little consideration given currently to re-assigning the shelf-life of whole blood in the current context in which it is being used. Most civilian centres who have re-implemented a whole blood programme in the pre-hospital setting do not store CSWB beyond 14 days, despite storage to 21 days or beyond being permitted by regulatory authorities. This may lead to wastage, unless recycling processes are put in place to produce other blood components from WB when it reaches the end of its shelf-life, such as that practiced in Pittsburgh during the initial study phase [3]. However, for some larger national blood providers, return of blood to stock holding inventory once it has been issued to hospitals is currently not permitted. Computer modelling could be used to evaluate the impact of revised inventories.

#### *Future areas for research*

In this paper, the term low litre has been used according to the use in different published papers. The challenge is, however, that this term is not well defined. In addition, the testing methods vary – also influencing the anti-A and anti-B titres. Also, anti-A and anti-B are both IgG and IgM – and there are different opinions concerning the importance of the immunoglobulin class. There have been several attempts to resolve these issues [54, 55]. However, further international agreement is required to standardize the methodology and definitions for low and high titres and to gather data on the safety of transfusing group O plasma to non-O recipients.

The optimal shelf-life of whole blood needs to be redefined since its current shelf-life is based on red cell viability and does not take into account platelet and plasma elements of whole blood and their deterioration on cold storage.

Studies are needed to understand the clinical and logistical benefits that whole blood may bring compared to component therapy, to define what patient populations might most benefit from WB and to define the maximal number of units that might safely be transfused before switching to component therapy. Recent observational studies suggest a benefit of whole blood [56, 57], but there is a need for well designed randomized trials to address these questions.

Local risk-based frameworks are needed to balance the timely and sufficient provision of whole blood, with the provision of a safe product for its intended use. These need to include considerations around the need or not for leucocyte depletion, selection of blood group and level of anti-A and B.

The pathogen inactivation of whole blood is of major clinical interest. We salute the ongoing studies by Allain

and others to optimize the potential of safe simple transfusion support to the communities who will most benefit [58].

## Summary

Early balanced transfusion may be lifesaving in patients with, or at risk of haemorrhagic shock. WB may be the most practical option in many emergencies. However, there are still unresolved questions related to the optimal preparation and storage of whole blood, and the advantages of cold-stored whole blood versus component therapy. The solutions are not just a matter for the transfusion community in developed countries with an established component portfolio. They have a wider application to global healthcare. The challenge to re-evaluate an old friend in a modern regulatory and scientific era provides new opportunities for good quality laboratory and clinical studies.

## Conflict of interest

The authors do not have any conflict of interest with the contents of this paper.

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ORIGINAL PAPER

# Blood supply sufficiency and safety management in Iran during the COVID-19 outbreak

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Vox Sanguinis

**Background** COVID-19 first appeared in Iran on 19 February 2020, and then spread rapidly over the country. In this article, we review the action plan of the Iranian Blood Transfusion Organization with respect to this disease.

**Method and materials** We collected data on blood donations and RBC inventory for the first 8 weeks of the outbreak. We also evaluated the trend of blood donations and RBC inventory and compared them with the data of the past year. We include a summary of actions taken by the National Committee on Management of COVID-19 outbreak.

**Results** Blood donations decreased from 33 275 to 23 465 units during the first 2 weeks of the outbreak with a corresponding decrease in the RBC inventory. But after that, donations gradually increased from 23 465 to 29 665 units. RBC inventory levels improved at the same time. Then, the Iranian New Year's holiday resulted in another downward trend. After the holiday, blood donations revived, along with the RBC inventory.

**Discussion** Although it appears that this virus cannot be transmitted through transfusion, changes in lifestyle had a significant impact on reducing blood supply. Following implemented measures, we saw an upward trend in blood donations and an adequate supply of RBC units in blood centres, helped by a reduction in demand by hospitals. Blood centres need to be more prepared to manage future viral disasters, especially in case of transfusion-transmissible infections.

**Key words:** blood collection, blood safety, donors.

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## Introduction

On 31 December 2019, China first reported to the World Health Organization (WHO) a pneumonia of unknown cause in the city of Wuhan [1]. The disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus spread rapidly, first in China then throughout the world. WHO declared the COVID-19 outbreak to be a pandemic on 12 March 2020 [2].

Blood component safety is a main concern in viral outbreaks, yet no evidence exists of transmission of respiratory viruses through blood transfusions. One study in China showed that 15% of patients with severe COVID-19 symptoms had RNA in their plasma [3]. However, the presence of infectious virus was not reported [4]. The main challenge for blood establishments is recruitment of healthy blood donors in a time of pandemic, ensuring the safety of staff and blood donors, and providing an adequate blood supply. Therefore, we need an emergency plan to manage the blood supply. To provide a safe environment for blood donation, The American Association of

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Blood Banks (AABB) has recommended certain measures and US blood centres can voluntarily implement them [4]. The WHO has released guidance on maintaining a safe and adequate blood supply during the COVID-19 outbreak [5].

Iran had its first case of confirmed positive COVID-19 on 19 February 2020. Subsequently, the disease spread rapidly throughout the country. Public events were then cancelled and schools, universities, shopping centres and holy shrines were closed down by the government. People were also encouraged to stay at home. Because of these measures, the Iranian Blood Transfusion Organization (IBTO) suffered a drop in the number of blood donors. There was also concern about the adequacy and safety of blood components. Another worry was the safety of staff members who had to deal with volunteers in the blood centres. In order to manage these concerns, the IBTO headquarters formed a National Committee on Management of COVID-19 outbreak.

This Committee constantly monitored the RBC inventory and blood donations, made decisions based on the results obtained through monitoring, and updated international and national evidence and facts. The aim of this article is to review the trend of blood donations and blood supply during the COVID-19 outbreak in Iran, illustrating the leadership of the National Committee on Management of COVID-19 outbreak and the resulting blood centre practices.

## Method and materials

### Data collection

We obtained data pertaining to the year 2019 from weekly reports received from all blood centres throughout the country. The data sent to the main headquarters of IBTO included the numbers of weekly blood donations and the available RBCs for distribution to hospitals (blood inventory). Blood centres extracted these data from provincial databases. The Donor Recruitment Department at the main headquarters verified and monitored the data.

We designed statistical software and implemented it shortly before the first case of COVID-19 was reported in Iran. The data entry started from 20 February 2020, the day after the first officially confirmed case of COVID-19 in Iran. The software enabled blood centres to send data on daily blood donations and RBC inventory levels.

In this article, we report the action plan and activities of National Committee on Management of COVID-19 outbreak in IBTO. We also evaluate the trend of weekly whole blood donations and RBC inventory throughout the country from 20 February to 19 April 2020 and compare it with the data of last year.

### Strategies to provide safe and adequate blood

Iranian Blood Transfusion Organization took the following actions to assure the availability of a safe and sufficient blood supply as well as to provide a healthy environment for its blood donors and personnel:

- The first step was to correspond with the Shanghai Blood Transfusion Centre in order to learn from their experiences with the management of blood centres during the COVID-19 outbreak. Special thanks to Dr. Ming for sending us their 'Recommendations for Blood Establishments regarding the Novel Coronavirus Disease' from Chinese Society of Blood Transfusion.
- A group consisting of faculty members of the Institute for Research and Education in Transfusion Medicine (IRETM) was formed to review and evaluate the latest findings and research about the effects of COVID-19 on blood transfusion.
- We approved and implemented mandatory changes in the eligibility criteria for blood donation in all blood centres.

  (a) A 28-day deferral after complete resolution of symptoms for blood donors diagnosed with COVID-19 or suspected respiratory infections.
  (b) A 28-day deferral for blood donors who have had close contact with COVID-19 patients.

The changes in eligibility criteria were inserted into the blood donor information software, and validation process was completed.

- To protect blood donors and staff, a station for measuring body temperature and mask distribution was established in each blood collection centre. We provided disinfecting materials for the reception area, physician's room, blood collection hall and refreshment area. Distancing was set up between the chairs of the reception area and blood collection beds. Posters instructed about the use of masks and disinfection of hands.
- Personnel were trained about methods for individual and public prevention of COVID-19. The training included issues on keeping social distance, cleaning surfaces and hands, and covering the mouth and nose.
- We held several webinars with the participation of all provincial managers and key staff members to discuss necessary actions and changes; insights and feedbacks were received.
- We activated an online blood donation appointment system throughout the country.

- We put out extensive information on the need for blood components and encouragement of eligible people to donate blood during the COVID-19 outbreak. This went out through mass media, social media and patient advocacy groups representing patients in need of blood transfusion. Some of the slogans used were as follows:

  (a) Our blood centres consider all safety issues and have adopted all new scientific guidelines.
  (b) Patients are awaiting your life-saving blood donations during COVID-19.
  (c) Excuses for coronavirus? No. Do not forget blood donation to save lives.
  (d) When out of your home, remember to donate blood at one of the blood centres near you.

- We provided Personal Protective Equipment (PPE) such as masks, gloves, sanitizers, and disinfecting materials for blood centres' staff and blood donors.
- A meeting was organized with members of the Association of Thalassaemia and Haemophilia Patients in order to assure them that blood supply is safe during the outbreak and to attract their cooperation in recruiting blood donors.
- We started plans to audit blood centres directly and without notice. Considering that it was not possible for auditors to be present in all blood centres due to travel limitations, we used alternative methods including a 'Blood Donor Auditors Program'.
- We involved regular and experienced blood donors in this program. Thus, in each province, three regular blood donors were identified who met the following criteria:
  (a) Adequate experience in blood donation.
  (b) Sufficient knowledge of the blood donation process.
  (c) Maintaining team spirit.

Finally, we chose one of the three nominated regular blood donors in each province. These donors were then informed by phone about the importance of this program and invited to participate in audits. They were trained to complete a checklist in the blood donation centres. They undertook the responsibility of making unscheduled visits to blood centres and filling out the checklist with precision and honesty. The completed checklists were forwarded to the IBTO main headquarters for analysis and corrective actions.

- We asked hospitals to minimize the number of RBCs in their inventories and encouraged them to implement patient blood management strategies.
- We arranged a national project to collect COVID-19 convalescent plasma (CCP) from all the eligible, recovered patients who have been free of symptoms at least for 28 days. This followed a national randomized clinical trial on treatment of COVID-19 patients with convalescent plasma which had been approved by the Research Council of IRETM. We took into consideration the recommendations of the USA Food and Drug Administration (FDA) and of European countries, based on the promising news of efficacy of CCP therapy.

## Results

The trend of weekly blood donations during the 8 weeks following the outbreak is shown in Fig. 1. This shows a decrease from 33 275 in the beginning to 23 465 blood units after the first 2 weeks of the outbreak. Although the number of blood donations decreased about 29·4%, the number of RBC units in inventory only decreased 19%. The trend of weekly blood donations then gradually increased from 23 465 to 29 665 blood units (26·4%) within the next two weeks. National RBC inventory levels reached a peak (35 747 blood units) at the same time.

The 3rd and 4th weeks of March are the New Year's holiday in Iran and schools, universities, and offices are closed. Therefore, a significant drop normally occurs in the rate of blood donations each year during this period. This year, the government adopted stricter policies and health regulations to manage people during the holidays because of the COVID-19 outbreak. As a result, IBTO was confronted with a significant reduction of blood donations again during the 3rd and 4th weeks of March. This showed in RBC inventory levels.

After the holiday, weekly blood donations recovered from 23 670 to 40 114 blood units (76·9%). Simultaneously, the trend of RBC inventory levels went up rapidly as well (Fig. 2).

## Discussion

We drew several conclusions after analysing the trend of weekly blood donations and RBC inventory levels. First of all,, there was a sudden drop of about 30% in donations in the beginning of COVID-19 outbreak. The percentage was variable among different blood centres based on the severity of the outbreak in each province. It was due to the fact that people were frightened by the outbreak and preferred to stay home. They were also afraid of being infected in blood centres. Wang et al. reported a drop of 67% at the beginning of the outbreak in Zhejiang

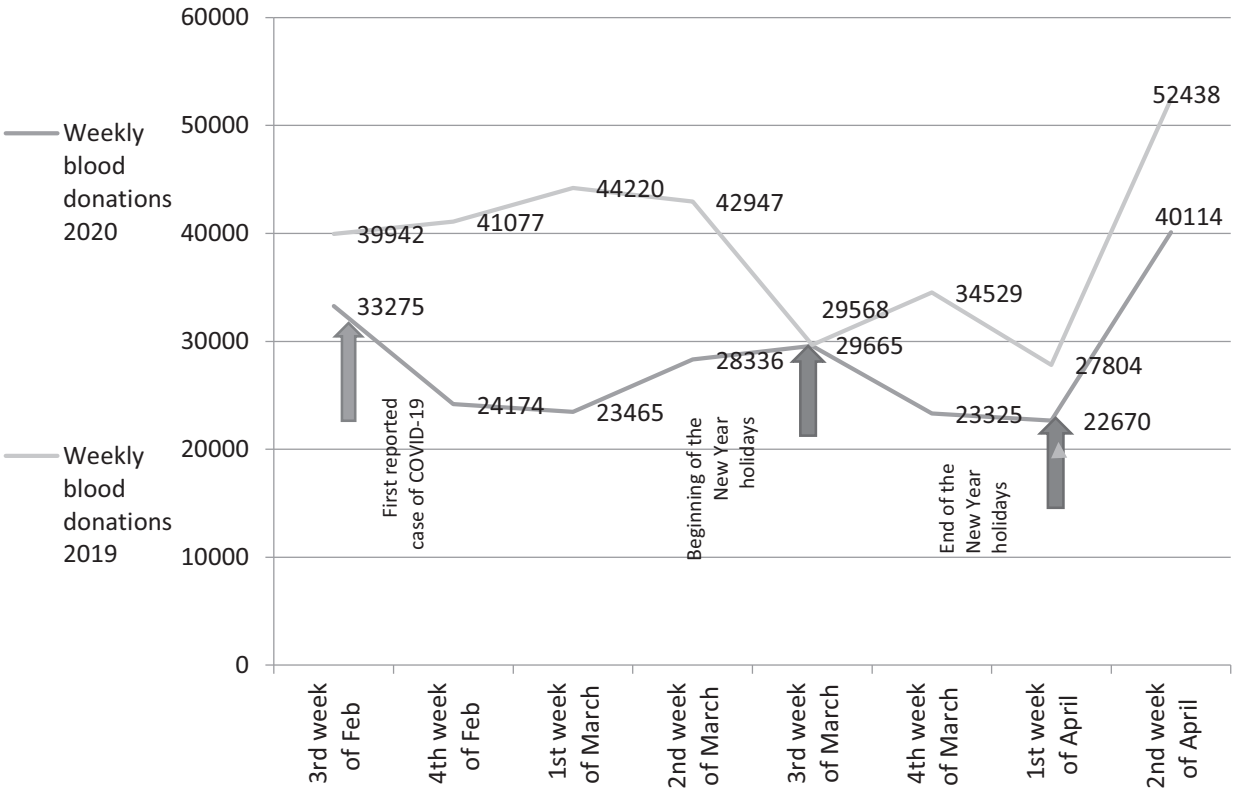


**Fig. 1** Weekly *blood donations in 2019 and 2020.*

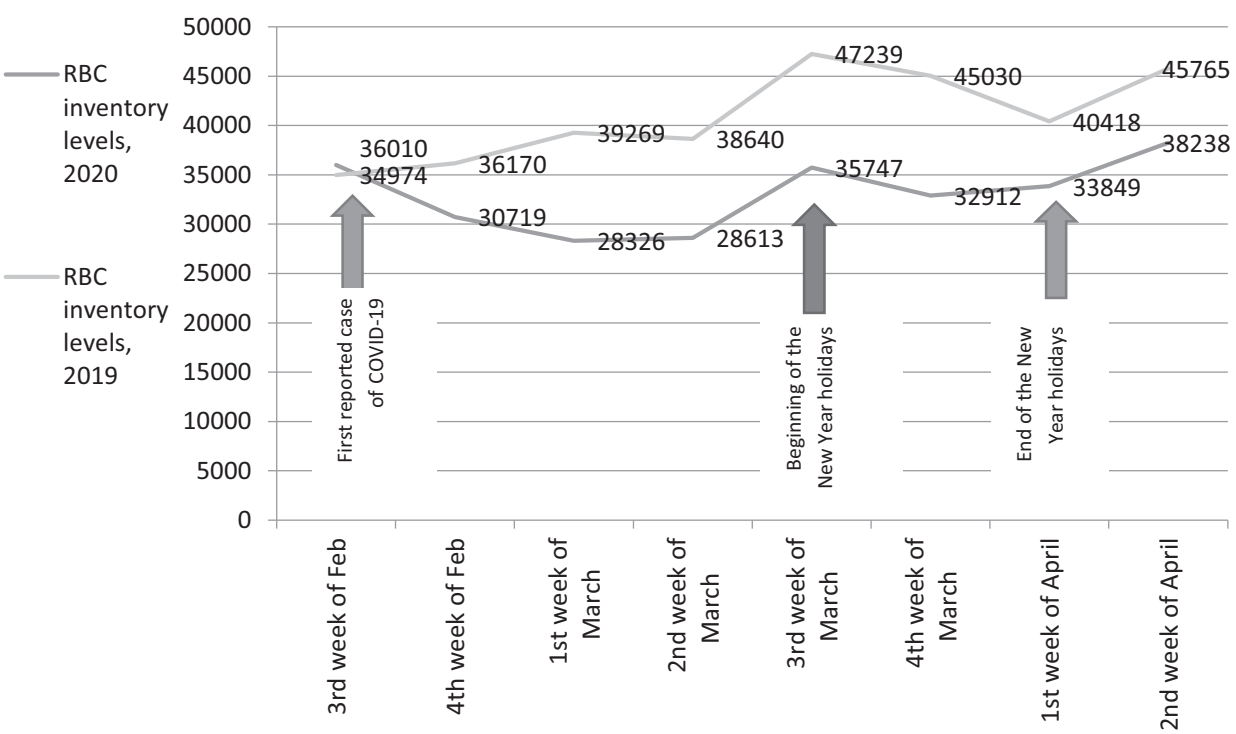


**Fig. 2** RBC *inventory levels in 2019 and 2020.*

province of China [6]. Franchini *et al.* [7] showed a 10% drop in blood donors during the first week of COVID-19 outbreak. Lee et al. found a 16·9% reduction in blood donation due to a decrease of donors in blood centres and cancellation of mobile sessions during SARS outbreak in 2003 [8].

Second, we saw an upward trend in weekly blood donations 2 weeks after the outbreak, while the prevalence and incidence of COVID-19 was increasing in Iran [9]. It shows that the National Committee on Management of COVID-19 outbreak in IBTO could manage the blood donor recruitment process and quickly build trust among the general population. As previously discussed, IBTO activities were focused on three aspects: (1) donor recruitment, (2) donor safety and (3) blood centre staff safety. All of these aspects had either a direct or indirect impact on blood donation.

Finally, although the number of blood donations was less than it had been at the same time in 2019, the number of stored RBCs did not drop below 28 000 units throughout the first 8 weeks of the outbreak. Based on IBTO's current policy, the acceptable RBC inventory is sufficient for 4 to 5 days. This amount is normally equal to the demand of 4 days of all hospitals throughout the country. Inventory levels remaining in the acceptable range may have been due to a simultaneous decrease in hospitals' demand during COVID-19; most patients other than COVID-19 were not admitted in order to prevent further spread of the virus. In addition, elective surgeries were cancelled by the Ministry of Health regulation. People suffering from chronic diseases also preferred to postpone their treatment. However, patients with haemoglobinopathies who needed regular blood transfusions did not have to avoid or postpone their transfusions.

There were many challenges in the CCP program. The main one was the limited availability of antibody test kits. Once these kits reached the market, 14 blood centres started to collect CCP based on the authorized protocol of IBTO. More blood centres are interested in joining this program. The accuracy of antibody tests was another challenge. The final challenge was recruitment of recovered patients as plasma donors, which is ongoing. Blood centres need to gain more experience in this field.

This study had a few limitations that need to be considered. A major limitation was that information pertaining hospital RBC requests were not included. These data existed in all blood centres throughout the country, but were not included in the weekly reports to headquarters, so we could not analyse them. Lacking knowledge of hospital requests made it difficult to interpret the data. Another limitation in this study is that Iran is a large country and the severity of COVID-19 varied in different parts of the country. Therefore, it was better to analyse the data by severity of disease in different part of the country.

In summary, blood donations were affected at the beginning of the COVID-19 outbreak. With all of the above-mentioned measures and participation of dedicated people, trend of weekly blood donations (with notable fluctuation) increased by 26·4% during the 3rd and 4th weeks compared with the first week of the outbreak. In addition, the RBC inventotry remained adequate in blood centres, mainly due to a reduction in blood demand by hospitals. Finally, SARS-CoV2 is not considered a transfusion-transmitted infection (TTI). However, it was a challenging problem for blood centres. It seems that in this era, blood centres need to be more prepared to manage future viral disasters, especially one involving a transfusion-transmissible infection.

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## Conflict of Interests

The authors declare no conflict of interest.

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ORIGINAL PAPER

# 28-day thawed plasma maintains $\alpha_2$-antiplasmin levels and inhibits tPA-induced fibrinolysis

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Vox Sanguinis

## Abstract

**Introduction** Evidence supports the use of plasma-first resuscitation in the treatment of trauma-induced coagulopathy (TIC). While thawed plasma (TP) has logistical benefits, the ability of plasma proteins to attenuate fibrinolysis and correct TIC remain unknown. *We hypothesize that TP retains the ability to inhibit tissue plasminogen activator(tPA)-induced fibrinolysis at 28-day storage.*

**Methods** Healthy volunteers underwent blood draws followed by 50% dilution of whole blood (WB) with TP at 28-, 21-, 14-, 7-, 5-, and, 0-day storage, normal saline (NS), and WB control. Samples underwent citrated tPA-challenge (75 ng/ml) thromboelastography (TEG). Plasminogen activator inhibitor-1 (PAI-1) and $\alpha_2$-antiplasmin ($\alpha_2$-AP) concentrations in thawed or stored plasma were determined.

**Results** In the presence of tPA, 28-day TP inhibited tPA-induced coagulopathy as effectively as WB. 28-day TP had a similar R-time, MA, and fibrinolysis ($P > 0{\cdot}05$ for all) compared to WB, while angle was enhanced ($P = 0{\cdot}02$) compared to WB. Significant correlations were present between storage time and clot strength ($P = 0{\cdot}04$) and storage time and fibrinolysis ($P = 0{\cdot}0029$). Active PAI-1 levels in thawed plasma were $1{\cdot}10 \pm 0{\cdot}54$ ng/mL while total PAI-1 levels were $4{\cdot}79 \pm 1{\cdot}41$ ng/mL. There was no difference of $\alpha_2$-AP levels in FFP ($40{\cdot}45 \pm 3{\cdot}5$ μg/mL) compared to plasma thawed for 14 ($36{\cdot}78 \pm 5{\cdot}39$ μg/mL, $P = 0{\cdot}65$) or 28 days ($45{\cdot}16 \pm 5{\cdot}61$ μg/mL, $P = 0{\cdot}51$).

**Discussion** Thawed plasma retained the ability to inhibit tPA-induced fibrinolysis over 28-day storage at 1–4°C. $\alpha_2$-AP levels were maintained in plasma thawed for 28 days and FFP. These *in vitro* results suggest consideration should be made to increasing the storage life of TP.

**Key words:** hemostasis, plasma, transfusion, traum.

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## Introduction

Hyperfibrinolysis is a lethal phenotype of trauma-induced coagulopathy (TIC) that is associated with a high rates of mortality from haemorrhage [1, 2]. As pre-hospital resuscitation with crystalloid has been shown to exacerbate fibrinolysis, recent research efforts have focused on the use of plasma-first resuscitation, which supports the use for correction of hyperfibrinolysis [1, 3, 4]. There are logistic challenges of early plasma administration that are well-documented, including time to thaw the plasma for use, appropriate length of storage, and transport of

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plasma [5]. These challenges have led to pre-treatment thawing of plasma and storage at 4°C for subsequent use in situations of haemorrhage requiring blood component transfusion in the injured patient [6].

Data shows that there is a 2·2% outdated wastage rate of plasma in the United States. This accounts for almost 130 000 units or wasted plasma each year at a cost of over $7 million [7]. Increasing the ability to store thawed plasma should reduce the wastage rate and further improve availability and accessibility to plasma in remote medical centres or austere environments. Currently, thawed plasma (TP) is stored at 4°C for up to 5 days to be available for use in exsanguinating haemorrhage [8]. Current 5-day TP lifespan recommendations are based on factor V, VII, and VIII activity levels in the thawed plasma [5, 9]. The key plasma proteins involved with ameliorating fibrinolysis and correction of TIC remain unknown however. Similarly, the changes in activity level of these proteins over time and how the change in protein activity affects haemostatic capacity is unknown. In a modified assay evaluating susceptibility to tissue plasminogen activator (tPA)-mediated hyperfibrinolysis, we have previously evaluated coagulation proteins and plasma proteins in fresh frozen plasma (FFP), freeze-dried plasma (FDP), and 5% albumin [10]. Ongoing studies of proteomics continue to identify specific proteins that may be involved with specific TIC phenotypes.

Tissue plasminogen activator is a serine protease known to catalyse the conversion of plasminogen to plasmin leading to the breakdown of clot while plasminogen activator inhibitor-1 (PAI-1) and $\alpha_2$-antiplasmin ($\alpha_2$-AP) act as the primary inhibitors of fibrinolysis [11]. Multiple mechanisms have been found to elicit resistance to fibrinolysis including human neutrophil elastase, fibrin structural modification via thrombin-activatable fibrinolysis inhibitor, or inhibitors of binding to plasminogen [12, 13]. Multiple studies have suggested that PAI-1 is the primary driver of fibrinolysis resistance in trauma [14–17]. However, in fresh plasma samples, these levels are extremely low [18]. While less research has focused on $\alpha_2$-AP, this is a potent inhibitor of fibrinolysis and in the injured patient and the bleeding associated with a deficiency of $\alpha_2$-AP occurs secondary to premature dissolution of a haemostatic plug prior to tissue repair [11, 19, 20]. This removal or inhibition of $\alpha_2$-AP has been shown to enhance fibrinolysis [11, 19, 20]. Conversely, high tPA activity levels drive hyperfibrinolysis in severely injured patients (injury severity score > 15) [21, 22]. We have previously described an *in vitro* assay combining whole blood with tPA to assess the impact of clot fibrinolysis with thrombelastography (TEG) for the evaluation of plasma resuscitation and fibrinolysis [3]. Furthermore, our group has evaluated the extended storage of up to 14 days of TP without changes in ability to inhibit tPA-mediated fibrinolysis [7]. Extending the ability to utilize TP beyond 14 days may further improve availability and accessibility to TP in remote hospitals and austere environments. Therefore, we hypothesize that TP would retain the ability to inhibit tPA-mediated fibrinolysis at 28-day storage at 1–4°C similar to FFP and $\alpha_2$-AP levels will be maintained during this same time period.

## Methods

### Materials

Human single-chain tissue plasminogen activator (tPA) from Molecular Innovations (Novi, MI) underwent dilution with 5% bovine serum albumin in phosphate-buffered solution (PBS) followed by separation into aliquots and storage at −80°C. AB blood type FFP was donated by Vitalant Mountain Division (Denver, CO). Normal saline was purchased from Baxter International (Deerfield, IL). $\alpha_2$-AP deficient plasma was purchased from Sekisui Diagnostics (Lexington, MA).

### Healthy volunteer blood collection

Blood samples were collected from eight healthy volunteers in 3·3-mL buffered sodium citrate (3·2%) tubes (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ) under the Colorado Multiple Institutional Review Board (COMIRB) protocol number 10-0477. Of the volunteers, 5/8 were men, ages 25–33, not pregnant or taking any medications at the time of blood draw ($n$ = 8).

### Plasma

Seven units of AB (Rh-) FFP were thawed and separated into one millilitre aliquots, stored in two millilitre centrifuge tubes, and flash frozen with liquid nitrogen. Samples were subsequently thawed in a 37°C water bath and stored at 1–4°C at the following intervals: 28, 21, 14, 7, 5 days prior to the experiment as well as thawed on day of experiment.

### Thrombelastography

Blood samples underwent a 50% dilution with the above TP intervals as well as immediately thawed FFP, normal saline (NS), and whole blood (WB) control. A 50% dilution produces a reliable fibrinolysis profile that can be used to test interventions (addition of TP). To determine fibrinolysis in $\alpha_2$-AP deficient plasma, blood samples underwent a 50% dilution with NS, 0-day TP, or $\alpha_2$-AP deficient plasma.

Citrated native (CN) and a tPA-challenge (75 ng/mL) CN TEG were run for each dilution as previously described using the TEG 5000 Thrombelastograph Hemostasis Analyzer (Haemonetics, Niles, IL) [3]. TEG properties including speed of clot initiation (R-time), rate of clot formation (angle), maximum clot strength (maximum amplitude, MA), and percent lysis at 30 min (LY30) were analysed.

### Plasminogen activator inhibitor-1 levels

A quantitative ELISA kit for active and total human PAI-1 levels was purchased from R&D Systems (Minneapolis, MN) and plasma levels were measured following the manufacturer's instructions. Because the initial levels of PAI-1 were significantly lower in thawed plasma than previously published values for fresh platelet-free or platelet-rich plasma [18], further testing of PAI-1 levels for duration of stored thawed plasma was not completed as these levels were unlikely to be relevant in the inhibition of tPA-induced fibrinolysis.

### $\alpha_2$-Antiplasmin levels

A quantitative ELISA kit for human $\alpha_2$-AP levels was purchased from Molecular Innovations, Inc (Novi, MI) and plasma levels were measured following the manufacturer's instructions. In brief, plasma was thawed at 28 and 14 days and stored at 4°C until day of experiment. A third group of plasma samples were thawed the day of the experiment. These plasma samples were subsequently run in duplicate and a 1:100 000 dilution was necessary for appropriate determination of $\alpha_2$-AP levels in thawed plasma.

### Statistics

Sample size was calculated using PASS14 (NCSS, LLC) based on an adequate power (0·9) for a non-inferiority paired study for principal outcome LY30. Based on previous analysis of 160 healthy volunteer tPA-challenge TEGs (75 ng/mL), the median LY30 was 8·3% with a standard deviation of 9·9% and 95th percentile of 27% (at which point treatment would be initiated for hyperfibrinolysis). A sample size of 8 pairs allowed for an equivalence test with limits −2·6 to 2·6 (5·2%) with 90% power using a 5% significance level.

TEG values are reported as median with interquartile ranges. PAI-1 and $\alpha_2$-antiplasmin values are reported as mean ± SEM. Statistical analysis was done using SPSS version 24 (IBM) and GraphPad Prism version 7.0a (GraphPad Software, Inc; La Jolla, CA). TEG values R-time, angle, MA, and LY30 had a skewed, non-normal distribution. Differences across dilution groups were detected using a non-parametric paired Friedman test and Dunn's multiple comparisons test to compare 28-day TP to immediately thawed FFP, whole blood, and normal saline. To determine whether a correlation between storage time and clot properties existed, a Spearman correlation was done for R-time, angle, MA, and LY30. $\alpha_2$-AP levels were normally distributed. Differences over thawed time were detected using a parametric paired RM one-way ANOVA test with Dunn's multiple comparisons for comparison of 28- and 14-day TP to immediately thawed FFP. Difference of plasma and $\alpha_2$-AP deficient plasma compared to WB were detected using one-way ANOVA test with Dunn's multiple comparisons test.

## Results

### Thawed plasma maintains or enhances TEG properties in the absence of tPA

Without the addition of tPA, thawed plasma enhanced or maintained TEG properties (Fig. 1) when added to WB compared to WB alone. FFP, 28-day TP, and NS added to WB had similar time to clot initiation compared to WB ($P > 0{\cdot}50$ for all comparisons). FFP and 28-day TP added to WB enhanced the dynamics of clot formation (angle) compared to WB ($P = 0{\cdot}01$ for both) whereas a normal saline dilution did not affect angle ($P > 0{\cdot}99$) compared to WB. Day 0 TP, day 28 TP, and NS added to WB had similar clot strength (MA) compared to WB ($P > 0{\cdot}09$ for all comparisons). Finally, the addition of day 0, day 28 TP, and NS to WB had similar fibrinolysis profiles compared to WB ($P > 0{\cdot}29$ for all comparisons).

### Thawed plasma maintains or enhances TEG properties in the presence of tPA

With the addition of tPA, thawed plasma again enhanced or maintained TEG properties (Fig. 2). Day 0 TP, 28-day TP, and NS added to WB had similar time to clot initiation compared to WB ($P > 0{\cdot}09$ for all comparisons). When added to WB, FFP and 28-day TP enhanced the dynamics of clot formation (angle) compared to WB ($P = 0{\cdot}003$ and $P = 0{\cdot}02$, respectively) whereas a normal saline dilution did not affect angle ($P > 0{\cdot}9999$) compared to WB. FFP and 28-day TP dilution had similar clot strength (MA) compared to WB ($P > 0{\cdot}9999$ for all comparisons) whereas NS dilution significantly impaired maximum clot strength compared to WB ($P = 0{\cdot}04$). Finally, dilution with FFP and 28-day TP had similar fibrinolysis profiles compared to WB ($P > 0{\cdot}3$ for both) while NS dilution enhanced hyperfibrinolysis drastically compared to WB ($P = 0{\cdot}0001$).

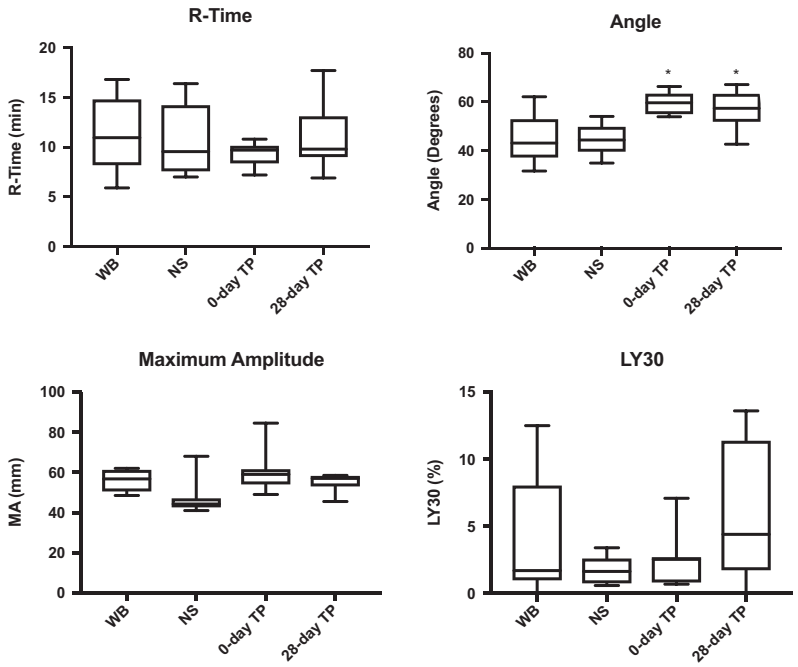


**Fig. 1** Box-plot of citrated native TEG properties of whole blood (WB), normal saline (NS) dilution, 0-day thawed plasma (FFP) dilution, and 28-day thawed plasma (TP) dilution. $*P < 0{\cdot}011$ compared to WB.

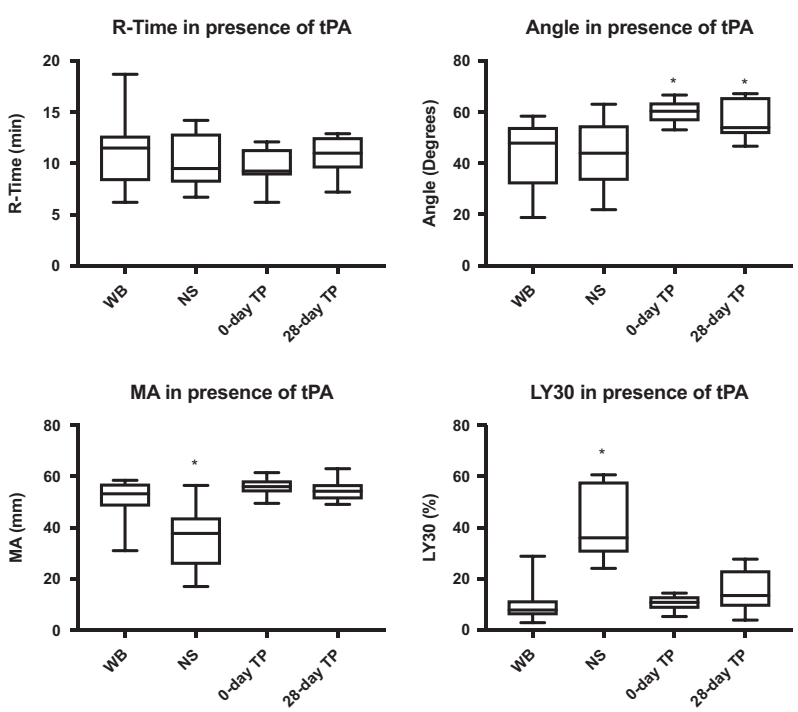


**Fig. 2** Box-plot tPA-challenge TEG properties of whole blood (WB), normal saline (NS) dilution, 0-day thawed plasma (FFP) dilution, and 28-day thawed plasma (TP) dilution. $*P < 0{\cdot}03$ compared to WB.

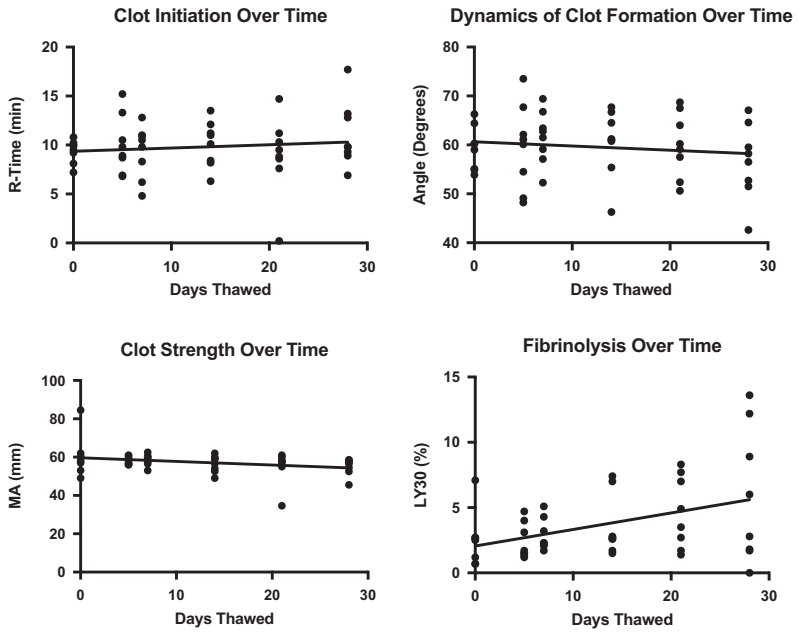


**Fig. 3** The was no significant correlation between time to clot initiation or dynamics of clot formation with storage time. Over 28 days of storage, there is weak but significant correlations between storage time and clot strength ($R^2$ = 0·086, $P$ = 0·0428) as well as storage time and fibrinolysis ($R^2$ = 0·1767, $P$ = 0·0029).

## Thawed plasma clotting properties over time

In the absence of tPA, there were differences in clotting properties over time (Fig. 3). There was no significant correlation between time to clot initiation or dynamics of clot formation with storage time ($P$ = 0·44 and $P$ = 0·39, respectively). However, over 28 days of storage there was weak but significant correlation between storage time and clot strength ($R^2$ = 0·086, $P$ = 0·04) as well as storage time and fibrinolysis ($R^2$ = 0·1767, $P$ = 0·003). These changes were not present over a 14-day time period ($P$ = 0·20 for MA and $P$ = 0·21 for LY30). There were no significant changes with storage time and clot properties including R-time ($P$ = 0·099), angle ($P$ = 0·1932), MA ($P$ = 0·44), and LY30 ($P$ = 0·11) in the presence of tPA. At 28 days, the median R-time was 9·8 min (IQR: 9–13·1 min), angle 57·35 degrees (IQR 51·8–63·3 degrees), MA 57 mm (IQR: 53–58·25 mm), and LY30 4·4% (IQR: 1·7–11·38%).

## PAI-1 levels

Thawed plasma levels for active and total PAI-1 were measured following thawing of plasma samples. Active PAI-1 levels in TP were 1·10 ± 0·54 ng/mL while total PAI-1 levels were 4·79 ± 1·41 ng/mL. Because these levels are significantly lower in thawed plasma than previously published values for fresh platelet-free or platelet-rich plasma [18, 23] (5·4–21·0 ng/mL for platelet-free plasma and 282·6 ng/mL in platelet-rich plasma), further testing for duration of stored thawed plasma was not completed.

## $\alpha_2$-Antiplasmin levels

$\alpha_2$-AP levels were not significantly different over the 28-day period of thawing. $\alpha_2$-AP levels in plasma thawed and stored at 4°C for 28 days, 14 days, and FFP are shown in Fig. 4. There was no difference of $\alpha_2$-AP levels in FFP (40·45 ± 3·5 μg/mL) compared to plasma thawed for 14 days (36·78 ± 5·39 μg/mL, $P$ = 0·6461) or 28 days (45·16 ± 5·61 μg/mL, $P$ = 0·5049).

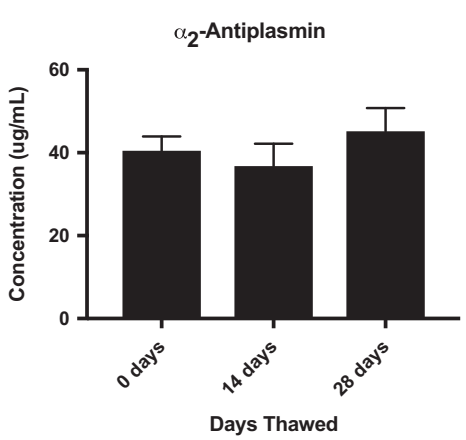


**Fig. 4** $\alpha_2$-antiplasmin levels of plasma thawed and stored at 4°C for different intervals.

**Table 1** tPA-challenged citrated native TEG properties.

| | R-Time (min) | Angle (degrees) | MA (mm) | LY30 (%) |
|---|---|---|---|---|
| WB | 10·75 (10·68–11·88) | 53·2 (45·13–61·78) | 58·5 (53·5–65·13) | 3·8 (1·25–7·3) |
| NS | 10·45 (10·03–11·2) | 49·7 (39·83–52·93) | 44·75 (41·75–48·38) | 23·65 (7·78–39·08)* |
| Plasma | 6·25 (5·8–6·73)* | 71·9 (70·33–72·48)* | 62·5 (59·38–64·5) | 4·6 (3·45–5·625) |
| $\alpha_2$-AP Deficient | 6·35 (5·03–6·73)* | 72·65 (69·9–74·18)* | 60·75 (59·88–66·38) | 3·55 (2·18–4·2) |

Values reported as median (interquartile range).
*$P < 0.05$ compared to WB.

### $\alpha_2$-Antiplasmin deficient plasma does not lead to enhanced fibrinolysis

Fibrinolysis is not enhanced in whole blood diluted with $\alpha_2$-AP deficient plasma. Again, thawed plasma enhanced or maintained TEG parameters similar to WB. $\alpha_2$-AP deficient plasma also enhanced or maintained TEG parameters, and specifically did not lead to enhanced fibrinolysis (Table 1, Figure 5). NS dilution of whole blood showed a significant increase in hyperfibrinolysis ($P = 0.0175$).

## Discussion

Dilution of whole blood with normal saline leads to significant abnormalities in the presence of tPA, most notably hyperfibrinolysis. Both 50% replacement of whole blood with FFP or 28-day thawed plasma retained near-normal TEG properties and did not lead to exacerbation of tPA-mediated hyperfibrinolysis as seen in a similar dilution with a crystalloid solution. While TEG properties remain near-normal up to 28 days after thawing of plasma, there is a weak but significant correlation between decreased clot strength and increased fibrinolysis over the time course of the 28-day experimental period. Furthermore, $\alpha_2$-AP levels are maintained over the 28-day period. However, an absence of $\alpha_2$-antiplasmin in plasma did not result in a significant increase in hyperfibrinolysis.

The dangers of hemodilution as they relate to TIC have been extensively evaluated [24–26]. As such, early trauma resuscitation has shifted from crystalloid to blood product administration, e.g. plasma [27]. There is an increased risk of hyperfibrinolysis in severely injured patients with each litre of pre-hospital crystalloid, [18] which has been confirmed *in vitro* [3, 7, 10, 23]. The reported data reveal: a maintained time to clot initiation after dilution with saline or plasma; the angle (dynamics of clot formation) is enhanced in WB diluted with plasma ± tPA; and MA (cloth strength) and fibrinolysis (LY30) are maintained with TP dilutions compared to WB ± tPA.

There are a number of anti-fibrinolytic proteins present in plasma that may mediate tPA-mediated fibrinolysis, including PAI-1 and $\alpha_2$-AP. When elevated, both serpins are associated with increased thrombotic activity [28]. PAI-1 is present in both the plasma and platelet α-granules and is released by thrombin to rapidly inhibit tPA activity [23]. Importantly, PAI-1 levels in platelet-free plasma are <10% of the PAI-1 concentrations in platelet-rich plasma [29, 30]. Previous studies have shown that normal levels of PAI-1 are 21·0 ng/mL in fresh platelet poor plasma [18]. The active (1·10 ng/mL) and total (4·79 ng/mL) levels of PAI-1 in our thawed platelet poor plasma samples were significantly lower than fresh plasma or serum [23]. Furthermore, fresh platelet-rich plasma samples were noted to have PAI-1 levels almost 10× greater than platelet poor plasma and almost 70× greater than the total PAI-1 concentration in our thawed plasma sample. Further, a recent study by Huebner *et al* evaluating the effects of PAI-1 on tPA-mediated fibrinolysis in platelet lysates showed that concentrations of PAI-1 of 8·7 ng/mL did not attenuate tPA-mediated

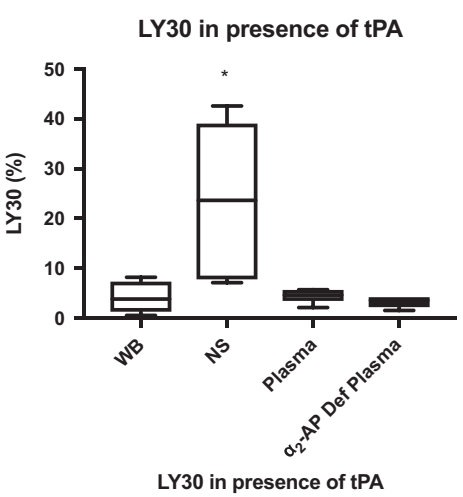


**Fig. 5** Box-plot tPA-challenge TEG properties of whole blood (WB), normal saline (NS) dilution, plasma dilution (plasma), and $\alpha_2$-AP deficient plasma ($\alpha_2$-AP Def Plasma) dilution. *$P = 0.0175$ compared to WB.

fibrinolysis [23]. This indicates that higher levels of PAI-1 are needed to efficiently bind tPA to inhibition of tPA-mediated fibrinolysis. As the levels of PAI-1 in our TP samples were much lower, we concluded that the levels of PAI-1 in our thawed plasma contributed negligibly to the inhibition of tPA-mediated fibrinolysis. It has been shown that longitudinal storage of frozen plasma does not lead to significant PAI-1 degradation up 59 months of storage [31]. However, no studies have evaluated the effects on PAI-1 levels in plasma after multiple freeze/thaw cycles. $\alpha_2$-AP, on the other hand, acts as a direct inhibitor of plasmin [11], and clinical bleeding due to $\alpha_2$-AP deficiency occurs secondary to premature dissolution of a haemostatic plug. Furthermore, inhibition or removal of $\alpha_2$-AP increases fibrinolysis [11, 19, 20]. In the reported study, $\alpha_2$-AP levels were maintained in plasma that had been thawed for 14 and 28 days versus freshly thawed plasma, and although the concentration was decreased versus fresh plasma [32]. Thus, we speculated that $\alpha_2$-AP may have an important role in the inhibition of tPA-mediated fibrinolysis. However, when WB is diluted with $\alpha_2$-AP deficient plasma, there is not an increase in hyperfibrinolysis compared to TP or WB, suggesting that $\alpha_2$-AP does not contribute to inhibiting tPA-mediated fibrinolysis. It is possible that in the injured patient, the physiologic, metabolic, and proteomic derangements that occur following injury are mitigated by the presence of $\alpha_2$-AP in plasma. There are a number of other fibrinolytic proteins that could be responsible for inhibition of tPA-mediated fibrinolysis. Recent studies from our group have focused on proteomic analysis in patient plasma and identified a number of proteins in hyperfibrinolytic patients that could be influenced with the administration of plasma [33], although individual proteins or metabolites could not be evaluated in this study. Interestingly, WB samples treated with TP and $\alpha_2$-AP deficient plasma had enhanced clot initiation compared to WB (Table 1), while WB that was treated with TP at different storages times did not (Fig. 2). These samples were drawn from the same volunteers, although the blood samples were drawn at temporally unique time points. It is unclear the aetiology of the enhanced clot initiation.

Recent work has identified prothrombotic extracellular vesicles in thawed plasma and perhaps these increase with storage time [34]. Other explanations include small differences in reagents that effected TEG parameters, physiologic changes of individuals between blood draws, or that volunteers had a more robust generation of thrombin and/or tissue factor at second blood draws that sensitized the patient to plasma addition to WB.

There are limitations to this study. While increased tPA activity has been repeatedly shown to be present in the plasma of trauma patients [21, 22], there are undoubtedly other clotting abnormalities that may impact the fibrinolysis profile. We acknowledge that the use of exogenous tPA, with significant haemodilution, does not completely replicate the plasma changes of trauma patients, but our addition of *in vitro* exogenous tPA to whole blood dilutions does produce a reliable fibrinolysis profile to study the effects of interventions. Furthermore, while PAI-1 and $\alpha_2$-AP levels were evaluated in this study, there are other proteins that could also affect fibrinolysis and may contribute to inhibiting tPA-mediated fibrinolysis after 28 days of storage. Given that PAI-1 levels in our thawed samples were so low, $\alpha_2$-AP, as the primary inhibitor of plasmin, was thought to impart the highest yield to focus this study. These experiments were performed with blood of healthy volunteers and likely do not reflect the full spectrum of metabolic and proteomic abnormalities seen with the physiologic stress of trauma. Further, our aliquoted samples of plasma went through more freeze-thaw cycles than clinically used plasma secondary to our aliquoting process. We acknowledge that this does not completely replicate what would happen in the clinical setting and may result in loss of protein function or changes in protein activity due to an extra freeze-thaw cycle.

In conclusion, there is preservation of haemostatic properties over a 28-day storage of TP by TEG in the presence of tPA, most notably the ability to maintain a fibrinolysis profile similar to that of WB in the presence of tPA. While PAI-1 levels are very low, levels of $\alpha_2$-AP are maintained in TP over 28 days of storage and were comparable to FFP. Further work evaluating the effects of TP on damaged endothelium may also help discern the mechanism by which plasma attenuates the coagulopathy of trauma. While further in vitro and clinical studies are needed to confirm the applicability, consideration should be made to increase the duration of stored TP as this will decrease the outdated wastage rate as well as increase the availability of TP in austere environments.

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ORIGINAL PAPER

# Whole blood haemostatic function throughout a 28-day cold storage period: an in vitro study

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Vox Sanguinis

**Background** In recent years, there has been renewed interest in whole blood (WB) transfusion, particularly in damage control resuscitation, in part due to the ability to provide the adequate ratio of blood components in a single transfusion. However, there is insufficient evidence to suggest that WB units maintain their haemostatic function during storage, which could compromise their quality and efficacy if transfused. Here, we evaluate the in vitro haemostatic function of stored WB units over a 28-day refrigeration period.

**Methods** Standard WB units were collected from healthy volunteers and stored at 4°C for 28 days. Samples were collected from each unit on several days throughout the storage period and tested for complete blood count (CBC), WB aggregation, clot kinetics as measured by thromboelastography (TEG), closure time and plasma-free haemoglobin.

**Results** Throughout the storage period, there were gradual, significant decreases in platelet count and function, including WB aggregation in response to collagen ($P < 0{\cdot}05$) and closure time with epinephrine ($P < 0{\cdot}0005$). Plasma-free haemoglobin increased substantially (by 163%) throughout the storage period. However, TEG results remained relatively stable for 3 weeks, indicating possible preservation of haemostatic function during that time.

**Conclusion** This study shows that clot kinetics (as measured by TEG) in WB units stored at 4°C are preserved for up to 21 days. However, high levels of free haemoglobin raise concern for the potential risks of transfusing stored WB. Clinical studies are required to evaluate optimal storage times and outcomes of patients resuscitated with WB as compared to blood components.

**Key words:** whole blood, transfusion, whole blood storage, haemostasis, platelet function.

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## Introduction

For over half a century, whole blood (WB) units have been separated into components, allowing for targeted blood product transfusion, reduced wastage rate and cost, and increased storage flexibility [1, 2, 3]. More recently, findings from both civilian and military studies have documented the prevalence of coagulopathy in trauma, for which the current US practice of balanced resuscitation with a ratio of 1:1:1 (plasma to platelets to RBC) was developed [4–6]. While this method dramatically improved patient outcomes as compared to the prior practice of infusing large quantities of crystalloid in massively bleeding patients, trauma-induced coagulopathy remains difficult to manage [7, 8], and there is conflicting evidence surrounding the optimal ratio of blood components in massive transfusion protocols [9–11]. Furthermore, the 1:1:1 component ratio results in a dilute mixture compared to WB, with a decreased haematocrit, platelet count and coagulation factor concentration [12].

There has been renewed interest in WB transfusion in recent years, particularly in the setting of damage control

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resuscitation, due to its physiologic ratio of components and ability to be collected and used emergently [13]. This has become a particularly effective solution in remote areas and combat zones, or smaller hospitals and clinics that do not have adequate blood bank supply of individual components. WB is often used in military hospitals in combat zones, where platelets especially are often in short supply, in part due to their short shelf life [14]. In these cases, fresh WB is often preferable to components as it is readily available and provides adequate coagulation factors, functional haemoglobin and platelets [15]. This contrasts with the prevalence of WB transfusion in civilian settings, which accounted for 0·15% of total civilian transfusions in 2011. Despite the increased general interest in WB transfusion, this rate did not increase by 2013 among AABB-member hospitals [16, 17].

There are insufficient data in the literature to sufficiently support the efficacy of WB transfusion as compared to blood components. A retrospective analysis of the 2009 National Trauma Data Bank based on data from 1745 patients from 567 US hospitals revealed that patients who received blood component transfusion were three times more likely to succumb to their trauma as compared to those who received WB transfusion, even with identical injury severity scores (ISS) [18]. Nevertheless, the large discrepancy between transfusion rates of blood components versus WB combined with the lack of randomized trials make it difficult to derive meaningful conclusions.

Laboratory analysis of the functionality of WB *in vitro* is also an important component in the determination of the efficacy of WB as a transfusion product. One study performed in 2013 revealed that refrigeration of WB for 21 days at 4°C helped retain haemostatic function as compared to WB stored at room temperature in terms of prothrombin time (PT) and partial thromboplastin time (PTT), clot kinetics as measured by thromboelastography (TEG) and thrombin generation [19]. Another recent study from 2018 outlined the effects of standard leucoreduction on haemostatic function on cold-stored (4°C) WB units up to 15 days; it found that platelet concentration, thrombin generation and viscoelastic properties measured via rotational thromboelastometry-Extrinsic Screening Test (ROTEM-EXTEM) were all significantly decreased after leucoreduction compared to nonleucoreduced WB [20]. Here, we evaluate the *in vitro* haemostatic potential of nonleucoreduced normal donor WB units that were cold-stored over the course of 28 days.

## Materials and methods

### Research subject participation

Participation in this study was approved by our internal Research Subject Review Board, and all volunteers signed an informed consent. Subjects were compensated monetarily for their time and travel.

### Whole blood unit collection

Healthy donors ($n$ = 6, 3 males) of 18 years of age and older (age range 22–60) provided informed consent for a single unit WB donation. Three of the six donors had group A, two had group O, and one had group B blood. Donors were free of anticoagulants, NSAIDs, and all other medications and supplements for at least 10 days prior to collection. One unit of 530 ml of WB was collected in a citrate phosphate dextrose adenine (CPDA-1) bag composed of polyvinyl chloride (PL 146) material (Fenwal, Inc., Lake Zurich, IL) from each donor. WB units were stored unagitated in a walk-in refrigerator set to 4°C for 28 days.

### Whole blood unit storage and processing

Each WB unit was stored in the collection bag at 4°C for 28 days. Testing was performed on days 0, 3, 7, 14, 21 and 28 from the day of collection. On each testing day, the bags were mixed via gentle inversion prior to sampling. Samples (15 ml each) were obtained directly from the collection bag via a sterile connection using a 30 ml Luer-Lok syringe (BD Biosciences, San Jose, CA). From days 0 to 28, a total of 90 ml of blood were removed from each collection bag, leaving 440 ml leftover in each bag at the conclusion of the study. A complete blood count (CBC) was obtained for each sample using a Sysmex Kx-21 (Sysmex Corporation, Kobe, Japan). In addition, on each day of testing 4 ml of WB was removed from the bag and split into two equal aliquots; 2 ml was centrifuged at 1500× $g$ 12 min to obtain platelet poor plasma (PPP) for coagulation testing, including prothrombin time (PT) and partial thromboplastin time (PTT). Plasma samples were stored at −80°C for future testing purposes.

### Thromboelastography

The clot kinetics of the WB samples were evaluated using thromboelastography (TEG) (TEG 5000 Thrombelastograph Hemostasis Analyzer, Haemonetics, Braintree, MA) according to manufacturer protocol. For each test, 1 ml of WB sample was pipetted into a 1·5 ml polyethylene tube containing 40 µl of kaolin (Haemoscope, Niles, IL) and mixed via gentle inversion. Next, 340 µl of the kaolin-treated WB sample was transferred into a disposable, prewarmed TEG cup containing 0·2 M stock calcium chloride (final concentration 10 mM) (Haemoscope, Niles, IL) and analysis was started.

### Whole blood aggregation

Whole blood impedance aggregation (Chrono-Log Model 700, Havertown, PA) using collagen (2 μg/ml), adenosine diphosphate (ADP, 10 μmol/ml) and arachidonic acid (AA, 10 μmol/ml) (all Chrono-Log Corporation) was performed on each day of testing. For each test, 450 μl WB and 450 μl normal saline (NS) were dispensed into a test cuvette. The cuvette was allowed to warm to 37°C, and the impedance probe was inserted into the cuvette. The baseline impedance was set, and the specific agonist was added, according to the manufacturer protocol. After the addition of the agonist, the test was started and allowed to run for 12 min. The lag time and area under the curve were calculated by the instrument software.

### Closure time

Closure time with epinephrine (CT-Epi) was measured via PFA-100 (Platelet Function Analyzer-100, Dade Behring, Deerfield, IL). Briefly, one test cartridge was used per sample, which was allowed to warm to room temperature for 15 min prior to use. The test cartridge was inserted into the instrument and 800 μl WB were dispensed into the cartridge. The test was then started per manufacturer protocol.

### Plasma-free haemoglobin

The plasma-free haemoglobin was assessed using the QuantiChrom Hemoglobin Assay Kit (BioAssay Systems, Hayward, CA). This is a colorimetric assay involving a 96-well plate in which haemoglobin is converted into a coloured end product and read at 400 nm using a spectrophotometer (ELx808 Absorbance Reader, BioTek, Winooski, VT). Briefly, undiluted PPP samples (50 μl each) were pipetted in duplicate into wells, followed by 200 μl of the assay reagent, and blank and calibrator wells were used as controls (the calibrator is equivalent to a free haemoglobin concentration of 0·10 g/dl), as well as in the final calculation of the free haemoglobin concentration.

### P50 of haemoglobin

P50, which is the partial pressure of oxygen at which haemoglobin (Hb) is 50 per cent saturated with oxygen [21], was also obtained for each sample. Physiologically, Hb has a variable affinity for binding to oxygen, which is evaluated via P50. From each WB sample, 50 μl was used to test p50 on the Hemox Analyzer (TCS Scientific Corporation, New Hope, PA) according to manufacturer protocol. The WB aliquot was diluted with 5 ml of the Hemox buffer solution, 20 μl of anti-foaming agent and 20 μl of bovine serum albumin (BSA) (all obtained from TCS Scientific Corporation). The sample was then inserted into the instrument and simultaneously oxygenated and allowed to equilibrate to 37°C. Samples were then de-oxygenated via the introduction of nitrogen over time. The oxygen dissociation curve was then recorded by the analyser.

### Statistical analysis

Day 0 samples from each donor were used as controls for all experiments. Due to variability between donors, per cent changes over the storage period were calculated for each test value of each donor. The average per cent change for each test was then calculated for all donors over the 28 day storage period. The Student *t*-test was performed in all pair testing statistical analyses, calculated from the raw values. A *P*-value of $<0{\cdot}05$ was considered to be statistically significant.

## Results

### Platelet counts significantly decreased over two week period

Platelet count markedly decreased starting from day 0 and continuing throughout the storage period, becoming statistically significant by day 14 (dropping from $158 \pm 47$ to $70 \pm 44 \times 10^3$, respectively, $P < 0{\cdot}05$) and worsening by day 21 ($59 \pm 34$, $P < 0{\cdot}005$). Significant decreases in WBC were also observed towards day 28 ($P < 0{\cdot}05$) (Table 1).

### Significant prolongations in prothrombin time (PT) and partial thromboplastin time (PTT) observed by the third day of storage

Both PT and PTT increased significantly by day 3 (from $12{\cdot}1 \pm 0{\cdot}8$ to $13{\cdot}3 \pm 0{\cdot}9$ seconds, and $32{\cdot}7 \pm 2{\cdot}7$ to $36{\cdot}8 \pm 3{\cdot}0$ seconds, respectively, $P < 0{\cdot}05$ each) and remained significant throughout the 28-day storage period to reach $16{\cdot}8 \pm 1{\cdot}5$ and $49{\cdot}0 \pm 8{\cdot}6$ seconds on day 28, respectively ($P < 0{\cdot}005$ each) (Table 1).

### Thromboelastography revealed impaired haemostatic function

Several viscoelastic properties as measured by TEG were found to gradually change throughout the storage period. While R time remained relatively stable, the K time increased, becoming significant by day 21 with a 33% increase as compared to day 0 ($P < 0{\cdot}05$). The maximum amplitude (MA) gradually decreased, becoming significant once it had decreased by 11% on day 21 ($P < 0{\cdot}05$). However, these changes were still within the normal TEG

**Table 1** CBC, PT and PTT results up to day 28

| CBC | Day 0 | Day 3 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|---|
| WBC ($10^3$/μl) | 4·8 ± 1·3 | 4·9 ± 1·3 | 4·6 ± 1·4 | 3·9 ± 2·1 | 3·2 ± 1·7 | 2·8 ± 1·6* |
| RBC ($10^6$/μl) | 3·9 ± 0·2 | 3·8 ± 0·5 | 3·7 ± 0·4 | 3·8 ± 0·6 | 3·7 ± 0·4 | 3·9 ± 0·5 |
| HGB (g/dl) | 12·2 ± 1·0 | 11·9 ± 1·7 | 11·5 ± 1·5 | 11·7 ± 2·0 | 11·5 ± 1·6 | 12·1 ± 1·9 |
| HCT (%) | 35·1 ± 2·9 | 34·9 ± 5·6 | 34·3 ± 4·9 | 35·9 ± 6·2 | 35·5 ± 5·3 | 37·3 ± 6·7 |
| MCV (fl) | 89 ± 3 | 91 ± 4 | 92 ± 4 | 94 ± 5 | 95 ± 6 | 95 ± 7 |
| MCH pg/cell) | 31·1 ± 1·1 | 31·0 ± 1.0 | 30·1 ± 1·1 | 30·7 ± 1·2 | 30·8 ± 1·2 | 31·0 ± 1·1 |
| MCHC (g/dl) | 34·8 ± 0·6 | 34·0 ± 1·2 | 33·5 ± 1·0* | 32·6 ± 1·1* | 32·4 ± 1·5* | 32·6 ± 1·7* |
| PLT ($10^3$/μl) | 158 ± 47 | 115 ± 65 | 105 ± 50** | 70 ± 44** | 59 ± 34** | 55 ± 48** |
| RDW (%) | 12·7 ± 0·3 | 12·6 ± 0·5 | 12·9 ± 0·5 | 12·9 ± 0·8 | 12·8 ± 0·9 | 12·7 ± 0·9 |
| PT (seconds) | 12·1 ± 0·8 | 13·3 ± 0·9* | 13·8 ± 1·4* | 14·7 ± 1·6* | 15·8 ± 1·7** | 16·8 ± 1·5** |
| PTT (seconds) | 32·7 ± 2·7 | 36·8 ± 3·0* | 38·3 ± 5·1* | 41·4 ± 5·7* | 45·4 ± 7·8* | 49·0 ± 8·6** |

Data shown in mean ± SD.

CBC, complete blood count; HCT, haematocrit; HGB, haemoglobin; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; PLT, platelets; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cells; RDW, red blood cell distribution width; WBC, white blood cells.

*$P < 0.05$.

**$P < 0.005$.

range (50–72 mm). No changes in α angle were observed throughout the storage period (day 0: 53·97 ± 9·74; day 28: 46·53 ± 4·78 degrees; $P = 0.136$). The LY30, which measures fibrinolysis, was significantly decreased by day 3 ($P < 0.05$). The coagulation index (CI), which is indicative of the clot firmness and stability, was significantly decreased by day 28 ($P < 0.05$), which implies worsening overall clot formation (Table 2).

### WB aggregation in response to collagen decreased significantly by day 3

Significant decreases in WB aggregation in response to collagen were observed starting on day 3 (78% decrease, $P < 0.05$). Marked decreases in aggregation in response to AA and ADP were also recorded throughout the storage period but were not statistically significant (Table 3).

### Closure time with epinephrine (CT-epi) was significantly prolonged by day 3

Compared to the normal CT-Epi for all donors at time of donation (day 0), a significant increase of 64% was seen by day 3 ($P < 0.005$). This persisted to reach a 95% increase by day 28 ($P < 0.001$) (Fig. 1).

### Significant changes were also observed for P50 and plasma-free haemoglobin

The oxygen dissociation as measured by P50 decreased gradually throughout the storage period, becoming significant after a 36% decrease on day 21 as compared to baseline ($P < 0.05$). The plasma-free haemoglobin increased substantially by day 3 (0·06 ± 0·03 g/dl, 38%) and throughout the storage period to reach 0·12 ± 0·03 g/dl by day 28 (163%), which was not statistically significant ($P = 0.16$) but clinically important (Table 4).

## Discussion

Within three days of storage at 4°C, we observed significant decreases in platelet count and function in terms of closure time and WB aggregation, in addition to prolongation in PTT. However, TEG data indicated the preservation of platelet function in terms of close to normal MA up to day 21, as well as conservation of coagulation factors and fibrinogen as measured by R time and α angle, which retained their function throughout the whole storage period. K time, which also represents the quality and quantity of fibrinogen, was retained until day 21. Likewise, the CI was increasing throughout the period, which denotes a marked shift towards hypercoagulability by the end of the storage period. These results indicate that the cold-stored WB was able to retain most of its haemostatic function until at least day 21, making it a potentially viable option for the management of trauma-induced coagulopathy within that timeframe if kept refrigerated. Furthermore, the preserved haemostatic function throughout the long storage period alone lends itself as a better option than the component storage of platelets, which only have a 5-day shelf life in the United States and have

**Table 2** TEG results up to day 28

| TEG | Day 3 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| R in min (%) (4–10 min) | 6·7 ± 1·8 | 6·6 ± 1·7 | 7·9 ± 1·7 | 8·1 ± 2·1 | 7·6 ± 1·6 |
| K in min (%) (1–3 min) | 2·6 ± 0·7 | 2·4 ± 0·4 | 2·9 ± 0·4 | 3·3 ± 0·5* | 4·2 ± 0·9** |
| Angle in degrees (%) (53–73 deg) | 50·5 ± 14·3 | 58·7 ± 4·2 | 52·1 ± 2.6 | 51·2 ± 4·5 | 46·5 ± 4·8 |
| MA in mm (%) (50–72 mm) | 54·1 ± 6·4 | 53·2 ± 3·1 | 52·2 ± 2·8 | 48·9 ± 2·8* | 44·3 ± 3·1** |
| LY30 (%) (0–8%) | 0·3 ± 0·6* | 0·0 ± 0·0* | 0·0 ± 0·0* | 0·0 ± 0·0* | 0·0 ± 0·0* |
| CI (%) (–3 to 3) | –2.7 ± 2·6 | –2·1 ± 1·1 | –3·7 ± 1·4 | –4·4 ± 1·7 | –5·4 ± 1·3* |

Normal ranges are shown for each parameter. Data shown in mean ± SD.
*$P < 0·05$.
**$P < 0·005$.

**Table 3** WB aggregation results up to day 28

| Agonist | Day 3 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| AA lag time | –43% | –6% | –24% | –34% | 19% |
| AA area under the curve | 75% | 29% | –46% | –4% | –38% |
| ADP lag time | –1% | 37% | 4% | 1% | 38% |
| ADP area under the curve | –50% | –40% | –48% | –52% | –67% |
| Collagen lag time | 199% | 212% | 188% | 233% | 227% |
| Collagen area under the curve | –78%* | –92%* | –80%* | –90%* | –92% * |

Data shown in per cent change.
*$P < 0·05$.

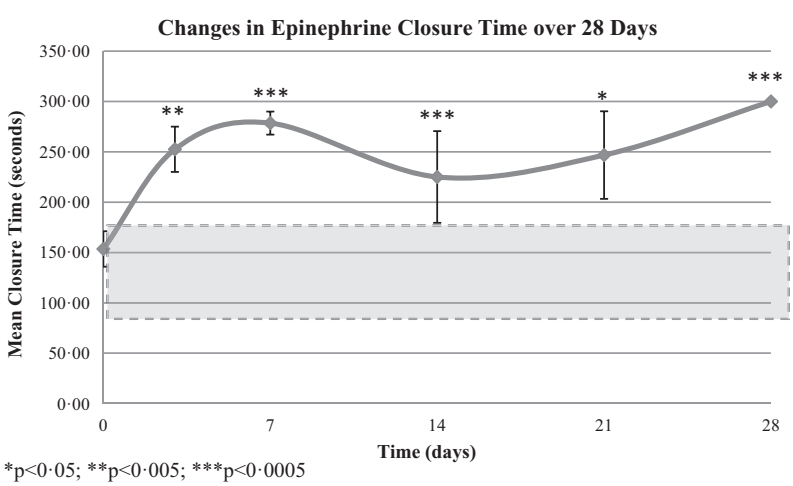


**Fig. 1** Epinephrine closure time results up today 28. Data shown in mean ± SD. Reference range also shaded in green. *$P < 0·05$; **$P < 0·005$; ***$P < 0·0005$

**Table 4** P50 and plasma-free haemoglobin results up to day 28

| Test | Day 3 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| P50 in mmHg (%) (26·7 mmHg) | 34·5 ± 12·7 (–5) | 24·0 ± 4·2 (–34) | 26·2 ± 10·1 (–28) | 23·4 ± 6·3 (–36)* | 19·7 ± 1·3 (–46)* |
| Free haemoglobin in g/dl (%) (<0·05 g/dl) | 0·06 ± 0·03 (38) | 0·06 ± 0·04 (36) | 0·08 ± 0·06 (72) | 0·08 ± 0·03 (80) | 0·12 ± 0·03 (163) |

Normal ranges shown for each test. Data shown in mean ± SD (per cent change).
*$P < 0.05$.

demonstrated impaired function in vivo [22]. Of course, further studies are required to ascertain whether WB retains its haemostatic function *in vivo*.

On the other hand, high levels of plasma-free haemoglobin, while not statistically significant, were detected by day 3 and were markedly increased by day 28 as compared to day 0. The abundance of free haemoglobin could cause clinical complications such as renal injury, inflammation, thrombosis, and acute and chronic vascular disease [23], and these risks would be multiplied after transfusion of multiple units of WB, which would be expected in a massively exsanguinating trauma patient. Previous studies have shown that average plasma-free haemoglobin concentrations for packed RBC units at 2, 26 and 40 days of storage are 0·017, 0·090 and 0·193 g/dl, respectively [24], indicating that our WB unit had an approximately 33% higher plasma-free haemoglobin concentration at 28 days as compared to an average 26-day-old RBC unit. In addition, the significant decrease in P50 by day 21 suggests an increase in oxygen binding affinity, thereby reducing the carrying capacity to the tissues. This would be another indication that cold-stored WB starts to have significant deleterious changes by 21 days that render it ineffective and potentially harmful if transfused after that point.

Our study was notably limited by a small sample size. In addition, our WB units were not leucoreduced, since the presence of WBC is not significant when measuring the haemostatic potential *in vitro*. However, it would be important to reproduce these results using leucoreduced WB units, as up to 80% of all RBC units transfused in the United States are leucoreduced [25]. In addition, our study lacked a proper control group, as the control for WB would be separate blood components, and it would be difficult to achieve a physiologically correct ratio using component units *in vitro*. We also chose to perform closure time with epinephrine only as opposed to both epinephrine and adenosine diphosphate (ADP). Closure time was used only to assess overall platelet function as opposed to a specific deficiency in response to either epinephrine or ADP, especially given that the WB units came from normal donors and thus the closure time with ADP would be expected to yield proportionally increased results as compared to epinephrine.

Despite increasing discussion of the potential return to WB transfusion in lieu of separate blood components in damage control resuscitation, there has been limited research surrounding its efficacy both *in vitro* and *in vivo*. Our limited *in vitro* data indicate that WB units can be considered more haemostatically efficient (via preserved TEG parameters) and less potentially harmful (via lower plasma-free haemoglobin levels) up to day 21 of storage. Further clinical studies are required to evaluate the optimal storage times as well as patient outcomes after resuscitation with WB as compared to blood components.

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## Conflicts of interest

The authors declare no conflicts of interest.

## Author contributions

Hannah McRae: wrote manuscript, devised and performed experiments, analyzed data. Ferhat Kara: assisted in performing experiments, data analysis, final manuscript review. Chelsea Milito: assisted in data analysis and statistics, final manuscript review. Christine Cahill: involved in study conception, final manuscript review. Neil Blumberg: involved in study conception, final manuscript review. Majed Refaai: study conception, assisted in data analysis and statistics, supervised research, assisted in manuscript preparation, final manuscript review.

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# Evaluation of the WHO global database on blood safety

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Vox Sanguinis

## Abstract

**Objective** While the Global Database on Blood Safety (GDBS) helps to monitor the status of adequate and safe blood availability, its presence alone does not serve as a solution to existing challenges. The objective of this evaluation was to determine the GDBS usefulness in improving the availability of adequate safe blood and its ability to function as a surveillance system.

**Methods** The GDBS was evaluated using methods set out by the Centers for Disease Control and Prevention (CDC) Guidelines for assessing surveillance systems. Six recommended tasks were used to evaluate if the GDBS met the requirements of a surveillance system in a public health context.

**Results** The majority of stakeholders engaged with GDBS found it was unique and useful. The GDBS answered all six questions essential for determining a blood safety surveillance system's usefulness. The GDBS fully met the needs to six of the eleven attributes used for evaluating the usefulness of a surveillance system.

**Conclusion** The GDBS is a unique global activity that provides vital data on safety of blood transfusion services across countries and regions. However, aspects of the GDBS such as timeliness of reporting and improvement of WHO Member States national blood information systems could enhance its effectiveness and potential to serve as a global surveillance system for blood safety.

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**Key words:** blood safety, WHO, transfusions, surveillance.

## Introduction

The right to an adequate and safe blood supply is one that the World Health Organization (WHO) has championed for over a decade [1]. However, in many low-resource countries, the morbidity and mortality associated with an inadequate supply of safe blood products remains high and has a direct impact on individuals and their families [2]. Blood transfusion is an essential component of health care which saves millions of lives each year. Every second, someone across the world needs blood for surgery, trauma, severe anemia or complications of pregancy [3,5,6]. An investment in a safe and adequate blood supply is therefore not only a responsibility of governments, but also a cost-effective investment in the health and economic wealth of every nation [4, 5].

The Global Collaboration for Blood Safety (GCBS) was a WHO-convened forum, established in 1995 in response to the Paris AIDS Declaration to fight HIV/AIDS [4]. The GDBS had the mission to promote the harmonization of all efforts to improve global blood safety and avoid duplication of activities [7].

The WHO Global Database on Blood Safety (GDBS) was established in 1998 and in 2001, the WHO Global Database on Blood Safety (GDBS) published its first report on blood safety in WHO Member States. Over the years, the GDBS has evolved from paper-based forms to electronic forms. Despite many initiatives and interventions, blood safety remains an important public health concern in

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Africa and other parts of the world where lack of availability of blood or provision of unsafe blood impacts morbidity and mortality [6]. One measure of a successful public health programme is the production, analysis, dissemination and use of reliable and timely information on health determinants and health status; however, the GDBS has struggled with the timely dissemination of information [7, 8].

Despite the GDBSs' operating for over a decade, it has never been formally evaluated for its impact on improving blood safety and public health globally. The aim of this paper is to evaluate the GDBS using the Centers for Disease Control and Prevention (CDC) Updated Guidelines for Evaluating Public Health Surveillance Systems (hereafter, Guidelines) as a framework to assess if the GDBS has the potential to function as a public health surveillance system [9].

## Methods

We used the CDC Guidelines as a framework to evaluate the usefulness of the GDBS as a global health tool and its ability to serve as a potential surveillance system. According to the CDC Guidelines, a surveillance system is defined as 'the ongoing systematic collection, analysis and interpretation of health data essential to the planning, implementation and evaluation of public health practice, closely integrated with the timely dissemination of this information to those who need to know' [9]. The CDC guidelines recommend the use of six required tasks in the performance of surveillance evaluations [9]:

- Task A. Engage the Stakeholders in the Evaluation
- Task B. Describe the system to be evaluated
- Task C. Focus the Evaluation Design
- Task D. Gather Credible Evidence Regarding the Performance of the System
- Task E. Justify and State Conclusions, Make Recommendations
- Task F. Ensure Use of Evaluation Findings and Share Lessons Learned

## Literature review

We conducted a literature review of the English-language peer reviewed academic research and grey literature (that is published reports by WHO). The internet and PUBMed was searched for reports published from 1995-2017 containing the words: Global Data Base on Blood Safety and Blood Safety Database. A review of existing literature did not reveal any similar or comparable global blood safety database or other comprehensive blood safety data sources or tools available for monitoring the availability of adequate and safe blood around the world.

### Patient and public involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. Patients were not invited to contribute to the writing or editing of this document for readability or accuracy.

## Results

### Public health information system evaluation

Since its establishment, the GDBS has collected vital data from WHO Member States, used to guide recommendations and strategies to improve global blood safety.

#### *Task A. Engage the stakeholders in the evaluation*

We engaged the GDBS technical advisor and the expert committee that oversees the GDBS status reports when conducting this evaluation. The expert committee consisted of representation from the six WHO regions. An informal interview of the expert committee was conducted with open ended questions. Due to time constraints, it was not possible to engage all member states for the evaluation. However, we were able to engage with a single member state during the course of the evaluation. The conclusions drawn from the stakeholder interviews were used primarily as anecdotal evidence.

#### *Task B. Describe the system to be evaluated*

The GDBS was designed to collect key data that facilitate to monitoring of blood transfusion services in all Member States of the WHO with the following objective: To collect and analyze data from all countries on blood and blood product safety as the basis for effective action to improve blood transfusion services globally. The GDBS yearly sends a standardized questionnaire consisting of 7 sections and 100 indicators to all health authorities in WHO Member States. In 2002, the questionnaire was revised and in 2005, the questionnaire was translated and issued in six languages (i.e., Arabic, Chinese, English, French, Russian and Spanish).

A programme logic model (Fig. 1) was developed by the authors to explain the system's objectives, resources, activities, outputs and outcomes. Blood donors and blood donation information is collected at the level of the blood banks; data are aggregated at the level of the National Blood Transfusion Service (NBTS) or by an organization or entity that is responsible for national blood safety

programs, and submitted to the national health authorities who report the information to GDBS. In some cases, the data maybe aggregated at the level of the national health authority and then reported to GDBS. A group of international experts from the transfusion medicine community analyses that data, and WHO generates and disseminates the report and findings to WHO Member States (Table. 1). The total cost of personnel and blood information systems per NBTS is dependent upon multiple factors such as size of country, population, existing systems and Gross Domestic Product. WHO and CDC currently fund the GDBS. However, at the Member State level funding for the NBTS is primarily from MOH and other donor sources.

### *Task C. Focus the evaluation design*

The intent of the evaluation was to understand if the GDBS, which was established to provide data on blood transfusion services in all WHO Member States, met this need and if its purpose could be expanded to that of surveillance system. CDC Guidelines suggest that meeting the following attributes: simplicity, flexibility, data quality, acceptability, sensitivity, positive predictive value, representativeness, timeliness and stability, are good indicators that a surveillance system will be more useful and complete for public health action [9]. However, due to the scope of the GDBS, and need to focus on characteristics that are essential for a useful surveillance system, six attributes were prioritized. The system attributes prioritized for this GDBS evaluation were usefulness, representativeness, simplicity, flexibility, stability and acceptability. We then proceeded to evaluate each attribute prioritized based upon how they provided evidence supporting the activity objectives (Table 1), which included collecting and analysing data from all countries, assessing global blood safety, best available information and monitoring trends in blood safety. The overall performance of each attribute was evaluated, using a rating system of; fully meets the needs, partially meets the needs, does not meet the needs, and not applicable.

### *Task D. Gather credible evidence regarding surveillance system performance*

A surveillance system is considered useful if it answers at least one of six questions (Table 2) [9, 10]. The GDBS exceeds the criterion set by the CDC Guidelines for usefulness as demonstrated in that it answers all six questions [9]. The GDBS was designed to collect and analyze data on national blood systems from all countries as the basis for effective action to improve access to safe blood and blood products and transfusions globally2. We reviewed all GDBS reports from 1998 to 2016, for criteria that answered or was related to the six questions.

*Evaluation of surveillance attributes*. Using a rating scale developed for this purpose, of fully meets needs (80-100%), partially meets needs (60–79%), does not meet needs (<59%) and not applicable, we evaluated the ten attributes (Table 3) of a public health surveillance system as stated in the CDC Guidelines [10].

*Acceptability was rated as 'fully meets the needs'*: On average 87% (77–93%) of all WHO Member States (195) fully participate in the process of reporting data into the GDBS [1, 5, 11–14].

*Data Quality was rated as 'partially meets the needs'*: The GDBS data quality was rated 'partially meets the needs' because at the central level, the data are cleaned with routinely conducted data checks. However, at the WHO Member State level data reported into the GDBS often times only represents data collected at the NBTS and excludes data on blood safety activity conducted in the private sector. Some WHO Member States have blood safety information systems that make the collection, analysis and report generation easier and more accurate, whereas others have less automated systems with a number of choke points for error.

*Simplicity was rated as 'partially meets the needs'*: The data information flow is simple at the level of WHO headquarters in Geneva and at the WHO Member State level. NBTS collect data that are reported to health authorities who subsequently report the data to WHO. However, the GDBS's simplicity is dependent on the existing funding, infrastructure and reporting mechanism of the individual NBTS of the Member State. For example, Member States whose NBTS depends on a paper-based system may find it difficult to provide the data required for the country to submit into the GDBS. Therefore, the rating is 'partially meets the needs', the system meets the needs when the mechanism for data flow exists within Member States.

*Flexibility was rated as 'fully meets the needs'*: Over the past decade, the GDBS has adapted to changing needs in the field of blood safety and public health, making significant changes at four different time points. The GDBS has also adapted to advances in technology by transitioning to web-based electronic data collection and availability of forms in multiple languages.

*Informatics was rated as 'partially meets the needs':* The GDBS was rated as 'partially meets the needs', because the data housed in the GDBS at headquarters are comprehensive, spanning 20 years and over 195 WHO Member States on secure servers. However, the data quality reported into the system is dependent upon the

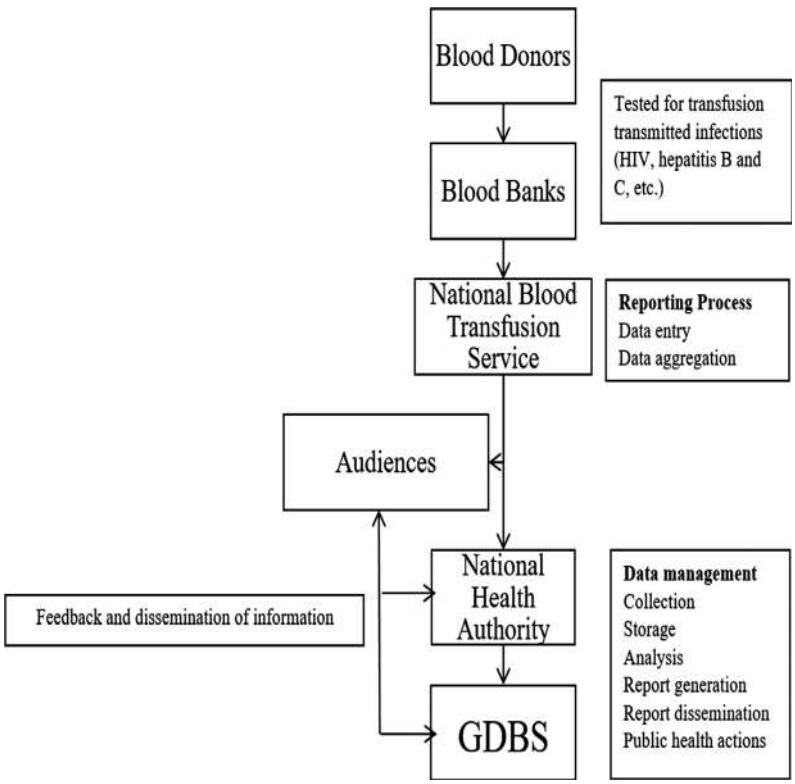


**Fig. 1** Simplified chart for data flow within the Global Database on Blood Safety (GDBS).

existing funding, infrastructure and health systems in WHO Member States. Unfortunately, there does not exist a universally agreed upon computerized blood bank information management system that all WHO Member States could use. Instead, WHO Member States and WHO regions use different commercial systems for data collection, tracking and monitoring.

*Positive Value Predictive (PVP) was rated as 'not applicable':* The PVP for the GDBS could not be calculated due to factors, such as the GDBS not capturing data on a single disease; multiple indicators being captured; testing algorithms between countries varying greatly.

*Representativeness was rated as 'fully meets the needs':* The objective of the GDBS is to provide information and guidance pertaining to the status of adequate and safe blood. The GDBS has a global reach extending over all six regions and including all 195 WHO Member States (2018). The 2016 GDBS status report included data from 180 countries representing a total population of 7 billion or 98·3% of the global population [1]. This does not take into consideration blood collected within the private sector and only refers to blood collections made in the public sector; however, it is representative of public sector data and adequate and safe blood availability in countries.

*Timeliness was rated as does 'not meet the needs'*: The need to provide an update on the status on adequate and safe blood at a regular interval is a critical objective of the GDBS. WHO Member States do not have a fixed timeline for reporting; instead, they are encouraged to report data on a yearly basis. Reporting times from WHO Member States into the central GDBS varied from one year to eighteen months. Thus, the variability in reporting leads to the inability to publish the data on a regular interval.

*Sensitivity was rated as 'fully meets the needs':* Sensitivity can be defined as the probability that a positive result occurs when the condition actually exist. Given the complexity of the GDBS and the large number of indicators monitored, sensitivity could not be calculated in the traditional sense.

Therefore, for the purpose of the calculating sensitivity, we used self-reported published data provided by NBTS from the President's Emergency Plan for AIDS Relief (PEPFAR) supported countries in the AFRO region as the gold standard and data reported into the GDBS from the same subset of countries. We used purposive selection of the countries based upon the accessibility of data, representation of national blood donations (100% of donations in the country are collected in the public sector with no blood collections conducted by the private sector), and

**Table 1** Programme logic model showing activity objectives, resources, activities and outputs: Global Database on Blood Safety

| | |
|---|---|
| Objectives | Collect and analyse data from all Member States on blood and blood product safety as the basis for effective action to improve blood transfusion services globally |
| | To assess the global situation on blood safety |
| | To obtain the best available information on blood transfusion services in each Member State |
| | To identify problems and needs in order to provide appropriate technical support |
| | To identify countries for priority assistance |
| | To monitor progress and trends in blood safety |
| Resources | Funding to support activity at central level (WHO) |
| | Funding to support activities both at central and country level (CDC) |
| | Funding at country level (MOH and NBTS) |
| | Technical Blood Safety Advisor |
| | Administrative staff at Member State health ministries |
| | Blood Transfusion Service Staff |
| | Supplies and Equipment |
| Activities | Disseminate data reporting forms to NBTSs |
| | Collect and aggregate data |
| | Provide reminders for delayed reporting |
| | Analyse, interpret data and generate reports |
| | Provide recommendations based upon emerging trends and evidence-based research |
| | Provide technical support to countries |
| Outputs | Recommendations for improving blood safety |
| | Assist Member States prioritize their needs in strengthening NBTS |
| | Provides yearly updates to CDC on priority countries |
| | Published updates available to public via WHO websites |
| Short-term outcomes | Early detection of changing trends in blood safety |
| | In some countries, only source of data on diseases such as hepatitis B and hepatitis C |
| Middle-term outcomes | Understanding of changing priorities and needs |
| | Identification of trends in transfusion-transmitted infections |
| | Adequate availability of blood |
| Long-term outcomes | Potential to identify shortages in global blood availability |
| | Potential to become a global surveillance system for blood safety |
| | Potential to identify emerging trends in blood safety and availability |

CDC, Centers for Disease Control and Prevention; MOH, Ministry of Health; NBTS, National Blood Transfusion Services; WHO, World Health Organization.

similarity of reported indicators. To calculate the sensitivity of the GDBS, we used select indicators as a proxy for the whole database. The selected indicators used were 'Units of Blood Donated' and 'Units Screened'.

The objective of this exercise was to estimate the ratio of the (blood donated: NBTS) to the (blood donated: GDBS. A ratio greater than 1·0 suggest that (blood donated: NBTS > GDBS), a ratio less than 1·0 indicates that the (blood donated: NBTS < GDBS), and a ratio of 1·0 means that (blood donated: NBTS = GDBS). In the following calculations, we are using a ratio of NBTS/GDBS. To demonstrate this, the ratio of (blood donated NBTS) to (blood donated reported to GDBS) in country A is 1·148, which means that self-reported data from NBTS recorded 14·8% more total blood donations than the GDBS. In contrast, the ratio in country B is 0·844, which suggests that the GDBS captured 15·6% (1 − 0·844 = 0·156 × 100 = 15·6%) fewer total donated blood units reported as compared with NBTS. In country C, the ratio was far less 0·3% (1 − 0·997 = 0·003 × 100 = 0·3%) which suggests that self-reported data from NBTS captured 0·3% less total donated blood units reported compared with GDBS. Six out of the nine countries had ratios of 1·0, suggesting that the majority of the countries captured by the NBTS and GDBS were reporting the same figures. Three countries detected differences across the indicator 'Blood donated'.

When using the same method to compare the ratio of the (Units Screened for TTIs: NBTS) to the (Units Screened for TTIs: GDBS) the findings were similar. The ratio of 'screened units' (NBTS: GDBS) in country A was 1·092, which means that the self-reported NBTS data recorded 9·2% more units screened than the GDBS. In contrast, the ratio in country B is 0·824, which suggests that the self-reported NBTS data captured 17·6 (1 − 0·824 = 0·176 × 100 = 17·6%) fewer units screened as compared with GDBS. In country C, the ratio was far less

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**Table 2** Measure of GDBS usefulness

| Questions from CDC[a] Guidelines | GDBS answers | Evidence[b] | Measure |
|---|---|---|---|
| Detects diseases, injuries, or adverse or protective exposures of public health importance in a timely manner | The GDBS detects diseases and adverse exposures of public health importance.<br>The GDBS monitors TTIs and hemovigilance. | Screening for transfusion transmitted infections increased from 54% in 1998, to 91% in 2016<br>Globally, 2013 it is estimated that 1.8 million blood donations collected were discarded due to transfusion-transmissible infection reactivity | √ |
| Detect trends that signal changes in occurrence of disease, including the detection of epidemics | The GDBS collects and analyzes data on four TTIs in all member states | In 1998-1999, 40% of LMIC did not screen for all four TTIs, while in 2016 only 7% did not screen for TTIs<br>In 2016, GDBS noted that the prevalence of the four TTIs in low-income countries was 1.08% (HIV), 3.74% (HBV), 1.03% (HCV), and 0.90% (syphilis) | √ |
| Lead to improved clinical behavioral, social, policy or environmental practices | The GDBS has led to implementation and adaption of the following policies: national blood policy, screening policy, and hemovigilance policy to name a few. | In 1998-1999, a total of 106 countries reported that national blood policy had been developed versus 145 countries in 2001-2002<br>In 2004-2005, only 49% had a policy on the clinical use of blood while in 2016, 70% had such a policy | √ |
| Provide estimates of the magnitude of morbidity and mortality, and identification of events under surveillance | The GDBS monitors the magnitude or transfusion-transmitted infections and adverse events. | In 2013, the GDBS 39% (70 of 180) countries reported data on adverse events | √ |
| Permit assessment of the effect of prevention and control programs | The GDBS collects and analyzes data on quality assurance and monitoring | In 2016, a total of 155 countries reported the existence of national standards for collection, testing, processing, storage and distribution of blood and blood products | √ |
| Stimulate research intended to lead to prevention and control | The GDBS stimulates research in the field of transfusion medicine intended to lead to prevention and control programs. | The GDBS has stimulated much research around the blood donors and the clinical use of blood | √ |

Abbreviations: GDBS = Global Database on Blood Safety; LMIC = Low and Middle Income Countries; NBTS = National Blood Transfusion Services; TTI = Transfusion TransmittedInfections √= yes meets criteria set by CDC guidelines

[a]German RR, Lee LM, Horan JM, et al. Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group. *MMWR Recomm reports Morb Mortal Wkly report Recomm reports*. 2001.

[b]World Health Organization. *Global Status Report on Blood Safety and Availability*. Geneva; 2016. http://www.who.int/bloodsafety/global_database/en/.

**Table 3** Summary of CDC[a] guidelines attributes, definition and GDBS rating

| Attribute | Definition | GDBS Rating |
|---|---|---|
| Acceptability | Willingness to participate in the activity | Fully meets the needs |
| Data Quality | Completeness and validity of the data | Partially meets the needs |
| Flexibility | Adaptability to changing information needs | Fully meets the needs |
| Informatics | User experience | Partially meets the needs |
| Positive Value Predictive | Proportion of reported cases that actually have the health-related event under surveillance | Not applicable |
| Representativeness | The occurrences of the health event over time and its distribution in the population | Fully meets the needs |
| Simplicity | Ease of activity operation | Partially meets the needs |
| Sensitivity | Proportion of reported cases of a disease detected by the system | Fully meets the needs |
| Stability | System reliability (i.e. the ability to collect, manage and provide data properly without failure) and availability (ability to be operational when needed) | Fully meets the needs |
| Timeliness | Speed between steps in as system | Does not meet the needs |
| Usefulness | Value, or practicality of the information generated | Fully meets the needs |

GDBS, Global Database on Blood Safety.

[a]German RR, Lee LM, Horan JM, et al. Updated guidelines for evaluating public health surveillance systems: recommendations from the Guidelines Working Group. *MMWR Recomm reports Morb Mortal Wkly report Recomm reports*. 2001.

0·996, which suggest that the NBTS captured 0·4% ($1 - 0{\cdot}996 = 0{\cdot}004 \times 100 = 0{\cdot}4\%$) fewer units screened than NBTS. Six out of the nine countries had ratios of 1·0, suggesting again that the majority of countries captured by the NBTS and GDBS are reporting the same figures with differences only in three out of the nine countries.

Overall, we see that for the indicators 'Blood donation' and 'Units screened', respectively, 92·8% and 91·2% of the time data captured by the self-reported NBTS and GDBS are reporting the same figures. Therefore, we could conclude that sensitivity of the GDBS is high given that six out nine countries report accurate data.

*Stability was rated as 'fully meets the needs'*: Since its inception and implementation, the GDBS has not experienced any interruptions or delays in accessibility of data with comprehensive reports published regularly at 2-year intervals covering data from a year. There have been no reported instances of loss of data or issues with the web-based system for data submission.

Five out of the ten attributes were rated as 'fully meets the needs', three were rated as 'partially meets needs', one was rated as 'does not meet needs', and one was 'not applicable'. Three of the priority attributes (flexibility, acceptability, stability) were rated as 'fully meets the needs' and simplicity was rated as 'partially meets the needs'.

### *Task E and F. Justify and state conclusions and recommendations, and ensure the use of the evaluation findings*

The GDBS was established in 1997 to address global concerns about the availability, safety and accessibility of blood transfusions. The CDC Guidelines were used to assess the GDBS' ability to function as a potential surveillance system. The findings from this review show that the GDBS meets six of the priority attributes that define a surveillance system as being useful. Overall, six out of the eleven attributes (fully met), three (partially met), one (not applicable), and only one (did not meet) the criteria by which the GDBS was evaluated. The results show that the GDBS is an effective public health activity that has the potential to serve as a surveillance system for capturing information concerning blood availability, safety, haemovigilance and accessibility. The GDBS also serves the role of providing effective feedback to the WHO Member States via analysis and recommendations prepared by experts from the transfusion medicine community. Major stakeholders described the GDBS as a unique activity that captures a wide range of information on all aspects of blood transfusion including, collection, screening, distribution and haemovigilance not only on a local level but at global one [15].

The GDBS exceeded the CDC Guidelines for the minimum requirement of a system's usefulness by successfully answering all six questions meant to establish if a surveillance system is useful. Further review also revealed that the GDBS was acceptable, flexible and stable and had a high representativeness among WHO Member States. According to the standards stated in the CDC Guidelines these attributes indicate that the GDBS activity is useful and provides vital information for public health action. Overall, the GDBS meets the needs of the stakeholders, although the timeliness and data quality could be improved.

## Discussion

Public health information systems are designed to provide specific information on a particular health condition(s)

that affect a large population. The data from such systems ideally can be used to monitor trends, identify outbreaks, provide recommendations, develop and implement public health programmes/activities. The GDBS despite monitoring changing trends of the availability of safe blood globally was not designed to serve as a surveillance system; however, after fifteen years of serving as the only global blood safety database in existence and given the needs of the future, we should consider expanding its use beyond that of a database, to that of a blood safety surveillance system. The impact of the GDBS could be made broader by adapting its use to the of a surveillance system which would allow for serial analysis of progress made in the field of blood safety. Multiple studies cite the GDBS as a source of data when evaluating the status of blood safety across multiple countries [18-22]. The GDBS remains the single most comprehensive source of blood safety and availability data that is collected globally and serves as a critical source for monitoring trends and progress made in the field of safe blood transfusions. The GDBS though not designed as a surveillance system definitely meets the six essential criteria of usefulness of public health surveillance systems. The GDBS evidently is a unique activity that collects information from all regions of the world while at the same time monitoring changing trends among diseases such as HIV, hepatitis B, hepatitis C and syphilis in blood donations and serves functions very similar to that of a proxy surveillance system. According to the GDBS status reports, HIV prevalence among blood donors in low-income countries form 11% in 2004–2005 to 1·08% in 2016 [1,13]. The impact of WHO blood safety policies have been captured by the GDBS in aspects of blood transfusions such as: nationally coordinated blood transfusion services; collection of blood from voluntary non-remunerated blood donors; testing and screening of all blood donors for blood grouping and TTIs; and reduction in unnecessary transfusions [23].

The data housed in the GDBS serve an important public health role in monitoring the safety and availability of blood and has the potential to serve as an important global surveillance system for blood safety. The GDBS has greatly influenced the availability of safe blood in the field of HIV transmissions, via policy, guidelines and advocacy for safe blood that results from the availability of data [24]. Research shows that almost 500,000 potential HIV infections via blood transfusions are averted in SSA through the adoption of simple blood safety measures in voluntary blood donation, blood donor selection and quality assured testing of donated blood [24].

Public health programmes can be evaluated for the effectiveness and impact of interventions, policies and public health strategy [16]. Since its establishment the GDBS has not been formally evaluated as to its impact on global blood safety as stated in its objectives. The CDC Guidelines provided a standardized framework for evaluating all attributes of the GDBS while also allowing the database to be evaluated as a surveillance system. Of the ten attributes evaluated, six fully met the needs of the system with only one attribute, timeliness not meeting the need. Paper-based systems which are used in some Member States are often too slow for analysis to guide urgent action and are difficult to maintain [17]. A well-designed electronic information system facilitates a streamlined data entry process or the direct digital capture of laboratory tests results; efficient data merge capabilities from multiple data sources; automated data quality checks; rapid search, retrieval and visualization capabilities; and early warning alerts for potential outbreaks [10, 17]. Increased commitments and investments by Member States into NBTS electronic information systems is needed for countries who struggle to provide complete and timely data. There is a critical need for governments and development donors to invest in support for information management systems for NBTS and mechanisms for improving data collection, donor and recipient tracking, and hemovigilance at hospitals 2. One of the benefits of routine collection of data from multiple Member States is the ability to use the data for making policy changes, targeted interventions and advocacy. However, despite decades of data collection by the GDBS, research has shown the need for systematic data capture, analysis, and data visualization methods which tend to hinder its goals and objectives [2,4,22]. The GDBS should consider modernizing its data capture and the use of data visualization, to improve its timeliness of reporting and use of data for advocacy.

## Conclusions

Our evaluation indicated that the GDBS met five out of the six criteria set forth by the CDC Guidelines for a surveillance system to be defined as useful. The GBDS also met eight out of the nine attributes used for evaluating the completeness of a public health surveillance system. Though the GDBS has been monitoring the availability, safety and accessibility of blood in all Member States globally for the past two decades successfully meeting its objectives, it may be time to strengthen the activity to that of a surveillance system.

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## Conflicts of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

## Authors' note

The findings and conclusions in this publication are those of the authors and do not necessarily represent the official position of the funding agencies.

## Author contribution

UK led the design of the study, guided the data collection and creation of tables and figures, and drafted the manuscript. DS, MC, MQ and BK provided critical guidance on the project to make it as relevant as possible and contributed to the drafting of manuscript. RS contributed to review data, table and figure creation, and analysis of the data for the manuscript. NB and IB participated in the drafting of the manuscript. FM supervised the data collection and drafting of the manuscript. The corresponding author attests that all listed authors meet the criteria for authorship and that no others meeting the criteria have been omitted. All authors have read and approved the final draft. UK is the guarantor.

## Data sharing

No additional data are available.

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**ORIGINAL PAPER**

# 10-year analysis of human immunodeficiency virus incidence in first-time and repeat donors in Brazil

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**Background and objectives** Incidence in first-time and repeat blood donors is an important measure of transfusion-transmitted HIV infection (TT-HIV) risk. This study assessed HIV incidence over time at four large blood centres in Brazil.

**Materials and methods** Donations were screened and confirmed using serological assays for HIV from 2007 to 2016, and additionally screened by nucleic acid testing from 2011 forward. Limiting antigen (LAg) avidity testing was conducted on HIV seroreactive samples from first-time donors to classify whether an infection was recently acquired. We calculated incidence in first-time donors using the mean duration of recent infection and in repeat donors using classical methods. Time and demographic trends were assessed using Poisson regression.

**Results** Over the 10-year period, HIV incidence in first-time donors was highest in Recife (45·1/100 000 person–years ($10^5$ py)) followed by São Paulo (32·2/$10^5$ py) and then Belo Horizonte (23·3/$10^5$ py), and in repeat donors was highest in Recife (33·2/$10^5$ py), Belo Horizonte (27·5/$10^5$ py) and São Paulo (17·0/$10^5$ py). Results from Rio de Janeiro were available from 2013 to 2016 with incidence in first-time donors of 35·9/$10^5$ py and repeat donors from 2011 to 2016 of 29·2/$10^5$ py. Incidence varied by other donor demographics. When incidence was considered in 2-year intervals, no significant trend was evident. Overall residual risk of TT-HIV was 5·46 and 7·41 per million units of pRBC and FFP transfused, respectively.

**Conclusion** HIV incidence in both first-time and repeat donors varied by region in Brazil. Clear secular trends were not evident.

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**Key words:** blood safety, donors, residual risk estimation, serological testing, transfusion- transmissible infections.

## Introduction

In Brazil, there are more than 3·5 million voluntary blood donations per year. Donors are differentiated as to whether they are providing replacement donation (donation in order to replenish the stock of blood because of the need for blood transfusion to a family member or person who has some relationship with the donor) and community donation (voluntary donation to support the overall blood supply) [1]. In Brazilian Public Health Service, blood centres most donations are from repeat donors (RD), but at the national level donations are equally distributed in first-time donors (FTD) and RD [1].

Trends in human immunodeficiency virus (HIV) incidence in blood donors and the residual risk of transfusion-transmitted HIV in Brazil are unknown. Donations with incident infection, particularly the ones only detected by nucleic acid testing (NAT), have an increased chance of being missed by current screening assays and could lead to transfusion transmission. The higher the incidence in the donor population, the greater the likelihood of donations in the pre-NAT and pre-seroconversion window periods. Therefore, monitoring incidence over time provides an assessment of changes in infection risk in donors as well as an indicator of potential public health concern.

Calculating incidence in repeat donors relies on classical methods [2]. However, incidence can also be calculated in first-time donors using cross-sectional approaches that rely on measures of HIV antibody maturation. Persons with recently acquired infection have lower anti-HIV IgG antibody avidity. The HIV-1 limiting antigen avidity enzyme immunoassay (LAg-Avidity EIA) measures antibody avidity in persons who have seroconverted and allows classification of infections as 'recent' or 'long-term'[3]. The objective of this study is to assess changes in HIV incidence in blood donors over time in different geographic locations and to monitor trends in HIV incidence in FTD and RD based on the demographic characteristics of the blood donor population in the different geographic locations.

## Materials and methods

### Study setting

We estimated HIV incidence in four large public blood centres in Brazil. Together these centres collect approximately 10% of all donations in the country [4]. Donor and donation data from 1 January 2007 to 31 December 2016 were included for Hemominas in Belo Horizonte, Hemope in Recife and Fundação Pró-Sangue in São Paulo. Hemominas, Hemorio and Fundação Pró-Sangue are located in the Southeast and Hemope in the Northeast of Brazil. Complete data for Hemorio in Rio de Janeiro were available from 1 January 2013 to 31 December 2016 for first-time donors and from 1 January 2011 to 31 December 2016 for repeat donors. This study was conducted as part of the NHLBI Recipient Epidemiology and Donor Evaluation Study (REDS)-II and -III Brazil program.

Donor and donation data during the study period were collected into a centralized database. The scope of the data captured in the REDS-II (from 2007 to 2012) and REDS-III (from 2013 to 2016) databases in Brazil has previously been described [5, 6]. Briefly, potential donors included all candidates for blood donation who answered screening questions that included the donor's health history, a brief physical examination with vital signs and a haematocrit/haemoglobin test. Donor eligibility was further assessed through a face-to-face interview with standardized questions, including HIV risk behaviours and risk factors for other transfusion-transmitted infections. RTI International, the data-coordinating centre for REDS-III, performed all statistical analyses.

### Laboratory methods

Samples from all donations were screened by two HIV chemiluminescence immunoassays (ChLIA) or EIAs in parallel from 2007 to 2011 and with one serological assay and NAT in minipool format of 6 donations per pool starting on various dates in 2011 at each site to 2017. Routine donation screening tests were completed according to standard operating procedures at each centre, and the specific serological assay reagents in use at each centre may have varied over time based on the procurement process used in Brazil. The NAT test for HIV, HCV and HBV is the Bio-Manguinhos NAT assay in a minipool format of 6 donations. The assay is the same for all Brazilian Public Health Service blood centres and is used to test about 60% of the overall blood supply in the country [7]. All screening test results were reviewed individually for each donor including the results from additional testing of samples obtained by the centres at the time of donor

return for confirmation testing and notification. If a final status could not be defined based on these results (e.g. EIA reactive and NAT negative or not available, with no additional routine testing results available), we performed Western blot (MP Diagnostics HIV Blot 2·2) on the donation sample at a central laboratory in Sao Paulo.

Available HIV EIA reactive samples were tested at Vitalant Research Institute (San Francisco, CA) using the LAg-Avidity EIA (Sedia BioSciences, Portland, OR). In accordance with the manufacturer's instructions for use, samples with an initial normalized optical density (ODn) value of ≤2·0 were retested in triplicate, with the median of the retest results constituting the final result. Any sample with a final ODn of ≤1·5 was classified as a recently acquired infection [8].

## Calculation of incidence among first-time donors

We defined a FTD to be someone with no history of donation at the participating blood centre. A FTD with an HIV-negative donation could also contribute to the RD analysis if that person donated at least one more time after their first donation. FTD with unknown or indeterminate HIV status or having long-standing infections (based on LAg) were excluded from the numerator and denominator of the incidence calculation. We used LAg results to derive incidence using a mean duration of recent infection (MDRI) of 129 days, which is the estimated MDRI for HIV clade B infection using an ODn threshold of 1·5 [9]. HIV incidence was computed as the number of recent infections over person years. Uninfected donors contributed 129 days each to the total time at risk (denominator), while recently infected donors contributed 64·5 days, based on the assumption of HIV infection occurring, on average, at the mid-point of the MDRI. Results were reported as the HIV infection rate per 100 000 person years ($/10^5$py). These estimates were adjusted for non-LAg avidity tested HIV-positive samples assuming the same proportion of recent and long-term infections as in the tested population. Wald 95% confidence intervals (CI) were calculated for each rate.

Incidence by blood centre was calculated overall and for each of the following 2-year periods: 2007–2008, 2009–2010, 2011–2012, 2013–2014 and 2015–2016. We generated these estimates individually for each centre and then in aggregate for all three centres. For donors from Rio de Janeiro, we calculated incidence for each of the last 2 time periods (data available starting in 2013).

## Calculation of incidence among repeat donors

Repeat donors were defined as any person who made at least two donations during the 10-year study period. Because our analysis was divided into five 2-year period, donors only contributed to incidence in the two-year intervals in which they had two or more donations. For example, a donor that made their first-ever donation in the second interval (2009–2010) would contribute to FTD person years in the second interval. If the donor made a second donation in the same interval, then the donor would also contribute person years to RD incidence. If the donor made two or more donations in the third interval (2011–2012), after making at least one in the second interval, then that donor would further contribute person years to RD incidence in the third interval.

Repeat donors with HIV infection are assumed to be infected at the mid-point of the inter-donation interval. The total individual inter-donation intervals for uninfected donors and half of the inter-donation intervals for infected donors are then summed to determine the total person years. The incidence rates are calculated as the number of infections divided by the py, reported as $/10^5$ py, and with associated Wald 95% CIs.

## Calculation of residual risk for first-time and repeat donors

The residual risk (RR) was estimated by multiplying the overall incidence estimate for 2015–2016 with a model-based estimate of the infectious window period ('risk day equivalents') with minipool NAT screening using the Bio-Manguinhos NAT assay. The model relies on virus doubling time during ramp-up phase viremia [9] the probability of non-detection by minipool NAT screening – estimated using the reported 50% and 95% limits of detection (LoD), calculated from analytic standards [7], the probability of infection when a single virion is present in the transfused product (per-virion infectiousness) [10] and transfused volume of plasma. The per-virion infectiousness is inferred from limited data generated using the simian immunodeficiency virus (SIV) Macaque transmission model for HIV infection [11]. We estimated a point estimate and plausible range for the residual risk of HIV transmission by packed red blood cell (pRBC) and fresh frozen plasma (FFP) transfusion, with an average of 20 and 200 ml of plasma, respectively [10, 12]. The plausible range is based on the lower and upper bounds of the incidence estimate confidence interval and a range of assumed per-virion infectivity levels. The upper end of the range was conservative since it assumed an infectious dose at which no animals in the SIV Macaque studies were infected [11].

## Statistical analysis

We analysed data using SAS 9.4 (SAS Institute, Cary, North Carolina, USA). Results are reported by FTD/RD

status and blood centre. Other potentially important variables influencing incidence included age and sex, type of donation (community or replacement), and calendar time interval. To assess which factors were independently associated with incident infection in multivariable analysis, we employed backward elimination using predictor variables associated with HIV infection at a level of $P \leq 0{\cdot}05$ from bivariable analyses. The final multivariable models include variables significantly associated with incident HIV infection in FTD and RD at a level of $P \leq 0{\cdot}05$. Confidence intervals for incidence adjusting for covariates were computed using multivariable Poisson regression. For each parameter included, the Poisson regression model estimates the confidence intervals, and the Wald chi-square statistic and associated *P*-value. The relative incidence (incidence rate ratio) for levels or categories of each predictor variable compared to the reference group within each variable are reported as exponentiated values to indicate excess risk in incidence in different levels of each categorical variable. Values above 1 indicate greater incidence relative to the reference group and values below 1 indicate lower incidence, adjusting for all other factors included in the model. HIV incidence rates were compared for the five 2-year calendar periods, and we used Poisson regression to assess linear trends in incidence over calendar time. The residual risk model was implemented in Python 3·7 (Python Software Foundation, Beaverton, OR, USA) and is publicly available [13].

## Ethical considerations

Study protocols were approved by the Federal Committee on Human Subjects (CONEP) of the Ministry of Health in Brazil as part of the REDS-II/III International Program, local ethical committees at each blood centre, and also the UCSF and RTI IRBs in United States.

## Results

The four blood centres together collected over 400 000 donations per year during the study period. The study population in Recife, Belo Horizonte and São Paulo comprised 930,180 (36·6%) FTD and 1,614,172 (63·4%) donations by RD (Table 1). Hemorio was not included in the 10-year analysis. We did include data from Hemorio consisting of 173 497 (73·5%) FTD between 2013 and 2016, and 62 447 (26·5%) RD between 2011 and 2016. The overall incidence of HIV among FTD was $34{\cdot}4/10^5$ py and among RD was $25{\cdot}1/10^5$ py. The HIV incidence varied by blood centre, from $45{\cdot}1/10^5$ py in Recife to $23{\cdot}3/10^5$ py in Belo Horizonte among FTD and from $33{\cdot}2/10^5$ py in Recife to $17/10^5$ py in São Paulo among RD (Table 2). There were no significant trends by 2-year intervals either overall or by individual blood centre (Fig. 1) for FTD or RD incidence rates.

### Incidence in first-time donors (FTD)

Among FTD the HIV incidence was highest in Recife ($45{\cdot}1/10^5$ py) followed by Sao Paulo ($32{\cdot}2/10^5$ py) and then Belo Horizonte ($23{\cdot}3/10^5$ py) (Table 2). Overall, Belo Horizonte had the lowest incidence in FTD donors in each 2-year period, except 2009–2010 ($36{\cdot}3/10^5$ py). When stratified by age, the highest HIV incidence was observed among blood donors < 24 years old in Recife at $53{\cdot}8/10^5$ py. In São Paulo and Belo Horizonte the highest incidence was among donors 25–34 years old ($43{\cdot}2/10^5$ py and $29{\cdot}5/10^5$ py, respectively). By 2-year interval, incidence varied among FTD donors, but no specific trend was evident. The results for Rio de Janeiro show incidence rates in FTD in the 2 later 2-year periods (2013–2014 and 2015–2016) to be similar in magnitude to those in Recife and Sao Paulo. HIV incidence in FTD donors was highest in Recife males, community donors and those < 24 years of age, all over $50/10^5$ py. Incidence in FTD male donors in Sao Paulo was also over $50/10^5$ py (Table 2). HIV incidence among FTD was higher among community donors compared with replacement donors in all sites. The left panel of Fig. 2 shows the age-stratified incidence rates for FTD. The results do not show any specific patterns or trends for FTD.

In the multivariable analysis, two factors were significantly associated with HIV incidence in FTD. The incidence risk ratio was 0·24 (95% CI 0·14–0·41) times lower in females compared to males and 2·39 (95% CI 1·54–3·70) times higher in community donors compared to replacement donors. No other factors including blood centre, age or 2-year interval were significantly associated with incidence in FTD.

### Incidence in repeat donors (RD)

Overall, the incidence in RD was highest in Recife followed by Belo Horizonte and São Paulo. The results for Rio de Janeiro show incidence rates in RD in the 3 later 2-year periods to be similar in magnitude to those of the other centres. The HIV incidence was higher among younger blood donors (<24 years old) and decreased with age at each of the three blood centres (Table 2). High variability was evident in each of the 2-year intervals when comparing age groups (Fig. 2). We found higher incidence of HIV among community as compared to replacement donors in Recife ($37{\cdot}7/10^5$ py vs. $24{\cdot}8/10^5$ py), Belo Horizonte (27·9 vs. $13{\cdot}3/10^5$ py) and São Paulo (17·5 vs. $8{\cdot}1/10^5$ py). The right panel of Fig. 2 shows the age-stratified incidence rates for RD with

**Table 1** Characteristics of the 2 544 352 donations by First-time and Repeat donors, 2007–2016

| Characteristics | First-time donor[a] | | Repeat donor | |
|---|---|---|---|---|
| | Number of donations | % | Number of donations | % |
| Overall | 930 180 | | 1 614 172 | |
| Blood Centre | | | | |
| Recife | 326 177 | 35·1 | 585 249 | **36·2** |
| Belo Horizonte | 230 259 | 24·8 | 330 413 | 20·5 |
| São Paulo | 373 744 | **40·1** | 698 510 | 43·3 |
| Type of donation | | | | |
| Community | 471 917 | 50·7 | 1 188 359 | **73·6** |
| Replacement | 458 263 | 49·3 | 420 869 | **26·1** |
| Missing[b] | 0 | 0 | **4 944** | **0·3** |
| Age (years) | | | | |
| ≤24 | 332 206 | 35·7 | 213 933 | 13·3 |
| 25-34 | 334 699 | 36·0 | 556 059 | 34·5 |
| 35-44 | 161 381 | 17·3 | 462 075 | **28·6** |
| ≥45 | 101 894 | 11·0 | 378 211 | **23·4** |
| Missing | 0 | 0 | 3 894 | 0·2 |
| Sex | | | | |
| Female | 400 634 | 43·1 | 431 776 | 26·7 |
| Male | 529 546 | 56·9 | 1 182 396 | 73·3 |
| Year | | | | |
| 2007-2008 | 186 128 | 20·0 | 318 099 | 19·7 |
| 2009-2010 | 191 392 | 20·6 | 339 138 | 21·0 |
| 2011-2012 | 194 300 | 20·9 | 331 319 | 20·5 |
| 2013-2014 | 194 330 | 20·9 | 317 548 | 19·7 |
| 2015-2016 | 164 030 | 17·6 | 308 068 | 19·1 |

[a]First-time donors included in the analysis are donors with no previous donation screening data at the participating blood centres.

[b]Repeat donor is those with two or more donations in each two-year estimation interval. Each donor only contributes person-time to those intervals where he or she made two or more donations.

lower incidence in older RD more evident in Recife and Belo Horizonte.

Multivariable analysis showed a significant difference between the blood centres. The incidence rate ratio in RD was 1·91 (95% CI 1·25–2·93) times higher in Recife and was also borderline significantly higher in Belo Horizonte compared with Sao Paulo (Table 3). Similar to FTD, the incidence risk ratio in RD was 0·31 (95% CI 0·18–0·54) times lower in females compared to males and 1·78 (95% CI 1·16–2·74) times higher in community donors compared to replacement donors. In addition, incidence rate ratios ranged from sixfold to threefold higher in age groups <24, 25–34, 35–44, respectively, compared to ≥45-year-old RD. Following multivariable adjustment, we did not observe incidence differences in RD by 2-year intervals.

### Residual risk of HIV transfusion transmission

We estimated contemporary residual risk of TT-HIV, after the adoption of MP6 NAT screening for HIV. We used a weighted average of first-time and repeat donor incidence, weighted according to the numbers of donations from each group at each centre for 2015–2016. The overall residual risk for HIV was 5·46 (plausible range: 3·07–8·47) and 7·41 (4·41–11·03) transmissions per million pRBC and FFP transfusions, respectively (Table 4). Additionally, residual risk in a best-case scenario (better sensitivity of the NAT assay, as reported using clinical samples) [7] was estimated at 2·26 per million pRBC transfusions, and in a worst-case scenario (a single virion in the product would cause transfusion-transmitted infection) was estimated at 8·88 per million pRBC transfusions.

## Discussion

In this study, we found incidence is higher in FTD compared to RD donors, consistent with data reported for other countries. However, the incidence rates in FTD and RD donors in Brazil are more similar in magnitude than reported for other countries [14] Incidence in both FTD and RD varied between the blood centres, type of

**Table 2** HIV Incidence per 100 000 Person Years and 95% Confidence Interval (CI) by characteristics for First-time and Repeat donors, 2007–2016

| Characteristics | Recife | 95% CI | Belo Horizonte | 95% CI | São Paulo | 95% CI | Rio de Janeiro | 95% CI |
|---|---|---|---|---|---|---|---|---|
| First-time donors | | | | | | | | |
| Time interval | | | | | | | | |
| All years[a] | 45·1 | 34·4–59·2 | 23·3 | 14·9–36·6 | 32·2 | 24·2–42.9 | N/A[b] | N/A |
| 2007–08 | 34·2 | 14·4–81·3 | 19·6 | 6·3–60·9 | 26·6 | 13·3–53·2 | N/A | N/A |
| 2009–10 | 63·1 | 34·2–116·3 | 36·3 | 16·3–80·9 | 17·3 | 7·2–41·5 | N/A | N/A |
| 2011–12 | 34·8 | 13·8–87·6 | 31 | 10·4–92·7 | 37·2 | 20·6–67·1 | N/A | N/A |
| 2013–14 | 57·5 | 25·5–129·9 | 18·8 | 0·6–561·6 | 59·8 | 37·2–96·1 | 36·14 | 17·2–75·8 |
| 2015–16 | 35·5 | 15·3–82·2 | 11·4 | 2·8–45·5 | 20·9 | 9·4–46·4 | 45·87 | 23·8–88·2 |
| Type of donation | | | | | | | | |
| Community | 64·0 | 40·6–100·8 | 38·9 | 19·7–76·8 | 37·7 | 27·7–51·4 | N/A | N/A |
| Replacement | 34·8 | 20·0–60·6 | 13·8 | 5·8–32·9 | 17·6 | 8·4–36·9 | N/A | N/A |
| Age (years) | | | | | | | | |
| ≤24 | 53·8 | 33·7–86·1 | 22·1 | 9·3–52·5 | 37·2 | 22·8–60·7 | N/A | N/A |
| 25–34 | 40·9 | 21·3–78·6 | 29·5 | 12·7–68·4 | 43·2 | 28·1–66·2 | N/A | N/A |
| 35–44 | 33·0 | 9–120·7 | 16·3 | 4·1–65 | 29·7 | 14·2–62·4 | N/A | N/A |
| ≥45 | 41·0 | 13·7–122·7 | 14·3 | 2–101·7 | 11·9 | 3–47·4 | N/A | N/A |
| Sex | | | | | | | | |
| Female | 20·6 | 8·2–52 | 7·5 | 2·4–23·2 | 11·4 | 5·7–22·9 | N/A | N/A |
| Male | 57·5 | 39·2–84·2 | 38·8 | 21·2–70·9 | 51·4 | 37·5–70·3 | N/A | N/A |
| Repeat donors | | | | | | | | |
| Time interval | | | | | | | | |
| All years | 33·2 | 26·0–42·3 | 27·5 | 19·2–39·4 | 17·0 | 12·4–23·2 | N/A | N/A |
| 2007–08 | 34·9 | 20·7–58·9 | 17·5 | 6·6–46·7 | 24·8 | 14·1–43·6 | N/A | N/A |
| 2009–10 | 37·2 | 22·4–61·7 | 36·5 | 19–70·1 | 10·4 | 4·3–24·9 | N/A | N/A |
| 2011–12 | 18·2 | 8·7–38·1 | 17·7 | 6·7–47·2 | 25·8 | 14·7–45·5 | 20·9 | 8·7–50·3 |
| 2013–14 | 33·7 | 19·6–58 | 40·4 | 20·2–80·8 | 18·4 | 9·2–36·8 | 43 | 21·5–86·1 |
| 2015–16 | 41·5 | 25·5–67·8 | 26·1 | 10·9–62·7 | 4·6 | 1·2–18·5 | 25·5 | 9·6–68·1 |
| Type of donation | | | | | | | | |
| Community | 37·7 | 31·3–45·4 | 27·9 | 21·6–36 | 17·5 | 14·7–20·8 | N/A | N/A |
| Replacement | 24·8 | 20·7–29·8 | 13·3 | 8·7–20·1 | 8·1 | 3·4–19·4 | N/A | N/A |
| Age (years) | | | | | | | | |
| ≤24 | 78·2 | 58·4–104·8 | 67·8 | 45·8–100·3 | 25·5 | 14·5–44·9 | N/A | N/A |
| 25–34 | 42·9 | 35·8–51·4 | 26·2 | 19·3–35·8 | 24·8 | 19·3–31·8 | N/A | N/A |
| 35–44 | 19·6 | 14·7–26·1 | 13·8 | 8·4–22·5 | 16·2 | 11·9–22 | N/A | N/A |
| ≥45 | 9·5 | 6–14·8 | 2·4 | 0·6–9·5 | 6·9 | 4·2–11·3 | N/A | N/A |
| Sex | | | | | | | | |
| Female | 17·2 | 11·6–25·7 | 8·6 | 5–14·9 | 6·3 | 4–9·9 | N/A | N/A |
| Male | 32·6 | 28·4–37·4 | 29·3 | 23·2–37 | 23·3 | 19·3–28 | N/A | N/A |

[a]Overall estimates for first–time donors using weighted averages.

[b]N/A - HIV-positive first-time blood donations in Rio de Janeiro were analysed only between the years 2013–2016 and for repeat donors were analysed between 2011 and 2016.

donation, and by donor demographic characteristics of age and sex.

Our 10-year analysis expands previous findings showing that HIV incidence was higher among community rather than replacement blood donors [6] and challenges WHO guidelines, which recommend community over replacement donation [1]. The conventional thinking is that replacement donors may feel compelled to donate and therefore may not fully answer screening questions intended to exclude donors with infectious disease risks hence leading to higher infection rates in replacement donors, but our results do not align with this expectation. The reasons why HIV incidence is higher in community donors in Brazil are not known. Previous results from our group have shown that test-seeking behaviour is higher among HIV-positive blood donors than donors with no infection [15]. In our previous study, although the proportion of test seekers was higher among replacement donors, this association was not maintained in multivariable analysis [16]. Therefore, test-seeking behaviour is

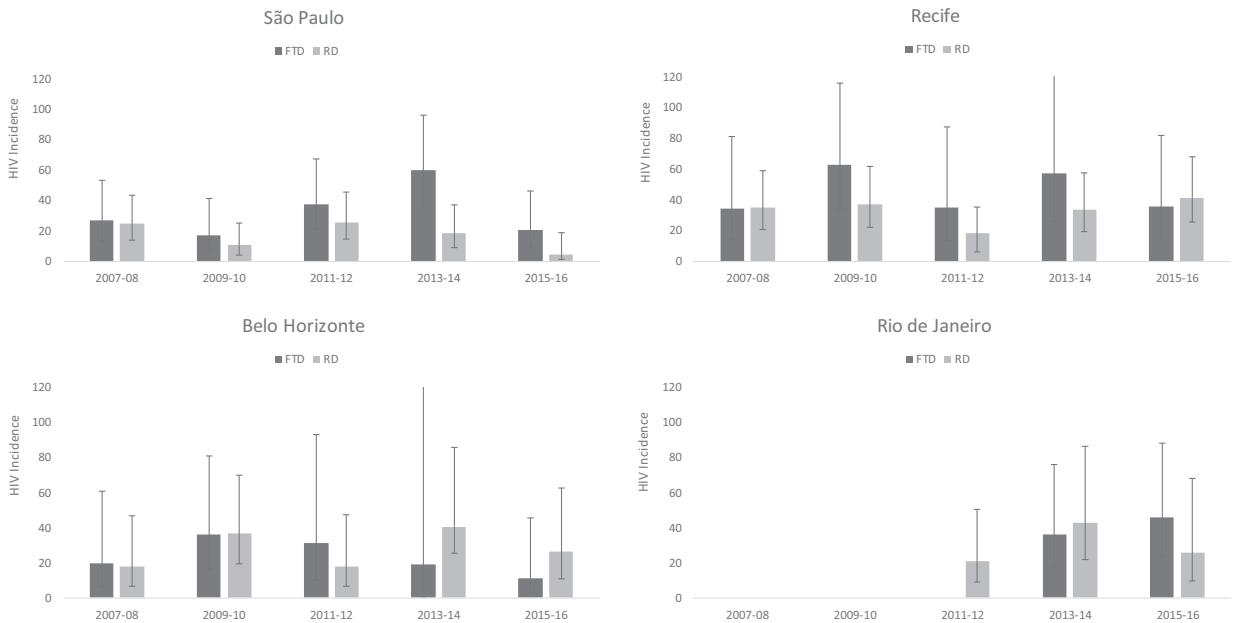


**Fig. 1** HIV Incidence per 100 000 Person Years and 95% Confidence Interval by Time Interval and Blood Centre for First-Time (FTD) and Repeat Donors (RD), 2007–2016.

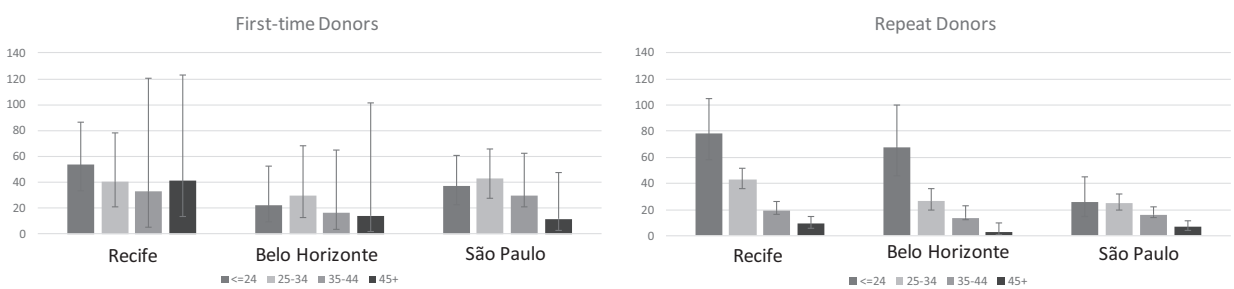


**Fig. 2** Comparison of HIV Incidence per 100,000 Person Years and 95% Confidence Interval by Age and Blood Centre for First-Time (FTD) and Repeat Donors (RD), 2007–2016.

not a simple explanation for why community donors had higher HIV incidence. Test-seeking behaviour is also associated with male gender, lower educational attainment and lower income [16]. Lack of knowledge of locations for free and confidential public HIV testing services as well as dissatisfaction with past experiences with HIV testing has been associated with test seeking at blood centres [17]. In our current study, we do not know if any of these factors contribute to incidence rates in FTD or RD.

New strategies in donor recruitment and screening are necessary to avoid the risk of HIV and others infectious diseases transfusion transmission. A possible model for Brazil would be to continue to recruit replacement donors, but to focus effort on conversion of those donors to community donors whether they are first-time or repeat donors [1]. However, this may be difficult to achieve without effective messaging strategies since the return rate of replacement FTD is around half that of community FTD [18]. Additionally, use of pathogen reduction has been shown to be effective to reduce risk of TTI, especially when it becomes available for all blood components. Currently, only plasma and/or platelet inactivation procedures are licensed by the US FDA, European Union and other countries [19]. At this time, pathogen reduction is not being considered for adoption by Brazilian Public Health Service blood centres.

Another aspect of our findings is the age distribution of HIV incidence among blood donors. It is expected that HIV incidence would be higher among young individuals, whether FTD or RD, due to a more active sexual life in younger ages. This pattern is observed among RD but not among FTD. Another relevant point to note is that in Recife and Belo Horizonte the young RD have a higher

**Table 3** Multivariable analysis of the HIV incidence of First-time (FTD) and Repeat donors (RD), 2007 to 2016

| | First-time donor | | | Repeat donor | | |
|---|---|---|---|---|---|---|
| **Characteristics** | **IRR[a]** | **Wald 95% CI** | ***P* value** | **IRR** | **Wald 95% CI** | ***P* value** |
| Blood Centre | | | | | | |
| Recife | 1·30 | 0·84–2·01 | 0·24 | **1·91** | **1·25–2·93** | **<0·01** |
| Belo Horizonte | 0·76 | 0·42–1·36 | 0·35 | 1·60 | 0·98–2·61 | 0·06 |
| São Paulo | 1 | | | 1 | | |
| Type of donation | | | | | | |
| Community | **2·39** | **1·54–3·70** | **<0·01** | **1·78** | **1·16–2·74** | **<0·01** |
| Replacement | 1 | | | 1 | | |
| Age (years) | | | | | | |
| ≤24 | 1·67 | 0·74–3·72 | 0·21 | **6·76** | **2·94–15·57** | **<0·01** |
| 25–34 | 1·79 | 0·80–3·98 | 0·15 | **6·64** | **3·05–14·48** | **<0·01** |
| 35–44 | 1·24 | 0·50–3·06 | 0·64 | **3·63** | **1·60–8·24** | **<0·01** |
| ≥45 | 1 | | | 1 | | |
| Sex | | | | | | |
| Female | **0·24** | **0·14–0·41** | **<0·01** | **0·31** | **0·18–0·54** | **<0·01** |
| Male | 1 | | | 1 | | |
| Year | | | | | | |
| 2007–2008 | 0·85 | 0·42–1·72 | 0·66 | 1·03 | 0·59–1·78 | 0·92 |
| 2009–2010 | 1·18 | 0·61–2·27 | 0·61 | 1·04 | 0·60–1·82 | 0·88 |
| 2011–2012 | 1·08 | 0·55–2·10 | 0·81 | 0,90 | 0·50–1·62 | 0·73 |
| 2013–2014 | 1·60 | 0·86–2·97 | 0·13 | 1·21 | 0·69–2·10 | 0·50 |
| 2015–2016 | 1 | | | 1 | | |

[a]Incidence rate ratio in the groups compared with the reference group designated by the 1 value.
Bolded parameters indicate those with statistical significance.

**Table 4** Residual risk estimate per 1 million donations for 2015 to 2016 and Risk-day equivalents per day for all centres combined

| | **Residual Risk Point Estimate (range) transmission/million transfusions** | **Risk-day equivalents Point Estimate (range) infectious window period/days** |
|---|---|---|
| RBC (20 ml) | 5·46 (3·07–8·47) | 7·96 (6·54–9·38) |
| FFP (200 ml) | 7·41 (4·41–11·03) | 10·79 (9·38–12·21) |

incidence than young FTD. One reason for this could be that 'at risk' young individuals are donating more frequently than those at lower risk in the same age groups. Efforts to understand the motivations to donate and the possibility of test seeking in this group may help blood centres to develop specific strategies to reduce donation from younger higher risk donors.

Estimation of residual risk is an important tool to assess whether reductions in TT-HIV risk are being achieved [20]. Previous research in the Northeast of Brazil for the period 2012-2014 from the State of Pará (with approximately eight million inhabitants) support the order of magnitude of the residual risk we report [21]. Our estimates show that residual risk of HIV transfusion transmission in Brazil is higher than in many other countries, such as Germany, reported as 0·52 [22], France 0·40 [23] and Canada 0·04 [24] per million RBC transfusions. At 5·21 per million RBC transfusions, similar residual risk results to ours have been reported for Italy [25]. In our study, we estimated RR for both RBC and FFP based on the amount of plasma in each component to help further define risk to recipients in Brazil.

Our study has limitations. First, we did not have complete data for Rio de Janeiro available for the entire study period, so we were unable to assess demographic factors or trends over time for that blood centre. A second limitation is that our study was conducted at four blood centres; as a result, we are not able to comment on trends in HIV incidence in blood donors in other regions Brazil. Despite these limitations, we believe the results from the four centres are indicative of general trends in HIV infection in donors in Brazil. The difference in the incidence of HIV in males compared to females was stable over time, and age and regional differences that are consistent

with the known epidemiology of HIV infection in Brazil were evident. A third limitation is that the proportion of first-time donors for whom we had samples we could test using LAg avidity was 72·2%, so almost three-quarters of first-time donors with HIV infection had LAg-avidity results. We believe this proportion of tested samples is sufficiently high to give us confidence our incidence calculations are accurate. We have no reason to be think that sample availability would be different between samples that tested recent versus longstanding on the LAg-Avidity assay, thus we do not believe our findings are biased. However, we may have reduced precision in the form of wider confidence intervals as a result not being able to test all samples from HIV positive first-time donors.

In summary, these incidence results show that a substantial number of HIV-infected donors are presenting to donate within 4 months of HIV acquisition in Brazil. Over the 10-year study period, this did not substantially change and consequently, the residual risk of HIV transmission has remained higher than in developed countries even after the introduction of NAT screening. Our findings suggest that it remains important to continue efforts in donor education and refinement of donor recruitment strategies that promote the disclosure of risk at the time of donation. Reducing donation among the cohort of donors with recently acquired HIV infection is the most assured way to reduce the risk of transfusion-transmitted infection, and particular attention is needed for RD, age groups and regions with the highest incidence of HIV.

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## Conflict of interest

The authors have disclosed no conflicts of interest.

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## Appendix

### The Recipient Epidemiology and Donor Evaluation Study (REDS-III) International Component Brazil is the responsibility of the following persons

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ORIGINAL PAPER

# Clinical outcomes of cardiac surgery patients undergoing therapeutic plasma exchange for heparin-induced thrombocytopenia

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Vox Sanguinis

**Background and Objectives** Heparin-induced thrombocytopenia (HIT) is an antibody-mediated condition that leads to thrombocytopenia and possible thrombosis. Patients with HIT who require cardiac surgery pose a challenge as high doses of heparin or heparin alternatives are required to permit cardiopulmonary bypass (CPB). Intraoperative therapeutic plasma exchange (TPE) is a valuable adjunct in the management of antibody-mediated syndromes including HIT. The clinical impact of TPE on thromboembolic events, bleeding and mortality after heparin re-exposure is not well established. We hypothesized that TPE with heparin re-exposure will not lead to HIT-related thromboembolic events, bleeding or increased mortality after cardiac surgery with CPB.

**Materials and Methods** We reviewed 330 patients who received perioperative TPE between September 2012 and September 2017.

**Results** Twenty four patients received TPE for HIT before anticipated heparin use for CPB. Most patients were males (79%) scheduled for advanced heart failure therapies. Three patients (12·5%) died within 30 days after surgery but none of the deaths were considered HIT-related. Thromboembolic events (TE) occurred in 3 patients within 7 days of surgery; of those, two were possibly HIT-related.

**Conclusion** Therapeutic plasma exchange with heparin re-exposure was not strongly associated with HIT-related thrombosis/death after cardiac surgery with CPB.

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**Key words:** cardiac surgery, heparin, plasma exchange, platelet factor 4, thrombocytopenia.

## Background

Heparin-induced thrombocytopenia (HIT) is a prothrombotic condition in which antibodies to the antigenic complex of heparin and platelet factor 4 antigen (anti-heparin/PF4) activate platelets to degranulate and aggregate in the microcirculation [1]. These events lead to thrombocytopenia and possible thrombosis [1]. Seroconversion occurs in 8–17% of patients requiring heparin for medical indications, and in approximately 50% of patients exposed to heparin during the course of cardiac surgery. Patients with HIT who require cardiac surgery pose a challenge as high doses of heparin or heparin alternatives are required to permit cardiopulmonary bypass (CPB). The risks of thrombosis associated with early heparin re-exposure during CPB must be balanced against the adverse effects (i.e. bleeding and circuit thrombosis) that are a consequence from the use of irreversible heparin alternatives such as bivalirudin [2–4] for which there are no reversal agents or adequate intraoperative laboratory monitoring strategies to date.

Intraoperative therapeutic plasma exchange (TPE) is a valuable adjunct in the management of antibody-

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mediated syndromes including HIT. TPE permits heparin use by removing immune complexes and HIT antibodies [5]. Intraoperative TPE allows the use of heparin rather than irreversible heparin alternatives. In a retrospective study of 11 HIT or heparin/PF4 seropositive patients undergoing TPE in preparation for cardiac surgery, a single TPE treatment reduced heparin/PF4 titres by 50–84%, and 7 of 9 patients had normal anti-heparin/PF4 levels after treatment [6]. Following re-exposure to heparin, no serious adverse complications of HIT or to TPE were noted.

Heparin-induced thrombocytopenia is associated with postoperative morbidity and mortality. Recent studies have shown that clinical complications of thrombosis and thrombocytopenia are highly correlated with antibody levels [7,8]. Studies have also shown that TPE effectively removes heparin/PF4 antibodies [6,9]. The clinical impact of TPE on thromboembolic events, bleeding and mortality after heparin re-exposure is not well established. We hypothesized that TPE with heparin re-exposure will not lead to HIT-related thromboembolic events, bleeding or increased mortality after cardiac surgery with CPB.

## Methods

After obtaining IRB approval, we retrospectively reviewed cardiac surgical patients undergoing TPE before CPB with heparin between September 2012–September 2017. We identified adult patients ≥18 years with a prior history of HIT (decrease in the platelet count of more than 50% from the highest platelet count value after the start of heparin with an onset 5–10 days after the start of heparin) or preoperative anti- heparin/PF4 antibody seropositivity (polyclonal ELISA OD >0·4; GTI PF4 EIA, GTI diagnostics, Waukesha, WI, USA) as their indication for TPE, which was performed as described in a previous study [6]. The American Society for Apheresis (ASFA) designates TPE for pre-CPB in HIT as category III (optimum role of apheresis therapy is not established. Decision making should be individualized) [10]. The level of evidence is grade 2C.

Therapeutic plasma exchange was performed using the COBE Spectra® (from 2012 to 2015) or Spectra Optia ® (2014 to 2017) after the induction of general anaesthesia. TPE was performed with plasma replacement using a standardized protocol to exchange 1·0 plasma volume (approximately 3500–4500 ml based on the patient's height, weight, gender and haematocrit [11]. The timing of TPE was dependent on the haemodynamic stability of the patient. Ideally, it was performed before heparinization (400 U/kg as a bolus) but if necessary, heparin was given, CPB initiated to stabilize the patient, and TPE performed during CPB as previously described [12]. Because heparin is removed during TPE, additional heparin was infused during the exchange based on the following estimation. Assuming a haematocrit of 0·25, we replaced heparin lost during TPE as calculated by 4 U/ml of plasma removed administered as a 4000-U bolus after every litre removed with any remainder at the completion of treatment.

Daily minimum platelet counts were abstracted 7 days before to 30 days after TPE. Arterial/venous thromboembolic (TE) events within 7 days of TPE and survival status up to 30 days were determined. Patient demographic and clinical variables were summarized by frequency (%) for categorical and mean (SD) or median [IQR] for continuous variables.

## Results

Patient demographic and descriptive data are summarized in Table 1. We reviewed 330 patients who received intraoperative TPE between September 2012 and September 2017 (Fig. 1). Of the 164 patients that underwent TPE during cardiac surgery using CPB, 24 patients received TPE for HIT before anticipated heparin use for CPB; the remainder received TPE in order to clear human leukocyte antigen (HLA) specific antibodies prior to heart or lung transplantation. All patients (24) had a history of HIT. Also, seven patients (29%) had a history of prior VTE, but it was not clear from the records if this was HITT based on timing therefore we did not include this. Of the 24, 12 patients had preoperative anti-heparin/PF4 antibody testing at our hospital; we had other patients transferred to us from outside hospitals with a history of HIT and a 'positive ELISA' although we did not have these data in our records.

Most patients (79·0%, 18 patients, Table 1) were males scheduled for advanced heart failure therapies (i.e. left ventricular assist device insertion or heart transplantation), they all received heparin for CPB. Twenty-one patients were anticoagulated with bivalirudin before surgery, one patient with argatroban and one patient with coumadin. Postoperative anticoagulation for the LVAD patients used bivaiirudin. The median preoperative HIT ELISA test optical density (OD) of 1·39 [0·67, 2·43]. Two patients with a positive polyclonal heparin/PF4 ELISA, were subsequently negative on the IgG specific assay.

Platelet counts typically reached a nadir on the day of surgery and steadily recovered for most patients during the first ten postoperative days (as shown in Fig. 2 and individually as Fig. S2). For the 24 patients, in the cohort the preoperative median was 160 x $10^3$/$mm^3$. The lowest daily median occurred on the day of surgery and had a median of 100 x $10^3$/$mm^3$. Thereafter, the median values

**Table 1** Demographic data

| Variable ($n$ = 24 patients) | Mean ± SD Percentage ($n$) |
|---|---|
| Age (years) | 55·2 ± 9·44 |
| Gender (Male) | 79% (19) |
| Procedure | |
| Heart transplant | 21% (6) |
| VAD insertion/replacement | 54% (13) |
| Other procedures | 22% (5) |
| Race | |
| African American | 37% (9) |
| Caucasian | 54% (13) |
| Other | 4% (2) |
| BMI | 32·0 ± 9·80 |
| Height (m) | 1·75 ± 0·09 |
| Weight (kg) | 98·3 ± 30·2 |
| Preoperative Anti-heparin/PF4 Antibody OD value | 1·39 ± 0·89 |
| Nonischaemic cardiomyopathy | 62% (15) |
| Ischaemic cardiomyopathy | 29% (7) |
| Coronary artery disease | 33% (8) |
| Mod-Sev Aortic stenosis/regurgitation | 50% (12) |
| Atrial fibrillation/flutter | 50% (12) |
| Diabetes | 42% (10) |
| Hypertension | 67% (16) |
| Smoker | 8% (2) |
| Hypercholesterolemia | 62% (15) |
| CKD | 42% (10) |
| ESRD | 4% (1) |
| Previous MI | 25% (6) |
| Previous stroke | 12% (3) |
| Prior VTE | 29% (7) |
| Peripheral Vascular Disease | 8% (2) |
| Mechanical circulatory support | 67% (16) |
| Preoperative IABP | 58% (9) |
| Preoperative VA ECMO | 8% (5) |
| Preoperative VV ECMO | 4% (2) |
| Preoperative LVAD | 25% (6) |
| Redo sternotomy | 29% (7) |

monotonically increased through postoperative day 10 to a median of 292 x $10^3$/mm$^3$. By day 16, all platelet counts were above 150 x $10^3$/mm$^3$.

Only 11 patients had a postoperative HIT ELISA retested. These patients had a preoperative median OD of 1·99 and a single, intraoperative TPE treatment reduced the OD titre to a median of 0·34. One patient had a significantly increased OD titre after TPE (0·9 preoperatively to 1·41 postoperatively), although this was an increase in the polyclonal ELISA only. The IgG specific ELISA was negative. There was no difference between pre and postoperative OD titres in two patients, and for the remaining seven, the OD titres were reduced by 35–85% from the preoperative value (Fig. 3). Similarly, seven patients decreased their postoperative OD to <0·4. The median [Q1, Q3] change from pre-to-post surgery is −1·57 [−2·01, 0·01], and the Wilcoxon Signed rank $P$-value is 0·037, suggesting a significant decrease in OD level in this patient group.

Three patients (12·5%) died within 30 days after surgery. One patient died after a haemorrhagic stroke with subsequent brain death. The platelet counts in the weeks preceding death were normal (Fig. S1) The second patient died from respiratory failure secondary to pneumonia. The third patient had multiorgan failure secondary to sepsis; this patient presented with a TE event associated with a drop in his platelet count after an initial recovery, that could have been HIT related. He died later after resolution of thrombocytopenia; the cause of death was unrelated to the prior TE event and was not TE-related. None of the deaths were considered HIT-related, as the platelet counts were normal at the time of death. For these three patients,

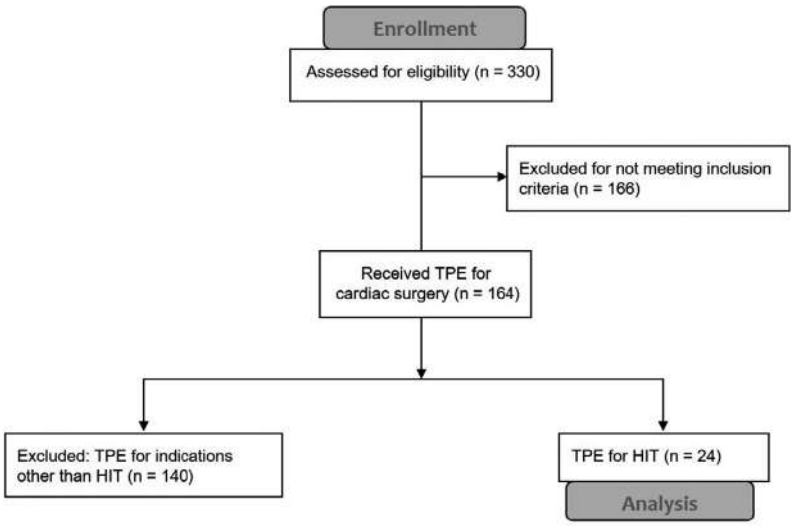


**Fig. 1** CONSORT 2010 flow diagram.

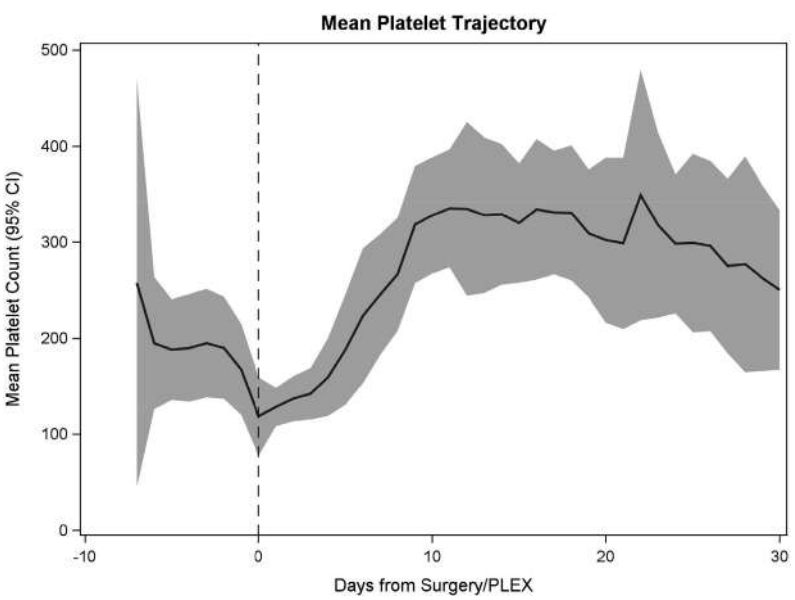


**Fig. 2** Platelet trajectories for all patients. This graph shows the daily mean platelet count. The band represents the 95% confidence interval.

the preoperative median OD was 2·47 [0·233–2·487]. After TPE, the median OD was 0·467 [0·151–0·933].

Thromboembolic events occurred in three patients within 7 days of surgery. Two were possibly HIT-related. One was a stroke with an ELISA OD four days before the TE of 0·47 and a PLT count of 54 x $10^3$. The second event was a deep venous thrombosis with an ELISA OD 1 day before the TE of 0·93 with a PLT count of 106 x $10^2$. A third TE occurred well after platelet count recovery to 330 x $10^3$ and was therefore unlikely HIT-related.

## Discussion

We present a series of 24 patients with current or recent HIT that were treated with TPE to remove heparin/PF4 antibodies and allow standard heparin anticoagulation for their complex cardiac surgery. These patients were successfully re-exposed to heparin, without intraoperative incident, to permit cardiopulmonary bypass. In our cohort, platelet counts reached a nadir on the day of surgery. Platelet counts typically decrease during cardiac surgery as a result of blood loss, transfusion,

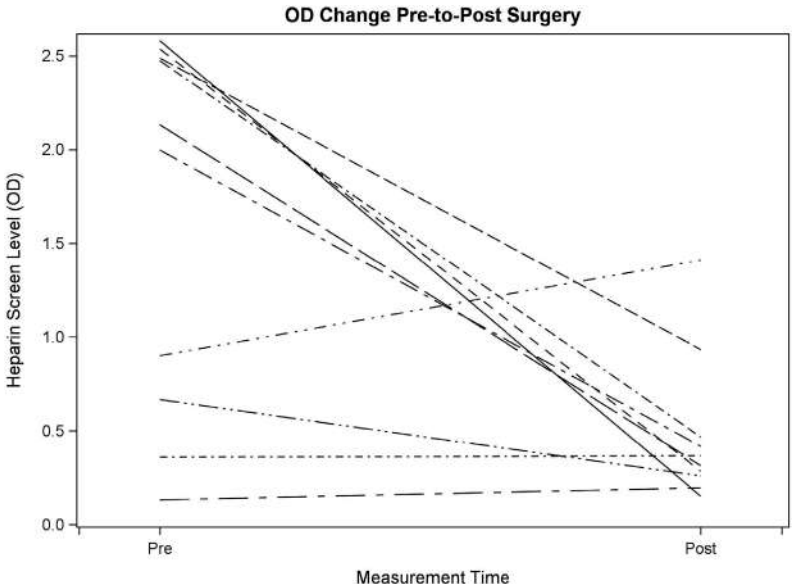


**Fig. 3** Median [Q1, Q3] change from pre-to-post surgery. The median [Q1, Q3] change from pre-to-post surgery is −1·57 [−2·01, 0·01]. The Wilcoxon Signed rank *P*-value is 0·037.

haemodilution and activation with sequestration in the extracorporeal circuit (primarily the fibres of the oxygenator). It is challenging to differentiate this from HIT, other than it is an expected observation with a typical nadir on the day of surgery, or the day after, with subsequent recovery. The postoperative platelet count profile seems to follow two specific patterns in the setting of HIT [13]. One of the patterns is biphasic and shows an initial recovery of the platelet count post bypass with a subsequent decrease in the count of at least 40% compared to the maximum postoperative value. The second pattern is monophasic with a low platelet count that persists in postoperative days 5–10. The Lillo-Le Loüet score was created to diagnose HIT after CPB and includes the postoperative platelet profile as a variable [14].

Anti-heparin/PF4 antibody titres were significantly reduced (by 35–85%) in the majority of the patients with available postoperative antibody testing data. A previous study also reported a reduction of 50%–84% after a single TPE treatment [6]. In our cohort, only two of the three TE events were possibly HIT-related, and none of the deaths were likely HIT-related. Therefore, TPE could be considered a reasonable option for patients with HIT requiring urgent cardiac surgery who were considered clinically unsuitable for heparin alternatives [2], due to complexity of surgery with anticipated long duration and therefore a large expected total bivalirudin dose in the setting of likely renal dysfunction and high bleeding risk.

None of the patients underwent additional TPE treatments aiming to achieve complete antibody removal, provided they had stable platelet counts postoperatively or could tolerate bivalirudin anticoagulation. Repeated postoperative TPE may be unwarranted due to risks of haemodynamic instability and exposure to additional blood products. However, it is unknown whether additional TPE based on repeat anti- heparin/PF4 testing could help further reduce TE complications in this morbid group. Serial PF4-dependent enzyme-immunoassay (EIA) testing for HIT antibodies may be useful especially if the IgG specific ELISA is unavailable. This is a simple, feasible method but is technically challenging as it only detects HIT antibodies indirectly [15].

There is no predetermined value for defining high-titre antibodies, and OD levels correlate poorly with antibody burden. The sensitivity and specificity for PF4/heparin optical density >0·40 is reported as 100% and 26% respectively [16], which means that some patients with positive test results in this assay may never develop HIT [17–19]. Some authors suggest that an OD >1·0–1·4 increases the risk of thrombosis [18,20]. In our study, of the eleven patients with postoperative OD data, 10 had OD below 1, while one patient had a postoperative OD >1·4. Interestingly, this patient neither presented with TE nor died in the first 30 days after surgery. The relationship between OD and antibody levels are expressed through a hyperbolic curve function [21]. At high ODs (>2–3), antibody saturation in the ELISA does not accurately reflect the antibody burden or actual titre. Serial dilutions of test samples over a wide range help to quantify the titre of antibody present.

Accuracy in the assessment of titres is particularly relevant for HIT TPE, as one exchange may not be sufficient to remove high-titre antibodies [22,23]. Some HIT patients with high ODs and high titres are likely to need more than one TPE to lower circulating PF4/H antibody burden. Functional assays such as a serotonin release assay (SRA) or heparin-induced platelet activation assay (HIPA), are more specific for immune-mediated HIT but take longer, are more technically demanding, and not widely available [23]. Achieving SRA negativity through multiple sessions of TPE can remove a sufficient amount of HIT antibodies despite persistent positive titres with ELISA [22]. In our study, the patients were not tested with functional assays after TPE, but previous studies have shown that the median time to a negative SRA after a single session is around 50 days [24,25]. To date, TPE is scarcely used for HIT in most institutions, mostly because it is not recognized as an option. In a recent survey [26], only 37% of the surveyed institutions used TPE for HIT for indications such as cardiovascular surgery and HIT-associated thrombosis. There were no major complications from TPE. Haemodynamic instability due to TPE or any plasma reaction was not apparent, although they may have been masked because patients were anaesthetized and often already maintained on vasoactive medications. There were no technical issues encountered. Hypocalcemia is expected and was prevented with concomitant intravenous calcium gluconate administration (3–4 g).

Intravenous immunoglobulin (IVIg) is emerging as a useful approach that can also help to treat patients with HIT. IVIg works by inhibiting HIT antibody-mediated platelet activation, apparently through competitive binding [24]. The inhibition is dependent on the constant domain of IgG (Fc) but not the antigen-binding portion (Fab), and the presence of the HH131 genotype. Other genotypes such as RR131 and HR131 responded favourably to high doses of IVIG, although not as well as the patients with the HH131 genotype. To date, there are no studies relating response to TPE to specific genetic phenotypes. Furthermore, IVIg had no effect on HIT antibody binding in a solid phase PF4-ELISA testing or in the SRA values, which were still strongly positive. In any case, IVIg treatment successfully achieved platelet count recovery [24]. Although laboratory assessments demonstrate antibody persistence after both TPE and IVIg, it is possible that the titre magnitude is not sufficient to trigger a pathological immune and platelet response. Another potential approach involves the concurrent use of cangrelor, a short-acting, intravenous P2Y12 inhibitor, and heparin for cardiopulmonary bypass in patients with HIT but there is limited literature at this time. [27]

This study is a case series without a control group and, consequently, has some limitations. It included a small number of patients, and not all patients had confirmatory tests (e.g. SRA). We based the diagnosis on PF4/heparin EIA only, which may be insufficient as only 50% of antibodies causing a positive EIA have clinical relevance. Most of the available SRA results were reported late during the hospital stay, which made them clinically unhelpful. We also did not check for antibody re-emergence which may be a possibility after re-exposure to heparin.

Bivalirudin for complex redo surgery has not been studied, and the bleeding risk in this setting maybe even higher. TPE permits standard anticoagulation for complex procedures and may be a feasible option for these types of procedures. Genetic assessments may also be useful in future studies to correlate the response to TPE to specific genotypes.

## Conclusion

Intraoperative TPE is one strategy to facilitate standard heparin anticoagulation during CPB in patients requiring urgent cardiac surgery in the setting of acute HIT. Modifying the protocol to plan for additional TPE based on repeat anti-heparin/PF4 testing could further reduce thromboembolic complications, depending on the results of future studies.

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## Conflicts of interest

The authors declare no conflict of interests.

## Author contributions

I. Moreno Duarte: Data collection, statistical analysis, data interpretation, manuscript writing. M. Cooter: Statistical analysis and graphics. O. A. Onwuemene: Manuscript writing, data interpretation. K. Ghadimi: Study design, Manuscript writing, data interpretation. I. J Welsby: Study design, Manuscript writing, data interpretation, final approval of the version to be published.

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## Supporting Information

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

Fig. S1. Platelet trajectories for patients with outcomes of interest (thrombotic events-TE/death).
Fig. S2. Platelet trajectories for all patients ($n$ = 24).

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# Registration errors among patients receiving blood transfusions: a national analysis from 2008 to 2017

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Vox Sanguinis

**Background and objectives** The key first step for a safe blood transfusion is patient registration for identification and linking to past medical and transfusion history. In Canada, any deviation from standard operating procedures in transfusion is an error voluntarily reportable to a national database (Transfusion Error Surveillance System [TESS]). We used this database to characterize the subset of registration-related errors impacting transfusion care, including where, when and why the errors occurred, and to identify frequent high-risk errors.

**Materials and methods** A retrospective analysis was conducted on transfusion errors reported to TESS by sentinel reporting sites relating to patient registration and patient armbands, between 2008 and 2017. Free-text comments describing the error were coded to further categorize into common error types. The number of specimens received in the transfusion laboratory was used as the denominator for rates to allow for comparison between hospital sites.

**Results** Five hundred and fifty-four registration errors were reported from 10 hospitals, for a global error rate of 5·4/10 000 samples (median 5·0 [interquartile range 3·7–7·0]). The potential severity was high in 85·7% of errors ($n$ = 475). The patient experienced a consequence in 10·8% of errors ($n$ = 60), but none resulted in patient harm. Rates varied widely and differed by nature across sites. Errors most commonly occurred in outpatient clinics or procedure units ($n$ = 160, 28·8%) and in emergency departments ($n$ = 130, 23·5%).

**Conclusion** Registration errors affect transfusion at every step and location in the hospital and are commonly high risk. Further research into common root causes is warranted to identify preventative strategies.

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**Key words:** blood safety, hemovigilance, quality control, quality management, transfusion medicine.

## Introduction

The blood transfusion chain is complex, frequently manual and error-prone. Due to transfusion's inherent hazards, patient identification errors are high risk to patients. Registration, the key first step in the transfusion chain, is essential for positive identification. New patient registration was identified as the most critical patient identification point [1], while correct registration at readmission is required for linking to past medical and transfusion history, including concordance with previous ABO and Rh typing and requirements for specialized components (e.g. antigen negative, irradiated products).

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Errors in sample collection and handling have been the subject of past studies [2–7], but there is a paucity of research relating to errors in patient registration. Registration errors can lead to loss of historical patient information and misidentification, failing to match for previously identified red blood cell alloantibodies, and accidental sharing of confidential personal health information [8]. As a consequence, registration errors account for up to 10% of ABO-incompatible transfusions [9]. These errors also affect the patient and are a financial burden due to requirements for sample recollection, delayed procedures and wasted products and components [10].

In Canada, transfusion errors are voluntarily reported to the Transfusion Error Surveillance System (TESS). The objective of this study was to describe registration errors in this multicentre national database at sentinel reporting sites over a 10-year period, aiming to characterize these errors, identify high-risk system critical failure points, and understand needs for system re-evaluation and re-design.

## Materials and methods

The TESS database is a web-based system which was initiated in 2005 by the Public Health Agency of Canada (PHAC), an agency of the Government of Canada, to log errors occurring at any point in the blood transfusion chain, including blood components and blood products [11]. Errors are defined as any unplanned deviations from standard operating procedures in transfusion [11]. Sentinel and non-sentinel hospital sites voluntarily report errors to TESS quarterly. Sentinel sites, but not non-sentinel sites, meet monthly by conference call and annually in person to discuss coding harmonization and process improvement. Error reporting is non-punitive. Sites are able to enter reports directly or to upload from their own databases and/or blood bank laboratory information system.

This database is a secure online platform accessible to the participating public health workers at regional, provincial, territorial and national levels within TESS. It was developed by and housed within the secure environment of the Canadian Network for Public Health Intelligence. All access to the TESS database is based on defined security and data can only be viewed by registered users within jurisdictional boundaries. All errors are anonymized during the reporting process and contain no patient data.

This is a retrospective analysis of errors reported into the TESS database that were categorized by the sites as 'incorrect/incomplete patient registration' (Miscellaneous [MS] 03) and/or 'patient armband not available/incorrect' (Sample Collection [SC] 10) during the reporting process (representing registration-related errors), entered between January 1, 2008 and December 31, 2017. Errors were either extracted by PHAC or sent directly to the research team by the TESS participating site. Data were available from 10 sentinel hospital sites in three Canadian provinces: Ontario, Quebec and British Columbia. Only sentinel sites were included due to the possibility of non-sentinel sites coding events incorrectly, because they do not participate in the same coding exercises as sentinel sites. Errors were analysed globally and by site for frequency, characteristics, variability, nature and consequences.

### Coding

Errors are coded during the reporting process by when and where in the blood transfusion chain they occurred and when and where they were discovered, days from error to discovery, time of day of occurrence and discovery, and role of the person involved in occurrence and discovery. Errors are considered near miss if they are discovered before infusion and do not affect the patient; and are considered to have reached the patient if the patient is affected in any way, including if the transfusion proceeded prior to error corrective action. The method of discovery is recorded for near miss errors; the outcome for the patient is recorded for patient-reaching errors (termed 'actual events' in TESS). The potential severity is recorded for all errors, assessed on a low–medium–high scale. High-potential severity errors are defined as errors which had the potential to cause serious injury or fatal outcomes (including errors described as 'patient registration incomplete/incorrect' or 'armband incorrect/not available'); medium potential severity errors are those with potential to cause minor or transient injury; low-potential severity errors have no potential for adverse effects [11]. Since 2016, the actual severity for actual events is also recorded on this scale. The hospital site (by numeric anonymous code) reporting the error is also recorded. PHAC records these sites' sizes on a small–medium–large scale based on number of red blood cell units transfused per year (<2000, 2000–10 000, >10 000 units) [11]. The number of patient samples received in the transfusion laboratory is recorded by site and year to allow the calculation of rates for benchmarking.

Error nature was further sub-coded centrally by the research team based on the free-text comments regarding the error description or its discovery details entered for each report. 14 sub-categories were created for the most common registration errors reported: Doppelgänger error,

name error, wrong date of birth (DOB), duplicate registration, missing armband, wrong armband, wrong provincial health insurance number (PHIN, used for Ministry of Health reporting and physician billing), comingling of more than one patient in a single hospital number, patients using another's identification (ID) either intentionally or in error, incorrect sex, missing medical record number (MRN, used for hospital records), wrong MRN, other and unknown (unable to further categorized given the details provided in the free-text comments). Name error was defined as incorrect or misspelled patient name. Previously described methods for coding errors involving similar files or patients were used as follows [8, 12]: Doppelgänger was defined as multiple records both containing information from more than one patient with similar identifiers; duplicate was defined as a single patient having multiple records at the same hospital site, causing a fragmentation of the record; comingling was defined as one record containing information from more than one patient. Each error could have up to three codes for events with more than one error.

### Statistical analysis

Descriptive statistics are reported. Categorical variables are summarized with percentages, with missing data included in the denominator. Normally distributed data are reported using mean and 95% confidence interval (95% CI). Non-parametric data are reported using median and interquartile range (IQR). For categorical data, the chi-square test was used and a $P$-value < 0·05 was considered statistically significant.

A generalized linear regression model with a log-link Poisson distribution was performed to analyse changes in error rates by year. A $P$-value < 0·05 was considered statistically significant. Least squares means were estimated from the regression model for rates by year, year and hospital size, year and hospital site, hospital size and hospital site.

The number of patient samples received in the transfusion laboratory was used as denominator data, where available, to calculate rates.

## Results

From 2008 to 2017, 554 registration errors were reported from 10 hospital sites in three provinces (Table 1). These included five sites in Province 1, three sites in Province 2 and two sites in Province 3. Four sites were small size, four sites were medium size, and two sites were large size. During this period, 1 022 556 patient samples were received in these sites' transfusion laboratories.

**Table 1** Errors reported to Transfusion Error Surveillance System, 2008–2017

| Error details | Errors reported (*n*) | % |
|---|---|---|
| | 554 | |
| Province | | |
| Province 1 | 197 | 35·6% |
| Province 2 | 174 | 31·4% |
| Province 3 | 183 | 33·0% |
| Year | | |
| 2008–2012 | 261 | 47·1% |
| 2013–2017 | 293 | 52·9% |
| Potential severity | | |
| High | 475 | 85·7% |
| Medium | 14 | 2·5% |
| Low | 54 | 9·7% |
| Unknown | 11 | 2·0% |
| Hospital size | | |
| Large | 250 | 45·1% |
| Medium | 277 | 50·0% |
| Small | 23 | 4·2% |
| Unknown | 4 | 0·7% |

### Missing data

Four error reports (0·7%) had missing site ID and could not be included in site or size calculations. These errors were all from Province 1 and were reported from 2009 to 2010. Days to discovery were missing in 66 reports (11·9%); these were all from four sites. Time and day of week of error occurrence were both missing from 12 reports (2·2%), time of discovery was missing from 26 reports (4·7%), the point of discovery was missing from 18 error reports (3·3%), and the person involved in the error was missing in 9 error reports (1·6%). The person involved was marked as 'other' in 113 reports (20·4%); 70 of these were determined by the research team to actually be a clerk, as free-text description listed them as admitting—a clerical task—leaving 43 remaining 'other' entries (7·8%). The person who discovered the error was unknown in 16 error reports (2·9%). Location of occurrence was missing from 15 error reports (2·7%); location of discovery was missing from 14 error reports (2·5%). The potential severity of the error was missing in 11 reports (2·0%). 23·6% of errors were classified as unknown nature due to the free-text entry for both event description and discovery description being left blank ($n$ = 131). This lack of free-text entry was not unique to any province, site or year. One site did not have free-text entry in any of its reports because they exclusively used an upload tool rather than direct entry. Sites had a

median of 25·0% of errors having unknown error (IQR 15·5%–33·3%). Two sites were outliers with a smaller proportion of unknown natures ($n$ = 24, 15·5%; $n$ = 1, 5·9%) while one site had a higher proportion ($n$ = 40, 42·1%) than the rest.

## Error rate

The global registration error rate per samples received in the transfusion laboratory was 5·4/10 000 (median 5·0 [IQR 3·7–7·0]). There was no significant change in error rate overall from 2008 to 2017 ($P$ = 0·5).

However, on analysis of year-by-year changes, there was an increase in error reports in 2014 (119 errors vs. median 51·5). This was not unique to any province or site and is not accounted for by new hospital sites or site dropouts from the TESS program.

## Site characteristics

Site had a highly significant impact on error rates ($P$ < 0·0001), as did hospital size between large and medium sites ($P$ < 0·0001) and between medium and small sites ($P$ = 0·002). Medium size hospitals had the highest error rates (mean 8·0/10 000 [95% CI 7·1–9·0] for medium, 4·4/10 000 for large [95% CI 3·9–5·0] and 4·0/10 000 for small [95% CI 2·7–6·1]).

Province had a highly significant impact on rate ($P$ < 0·0001). Specifically, Province 1 had the highest error rates compared to the other provinces (8·3/10 000 for Province 1 [95% CI 7·2–9·6], 4·9/10 000 for Province 2 [95% CI 4·9–5·7], 4·7/10 000 for Province 3 [95% CI 4·1–5·5]).

There was no significant change in error rate from 2008 to 2017 by hospital size ($P$ = 0·34), by province ($P$ = 0·33) or by site ($P$ = 0·06).

## Time

The median time between the error and discovery was 0 days (IQR 0-1). Time of occurrence and time of discovery were not significantly different ($P$ = 0·59). 51·6% of errors occurred during the day, between 8 AM and 4 PM ($n$ = 286); 52·7% of all errors were discovered during this time interval ($n$ = 292) (Table 2). Errors were most frequently made ($n$ = 176, 31·7%) and discovered ($n$ = 173, 31·2%) in the morning between 8 AM and 12 PM. Most errors occurred on weekdays ($n$ = 418, 75·5%) rather than weekends or holidays ($n$ = 124, 22·4%).

In the transfusion chain, errors were typically discovered before laboratory sample testing ($n$ = 222, 40·1%); 17·0% were discovered during or after patient test verification, before or during cross-match or processing ($n$ = 94); 16·2% were discovered after product issue but before infusion ($n$ = 90).

## Health care worker

The person involved in the error was most frequently a hospital clerk ($n$ = 284, 51·3%) or a nurse ($n$ = 150, 27·1%), while errors were typically discovered by a medical laboratory technologist or assistant ($n$ = 435, 78·5%) (Table 2).

## Location

Errors most commonly occurred in outpatient clinics or procedure units ($n$ = 160, 28·9%) and in emergency departments ($n$ = 130, 23·5%) (Table 2). In the hospital, the least errors occurred in operating rooms ($n$ = 14, 2·6%). Errors were most often discovered in the transfusion service ($n$ = 426, 76·9%); 7·2% were discovered in outpatient clinics and procedure units ($n$ = 40).

## Nature

Error nature was variable across sites (Fig. 1). The most frequent reports were name errors ($n$ = 134, 31·7%), duplicate patient registrations ($n$ = 124, 29·3%) and missing armbands ($n$ = 45, 10·6%) (Table 2). Examples of common name errors included: spelling errors, discrepancies between the name in the hospital system and on the provincial health insurance card, no notification of name change when provisional names (e.g. Unidentified, Andrew) were updated, incorrect assignment of provisional names (i.e. assigning a name of the wrong sex) and simply 'incorrect name' without further description. The least common reports were wrong armbands ($n$ = 3, 0·7%), patients using another individual's identification ($n$ = 7, 1·7%) and incorrect sex ($n$ = 7, 1·7%).

## Consequences

The potential severity of the error was high in 85·7% of cases ($n$ = 475), medium in 2·5% ($n$ = 14, e.g. incorrect Provincial Health Insurance Number) and low in 9·8% ($n$ = 54, e.g. spelling error in name). Actual severity data were collected starting in 2016 for Provinces 2 and 3, and 2017 for Province 1, a period during which 50 events occurred and 13 reached the patient (Table 3). The actual severity was medium in one case (7·7%), and low in twelve cases (92·3%). The medium severity error was due to incorrect sex which was corrected just before surgery,

**Table 2** Characteristics of errors reported to Transfusion Error Surveillance System, 2008–2017

| Error details | Errors reported (*n*) | % |
|---|---|---|
| | 554 | |
| Discovered during | | |
| Before testing patient sample | 222 | 40·1% |
| During/after patient test verification or during cross-match/processing | 94 | 17·0% |
| After issue before infusion | 90 | 16·2% |
| After cross-match/processing before or at issue | 49 | 8·8% |
| Other | 26 | 4·7% |
| Subsequent patient sample test or event did not involve a product | 21 | 3·8% |
| After infusion | 16 | 2·9% |
| Product check-in | 10 | 1·8% |
| Quality assurance review | 8 | 1·4% |
| Unknown | 18 | 3·2% |
| Location occurrence | | |
| Outpatient clinic/procedure unit | 160 | 28·9% |
| Emergency | 130 | 23·5% |
| Laboratory service | 82 | 14·8% |
| Medical/Surgical ward | 50 | 9·0% |
| Intensive care unit | 44 | 7·9% |
| Obstetrics | 37 | 6·7% |
| Operating room | 14 | 2·5% |
| Transfusion service | 12 | 2·2% |
| Supplier/Service provider | 10 | 1·8% |
| Unknown | 15 | 2·7% |
| Location discovery | | |
| Transfusion service | 426 | 76·9% |
| Outpatient clinic/procedure unit | 40 | 7·2% |
| Emergency | 18 | 3·2% |
| Medical/Surgical ward | 16 | 2·9% |
| Operating room | 15 | 2·7% |
| Intensive care unit | 14 | 2·5% |
| Supplier/Service provider | 5 | 0·9% |
| Obstetrics | 3 | 0·5% |
| Laboratory service | 3 | 0·5% |
| Unknown | 14 | 2·5% |
| Error nature | | |
| Name error | 134 | 24·2% |
| Duplicate registration[1] | 124 | 22·4% |
| Missing armband | 45 | 8·1% |
| Wrong DOB[2] | 33 | 6·0% |
| Other | 21 | 3·8% |
| Wrong MRN[3] | 20 | 3·6% |
| Missing MRN | 19 | 3·4% |
| Doppelgänger[4] | 19 | 3·4% |
| Wrong PHIN[5] | 16 | 2·9% |
| Comingling[6] | 9 | 1·6% |
| Patient using another's identification | 7 | 1·3% |
| Incorrect sex | 7 | 1·3% |
| Wrong armband | 3 | 0·5% |
| Unknown | 131 | 23·6% |
| Person involved in the error | | |
| Clerk | 284 | 51·3% |
| Nurse | 150 | 27·1% |
| Medical laboratory technologist/assistant | 60 | 10·8% |
| Physician | 8 | 1·4% |
| Other | 43 | 7·8% |
| Unknown | 9 | 1·6% |
| Discoverer | | |
| Medical laboratory technologist/assistant | 435 | 78·5% |
| Nurse | 50 | 9·0% |
| Quality assurance/Supervisor/Transfusion Safety Officer | 38 | 6·9% |
| Clerk | 3 | 0·5% |
| Physician | 3 | 0·5% |
| Other | 9 | 1·6% |
| Unknown | 16 | 2·9% |
| Subsequent events | | |
| Record corrected | 192 | 34·7% |
| Patient sample recollected | 112 | 20·2% |
| Product destroyed | 21 | 3·8% |
| Product retrieved | 11 | 2·0% |
| Additional testing | 10 | 1·8% |
| Discovery method | | |
| Request/sample receipt process check | 239 | 48·4% |
| Bedside check | 51 | 10·3% |
| Notification by ward | 45 | 9·1% |
| Testing process check | 38 | 7·7% |
| Return to inventory | 31 | 6·3% |
| Issuing process checks | 24 | 4·9% |
| Quality assurance review/quality control/administrative check | 24 | 4·9% |
| Customer inquiry/complaint | 13 | 2·6% |
| Notification by supplier | 11 | 2·2% |
| Transfusion/inventory audits | 4 | 0·8% |
| Review of documentation of transfusion | 2 | 0·4% |
| Miscellaneous | 12 | 2·4% |
| Consequence | | |
| Transfusion delayed | 46 | 8·3% |
| Product transfused - no reaction | 8 | 1·4% |
| Procedure delayed or cancelled | 6 | 1·1% |

[1]Two files for one patient.
[2]Date of birth.
[3]Medical record number.
[4]Mingling of information for two patients in two files.
[5]Provincial health insurance number.
[6]Two patients' information in one file.

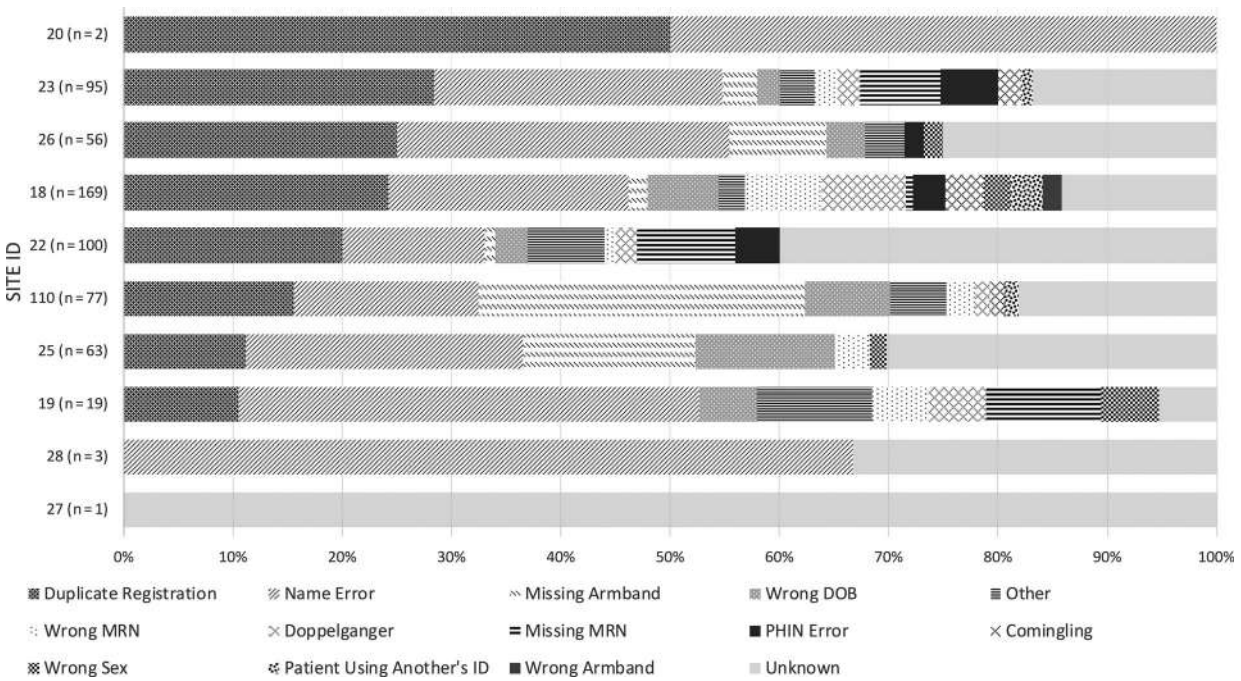


**Fig. 1** Error nature by site, 2008–2017.

requiring a new sample to be drawn. This caused unmatched blood to be issued for surgery while the sample was being tested and a 45-minute delay in issuing crossmatched blood.

10·8% of errors reached the patient ($n$ = 60); 89·2% were a near miss ($n$ = 494) (Table 2). The most frequent consequence of errors reaching the patient was a delayed transfusion ($n$ = 46, 76·7%). Eight events involved the product being transfused with no transfusion reaction (13·3%), while six caused a procedure to be delayed or cancelled (10·0%). Examples include: (1) Patient with known anti-Jk$^a$ being transfused Jk$^a$ antigen unknown blood due to a duplicate registration and an undetectable antibody (no transfusion reaction reported); (2) Nurse noting on transfusing the second of two units that the patient had no armband and that the bedside check (and the sample collection check) had been omitted for the first unit requiring re-draw of an additional sample before proceeding with the second unit.

The patient record was corrected in 34·7% of errors ($n$ = 192) (Table 2). The patient sample was recollected in 20·2% of errors ($n$ = 112). The product was destroyed in 3·8% of errors due to the unit being unable to be returned to inventory, due to temperature or time deviations at point of return to the transfusion service ($n$ = 21). The product was retrieved and able to be returned to available inventory in 2·0% of events ($n$ = 12). Ten patients required additional testing (1·8%).

### Discovery

For the 494 errors that were a near miss, the most common discovery method was through a process check during the receipt of a test request and/or product order ($n$ = 239, 48·4%); 10·3% of near miss errors were discovered during a bedside check, including during sample collection or transfusion ($n$ = 51); 9·1% were discovered due to a notification from the hospital ward ($n$ = 45) (Table 2).

## Discussion

To our knowledge, this is the first multi-site analysis of hospital registration errors impacting transfusion. This study highlights five important areas for attention. First, registration errors are potentially an under-recognized cause of potentially high-severity transfusion errors. Second, registration error rates and nature of these errors vary widely across sites. Third, registration errors commonly occurred in outpatient areas and emergency departments. Fourth, the most common consequence is a delay in transfusion. Last, missing data in TESS reports were common and warrants system re-design to ensure complete data entry for all uploaded events.

Previous literature on all types of errors reported to TESS shows a global potential high-severity rate of between 14% and 17% [11, 13]. In the subset of

**Table 3** Characteristics of errors reported with actual patient harm to Transfusion Error Surveillance System, 2016–2017

| Error details | Errors reported (*n*) | % |
|---|---|---|
| | 13 | |
| Actual severity | | |
| Low | 12 | 92·3% |
| Medium | 1 | 7·7% |
| Location occurrence | | |
| Emergency | 6 | 46·2% |
| Outpatients clinic/procedure unit | 4 | 30·8% |
| Operating room | 1 | 7·7% |
| Medical/Surgical ward | 1 | 7·7% |
| Transfusion service | 1 | 7·7% |
| Person involved in the error | | |
| Clerk | 7 | 53·8% |
| Medical laboratory technologist/assistant | 4 | 30·8% |
| Nurse | 2 | 15·4% |
| Error nature | | |
| Name error | 3 | 23·1% |
| Missing armband | 3 | 23·1% |
| Duplicate registration | 1 | 7·7% |
| Wrong date of birth | 1 | 7·7% |
| Incorrect sex | 1 | 7·7% |
| Unknown | 4 | 30·8% |
| Subsequent events | | |
| Additional testing | 5 | 38·5% |
| Patient sample recollected | 4 | 30·8% |
| Record corrected | 3 | 23·1% |
| Product retrieved | 1 | 7·7% |
| Consequence | | |
| Product transfused - no reaction | 5 | 38·5% |
| Procedure delayed or cancelled | 5 | 38·5% |
| Transfusion delayed | 3 | 23·1% |

registration-related errors in this analysis, 85·7% were potentially high severity. A previous Canadian single-site study by our team on all error types also shows just 4% of errors reaching the patient [14]; 10·8% reached the patient in this registration error study. Thus, registration errors may be higher risk to patients and less likely to be detected prior to transfusion than other transfusion-related errors. However, of 13 errors reaching the patient for which actual severity data were available, none were high severity. The discrepancy between the generally high-potential severity and low actual severity may be explained by the quick identification (usually same day in this study) of errors.

Registration error rates varied by an order of magnitude across sites, with medium size hospitals reporting the highest rates. It is possible that larger sites, being more accustomed to high-transfusion and high-registration rates, have more experience preventing errors or more existing interventions in place, while smaller sites, with less traffic, have greater capacity to avoid errors; medium size sites may have higher error rates due to less resources for prevention and high volumes of patients. However, it is also likely that this is due to small sample size. The doubling of errors during the year 2014 with no discernible cause also highlights the variability of error occurrence over time and the need for long periods of data collection. The nature of errors varied by site. The most common registration errors across sites were name errors and duplicate registrations, which concurs with the findings of several other studies [7, 14–16]. The heterogeneity of errors across sites suggests that root causes for these errors or existing preventative measures will need to be studied by site; the absence of some error types or lower rates at some sites suggest transfusion chain system improvement exist and could be adapted at other sites, and that these improvements will need to be tailored at the site level.

Outpatient areas and emergency departments were identified as concerning areas for targeting registration process improvements. Other studies investigating sample collection and handling errors have found frequent errors in the emergency department, operating room and inpatient wards, but not outpatient units [2–4]. This indicates that outpatient areas may be uniquely at risk for registration errors over other error types. Clerks, including admitting clerks, were involved in 51·3% of registration errors, suggesting that registration may be underprioritized in high-volume outpatient units, including outpatient sample collection clinics and office visits. However, we did not have access to denominator data for the number of registrations by hospital location, so true rates by location could not be calculated. It is likely that outpatient areas and emergency departments, as points of first patient contact with first blood sample draw, experience more registration than other areas of the hospital. This could be a potential reason for higher volumes of errors, compared to other hospital areas.

A minority of errors reached the patient; the most common consequence for the patient was a delayed transfusion or procedure. Of 13 errors that reached the patient during the period in which actual severity data were available, none resulted in patient harm. Similarly, a recent transfusion report with a larger sample size showed one out of 51 actual events caused by registration errors led to major morbidity, compared to one out of 50 actual events caused by errors in pre-transfusion testing [17].

Not all of the fields in each report were completed, creating a large amount of missing data. In particular, there was a lack of data for determining error nature. Part of the reason for this is that rather than entering reports directly into TESS, some sites upload existing databases,

meaning any blanks or missing fields in their databases appear as unknowns when extracted. This suggests an important potential process improvement for TESS: given that all fields are mandatory to be filled out, and directly entered reports cannot be submitted with any missing data, the system should also not allow reports that have any blanks to be uploaded until all errors are checked and corrected. There was also a large number of entries with the person involved in the error listed as 'other', though many of these turned out to be employees in clerical positions, indicating a need for re-training of error reporting technologists at all sites. This problem is not unique to TESS; the United Kingdom's Serious Hazards of Transfusion reported missing data on human factors in 11% of error reports in 2018 and many which may have been incorrectly reported [17].

This study had several limitations. Sites and provinces with higher rates could reflect higher detection and reporting capabilities rather than inferior safety. Some of the 10 sites joined TESS between 2008 and 2017, meaning there is inconsistency in reporting across this time period. Any degree of under-reporting cannot be detected in this study and may cause artificially low-error rates. Registration errors detected by the Transfusion Service and errors reported in TESS represent only a fraction of errors that exist and that pose a risk to patients. Participating TESS sites do not report these errors in a consistent manner; some may not receive systematic notices of all registration errors, and those that do may not have entered them (e.g. because of volume and missing date and time information that should be included in the error report). Missing data, as mentioned, affected results for categorical data. True denominator data (number of patients registered and armbands generated) were not available so number of samples received in the blood bank was used as a proxy. Rates could not be calculated for any variable other than year or site, as these denominator data were only available for year and site.

## Conclusion

This study provides an analysis of registration errors impacting transfusion care that were reported to a national hemovigilance programme. Overall, 85·7% of registration errors have potentially serious consequences for patients. Further research is warranted to understand the root causes for the drivers of these potentially serious errors, to assist with re-design of patient registration processes and systems.

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## Conflicts of interest

The authors have no conflicts of interest to declare.

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ORIGINAL PAPER

# Elimination of pretransfusion RhD typing at Mackay Memorial Hospital, Taiwan—30-year experience (1988–2017)

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Vox Sanguinis

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**Background** The frequency of the RhD negative (D-) phenotype among the population of Taiwan is only 0·34% and so anti-D is a relatively rare antibody. Routine pretransfusion D typing of patients at Mackay Memorial Hospital (MMH) was discontinued in 1988, and this report is a look back and retrospective evaluation over 30-years (1988–2017).

**Study Design and Methods** The incidence of anti-D among patients at MMH during the periods 1984–1988 (when D typing was performed) and 1988–2017 (when D typing was not performed) was reviewed. Also, the incidence of anti-D among both MMH patients and voluntary blood donors at the Taiwan Blood Foundation was compared. The importance of anti-'Mi$^{a}$' in Taiwan is also discussed.

**Results** The incidence of anti-D relative to other Rh antibodies among MMH patients when D typing was performed and D typing not performed has remained relatively unchanged (5%). The frequencies of anti-D and anti-'Mi$^{a}$' among 38 537 patients who were transfused at MMH during 2008–2017 were found to be 0·06% and 2·6%, respectively. During the same period, among 3 510 131 blood donors at Taiwan Blood Foundation, the frequencies of anti-D and anti-'Mi$^{a}$' were 0·004% and 0·2%, respectively.

**Conclusion** The elimination of D typing of patients at MMH has proven to have been a correct and logical decision. D- patients, if they do not carry anti-D, can thus be safely transfused with D+ red cells.

**Key words:** anti-'Mi$^{a}$', anti-D, anti-D induced by the $D_{el}$ phenotype, blood transfusion in D-, Taiwan.

## Introduction

Modification of 'standard Western pretransfusion testing' for use in Taiwan [1] has been a difficult task, as it is considered the global gold standard. However, for the past 30 years, (since 1988) MMH has not routinely performed D typing of patients requiring transfusion because 99·66% of the population of Taiwan are D+. Prior to 1988, undertransfusion of D– patients often occurred because D– blood was often unavailable due to blood donation being still in its infancy, resulting in several fatal outcomes, especially in emergency situations. In 1988, MMH made the courageous decision to discontinue routine pretransfusion D typing of patients in order to reduce the fear surrounding the D– phenotype among Taiwan's general population and also to help inform medical personnel that transfusing D+ blood to D– patients who did not carry anti-D was safe. In addition, Prof. M. Contreras of the North London Blood Centre replied to our letter of concern as follows: 'After a long discussion with Prof. Mollison. . .it is not logical to type for D when E-typing and c-typing are not done. . .. My conscience is quite clear when I give you this advice that it is ethical to discontinue routine Rh(D) typing of recipients and

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antenatal patients'. (Contreras, personal communication, 1985). In addition, at that time we were trying to save limited medical resources by eliminating unnecessary testing (National Health Insurance was established in 1995 and offered reimbursement to hospitals for D typing). From 1988–2018, pretransfusion testing at MMH consisted of ABO grouping, antibody screening and major cross-matching by the manual Polybrene method. No D typing was performed [1,2]. MMH is a general hospital and also a teaching medical centre with 1130 beds, usually having 90% bed occupancy rate. During 2008–2017, 30 000–32 000 units of RBC were transfused each year (every unit was made from 250 ml whole blood). The Taiwan blood donation centre usually delivers only D+ blood to hospitals if there is no special request for D– blood. On the other hand, RhD typing is a routine test in the prenatal care of our Obstetric Department for any female patient who wants to have a baby.

This report is a look back evaluation of our policy to discontinue D typing over the past 30 years by analysing the incidence of anti-D among both patients and blood donors. However, it is of interest to mention that at the end of 2018, the blood bank at MMH started to automate and D typing was reintroduced due to the purchase of a Western style analyser which included both ABO and D typing reagents together. In addition, Taiwan has in recent years gradually become a global village and many Caucasians now live in Taiwan. Therefore, since the end of 2018, D typing has once again become part of the routine pretransfusion testing protocol at MMH. However, we hope that our 30 years' experience might be helpful to other Southeast Asian countries whose populations are genetically closely related to Taiwan and also have a high incidence of both D and 'Mi$^{a}$' (Miltenberger) phenotypes [3,4].

## Materials and methods

The incidence of anti-D among patients at MMH during the periods 1984–1988 (D typing performed) and 1988–2017 (D typing not performed) was reviewed. In addition, the incidence of anti-D in both patients (MMH) and blood donors (Taiwan Blood Foundation) [5] was compared. The clinical significance of both anti-D and anti-'Mi$^{a}$' (the most common alloantibody in Taiwan) among both patients and blood donors was also analysed.

## Results

From Table 1, it can be seen that the relative frequency of anti-D vs. other Rh system alloantibodies (5%) among hospital patients at MMH has remained unchanged whether D typing was performed routinely (1984–1988) or not performed (1988–2017). In fact, it actually decreased to 2% during the most recent 10-years period (2008–2017). A similar result (5%) was observed among blood donors and interestingly was obtained during the period when all hospitals in Taiwan, except MMH, were all routinely performing pretransfusion D typing of patients.

From Table 2, it can be seen that during the period 2008–2017 among 38 537 patients at MMH, 1002 cases of anti-'Mi$^{a}$' (2·6%) were detected compared with only 22 cases of anti-D (0·06%) [6]. During the same period among 3 510 131 blood donors at Taiwan Blood Foundation, 7015 cases of anti-'Mi$^{a}$' (0·2%) were detected compared with only 146 cases of anti-D (0·004%) [5] indicating that anti-D is a relatively low-frequency alloantibody compared with other clinically significant alloantibodies. See the complete list of alloantibodies in Table 2.

Among 22 D- patients who had anti-D during 2008–2017 at MMH, 10 cases were induced by transfusion (Table 3): nine cases were the result of transfusion of D+ blood and one case was due to the transfusion of 2 units of the $D_{el}$ phenotype. This latter case occurred in an 81-year-old lady with upper G-I bleeding. Anti-D was detected 3 months after the transfusion of 4 units of D- blood and 2 units of $D_{el}$ positive blood. Of the remaining 12 D– patients with anti-D, 10 patients had all been previously sensitized prior to transfusion at MMH; one patient had passively received anti-D from transfusion of D- blood carrying anti-D and one patient due to Rhogam administration. During the 10-year period from July 1992 to June 2002, five cases of transfusion-induced anti-D occurred at MMH [7].

Since anti-'Mi$^{a}$' and anti-E are the most common alloantibodies detected among patients at MMH, it is not surprising to discover that anti-E/E+c and anti-'Mi$^{a}$' are also commonly incriminated in haemolytic disease of the newborn (HDNB). During the period 2008–2017 at MMH, the causative antibodies of HDNB were found to be anti-E/E+c (16 cases), anti-Jk$^{b}$ (three cases), anti-'Mi$^{a}$'/'Mi$^{a}$'+E (two cases) and one case each of anti-C+ e and anti-D. Not surprisingly, anti-D rarely causes HDNB among Taiwanese.

## Discussion

The D– phenotype is rare among the population of Taiwan with a frequency of 0·34% (Taiwan Blood Foundation). Since the discontinuation of routine pretransfusion D typing of patients at MMH 30 years ago, it is likely that almost all D- patients since then have been transfused with D+ blood. However, the incidence of anti-D did not increase during this period and has actually decreased

**Table 1** Comparison of the incidence of anti-D vs. other Rh system alloantibodies among MMH patients, before (1984–1988) and after (1988–2017) discontinuation of routine pretransfusion D typing, with that among blood donors

| Year | Routine D typing | Number of cases of anti-D vs. other Rh system alloantibodies | % |
|---|---|---|---|
| 1984–1988[a] [4] | Yes | 5/103 | 5 |
| 1992–1996[a] [4] | No | 4/102 | 4 |
| 1999–2001[a] [4] | No | 10/194 | 5 |
| 2008–2017[a] | No | 22/1404 | 2 |
| 2008–2017 [5](blood donors) | Yes | 146/2792 | 5 |

[a]Patients at MMH.

**Table 2** Alloantibodies encountered during the most recent 10 years (2008–2017) in patients at MMH and among blood donors at the Taiwan Blood Foundation

| MMH [6] Total no. of patients: 38 537 | | Taiwan Blood Foundation [5] Total no. of blood donors: 3 510 131 | |
|---|---|---|---|
| Anti- | No. | Anti- | No. |
| 'Mi$^{a}$' | 1002 | 'Mi$^{a}$' | 7015 |
| E | 906 | P1 | 3159 |
| c | 367 | Le$^{a}$ | 2440 |
| P1 | 257 | E | 2281 |
| Le$^{a}$ | 198 | Le$^{b}$ | 1660 |
| M | 193 | M | 1537 |
| C | 73 | c | 283 |
| Le$^{b}$ | 70 | Wr$^{a}$ | 165 |
| Di$^{a}$ | 60 | D | 146 |
| e | 58 | S | 138 |
| Jk$^{a}$ | 58 | e | 116 |
| Jk$^{b}$ | 42 | Di$^{a}$ | 97 |
| Wr$^{a}$ | 40 | C | 94 |
| D | 22 | Fy$^{b}$ | 43 |
| Fy$^{b}$ | 19 | N | 40 |
| S | 18 | G | 13 |
| Bg$^{a}$ | 16 | Jk$^{a}$ | 8 |
| N | 13 | Jk$^{b}$ | 4 |
| Bg$^{b}$ | 7 | Le$^{bh}$ | 4 |
| Fy$^{a}$ | 1 | Jk3 | 3 |
| Jk3 | 1 | Ce, Cw, Di$^{b}$, M$^{g}$, Pr | 2 |
| i | 1 | V, PP1P$^{k}$, Vw, He, Kp$^{a}$, Ku, Lan, Jr$^{a}$, LW, Lw$^{a}$ | 1 |
| Total | 3422 (8·9%)[a] | Total | 19 266 (0·55%)[a] |

Antibody screening and identification were performed by the manual Polybrene method at MMH and on an Olympus PK7300 analyser (0·3% bromelinized screening cells on a specially designed microplate) at Taiwan Blood Foundation. Anti-I/HI and cold agglutinins were excluded.
[a]Frequency (%) of alloantibodies encountered in patients and blood donors.

during the most recent 10 years (2008–2017), as shown in Table 1. Interestingly, since the discontinuation of routine pretransfusion D typing of hospital patients at MMH, the relative incidence of anti-D versus other Rh alloantibodies (5%) among patients has been found to be identical to that among Taiwanese blood donors (5%) as shown in Table 1. This relative incidence of anti-D versus other Rh alloantibodies among blood donors (3 510 131 donors) is more representative of the actual incidence of anti-D among the general population of Taiwan when all hospitals apart from MMH performed pretransfusion D typing of patients routinely. Therefore, anti-D is not as important in the Taiwan population as it is in Caucasian populations and it appears to be unnecessary to transfuse D– patients in our population with D- blood when they do not carry anti-D.

**Table 3** Ten cases of transfusion-induced anti-D detected during 2008–2017 at MMH

| Case | Anti- | Sex | Age (year) | Transfused[a] | MP[b] | LIAT |
|---|---|---|---|---|---|---|
| 1 | D + C+Jk[a] | F | 44 | 2U | 3+ | 4+ |
| 2 | D + C+E | M | 76 | 4U | 3+ | 3+ |
| 3 | D + E | F | 5M | 4U | 1+ | Negative |
| 4 | D | F | 92 | 16U | 4+ | NA |
| 5 | D + E | M | 52 | 2U | 1+ | ± |
| 6 | D | F | 83 | 2U | 1+ | ± |
| 7 | D | F | 81 | 10U | 2+ | NA |
| 8[c] | D | F | 81 | 2U | 2+ | NA |
| 9 | D + C | M | 52 | 5U | 1+ | NA |
| 10 | D | M | 38 | 2U | 2+ | 3+ |

LIAT, LISS indirect antiglobulin test.

[a]Units of packed RBC transfused.

[b]Manual Polybrene method.

[c]Patient was transfused with 2 units of $D_{el}$ phenotype blood.

With regards to the $D_{el}$ phenotype, as in case 8 in Table 3, it can be seen that transfusing D– patients with 'supposedly' D– blood does not necessarily prevent them from producing anti-D due to the presence of the '$D_{el}$ phenotype' [8,9] in the Taiwan population [10]. The $D_{el}$ phenotype is an extremely weak D phenotype in which the D antigen is only detectable by adsorption and elution with anti-D [11] and has a frequency of 32·6% among serologically D– individuals in Taiwan [12]. In case 8, the patient was transfused with 2 units of the $D_{el}$ phenotype resulting in the production of anti-D. The genetic marker *RHD1227A* for the $D_{el}$ phenotype was recently found to be present in all $D_{el}$ individuals [13]. Since 2017, a rapid genotyping assay for the detection of the *RHD1227A* allele is now performed routinely for all D– blood donors (with an additional confirmatory adsorption and elution test with anti-D on all first time D-donors) [14]. The $D_{el}$ phenotype in Taiwan is now considered as D+ and all such units are labelled D+.

In addition, because of the rarity of the D- phenotype in Taiwan it has in the past caused unnecessary anxiety and fear among the general population. This irrational fear has resulted in significant and harmful under transfusion in many cases. D– patients, if they do not carry anti-D, can thus be safely transfused with D+ red cells.

Since the discovery in 1987 of the GP.Mur phenotype and the corresponding antibody anti-'Mi[a]' among the Taiwan population, the GP.Mur phenotype has become the clinically most important blood group in Taiwan [12]. The GP.Mur phenotype has therefore been included in antibody screening cells in Taiwan since 1990. In addition, anti-'Mi[a]' and anti-E have proven to be the most common and clinically significant alloantibodies (see Table 2). Anti-'Mi[a]' has caused both intravascular haemolytic transfusion reactions and severe HDNB [15,16]. The Taiwan Blood Foundation has successfully produced monoclonal anti-Mi[a], anti-Mur and anti-Mut [17] making it possible to perform mass screening of blood donors. The frequency of the GP.Mur phenotype in Taiwan was found to be 4·71% after the screening of 704 833 donors in 2018 (Taiwan Blood Foundation, 2019).

Since the D- phenotype is rare throughout Southeast Asia, Taiwan's experience may be of benefit to other countries in this region.

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## Conflict of interests

The authors declare no conflict of interests.

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# Development and evaluation of stem cell collection procedure diagrams to support the education and recruitment of committed stem cell donors

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**Background** Diagrams which allow potential unrelated stem cell donors to visualize the stem cell collection process were hypothesized to support the recruitment and education of committed stem cell donors.

**Study design and methods** A series of bone marrow and peripheral blood stem cell collection procedure diagrams were developed, featuring young adult male donors of varied ethnic backgrounds. Post-implementation, surveys were conducted to evaluate stakeholder perspective on the diagrams' utility. A quality improvement project was conducted at five stem cell drives from 2017 to 2018 at which recruiters did or did not show the diagrams to potential donors. Following the drives, registrants were invited to complete a survey exploring their experience, knowledge and attitude towards donation.

**Results** The diagrams were implemented in Canada in 07/2016. Of 293 participating registrants (24·7% non-Caucasian males) recruited at five drives between 2017 and 2018, 76% ($n$ = 197) were shown the diagrams. Participants who were shown the diagrams were significantly more likely to report that the recruiters appeared very knowledgeable (89% vs. 76%, $P$ = 0·019) and to report improved self-reported knowledge of stem cell donation ($P$ = 0·010) compared to participants not shown the diagram. Data are also shown demonstrating that stakeholders in donor recruitment used and valued the diagrams and that use of the diagrams was associated with improved donor recruitment outcomes in Canada.

**Conclusion** This report is the first evaluation of stem cell collection diagrams in the literature. The diagrams are relevant to donor registries, recruitment organizations and transplant centres worldwide, and their use may support efforts to educate and recruit committed, ethnically diverse donors.

**Key words:** donor recruitment, informed consent, donor, donation, bone marrow, peripheral blood stem cell.

## Introduction

Allogeneic stem cell transplantation is a potentially curative therapy for a variety of blood, immune and metabolic diseases; however, the majority of patients do not have a suitable matching donor in their family, and require an alternative donor. Matched unrelated donors remain the most common alternative donor choice, though haploidentical donors have been increasingly used in recent years [1, 2]. Unrelated donors are typically recruited either online or at stem cell drives, at which they provide informed consent and a tissue sample for human leucocyte antigen (HLA)-typing. Registrants' HLA typing is listed in an international database, and transplant physicians can search the global inventory using their national registry.

Despite over 35 million unrelated donors around the world today [3], many patients are unable to find a suitable matching unrelated donor. This is especially true for ethnic and racial minority groups, many of which experience lower rates of finding HLA-matched donors, both within [4–7] and outside [8–10] of North America. This is due to the combination of smaller donor pools, disproportionate representation on individual registries and on the worldwide network, and ethnic/racial differences in genetic diversity and in attrition from the registry [11, 12]. Younger donors are needed as they are associated with improved survival in transplant recipients and can remain on the registry longer once they have signed up [13, 14]. The transplantation community has also demonstrated a preference for selecting male donors [15], due to the reduced risk of chronic graft-versus-host disease in recipients and the higher cell count yields with peripheral blood stem cell collections, although a recent study showed that donor age was the only variable which impacted on recipient survival [16, 17].

Securing informed consent for unrelated haematopoietic stem cell donors is an important ethical and legal obligation. Moreover, several studies have found that donors who felt less informed at various points in the donor recruitment, evaluation and workup process were more ambivalent about donation and more likely to withdraw if asked [18–20]. This work suggests that increasing efforts to educate donors about the donation procedure could help to reduce attrition.

Infographics are often used to convey medical information, and previous studies have shown that infographics can increase readers' attention, comprehension, recall and adherence [21]. We hypothesized that diagrams which allowed potential donors to visualize the stem cell collection process could enrich the informed consent process and support the recruitment and education of quality stem cell donors. Here, we describe the development of stem cell collection procedure diagrams, and their implementation at stem cell drives spearheaded by the Canadian donor recruitment organization Stem Cell Club [22]. Our aim was to develop a resource to help recruiters and healthcare professionals realize World Marrow Donor Association (WMDA) standards, especially standards 3·04 and 3·05 [23]. We show data evaluating Stem Cell Club's donor recruitment outcomes prior to and following implementation of the diagrams; recruiter perspective of the diagrams; and the impact of the diagrams on registrant knowledge and attitudes.

## Methods

### Stem cell collection procedure diagram development

A graphic artist was retained to develop the diagrams based on preliminary designs provided by the authors. This work was informed by the seven G.R.A.P.H.I.C. principles for public health infographic design [24]. Accompanying text was written to be understandable to the lay-person. The artist was instructed to feature the most needed donor-demographics: ethnically diverse, young adult males. Diagrams were reviewed for accuracy by actively practising transplant haematologists and for appeal by community representatives from the respective ethnic groups.

In total, four versions of the bone marrow and peripheral blood stem cell collection procedure diagrams were developed, featuring Black (Fig. 1A,B), Chinese (Figure 1C,D), South Asian (Fig. 1E,F) and Indigenous Peoples of Canada (Figure 1G,H) young adult male donors. The peripheral blood stem cell collection procedure diagrams (Fig 1A,C,E, G) highlight pre-procedure GCSF administration and day-of-donation apheresis. The bone marrow collection procedure diagrams (Fig 1B,D,F,H) illustrate marrow being harvested from the posterior superior iliac spine via a bone marrow aspiration needle. A zoom-in of the needle inside

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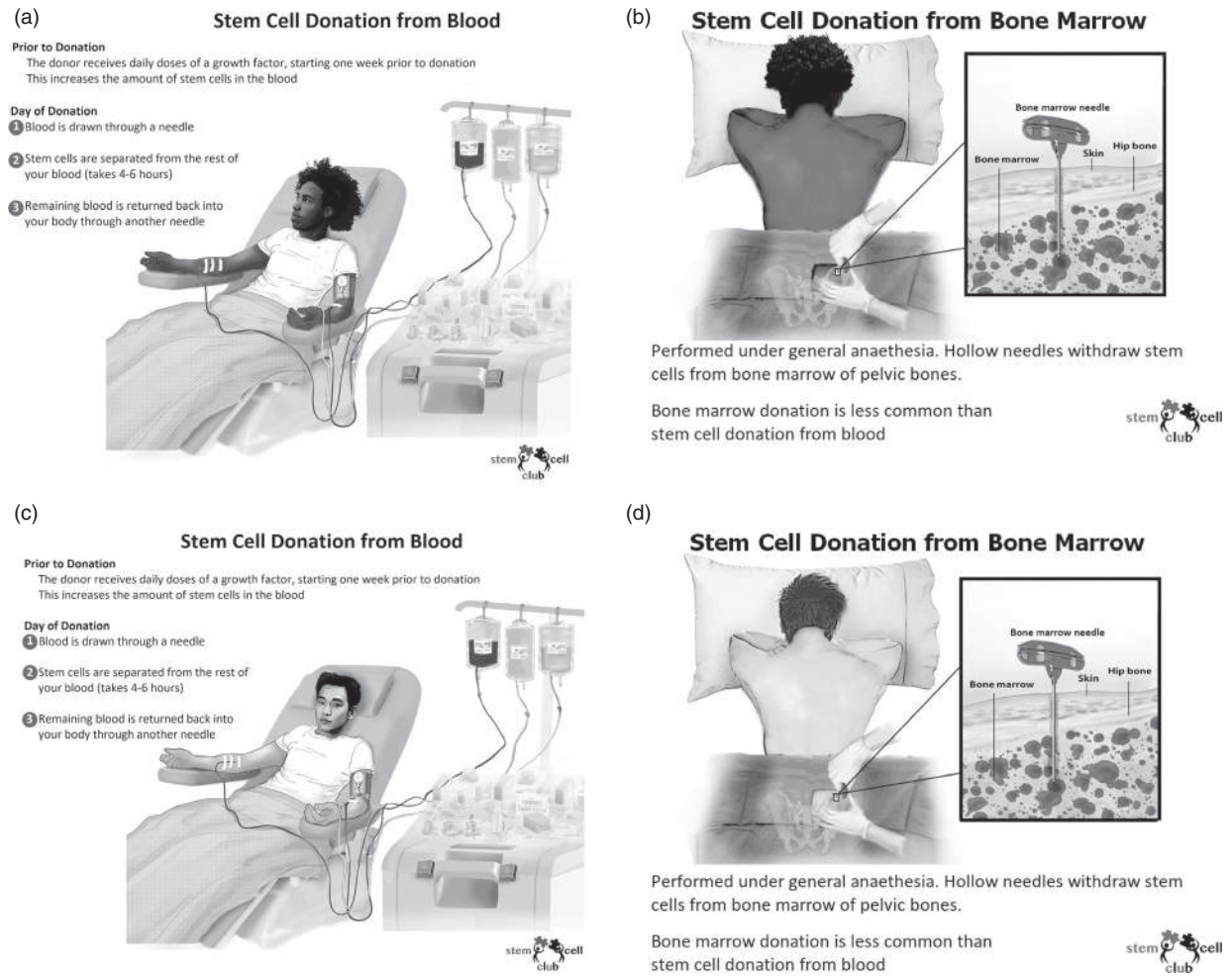


**Fig. 1** Bone marrow and peripheral blood stem cell collection procedure diagrams, featuring Black (Figure 1A,B), Chinese (Figure 1C,D), Indian (Figure 1E,F) and Indigenous (Figure 1G,H) young adult male donors. The peripheral blood stem cell collection procedure diagrams (Figure 1A,C,E,G) highlight pre-procedure GCSF administration and day-of-donation apheresis. The bone marrow collection procedure diagrams (Figure 1B,D,F,H) illustrate marrow being harvested from the posterior superior iliac spine via a bone marrow aspiration needle, a procedure performed under general anaesthetic. A zoom-in of the needle inside the marrow is shown. These diagrams are available in colour at www.stemcellclub.ca/supplies.html

the marrow is shown. The diagrams were published online to www.stemcellclub.ca in 07/2016 and made available for the transplantation community to access and use.

## Implementation of stem cell collection procedure diagrams

Starting in 07/2016, the stem cell collection procedure diagrams were built into Stem Cell Club's stem cell drives [25]. The Stem Cell Club checklists (available at stemcellclub.ca/supplies.html) were modified, requiring recruiters to bring these diagrams to each stem cell drive and show them to all new registrants. Furthermore, Stem Cell Club's training modules (available at http://stemcellclub.ca/training/index.html) were updated to include these diagrams, and a series of recruiter training videos was developed in 2016 to complement these modules. One of these videos discusses informed consent, and features the use of these diagrams (https://youtu.be/PVwJ3ogkrOg; also available at stemcellclub.ca/training).

## Donor recruitment outcomes before and after implementation of the diagrams

We set out to determine the number, demographics and availability of the donors recruited in the 18 month periods prior to (January 2015-June 2016) and following (July 2016-December 2017) implementation of the stem cell collection diagrams at drives spearheaded by Stem Cell Club. Donors were tracked through the Canadian Blood Services Stem Cell Registry Stem Cells National System Solution software. We also tabulated how many

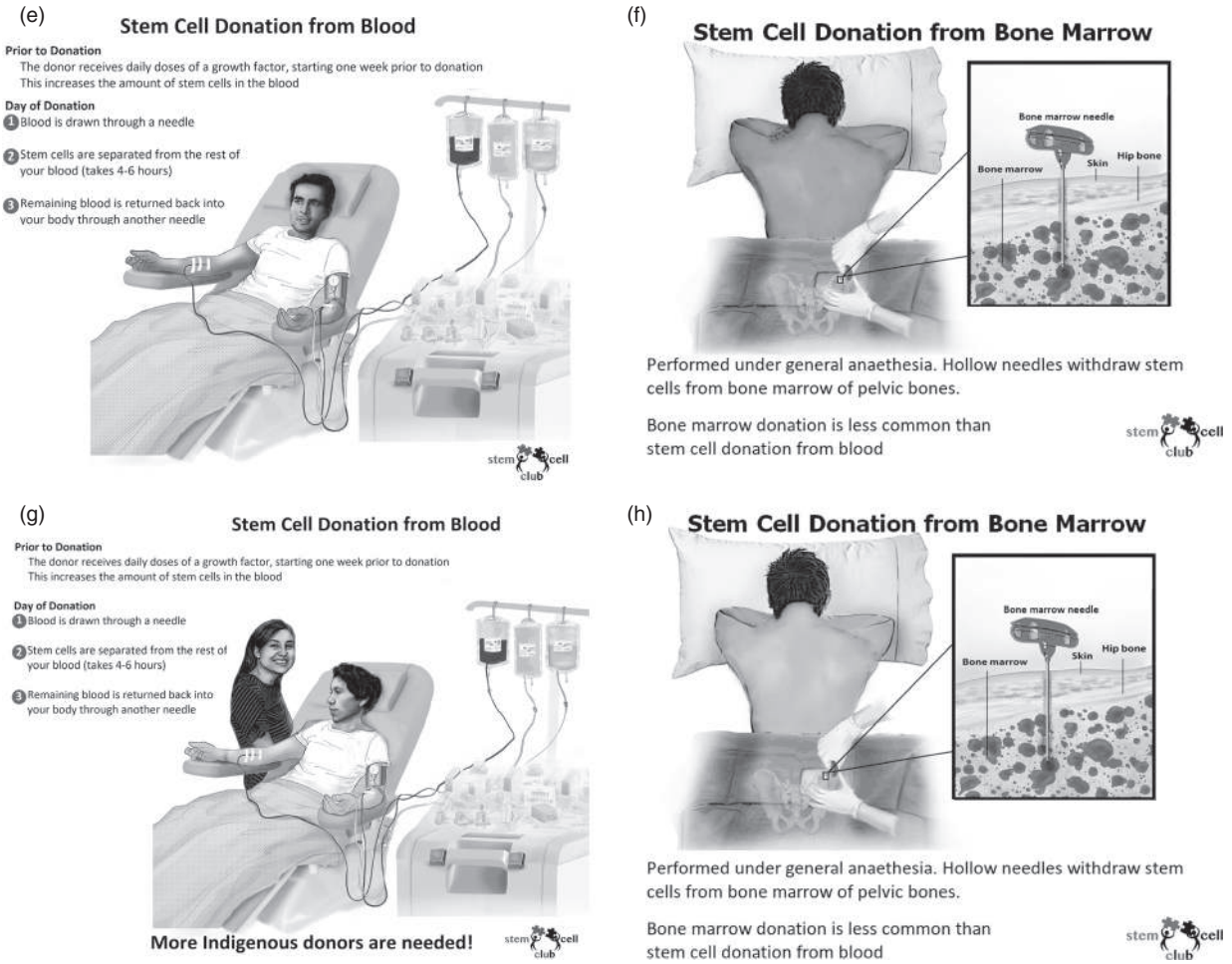


**Fig. 1** (Continued)

in each time horizon had been requested for verification typing (VT), the proportion unavailable at VT (due to loss of interest of the donor or inability of the registry to contact or locate the donor) and the number of donors who proceeded to stem cell donation.

## Evaluating recruiter perspective

Six months following implementation of the stem cell collection procedure diagrams, Stem Cell Club recruiters across Canada were invited by email to participate in a SurveyMonkey survey (https://www.surveymonkey.com/) to assess their perspective on the diagrams; survey questions employed Likert scales.

## Exploring the impact of the diagrams on registrant knowledge and attitudes

We set out to investigate whether there were differences in the knowledge or attitudes of registrants who either were or were not shown the stem cell collection diagrams as part of their recruitment as donors. This work was conducted as a quality improvement project according to criteria of the Hamilton Integrated Research Ethics Board [26]. The project was conducted at five stem cell drives which took place between 03/2017 and 04/2018 in Ontario or British Columbia. Ahead of each drive, participating recruiters (5–8 per drive) were asked to sign up for shifts to confirm their participation. All recruiters completed Stem Cell Club's updated recruiter training programme, which introduced and discussed use of the diagrams. A numbered list of participating recruiters was generated, in order of when they confirmed their participation. A random number generator (https://www.calculator.net/random-number-generator.html) was then used to select four participating recruiters to receive a copy of the diagrams to use at the drive. These four recruiters were instructed to show the diagrams to potential donors, and, where possible, to specifically approach ethnic demographics matching that of the

characters portrayed in the diagram they carried; other recruiters did carry or use these diagrams, and relied on face to face discussion to explain the donation process. Recruiters were informed that a survey was being conducted at the drive to examine registrant experience, but were not explained the nature of the quality improvement project ahead of time, and were not shown the survey questions.

Newly registered stem cell donors at these drives were invited immediately after recruitment to provide their emails to participate in an anonymous survey. Registrants were told that the survey objective was to explore registrant experience at the stem cell drive and that they would receive a $10 gift card after completion of the survey for their time. Surveys were administered via SurveyMonkey (www.surveymonkey.com) immediately following the drive. In total, two reminder emails were sent to registrants who did not complete the survey. Surveys asked registrants to provide or withdraw consent to be included in the analysis. After survey completion, participants were directed to a second, nonanonymous survey asking for an email address to which the $10 honorarium would be sent.

Participating registrants were asked to report their sex and ethnicity. The Simmons Ambivalence Scale (SAS) was employed to assess registrant ambivalence at the time of registration. This seven-item scale has been used to assess ambivalence in previous studies of marrow donors [18, 27]. We dichotomized the responses for each item to reflect whether participants expressed any ambivalence (score = 1) or no ambivalence (score = 0). We employed a 14-question, true or false, informed consent quiz which we previously designed [22] to assess registrants' knowledge according to World Marrow Donor Association-suggested procedures for informed consent at the time of registration [28]. Registrants also were asked whether they had any unanswered questions after the drive, and they rated their knowledge of stem cell donation before and after the drive and their perception of recruiter knowledge. These metrics of registrant experience have been shown previously to impact on ambivalence [18].

Mean informed consent quiz score, SAS score and markers of registrant experience were compared between the participants who were or were not shown the stem cell collection diagrams at the drive. Categorical variables were compared using the chi-square or Fisher exact test. Mean informed consent and SAS scores between groups were compared using two-tailed t-tests. All statistical analyses were performed using SPSS v.20.0 (IBM Corp., Armonk, NY, USA).

## Results

### Donor recruitment outcomes before and after implementation of the diagrams

In the 18 months prior to implementation of the stem cell collection procedure diagrams (01/2015–06/2016), stem cell drives spearheaded by Stem Cell Club recruited 2148 stem cell donors at 71 stem cell drives; 55% ($n$ = 1190) were male and 59% ($n$ = 1265) were non-Caucasian (Table 1). Forty-one donors were requested for verification typing, of whom 41·4% were unavailable and none went on to donate stem cells. In the 18 months following implementation (07/2016–12/2017), 4601 donors were recruited at 89 stem cell drives; 49% ($n$ = 2238) were male and 56% ($n$ = 2578) were non-Caucasian. Fifty-six donors were requested for verification typing, of whom 30·3% were unavailable and 7 went on to match to a patient and donate stem cells (Table 1).

### Recruiter perspective on stem cell collection diagrams

Seventy-six Stem Cell Club donor recruiters based in 17 cities in 5 provinces across Canada participated in the online survey conducted six months following implementation of the diagrams at drives. Participants reported prior experience at a median of 3 stem cell drives (range 1–15). Most agreed or strongly agreed that: the diagrams were available for recruiters to use at every drive (99%); every registrant was shown the diagrams (91%); and the diagrams made it easier to explain the stem cell donation process (96%) and supported efforts to recruit ethnically diverse donors (85%) (Fig. 2A).

### Impact of diagrams on registrant knowledge and attitudes towards donation

Five-hundred and one registrants (34% non-Caucasian males) were recruited at the stem cell drives conducted as part of the quality improvement project, of whom 293 (24·7% non-Caucasian males) participated in the online survey following the drive, reflecting a 58·5% participation rate. Sixty-seven per cent ($n$ = 197) of survey participants reported being shown the stem cell collection procedure diagram, and 33% ($n$ = 96) reported they were not shown the diagram. There was no significant difference in the proportion of non-Caucasian males in the groups who were or were not shown the diagrams (25·3% vs. 24·5%, $P$ = 0·886). Of those registrants who were shown the diagrams ($n$ = 197), most (91–93%) agreed or

strongly agreed that they helped them understand the respective collection procedures (Fig. 2B). Participants who were shown the diagrams were more likely to agree or strongly agree that the recruiters appeared knowledgeable compared to participants not shown the diagram (89% vs. 76%, $P = 0{\cdot}019$, Fig. 3A).

**Table 1** Stem Cell Club donor recruitment outcomes

| | **January 2015 – June 2016 (Prior to implementation of stem cell collection procedure diagrams)** | **July 2016 – December 2017 (Following implementation of stem cell collection procedure diagrams)** |
|---|---|---|
| Number of stem cell drives run by Stem Cell Club | 71 | 89 |
| Total donors recruited by Stem Cell Club | 2148 | 4601 |
| Total males recruited by Stem Cell Club[1] | 1190 (55%) | 2238 (49%) |
| Total non-Caucasian people recruited by Stem Cell Club[1] | 1265 (59%) | 2578 (56%) |
| Verification Typing (VT) Requests[2] | 41 | 56 |
| % Donors unavailable[3] | 41·4% | 30·3% |
| Number of donors who matched to patient and donated stem cells | 0 | 7 |

[1]Per cent shown is per cent of total donors recruited during the time horizon.
[2]Number if VT requests for donors recruited during the time horizon, as of April 2020.
[3]Number of donors who lost interest in donation or were not contactable or locatable divided by total VT requests.

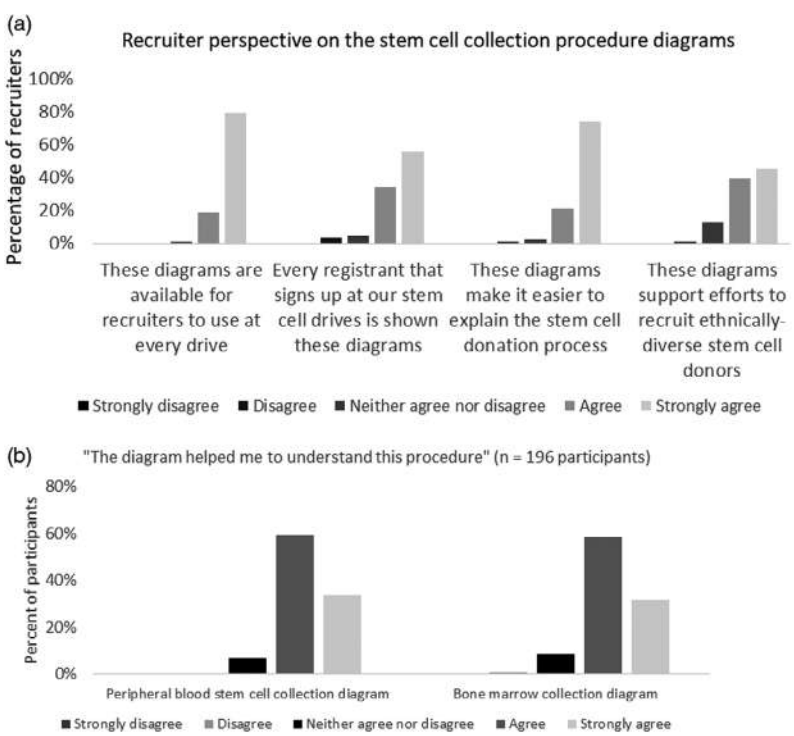


**Fig. 2** Recruiter (Figure 2A) and registrant (Figure 2B) perspective on the stem cell collection procedure diagram. Figure 2A: Results from an online survey of Stem Cell Club donor recruiters conducted six-month post-implementation of the diagrams at stem cell drives ($n = 76$ participants from 17 cities in 5 provinces across Canada. Figure 2B: Results from an online survey of newly recruited stem cell donors who were shown the stem cell collection procedure diagrams at the stem cell drive ($n = 197$ participants; 24·5% non-Caucasian males). Participants were recruited at one of five stem cell drives run by Stem Cell Club chapters in Ontario or British Columbia from 04/2017 to 03/2018. Surveys were completed within two weeks of recruitment as donors.

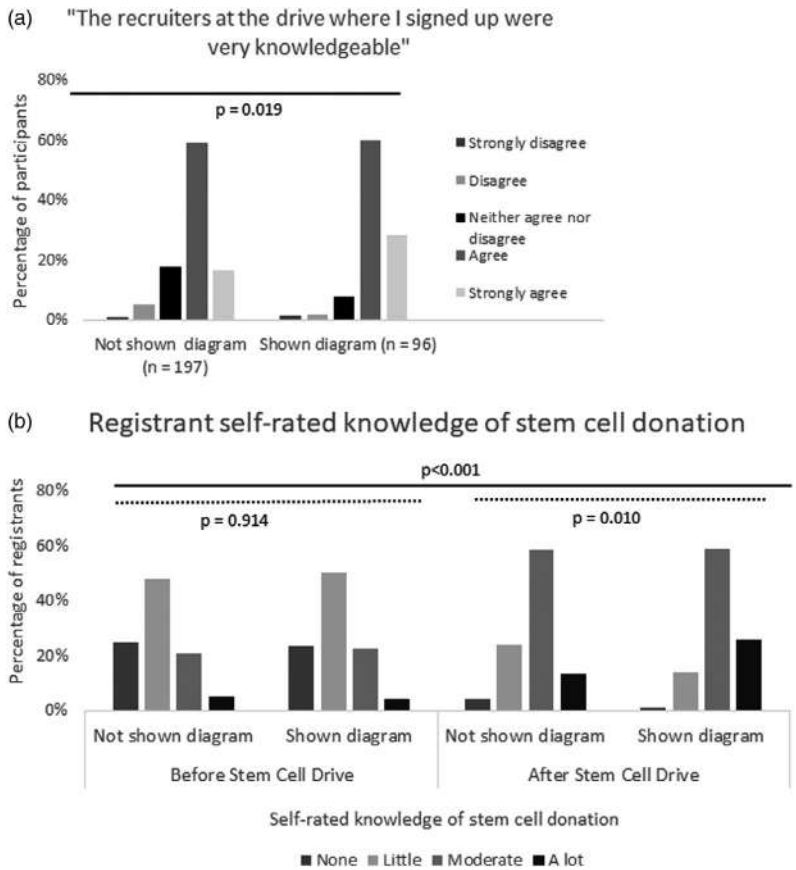


**Fig. 3** Impact of stem cell collection procedure diagrams on registrant perception of recruiter knowledge (Figure 3A) and self-reported knowledge of stem cell donation prior to and following the stem cell drive (Figure 3B). A quality improvement project was conducted at a series of five stem cell drives run by Stem Cell Club chapters in Ontario or British Columbia from 04/2017 to 03/2018. At these drives, four recruiters carried and used the stem cell collection procedure diagrams while securing informed consent, and the remainder ($n$ = 1–4) secured informed consent without these diagrams. Two-hundred ninety-three registrants participated in the survey, reflecting a 58% participation rate. Answers are stratified according to whether participants were shown the stem cell collection procedure diagrams ($n$ = 197) or not ($n$ = 96). Figure 3A: Participants who were shown the diagrams were significantly more likely to agree or strongly agree that recruiters at the drive were knowledgeable, compared to participants who were not shown the diagrams ($P$ = 0·019 by Fisher's exact test). Figure 3B: Participants' self-reported knowledge of stem cell donation was greater following the stem cell drive compared to prior to the drive ($P$ < 0·001 by chi-squared test). There was no significant different in self-reported knowledge of stem cell donation prior to the drive ($P$ = 0·914 by Fisher's exact test); however, self-reported knowledge following the drive was significantly increased in those who were shown the diagrams ($P$ = 0·010 by Fisher's exact test).

Overall, participants' self-reported knowledge of stem cell donation significantly improved following the drive compared to prior to the drive (26% reporting a moderate amount or a lot of knowledge about stem cell donation prior to the drive vs 80% following the drive, $P$ < 0·001) (Fig. 3B). There was no significant difference in self-reported knowledge prior to the stem cell drive in the group who was shown the diagrams compared to the group who was not ($P$ = 0·914). However, participants self-reported knowledge following the stem cell drive was greater in the group who reported seeing the diagrams compared to those who did not (85% vs. 74% reporting moderate or a lot of knowledge about stem cell donation following the drive, $P$ = 0·010). There were no significant differences between groups in mean scores on the 14 question informed consent quiz (Table 2) or the seven question Simmons Ambivalence Scale (Table 3).

## Discussion

We report the development stem cell collection procedure diagrams which feature a range of ethnically diverse young adult male donors donating peripheral blood stem cells or bone marrow, and their implementation at stem cell drives run by the Canadian donor recruitment organization Stem Cell Club. To our knowledge, these are the

**Table 2** Registrant knowledge about donation

| Informed consent quiz question | Correct answer | Percentage correct | |
|---|---|---|---|
| | | Not shown diagram ($n$ = 96) | Shown diagram ($n$ = 196) |
| Everyone can register to donate stem cells | False | 57·29% | 64·97% |
| Stem cell donors can direct their donations to a patient of their choice | False | 83·16% | 82·14% |
| Swabs collected at stem cell drives are stored for future additional testing | True | 91·58% | 89·80% |
| Everyone who registers as a donor will have the opportunity to donate stem cells | False | 75·79% | 72·96% |
| All stem cell donations involve surgery under anaesthetic | False | 93·68% | 91·33% |
| Donors could be saving the life of a patient who could be in any country, anywhere in the world | True | 78·95% | 79·08% |
| Donors are informed of the identity of the person who will receive their stem cells before the donation | False | 67·37% | 69·90% |
| Once committed to save a patient's life, donors are not allowed to withdraw from the programme | False | 92·63% | 94·39% |
| Donors who have blood-born and transmissible diseases (such as HIV or Hepatitis) are allowed to donate stem cells | False | 92·63% | 92·86% |
| Potential donors need to inform Canadian Blood Services when their contact information changes | True | 87·37% | 92·35% |
| Donors are paid in exchange for their stem cells | False | 95·79% | 96·43% |
| Common side effects of stem cell donation include pain, nausea, fatigue and difficulty sleeping | True | 73·68% | 77·04% |
| Prior to donating stem cells from blood, the donor is given injections of a growth factor to move stem cells from their bone marrow into their blood | True | 68·42% | 76·02% |
| A registrant's medical information (HLA markers, medical history) will be shared with other stem cell donor databases around the world, but their personal information (name, contact information) will be kept strictly confidential | True | 86·32% | 86·73% |
| Mean score on informed consent quiz | | 81·76%* | 83·29%* |

*$P$ = 0·717 by two-tailed $t$-test.

**Table 3** Registrant ambivalence towards donation

| Simmons ambivalence scale question | Answers demonstrating any ambivalence | Percentage expressing any ambivalence | |
|---|---|---|---|
| | | Not shown diagram ($n$ = 96) | Shown diagram ($n$ = 196) |
| How hard a decision was it for you to register as a potential stem cell donor? | Moderate, Hard | 28·42% | 31·63% |
| Did you know right away that you would do it or did you think it over? | Had to think it over | 23·15% | 37·25% |
| Many donors have doubts and worries about registering as a donor, even though they go through with it. Did you ever have doubts about registering as a potential donor | Yes | 36·84% | 48·97% |
| How would you feel if you found out that you couldn't donate for some reason? | Relieved | 0% | 5·61% |
| How strongly do you agree or disagree with the statement "I sometimes feel unsure about whether I would go through with donating."? | Agree, Strongly agree | 29·47% | 42·85% |
| How strongly do you agree or disagree with the statement "I would want the transplant patient to get stem cells from someone else instead of from me."? | Agree, Strongly agree | 9·47% | 10·71% |
| How strongly do you agree or disagree with the statement "I would really want to donate myself even if someone else could do it."? | Disagree, Strongly disagree | 18·94% | 14·79% |
| Mean simmons ambivalence score | | 1·50* | 1·92* |

*$P$ = 0·43 by two-tailed t-test.

first stem cell collection diagrams which have been evaluated and published in the literature. We show (1) recruiter perspective that the diagrams support the education of stem cell donors and the recruitment of ethnically diverse donors; (2) newly registered donors' perspective that the diagrams help them to understand the stem cell collection procedures; (3) data supporting that registrants who are shown the diagrams have significantly improved scores on key performance indicators which have previously been shown to be associated with reduced ambivalence (perception of recruiter knowledge and self-reported knowledge of stem cell donation); and (4) data supporting strong donor recruitment outcomes following implementation of these diagrams by the donor recruitment organization Stem Cell Club, including successful targeted recruitment of ethnically diverse males during a period of time in which Stem Cell Club's annual donor recruitment doubled.

Attrition remains a challenge for donor registries around the world. In Canada, almost half of all donors on the Canadian Blood Services Stem Cell Registry who have matched to a patient do not go through with donation [29]. Similar rates of attrition have been reported with Anthony Nolan of the United Kingdom and with the National Marrow Donor Program of the United States [12, 30]. Multiple studies have shown that donor attrition is considerably higher in ethnic minorities [12, 20, 30]. In our study, a better understanding facilitated by the use of stem cell collection diagrams may have contributed to the observed decreased proportion of donors unavailable at VT after implementation compared to prior (30·3% vs. 41·4% respectively; Table 1). These diagrams could therefore be implemented as part of a multi-pronged effort to improve donor awareness and commitment, including in ethnic minority groups.

Our work has several limitations: first, recruiters self-selected to participate in the survey administered six-month post-implementation of the diagrams; those with negative feedback may have been less likely to participate. Second, the quality improvement project surveys of newly registered donors included 293 participants recruited at five stem cell drives at which 501 donors were recruited, reflecting a 58·5% participation rate. This incomplete participation could have introduced bias into the survey; however, it is reassuring that the proportion of non-Caucasian males is similar in the overall sample and in the survey participants. Registrants also self-selected to participate in this survey; however, the use of a survey incentive may have mitigated potential bias. Third, the quality improvement project had a number of potential confounders, including lack of randomization of registrants who were approached by the recruiters. Further, the surveys were completed within two weeks following the drive and relied on registrants' recall of whether or not they had been shown the diagrams.

In addition to their use at time of recruitment of unrelated donors, these diagrams could be employed at a later stage in the donation process. The World Marrow Donor Association (WMDA) standard 3·11 states that valid informed consent must be secured from volunteer donors at time of workup. The diagrams could also be included as part of donor retention efforts and adapted to counsel matched sibling or haploidentical donors. They are relevant to donor registries, recruitment organizations and transplant centres around the world.

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## Conflicts of interest

The authors have no conflicts of interest to report. DA is a paid medical consultant with Canadian Blood Services (CBS). MG, JW, DM, TP and HE are employed by CBS. WF receives grant funding from CBS.

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LETTER TO THE EDITOR

# The protective effect of O blood type against SARS-CoV-2 infection

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ABO antigens, which are ubiquitously expressed on the surface of human cells and tissues, have been implied in a wide array of diseases, first of all cardiovascular disorders [1,2]. Recent evidence has suggested a relationship between the ABO blood group and the susceptibility of developing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [3,4]. In particular, it has been hypothesized that individuals belonging to O blood type are less susceptible to SARS-CoV-2 infection than those belonging to non-O blood groups [4]. The reason for this phenomenon could reside in the presence in O blood group subjects of IgG anti-A isoagglutinins which would prevent the binding of SARS-CoV-2 to its receptor thereby inhibiting the virus entry into the targeted human cells [5]. To verify the protective effect of O blood type against SARS-CoV-2 infection, we have compared the ABO blood group distribution of all donors of convalescent plasma (CP) with that of healthy uninfected periodic volunteer blood donors. During the period between 25 March 2020 and 22 June 2020, 447 consecutive CP donors were enrolled at the University Hospital of Pavia and the City Hospital of Mantova, Lombardy region, Italy. All CP donors were of Caucasian origin and were recovered (clinical resolution of symptoms from at least 14 days with two consecutive negative SARS-CoV-2 RT-PCR nasal swabs) from coronavirus disease 2019 (COVID-19). These subjects were compared with an historical series of 16 911 healthy blood donors from the same geographical area (Table 1, upper section). The two analytical population were comparable in terms of age, whereas as expected an extra representation of males was observed in CP donors. Indeed, in accordance with the Italian transfusion law for the prevention of transfusion-related acute lung injury (TRALI), only nulliparous women can donate CP. The prevalence of O blood type in CP donors was significantly lower than that observed in healthy blood donors of Mantua and Pavia (Table 1). According to these data, the relative risk (and 95% confidence interval) of having experienced SARS-CoV-2 infection in O blood type subjects is estimated as 0·74 (0·6–0·90), thus suggesting the protective role of O blood type towards SARS-CoV-2 contraction. When considering in CP donors the clinical course of the disease, O blood type did not appear as a modulator of the severity of COVID-19 (Table 1 bottom section). Interestingly, considering O and B blood groups together and comparing them with the other blood types, the O blood type-related protective effect disappeared (Table 1). This finding could be in contrast with the hypothesized anti-SARS-CoV-2 activity of anti-A isoagglutinins, which are

**Table 1** Comparison of characteristics of observed samples.

| Characteristics | CP donors | Healthy blood donors | *P value*[b] |
|---|---|---|---|
| Number | 447 | 16 911 | |
| Mean age (y, ±SD) | 47·7±12·1 | 47·1 ±14·3 | 0·41 |
| N Males/Females | 385/62 | 10 321/6590 | |
| %Males | 86·1 | 61·0 | <0·0001 |
| ABO blood group | | | |
| O | 162 (36·2%) | 7,375 (43·6%) | 0·002[c,d] |
| Non-O | 285 (63·8%) | 9,536 (56·4%) | |
| A | 207 (46·3%) | 7,209 (42·6%) | |
| B | 54 (12·1%) | 1,620 (9·6%) | |
| AB | 24 (5·4%) | 707 (4·2%) | |

| | O blood type | Non-O blood type | *P value*[c] |
|---|---|---|---|
| O vs. non-O blood type comparisons in CP donors | | | |
| Asymptomatic COVID-19[a] | 5/162 (3·1%) | 6/285 (2·1%) | 0·51 |
| Non-severe COVID-19[a] | 145/162 (89·5%) | 259/285 (90·8%) | 0·63 |
| Severe COVID-19[a] | 12/162 (7·4%) | 20/285 (7·1%) | 0·87 |

CP, convalescent plasma; SD, standard deviation; y, years.
[a]Patients with SARS-CoV-2 pneumonia requiring hospitalization with mechanical (invasive or not invasive) respiratory support. All the other cases were classified as non-severe.
[b]CP donors vs. healthy blood donors.
[c]O blood type versus non-O blood type.
[d]O and B blood types versus other blood types: $P = 0·06$.

present also in B group individuals. However, the neutralizing effect could be a characteristic of anti-A isoagglutinins belonging to IgG class (predominant in O blood type individuals) and not of those belonging to IgM class (predominant in B blood group subjects).

In conclusion, we documented for the first time the association between ABO blood type and COVID-19 in a homogeneous population of CP donors recovered from SARS-CoV-2 infection, having O blood type subjects a reduced predisposition to become infected.

## Conflict of interest

The authors declare no conflict of interests.

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LETTER TO THE EDITOR

# Lack of association between ABO blood groups and susceptibility to SARS-CoV-2 infection

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Studies in China, the USA and Turkey, summarized by Focosi [1], described an association between risk for SARS-CoV-2 infection and ABO blood groups, with the results converging to a higher rate of infection among type A and lower among type O subjects. These data are reinforced by an elegant study employing genome-wide association, which identified a region on chromosome 9 corresponding to the ABO locus, with frequency misbalance between severe COVID-19 patients and healthy blood donors from Italy and Spain [2].

The biological mechanism behind the increased susceptibility of type A and resistance by type O individuals to SARS-CoV-2 infection has been related to anti-A isohaemagglutinin, which may prevent the binding of the viral spike protein to the cellular receptor ACE2 [3].

We sought to evaluate the relationship between blood group types and SARS-CoV-2 laboratory results from our database. Up to 22 June 2020, 196 897 and 256 471 real-time PCR (RT-PCR) and serological tests respectively were performed at Dasa, the largest clinical pathology laboratory in Brazil.

Among patients submitted to SARS-CoV-2 testing, we identified 6457 who had a concomitant ABO blood group typing result, being 4353 tested by RT-PCR and 2275 for COVID-19 antibodies, while 171 did both (Table 1). Most patients submitted to RT-PCR were hospitalized, as testing due to reagent shortage was prioritized to them, while serology did not require a prescription and was performed by asymptomatic individuals and patients presenting a broader range of clinical manifestations. However, results did not differ when analysing in separate RT-PCR and serology patients (data not shown). We also retrieved historical ABO blood group typing from our records, representing 1 813 237 patients. The program 'R' (R Foundation for Statistical Computing, Vienna, Austria) was used to perform Pearson's chi-squared test.

Our data failed to reproduce the skewed frequency of ABO blood group types reported by the studies above. Though we do find a trend for a higher frequency of A and lower of O type comparing SARS-CoV-2 patients with the general population attended by the laboratory, these differences are quite small and did not reach statistical significance. Essentially, 30% of the patients in our cohort had one or more laboratory markers indicating previous or current SARS-CoV-2 infection, irrespective of the blood group (Table 2). The absence of a relationship between ABO blood type and susceptibility to SARS-CoV-2 infection was also reported in another US study [4], which suggested that ethnicity may have biased previous analysis. Noticeably, a higher frequency of type A was found among COVID-19 patients in non-Hispanic White subjects but not in Black or Hispanic subjects [5], who are certainly more related to the genetic background of Brazilians. In conclusion, ABO blood group types do not seem to significantly impact on the risk for SARS-CoV-2 infection among a representative population from Brazil.

**Table 1** Frequency of ABO blood group types in the general patient's population, SARS-CoV-2 testing patients and SARS-CoV-2-reactive patients

| | Patients in general[#] | | SARS-CoV-2 suspects | | SARS-CoV-2 positives*,[#] | |
|---|---|---|---|---|---|---|
| Blood group | | | | | | |
| O | 843 108 | 46·5% | 3002 | 46·5% | 913 | 44·8% |
| A | 682 074 | 37·62% | 2505 | 38·8% | 816 | 40·1% |
| B | 217,970 | 12·02% | 713 | 11·0% | 237 | 11·6% |
| AB | 70 085 | 3·87% | 237 | 3·7% | 71 | 3·5% |
| Total | 1 813 237 | 100% | 6457 | 100% | 2037 | 100% |

*Either by RT-PCR ($n$ = 1589), serology ($n$ = 442) or both ($n$ = 6).
[#]Chi-square for patients in general x SARS-CoV-2-positive patient comparison, $P$-value = 0·1405.

**Table 2** SARS-CoV-2 reactivity (serology and/or qPCR) according to ABO blood group

| Blood group | SARS-CoV-2+ | SARS-CoV-2- | Total | % COVID+ |
|---|---|---|---|---|
| O[a] | 913 | 2089 | 3002 | 0·30 |
| A[a] | 816 | 1689 | 2505 | 0·32 |
| B | 237 | 476 | 713 | 0·33 |
| AB | 71 | 166 | 237 | 0·29 |
| TOTAL | 2037 | 4420 | 6457 | |

[a]Chi-square for A x O blood group comparison, $P$-value = 0·085.

## Conflict of interests

The authors declare no conflict of interests.

## Funding

None.

## Data availability statement

Data sharing requests should be sent to José Eduardo Levi (jose.levi.ext@dasa.com.br).

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| 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 |