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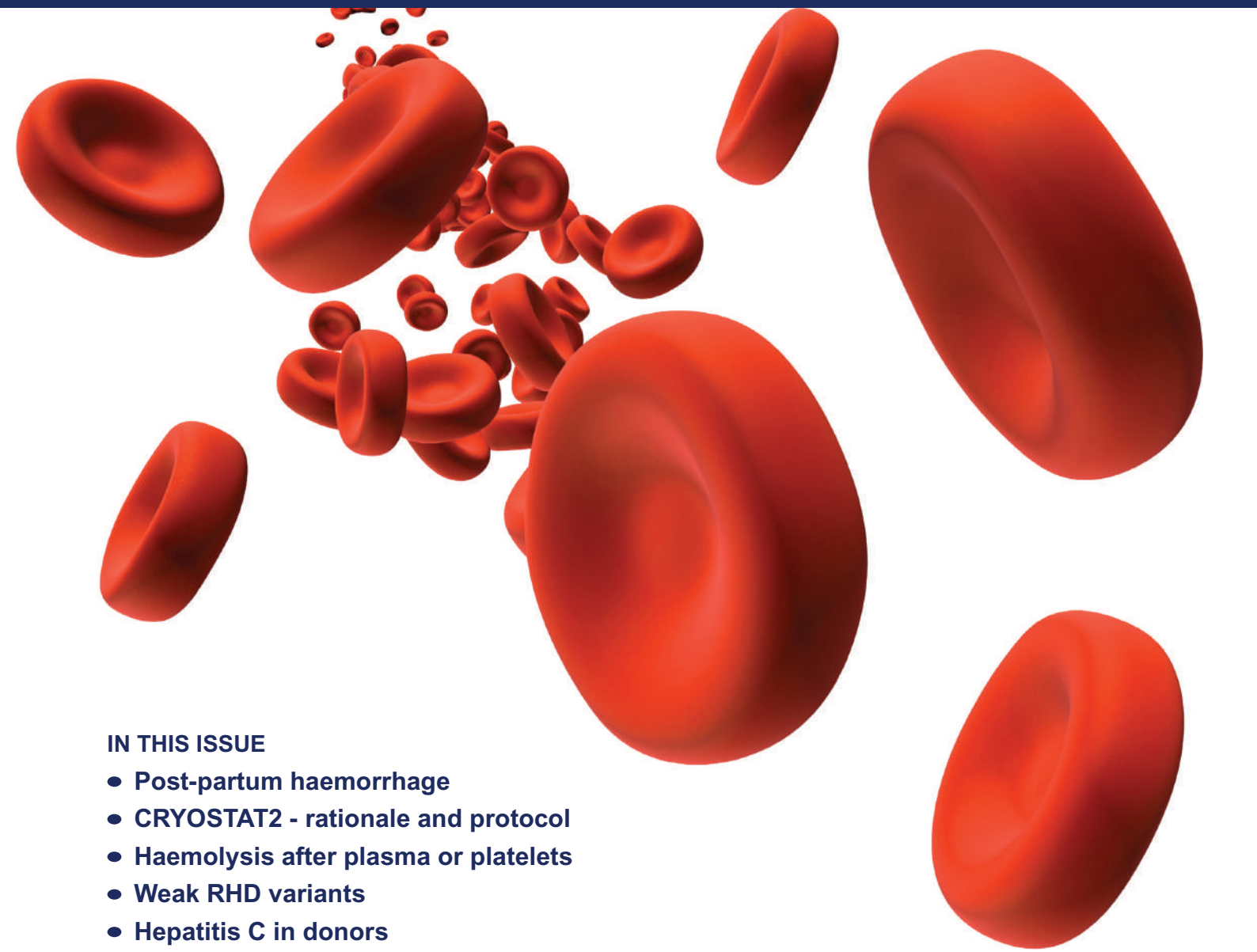

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REVIEW

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Comparison of European recommendations about patient blood management in postpartum haemorrhage

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Abstract

Postpartum haemorrhage (PPH) is the leading cause of maternal mortality and morbidity worldwide. Some documents with practical recommendations for the management of PPH do not include the most updated directives. This review offers a quality comparison of the recommendations stated in Europe since 2015. A literature search was conducted to identify the documents published in Europe from 2015 to 2020 containing recommendations about management of PPH. The search returned 10 publications. A narrative synthesis and a summary of the information about PPH definition and its management were performed. Differences in the definition of PPH were identified: some documents considered the delivery procedure, and many publications included severity criteria. The therapeutic goal for red blood cells transfusion ranged from 6 to 9 g/dl. There were divergences in the need for considering haemostatic results before fresh frozen plasma transfusion. The therapeutic goal of platelet transfusion ranged from 50×10^9 to 100×10^9 μ/L. There was a wide consensus about the therapeutic goal of fibrinogen replacement (>2 g/L), but not about its use in an unmonitored or pre-emptive manner. Most publications included therapeutic approaches such as tranexamic acid and recombinant factor VII activated, but not prothrombin complex concentrate or coagulation factor XIII. The recommendations about PPH management offered in European documents are heterogeneous and have changed over time. The standardisation of all them could be useful to make progress in PPH clinical management and research which, in turn, could strongly impact in patient outcomes.

KEYWORDS
guidelines, hemostasis, medical, postpartum hemorrhage, pregnancy, societies

1 | INTRODUCTION

Maternal mortality consists of the death of women during pregnancy or within 42 days after delivery or end of pregnancy.[1] In 2015, a total of 275 000 cases of maternal mortality were estimated worldwide, and one third of them were due to haemorrhages. From 2000 to 2017, the incidence of the world maternal mortality was 211 women per 100 000 live births, and ranged from 11/100000 in high income countries to 460/100000 in low income countries.[2]

According to the WHO, postpartum haemorrhage (PPH) is the loss of 500 ml of blood or more within 24 h after delivery, and it is categorised as severe if the loss exceeds 1000 ml.[3] PPH can occur in the

TABLE 1 List of records considered in this analysis

Title	Institution	Type of committee	Country	Year	Type of document
Interventional Algorithms for the Control of Coagulopathic Bleeding in Surgical, Trauma, and Postpartum Settings: Recommendations from the Share Network Group[17]	SNWG	Portuguese algorithm action team (immunohemotherapy, haematology and anaesthesiology)	Portugal	2016	Recommendations document
Management of coagulopathy associated with postpartum haemorrhage: guidance from the SSC of the ISTH[10]	ISTH	Subcommittees on Women's Health Issues in Thrombosis and Haemostasis and on Disseminated Intravascular Coagulation	United Kingdom	2016	Guideline
Postpartum haemorrhage: guidelines for clinical practice from the French College of Obstetricians (CNGOF): in collaboration with the French Society of Anesthesiology and Intensive Care (SFAR)[12]	CNGOF	Steering committee (obstetricians, anaesthesiologists and midwives)	France	2016	Guideline
Prevention and management of postpartum haemorrhage[13]	RCOG	Scottish Committee of the Royal College of Obstetricians and Gynaecologists	United Kingdom	2017	Guideline
The daily-practiced post-partum haemorrhage management: an Italian multidisciplinary attended protocol[14]	IMG	Italian multidisciplinary team of specialists adopting a modified Delphi method	Italy	2017	Protocol
Recomendações Portuguesas para a Abordagem Multidisciplinar da Hemorragia Obstétrica[15]	MCG	Multimodal group (obstetrics, anaesthesiology, immunohemotherapy and haematology) with Portuguese Society of Anaesthesiology	Portugal	2018	Recommendations document
Peripartum Haemorrhage: Haemostatic Aspects of the New German Guideline[18]	ASCS	DACH group with German Society of Anaesthesiology and Intensive Care Medicine and Society of Thrombosis and Haemostasis Research	Germany	2018	Guideline
Guideline on the Management of Postpartum Haemorrhage, HSE Home Birth Service[16]	HSE	Sub-group for the Clinical Governance Group for the HSE Home Birth Service	Ireland	2018	Guideline
Patient blood management in obstetrics: prevention and treatment of postpartum haemorrhage. A NATA consensus statement[1]	NATA	Multidisciplinary group from NATA, FIGO, EBCOG and ESA	Europe	2019	Consensus document
Patient blood management (PBM) in pregnancy and childbirth: literature review and expert opinion[11]	PBMO	Multidisciplinary group of Swiss experts	Switzerland	2020	Consensus document

Abbreviations: ASCS, Association of the Scientific Medical Societies; CNGOF, French College of Gynaecologists and Obstetricians; DACH, Germany, Austria and Switzerland Group; EBCOG, European Board and College of Obstetrics and Gynaecology; ESA, European Society of Anaesthesiology; FIGO, Federation of Gynaecology and Obstetrics; HSEWG, Health Service Executive; IMG, Italian multidisciplinary Group; ISTH, International Society on Thrombosis and Haemostasis; MCG, Multidisciplinary Consensus Group; NATA, Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis; PBMO, Patient Blood Management Obstetrics; RCOG, Royal College of Obstetricians and Gynaecologists; SNWG, Share Network Group.

absence of evident risk factors; however, uterine atony, placenta issues, uterus or cervical trauma and blood coagulation disorders related to thrombin dysfunction have been described as potential causes of PPH.[4,5] PPH is the leading cause of maternal mortality and morbidity worldwide,[1,6] and most PPH-related deaths occur within 24–48 h from delivery.[7] Nevertheless, many cases of PPH-related deaths could be prevented with timely diagnosis and management,[6] and two out of three cases are estimated to occur due to substandard care.[8]

Patient blood management (PBM) entails a series of measures and methods to reach better outcomes for patients through the maintenance of haemoglobin concentration, the optimization of haemostasis, the minimization of blood loss (BL), and the limitation of blood transfusion.[1] Although vast benefits from implementing PBM in clinical practice have been demonstrated, some non-evidence based misconceptions that limit its global implementation still endure,[9] and they should be overcome.

In light of all this, multidisciplinary approach is increasingly applied in the management of PPH where each element of the team needs to follow the most updated recommendations.

In the European setting, some different documents have proposed practical recommendations to support the PBM in patients with PPH, in accordance with the available evidence, as well as with the knowledge and experience of experts. However, it is not clear whether these documents compile the most updated practices and recommendations. The aim of this review was to perform a quality comparison between several European recommendations since 2015, and identify important differences between them.

TABLE 2 Definition of PPH and its types

	PPH	Primary PPH	Secondary PPH	Minor PPH	Major/severe PPH
SNWG (2016)[17]	· VBL >500 ml (24 h) · CBL >1000 ml (24 h)				
ISTH (2016)[10]	ND				
CNGOF (2016)[12]				BL ≥500 ml	BL ≥1000 ml
RCOG (2017)[13]		Check minor and major PPH values	Abnormal or excessive bleeding from the birth canal between 24 h and 12 weeks postnatal	BL = 500–1000 ml (24 h)	BL ≥1000 ml · Moderate = 1001–2000 ml · Severe >2000 ml
IMG (2017)[14]				BL = 500–1000 ml hemodynamically stable (alert PPH)	BL ≥1000 ml, hemodynamically unstable
MCG (2018)[15]				· VBL ≥500 ml · CBL ≥1000 ml	VBL >1000 ml · Mild = 1000–2000 ml · Severe >2000 ml
ASCS (2018)[18]		· VBL ≥500 ml (24 h) · CBL ≥1000 ml (24 h)	· VBL ≥500 ml (24 h–12 weeks after delivery) · CBL ≥1000 ml (24 h–12 weeks after delivery)		
HSE (2018)[16]		BL ≥500 ml (24 h)	24 h-6 weeks after delivery	BL = 500–1000 ml	BL ≥1000 ml · Moderate 1000–2000 ml · Severe >2000 ml
NATA (2019)[1]		BL >500 ml (24 h)			· Severe >1000 ml (24 h) or BL accompanied by signs/symptoms of hypovolaemia · Massive life-threatening >2500 ml or hypovolemic shock
PBMO (2020)[11]	ND				

Abbreviations: ASCS, Association of the Scientific Medical Societies; BL, Blood Loss; CBL, Caesarean delivery Blood Loss; CNGOF, French College of Gynaecologists and Obstetricians; HSEWG, Health Service Executive; IMG, Italian multidisciplinary Group; ISTH, International Society on Thrombosis and Haemostasis; MCG, Multidisciplinary Consensus Group; NATA, Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis; ND, Not discussed; PBMO, Patient Blood Management Obstetrics; PPH, Post-partum Haemorrhage; RCOG, Royal College of Obstetricians and Gynaecologists; SNWG, Share Network Group VBL, Vaginal delivery Blood Loss.

2 | MATERIAL AND METHODS

A literature search was conducted to identify the documents including recommendations for the clinical management of PPH published in Europe from the 1st of January 2015 to the 31st of December 2020. The following terms were used in both PubMed and Google browsers: "postpartum haemorrhage" and "bleeding management." Only guidelines and documents offering recommendations developed by committees, consortia or working groups were selected. Only documents in either English or Portuguese were contemplated. The authors of this publication performed the search and retrieved the publications.

The literature search returned 10 records, including two consensus documents, two recommendations documents, five guidelines and one protocol (Table 1). Three documents were published in 2016, two in 2017, three in 2018, one in 2019, and one in 2020.

The selected publications were reviewed and the key information was extracted from each one. A narrative synthesis and a summary of the information found was performed. The parameters analysed were those related to PPH definition and haemostatic management: red blood cells (RBCs) transfusion, fresh frozen plasma (FFP) transfusion, platelets (PLTs) transfusion; fibrinogen (FI) replacement, prothrombin complex concentrate (PCC), coagulation factor XIII (FXIII) concentrate, massive transfusion protocol (MTP), tranexamic acid (TXA), recombinant factor VII activated (rFVIIa), cell salvage use, and other recommended PBM strategies (Table 2).

3 | RESULTS

3.1 | Postpartum haemorrhage definition

The different PPH definitions provided by the documents reviewed are synthetized in Table 2. Two publications did not discuss PPH definition.[10,11] The rest of the publications used BL for defining PPH, and some of them offered criteria for the definition of major or severe

PPH.[1,12–16] Three publications considered the delivery procedure (vaginal or caesarean) when defining PPH and its severity.[15,17,18]

Some changes have been notice over the years. Since 2016, minor PPH had been defined as an BL greater than 500 ml within the first 24 h after delivery, while major/severe PPH as greater than 1000 ml.[12] The boundaries of caesarean PPH were set at a BL greater than 1000 ml in three publications.[15,17,18]

Some documents also classified PPH as primary when the onset was occurring within the first 24 h after the delivery, and secondary if it was occurring from 24 h to 12 weeks after the delivery,[13,18] or from 24 h to 6 weeks after delivery.[16]

NATA guidelines, the most recently published guidelines including PPH definition, classified PPH in three types according to BL, its onset and other clinical signs, regardless of the mode of delivery. Primary PPH applied for BL greater than 500 ml within 24 h after delivery, irrespective to the delivery modality. Severe PPH applied for BL greater than 1000 ml within 24 h after delivery or BL accompanied by signs or symptoms of hypovolemia. Massive life-threatening PPH applied for BL greater than 2500 ml or hypovolemic shock.[1]

3.2 | Postpartum haemorrhage management

3.2.1 | Red blood cells transfusion

Not every document offered recommendations about the criteria to initiate RBCs transfusion or the therapeutic goal to achieve (Table 3). The latest recommendations pointed to more restrictive strategies in terms of haemoglobin therapeutic goals: values of 8 g/dl by the CNGOF in 2016[12]; values of 7 g/dl, by the SNWG in 2016,[17] the MCG in 2108[15] and the NATA in 2019[1]; values of 7–9 g/dl, by the ASCS in 2018[18]; and, finally, values of 6 g/dl were recommended by the PBMO in 2020.[11] This last document stated that the performance of RBCs transfusion should depend on the patient's symptoms when haemoglobin is between 6 and 8 g/dl.

3.2.2 | Plasma transfusion

FFP transfusion was contemplated for correcting coagulopathy in most of the documents revised in this publication (Table 3). The criteria to transfuse FFP was that activated partial thromboplastin time (aPTT), prothrombin time (PT) or the International Normalised Ratio (INR) should be 1.5 times the normal value or higher in some publications.[1,10,13,15] Recommended doses ranged from 10[15] to 30 ml/kg.[14] Some publications stated that FFP should be given if coagulopathy is suspected, even when haemostatic results are not available, and that the amount of FFP should be calculated according to the number of RBCs bags infused.[10,12,13,15,19] The latest recommendations suggested the use of FFP not only for coagulopathy correction but also for volume resuscitation, in situations of severe hypovolemia and concomitant coagulopathy.[11]

3.2.3 | Platelets transfusion

There is no consensus regarding the therapeutic goal of PLTs transfusion (Table 3). A therapeutic goal of 50 x 10^9 μ/L was recommended by CNGOF and HSE.[12,16] A goal of 75 × 10^9 μ/L was considered by SNWG and ISTH in 2016[10,17]; by RCOG in 2017[13]; by MCG in 2018[15]; and by NATA in 2019.[1] Only ASCS recommended a therapeutic goal of 100 x 10^9 μ/L, in 2018.[18] IMG did not define a therapeutic goal[14] and PBMO did not address PLTs transfusion.[11]

3.2.4 | Fibrinogen replacement

The therapeutic goal of FI replacement therapy differs from one document to another (Table 3). Most publications recommended levels above 2 g/L or a value of FIBTEM A5 > 12 mm.[1,10,12–14,18] Exceptionally, Portuguese groups recommended to reach 3 g/L or more, or a value of $FIBTEM_{MCF}$ ≥ 18 mm.[15,17]

Some documents coincided with performing the replacement with 25–60 mg/kg of FI concentrate,[14,15,17,18] and IMG also contemplated the use of cryoprecipitate as second-line treatment (1 unit/10 kg of body weight).[14] On the contrary, RCOG recommended FI replacement exclusively with cryoprecipitate.[13]

IST and PBMO recommended against the use of FI concentrate in an unmonitored or preventive manner,[10,11] while SNWG and MCG recommended it in the presence of a BL of 1.5 L or higher.[15,17]

3.2.5 | Prothrombin complex concentrate

The use of PCC in the management of PPH is not a current widespread practice and, consequently, more than half of the reviewed publications did not discuss it (Table 3). Only SNWG, MCG and ASCS contemplated this therapeutic approach. Portuguese groups suggested the use of PCC to exclusively correct the coagulation time when BL volume is 150%–200% or higher, when aPTT and PT are higher than 1.5 times the normal value or when clotting time is prolonged; the recommended dose of PCC was 20–30 IU/kg.[15,17] ASCS guidelines recommended a dose of 25 IU/kg without stating specific criteria.[18] Both ISTH and NATA recommended against the use of PCC in the management of PPH because of its safety concerns and the lack of evidence to support its efficacy.[1,10]

3.2.6 | Coagulation factor XIII concentrate

The majority of the publications in study did not contemplate the use of FXIII (Table 3). However, SNWG recommended its use at a dose of 30 IU/kg in a situation of low clot strength evidence, even with adequate levels of FI,[17] and ASCS guidelines mentioned its use at a dose of 15–20 IU/kg if needed, but did not specified any clinical criteria.[18]

TABLE 3 Recommendations regarding PPH haemostatic control

	SNWG (2016)[17]	ISTH (2016)[10]	CNGOF (2016)[12]	RCOG (2017)[13]	IMG (2017)[14]	MCG (2018)[15]	ASCS (2018)[18]	HSE (2018)[16]	NATA (2019)[1]	PBMO (2020)[11]
RBCs	Hb TG >7 g/dl	ND	Hb TG >8 g/dL Based on clinical signs of PPH severity without waiting for lab results	No firm criteria for initiation Decision based on both clinical and haematological assessment	If haematocrit ≤21%: 4 bags of packed red blood cells blindly	Hb TG >7 g/dl	Hb TG = 7–9 g/dl	NSR	Hb TG >7 g/dl (except massively bleeding patients with symptoms of anaemia)	Hb TG ≥6 g/dl Consider clinical situation and symptoms if 6–8 g/dl
FFP	12–15 ml/kg initially, up to 20 ml/kg, to correct coagulation time	15 ml/kg if the PT/aPTT >1.5× normal Against using FFP before haemostatic tests are available until 4 RBCs have been infused.	Give FFP without waiting for lab results	12–15 ml/kg If PTT or INR >1.5× normal If no haemostatic results are available If suspected coagulopathy	20–30 ml/kg If PTT or INR >1.5× normal	10–5 ml/kg to correct coagulopathy without waiting for lab results or to correct coagulation time (aPTT and PT >1.5 × normal)	20 ml/kg to correct coagulopathy	4 U/every 4–6 units of RBCs transfused	15–20 ml/kg If aPTT and PT >1.5× normal/ EXTEM CT prolonged If lab results are late after administration of 4 RBCs, at least in a 1FFP:2RBCs ratio	For volume resuscitation in situations with severe hypovolemia and concomitant coagulopathy. Timely organisation of FFP is recommended
PLTs	PLTs TG ≥ 75 X 10^9/L	PLTs TG ≥ 75 X 10^9/L Based on laboratory monitoring and against 1:1:1 RBCs:FFP:PLTs ratios. Consider if the PLTs count is unknown after 8 RBCs.	PLTs TG > 50 X 10^9/L	PLTs TG >75 X 10^9/L	No PLTs TG 1 U for every 8 bags of RBCs	PLTs TG >75 X 10^9/L	PLTs TG ≥ 100 X 10^9/L	PLTs TG > 50 X 10^9/L	PLTs TG >75 X 10^9/L Standard dose of platelets: 5–10 ml/kg	ND
FI	TG ≥3–4 g/L ($FIBTEM_{MCF}$ ≥ 18 mm) Initial dose CF: 30–60 mg/kg	TG ≥2 g/L Against the use in an unmonitored or pre-emptive manner	TG ≥2 g/L	TG >2 g/L Cryoprecipitate should be used for FI replacement	TG >2 g/L Early supplementation with CF 30–50 mg/kg or cryoprecipitate (1 unit per 10 kg)	TG >3 g/L ($FIBTEM_{MCF}$ > 18 mm) If ongoing bleeding (>1.5 L), CF:25–50 mg/kg	TG ≥2 g/L (FIBTEM A5 > 12 mm) Administration of CF 30–60 mg/kg	ND	TG ≥2 g/L (FIBTEM A5 > 12 mm)	FI should be given in severe PPH, according to POC tests results
PCC	To correct coagulation time if: BL ≥150%–200% volemia aPTT/PT >1.5× normal/EXTEM CT prolonged PCC: 20–30 U/kg	Against the use outside of clinical trials	ND	ND	ND	To correct coagulation time if: BL ≥150–200% volemia aPTT and PT >1.5× normal/EXTEM CT prolonged PCC: 20–30 U/kg	1000–2500 IU (25 IU/kg BW) if needed	ND	Against the use outside of clinical trials	ND
FXIII	30 U/kg if available and if low clot strength (FXIII activity <60%)	ND	ND	ND	ND	ND	15–20 IU/kg if needed	ND	ND	ND
TXA	20–25 mg/kg if clinical signs of coagulopathy	1 g if PPH ongoing	1 g and repeat if required if PPH is secondary to refractory uterine atony Against the use to prevent PPH during CD	Should be given	30 mg/kg Early use	· VBL: 20–25 mg/kg or 1 g (10 min) · CBL: 0,5–1 mg before CF	15–30 mg/kg if clinical signs of fibrinolytic Activity. Repeat if required, before CF	ND	1 g ASAP within the first 3 h after PPH onset. Against the routine use for PPH prevention	Recommended as the first line of treatment for women with increased BL during birth.
rFVIIa	90–120 μg/kg if everything fails	60 μg/kg if ongoing PPH unresponsive to standard treatment or to prevent hysterectomy (CF >2 g/L and PLTs >50×10^9/L)	If everything fails.	Against the routine use in the management of major PPH unless as part of a clinical trial	60–90 μg/kg (may be repeated within 15–30 min) if unresponsive ongoing PPH	90-120 μg/kg as last-line therapy after all other parameters optimised	90 μg/kg if everything fails	ND	If everything fails	If life-threatening PPH

(Continues)

TABLE 3 (Continued)

	SNWG (2016)[17]	ISTH (2016)[10]	CNGOF (2016)[12]	RCOG (2017)[13]	IMG (2017)[14]	MCG (2018)[15]	ASCS (2018)[18]	HSE (2018)[16]	NATA (2019)[1]	PBMO (2020)[11]
MTP	ND	A quality control protocol should be agreed with the haematology department	ND	Full protocol for monitoring and investigation in major PPH (BL greater than 1000 ml) and ongoing haemorrhage or clinical shock	If critical haemorrhage with signs of hemodynamic instability and hypoperfusion	Protocol if life-threatening PPH	Essential for hospitals with obstetric department to develop MTP	ND	If BL >2.5 L, and in the presence of massive PPH	ND
Cell Salvage	ND	ND	ND	For emergency use in PPH associated with CD and with ED	ND	Usage of intra-operative cell salvage	Usage of intra-operative cell salvage in elective CD	ND	Usage of intraoperative cell salvage (with leucocyte-depletion filters in the infusion line)	In individual situations with anticipated high BL. Jehovah's witnesses Against the use as a routine procedure for CD

Abbreviations: aPTT, Activated Partial Thromboplastin Time; ASCS, Association of the Scientific Medical Societies; BL, Blood Loss; CBL, Caesarean delivery Blood Loss; CD, Caesarean delivery; CNGOF, French College of Gynaecologists and Obstetricians; EXTEM CT, Clotting time measured by EXTEM® test; FFP, Fresh Frozen Plasma; FI, Fibrinogen; $FIBTEM_{MCF}$, Maximum Clot Firmness measured by FIBTEM® test; FIBTEM A5, Clot amplitude at 5 min measured by FIBTEM® test; FXIII, Coagulation Factor XIII; Hb, Haemoglobin; HSEWG, Health Service Executive; IMG, Italian multidisciplinary Group; INR, International Normalized Ratio; ISTH, International Society on Thrombosis and Haemostasis; MCG, Multidisciplinary Consensus Group; NATA, Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis; MTP, Massive transfusion protocol; ND, Not discussed; NSR, No specific recommendations; PBM, Patient Blood Management; PBMO, Patient Blood Management Obstetrics; PCC, Prothrombin Complex Concentrate; PLTs, Platelets; POC, Point-of-care; PPH, Post-partum Haemorrhage; PT, Prothrombin Time; RBCs, Red Blood Cells; RCOG, Royal College of Obstetricians and Gynaecologists; rFVIIa, Recombinant activator factor VII; SNWG, Share Network Group; TXA, Tranexamic acid VBL, Vaginal delivery Blood Loss; VD, Vaginal delivery.

3.2.7 | Tranexamic acid

The use of TXA during PPH was addressed in all the publications but HSE guidelines (Table 3). IMG, MCG, NATA and PBMO recommended the administration of TXA as soon as possible after PPH onset.[1,14,15] MCG emphasised the importance of giving it before administering clotting factors, and recommended different doses for vaginal and caesarean delivery.[15] Proposed therapeutic doses ranged from 15 to 30 mg/kg (1–2 g).[1,10,12,14,15,17,18]

3.2.8 | Recombinant factor VII activated

All the documents recommended the use of rFVIIa in sight of a failure of all the other management options and as a strategy for a life-threatening situation. The recommended doses ranged from 60[14] to 120 μg/kg.[15,17] RCOG recommended against the routine use of rFVIIa in the management of major PPH unless as part of a clinical trial.[13]

3.2.9 | Massive transfusion protocol

The importance of having a MTP came to the surface in 2016 (Table 3). ISTH, RCOG, IMG, MCG, ASCS and NATA highlighted the importance of having a quality MTP for patients with life-threatening PPH. RCOG mentioned it for patients with BL greater than 1000 ml[13]; IMG, for patients with critical haemorrhage and signs of hemodynamic instability and hypoperfusion[14]; and NATA considered it in the presence of massive PPH.[1]

3.2.10 | Cell salvage

The use of cell salvage is recommended in some of the newest publications included in this review (Table 3), mostly in individual intraoperative emergency situations.[1,11,13,15,18] NATA recommended its use with leucocyte-depletion filters in the infusion line.[9] PBMO recently stated that it is not advised as a routine procedure for caesarean delivery,[11] while ASCS mentioned its use in elective caesarean delivery in 2018.[18] Other specific contemplated situations are a high risk of PPH, unavailability of packed RBCs,[1] situations with anticipated high BL, or Jehovah's witnesses.[11]

3.2.11 | Other patient blood management strategies

Some PBM strategies for patients with PPH other than those summarised above were addressed in the revised publications (Table 4). The majority referred to the importance of point-of-care (POC) tests (or laboratory monitoring available within 30 min).[1,10,12–15,17,18] CNGOF, MCG, NATA and PBMO emphasised the need for preventing severe antenatal anaemia[1,11,12,15]; RCOG, ASCS and HSE highlighted

TABLE 4 Other recommended PBM strategies

	POC tests	Management algorithm/lab monitoring	Multidisciplinary approach	Prevention/correction of severe antenatal anaemia	Other
SNWG (2016)[17]	√				
ISTH (2016)[10]	√	√			
CNGOF (2016)[12]	Lab monitoring			With iron supplements	Maternities with blood bank Blood available within 30 min
RCOG (2017)[13]	√	√	√		
IMG (2017)[14]	√				
MCG (2018)[15]	√	√		√	ABCDE methodology Further stabilisation on ICU
ASCS (2018)[18]	√		√	√	Cross-matching Haemostatic agents available
HSE (2018)[16]			√		
NATA (2019)[1]		√		√	Prevention/Treatment of severe anaemia before and after postpartum haemorrhage
PBMO (2020)[11]				√	Prevention/reduction of perioperative/peripartum RBCs loss Optimising postoperative/postpartum treatment of anaemia, including restrictive use of RBCs

Abbreviations: ASCS, Association of the Scientific Medical Societies; CNGOF, French College of Gynaecologists and Obstetricians; HSEWG, Health Service Executive; ICU, Intensive care unit; IMG, Italian multidisciplinary Group; ISTH, International Society on Thrombosis and Haemostasis; MCG, Multidisciplinary Consensus Group; NATA, Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis; PBMO, Patient Blood Management Obstetrics; POC, Point of Care; RBCs, Red blood cells; RCOG, Royal College of Obstetricians and Gynaecologists; SNWG, Share Network Group.

the relevance of multidisciplinary teams[13,16,18]; and ISTH, RCOG, MCG and NATA included the necessity for institutional protocols.[1,10,13,15] At last but not least, MCG mentioned the ABCDE Method and the importance of the further stabilisation on ICU.[15]

4 | DISCUSSION

The general implementation of PBM reduces the risk of perioperative bleeding, morbidity and mortality, the requirement of blood transfusion, the duration of hospitalisation and haemorrhage related costs.[20–23] In obstetrics, with a PPH risk inherent to pregnancy and partum, PBM is particularly relevant. The incidence of maternal deaths is obviously higher in low-income countries due to limited resources, but it is also rising in high-income countries,[24] partially due to poor resuscitation management of patients with PPH,[25,26] atonic bleeding rise,[27] use of oxytocin for induction or augmentation of labour,[28] and caesarean surgeries "by choice."[29]

PBM is probably more challenging in obstetrics than in other specialties, in part due to the lack of large well-designed clinical studies in the field, the still valid but wrong consideration of blood transfusions as the first line treatment to correct low haemoglobin values, and the hemodynamic changes that women experience during pregnancy. Many guidelines on PPH management have been released, but the lack of published solid clinical results has led to the formulation of different recommendations about PBM by different scientific societies and international groups.[30] To analyse such differences and show the changes over time, this work reviewed and compared the recommendations offered by European societies and working groups from 2015 to 2020.

PPH definition has been redefined over time and, currently, there is not an internationally accepted definition. The latest classification considers not only BL volume, but also the onset of PPH and clinical signs (including pre-delivery haemoglobin concentration and blood flow rate) and ignores the type of delivery.[1] In fact, PBM is better described in planned obstetric surgeries, but it should be standardised for any obstetric procedure with excessive bleeding risk, including vaginal or non-elective caesarean deliveries.[11] The lack of consensus in the definition of PPH entails a greater difficulty in the comparison of different therapeutic strategies and the outcomes obtained, and its homogenization would suppose a benefit for the benchmarking between institutions and different countries.

Haemoglobin threshold below which RBCs transfusion is indicated is a parameter that has changed over time. Several randomised clinical trials and systematic reviews in fields other than PPH show that patients should be ideally classified in massive and non-massive

bleeders,[1] and that RCBs transfusion should only be considered when haemoglobin concentration is lower than 7 g/dl.[31] Consequently, prevailing recommendations of haemoglobin values greater than 7–8 g/dl in 2016[12,17] have moved forward to values of 6 g/dl in 2020,[11] also contemplating the clinical situation and the symptoms in those patients with values between 6 and 8 g/dl.

FFP transfusion is also a controversial issue in PBM, due to the very little data published in non-massive PPH. Some documents have agreed that it should only be used to correct thrombin generation time.[1,10,14,17] Other documents have recommended it for correcting coagulopathy in sight of the first signs of bleeding, although laboratory results are not available.[12,13,15,16,18] ISTH[10] and some late recommendations such NATA 2019[1] advocated for FFP after the transfusion of 4–6 units of RBCs. However, the same year an analysis of more than 32 000 deliveries over more than 4 years was published and coagulopathy seemed to be not present in all cases of obstetric haemorrhage and could not be predicted solely by blood loss. These results show that switching from this "ratio-driven" approach to an algorithm-based one significantly improved maternal outcome, avoiding the exposition of women to unneeded blood products. The viscoelastic testing seems to be the better approach due to its early identification and individualised treatment of coagulopathy.[32] Finally, PBMO stated that FFP should be used for volume resuscitation in situations of severe hypovolemia and concomitant coagulopathy.[11]

Proper PLTs concentration is associated with higher survival in patients with major haemorrhage.[33] The early transfusion of PLTs is supported, for instance, in patients with trauma[34] but the available evidence in severe ongoing PPH is still scarce. Consequently, the documents reviewed offered different thresholds below which PLTs should be transfused: from 50×10^9/L[12,16] to 75×10^9/L.[1,10,13,15]

FI seems to be an important predictor of severe PPH,[35,36] and the use of FI concentrate is associated with reduced bleeding and lower need for transfusion in patients with PPH. The availability of this concentrate varies depending on each country licensing; while some countries only contemplate its indication in fibrinogen inherited deficiency, other countries contemplate its use in acquired haemorrhages such as PPH. Although the evidence available mostly comes from case series, retrospective register investigations, or uncontrolled, non-randomised studies,[37] almost all the documents here reviewed recommended it.[1,10,12–15,17,18] Plasmatic FI values for considering FI administration are diverse among countries, and Portuguese guidelines refer to the highest threshold (>3 g/dl),[15,17] while a threshold above 2.0–2.5 g/L has been described as sufficient for haemostasis in literature.[32,38] A low plasma FI during haemorrhage has been shown to be associated with an increased risk of progression to severe PPH.[36] Conversely, the pre-emptive use of FI is not universally recommended, probably because its efficacy has not been yet proven.[32,39] Several studies are currently ongoing to provide evidence in this sense.

Recommendations regarding PCC for the control of PPH have changed over the years. Until 2019, the documents discussing it, except ISTH guidelines, did so in order to correct the coagulation time under an accurate analysis.[15,17] In 2019, NATA recommended against its use and, in order to generate new efficacy and safety data, limited it to clinical trials.[1] The administration of FXIII concentrate was also poorly mentioned in both newest and oldest documents. The SNWG was the study group that better discussed this issue, in 2016, and advised it in order to strengthen the clot despite adequate FI levels.[17] On the contrary, based on the WOMAN trial results,[40] the recommendation of early administration of TXA after PPH onset became stronger in the documents subsequently published,[1,11,14,15] although it was already included in previous guidelines. In fact, NATA highlighted the importance of administering TXA within the first 3 h after PPH onset,[1] and also referred to the avoidance of routine use of TXA for PPH prevention in the setting of a caesarean delivery, with the exception of cases of antepartum bleeding and increased risk of PPH.[1] Interestingly, according to secondary analysis of the WOMAN trial performed with a sample of women in Nigeria, one fourth women had findings suggestive of hyper-fibrinolysis.[41] Nevertheless, in the Netherlands, evidence of hyper-fibrinolysis was found to be rare when evaluated after 800–1500 ml of blood loss following child birth.[42] Consequently, the generalisation of the WOMAN trial results is being questioned, especially for high-income countries.

MTP and cell salvage implementation are interventions addressed by most recent guidelines and the agreement regarding their appropriateness is high.[1,11,13,15,18] The use of cell salvage has been widely included in guidelines since 2016, and its use has revealed to be safe and cost-effective in autologous blood recovery.[43] Moreover, some publications give solid evidence and advocate its use in PPH.[43–45]

Finally, the current work compiled other PBM strategies contemplated in the documents reviewed. The use of POC tests was the approach most often included. Another commonly addressed issue was the prevention of postpartum anaemia and the correction of antenatal anaemia.[1,11,12,15,18] In this sense, identification of anaemia and its cause, its treatment, and the early treatment of iron deficiency are fundamental. To support this, some studies have concluded that oral iron supplementation during pregnancy can reduce the risk of anaemia during pregnancy and postpartum,[46–48] and the WHO has recommended an alimental iron supplementation of 30–60 mg/day to prevent iron deficiency and iron deficiency anaemia for all pregnant women.[49] Besides, the importance of having a clear-cut PPH algorithm/protocol was increasingly highlighted.[1,10,13,15]

5 | CONCLUSIONS

Recommendations regarding the clinical management of patients with PPH offered in the different European documents reviewed in this publication are heterogeneous and have changed over time. The definition of PPH should be homogenised along the different guidelines and documents in order to ease the comparison of therapeutic strategies available and to perform further research. The standardisation of the aims of the different therapeutic strategies, as well as the thresholds and criteria to indicate them would probably impact in patients' clinical outcomes. The current recommendations in terms of PBM of PPH need to be reviewed and adapted to the latest available

evidence, in order to reduce the need for blood transfusion, improve the care of patients with PPH and increase survival.

AUTHOR CONTRIBUTIONS

Rosa Leal has participated in the design of the study, data collection and data analysis. Dra. Filipa Lança has participated in the design of the study and the review of the manuscript

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CONFLICT OF INTEREST

Rosa Leal works at the Medical Department of CSL Behring and Dra. Filipa Lança has no conflicts of interest to declare.

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ORIGINAL ARTICLE

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Individual and environmental determinants of serum ferritin levels: A structural equation model

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Abstract

Background and Objectives: Serum ferritin levels are increasingly being used to assess iron stores. Considerable variation in ferritin levels within and between individuals has been observed, but our current understanding of factors that explain this variation is far from complete. We aim to combine multiple potential determinants in an integrative model, and investigate their relative importance and potential interactions.

Methods: We use ferritin measurements collected by Sanquin Blood Bank on both prospective ($N = 59\,596$) and active blood donors ($N = 78\,318$) to fit a structural equation model with three latent constructs (individual characteristics, donation history, and environmental factors). Parameters were estimated separately by sex and donor status.

Results: The model explained 25% of ferritin variance in prospective donors, and 40% in active donors. Individual characteristics and donation history were the most important determinants of ferritin levels in active donors. The association between environmental factors and ferritin was smaller but still substantial; higher exposure to air pollution was associated with higher ferritin levels, and this association was considerably stronger for active blood donors than for prospective donors.

Discussion: In active donors, individual characteristics explain 20% (17%) of ferritin variation, donation history explains 14% (25%) and environmental factors explain 5% (4%) for women (men). Our model presents known ferritin determinants in a broader perspective, allowing for comparison with other determinants as well as between new and active donors, or between men and women.

KEYWORDS
air pollution, ferritin testing, iron metabolism

1 | INTRODUCTION

Iron is essential for human life, but both iron deficiency and iron overload can cause various adverse health effects. Therefore, iron homeostasis is tightly regulated in humans. In case of insufficient availability of iron in the circulation, recycling of old red blood cells is increased and hepcidin is downregulated both to increase dietary iron absorption and release iron stored in ferritin.[1,2] Haemoglobin levels

have long been the standard method to assess iron status. However, haemoglobin levels can remain sufficient for some time, even when iron stores are dwindling; this is known as iron deficiency non-anaemia.[1]

In contrast to haemoglobin, serum ferritin levels reflect the amount of stored iron.[1] Therefore, they are increasingly used to assess individuals' iron stores when these are at risk, for instance after traumatic blood loss, during pregnancy, or in blood donors.[3] Sanquin, the national blood service in the Netherlands, started measuring ferritin levels in each new donor, and subsequently after every fifth donation, in October 2017. Donating blood has a substantial impact on ferritin levels. Ferritin levels are lower among blood donors than in the general population: cross-sectional studies report lower ferritin levels in donors with a higher number of whole blood donations and a large randomised trial showed that ferritin levels indeed decline with more frequent blood donations.[4,5] Among new donors, large variation in ferritin levels is observed.[4] It is well established that individual characteristics such as sex and age are relevant: women in general, but pre-menopausal women in particular, have considerably lower ferritin levels than men.[4,6,7] Higher body mass index (BMI) is associated with higher ferritin levels.[8] In recent decades, many other factors that affect iron status have been identified: diet,[9,10] genetics,[11,12] ethnicity,[13] and iron supplementation, which is mostly studied among blood donors.[14,15]

Ferritin is also a known acute-phase protein that is elevated in inflammatory conditions, complicating its diagnostic value in individuals with conditions such as inflammatory bowel disease or chronic heart failure.[16] This could also explain the association between BMI and ferritin levels, as adipose tissue is known to promote systemic inflammation.[17] Additionally, exposure to environmental pollutants has been linked to disordered iron homeostasis,[18,19] and ambient particle matter (PM) concentration is correlated with ferritin levels.[19] The biological mechanism behind this is still unclear, but it is postulated that iron attaches to the PM rather than to cell nuclei, effectively creating a functional deficiency.[18,19] In turn, mechanisms start upregulating iron uptake and recycling in an attempt to meet the iron requirement of the cells, thereby altering iron homeostasis. Another suggested mechanism is that when pollutants enter the lungs, iron is transported away from the surface of the lung tissue and stored in ferritin complexes, in order to avoid chemical reactions between iron and the pollutant.[18] Other potential environmental determinants are neighbourhood characteristics, including population density and socio-economic status, which are consistently shown to be related to body weight[20] and blood parameters.[21]

Previous studies on ferritin levels have focused on studying the association with variables in a limited setting, for example, characteristics such as age and BMI, donation-related variables, or environmental pollutants. In this paper, we propose a novel framework that integrates multiple settings, using a structural equation model. By grouping relevant explanatory variables into constructs, we describe relationships with ferritin on a more general level. This enhances the insight into various mechanisms that influence ferritin levels, which is valuable to those who use these as a diagnostic tool. We explore associations between ferritin levels and individual characteristics, donation behaviour and environmental factors, in a large group of newly registered and active whole blood donors.

2 | METHODS

For this cross-sectional study, data collected by Sanquin and the Geoscience and health cohort consortium (GECCO) were analysed. Sanquin is by law the only blood service in the Netherlands, collecting over 400 000 whole-blood donations each year, with collection sites geographically well-distributed throughout the country. Several eligibility criteria exist to ensure the safety of the donors and recipients and the quality of the blood product. Donors must be aged between 18 and 79 years old, and a pre-donation screening visit takes place before the first 500 ml whole blood donation, which includes blood sampling for blood type and infectious disease testing, as well as initial haemoglobin and ferritin measurements. We will refer to these prospective donors, who have not donated yet, as 'new donors'.

Before every donation, a donor screening is performed, including a donor health questionnaire and measurements of blood pressure, pulse rate and haemoglobin levels to assess whether the donor is eligible to donate. Haemoglobin levels need to be at least 7.8 mmol/L for women and 8.4 mmol/L for men. This is measured by point-of-care testing with a photometer (HemoCue, Angelholm, Sweden). Ferritin levels, are measured in serum samples, using the Architect i2000 (Abbott Diagnostics, Chicago, IL), after the pre-donation screening visit and after every fifth whole blood donation. As such, ferritin measurements are only available in case of successful whole blood donations, and for new donors whose venous samples are taken as part of the pre-donation screening visit.

2.1 | Data

This study included all new and active whole blood donors who gave consent to the use of their data for scientific research (consent given by >99% of all donors) and for whom ferritin measurements were available between 1 October 2017 and 31 December 2019. If multiple ferritin measurements were available for a donor, only the first measurement was used. Information on donors and donation histories was extracted from the blood bank information system (ePROGESA, MAK-SYSTEM International Group, Paris, France). Variables used were sex, age, height, weight, time since previous successful donation, the number of successful donations in the previous 2 years, donor status (new or active donor), and ferritin levels. BMI was calculated from self-reported donor height and weight. Sanquin does not register donor ethnicity, but Duffy negative phenotype was included to function as a proxy for sub-Saharan African descent.

Environmental exposure variables of various characteristics were obtained from the Geoscience and health cohort consortium (GECCO).[22] The exposure data were operationalised based on publicly

TABLE 1 Grouping of variables into constructs for each model

Variable	Model A	Model B	Model C	Model D
Age	Individual characteristics	Individual characteristics	Individual characteristics	Individual characteristics
Weight				
Height				
BMI				
Duffy phenotype				
Time since previous donation[a]	Donation history	Donation history		
Number of previous donations[a]				
Population density	Environment	Environment	Environment	Environment
Temperature				
Socio-economic status				
Ozone	Pollution		Pollution	
PM2.5				
PM10				
Soot				
NO_2				

Note: All models contain the same observed variables but differ in how these are grouped into latent constructs.
[a]Only available for active donors.

available data. Data from 30 weather stations in the Netherlands—obtained from the Royal Netherlands Meteorological Institute (KNMI)—were used to estimate temperature at a spatial resolution of 1 km. Three options for the measurement level were considered (minimum, average, and maximum daily temperature), as well as three time spans (day, week or month before donation), resulting in nine options in total. The combination that showed the highest correlation with ferritin was included in the final model.

Daily concentrations for particulate matter (PM) 2.5, PM10, NO_2, ozone and soot levels were obtained via the Dutch National Institute for Public Health and the Environment (RIVM), for the years 2017–2019. These variables were imputed on a spatial resolution of 1 by 1 km. Neighbourhood socio-economic status (SES) scores and population density from 2017–2019 were acquired from Statistics Netherlands (CBS), both available on 6-digit postal code level. SES scores are based on percentiles of income, education level and vocational history of households, with a score of 0 being exactly the national average, and positive scores being above average. All spatio-temporal variables were matched with donor and donation data based on donation date and donor postal code. Lastly, the date and time of each donation were included as potential factors to account for seasonal and diurnal variation, as they are known to affect haemoglobin levels and may also affect ferritin levels.

To check for a possible confounding effect of smoking on environmental variables, we analysed the correlation between the percentage of smokers per municipality (data from Statistics Netherlands) and all environmental variables described in the above paragraph.

There were no missing data for environmental datasets from the RIVM and CBS. Donors with no ferritin measurement were excluded from the analysis. There were no missing data for the other donor or donation level variables.

2.2 | Statistical analysis

Structural equation modelling (SEM) was used to investigate which variables relate to serum ferritin and to what extent. Briefly, observed variables and latent constructs are distinguished in SEM. Latent constructs cannot be measured or observed directly, but are inferred from the observed variables. One or more hypothesized sets of relationships and correlations between variables and constructs are specified a priori and shown in a path diagram. For each relationship, a parameter is estimated that indicates its strength. Estimates are obtained by numeric optimization of a fit criterion, using maximum likelihood estimation. A more detailed overview of this method is provided in Appendix A.

We compared four ways to divide the 15 variables included in the analysis into latent constructs, as shown in Table 1. Date and time of the donation were added to the model separate of the constructs, and as such are not included in Table 1. Model A contains four latent constructs, and in models B, C and D different sets of constructs are combined. Confirmatory factor analysis (CFA) was used to test the validity of the specified measurement models, that is, the hypothesized relationships between the latent constructs and their observed variables. The overall fit of the models was assessed by the Tucker-Lewis Index (TLI) and the root mean square error of approximation (RMSEA). A rule of thumb is to exclude variables for which the absolute value of the standardised factor loading is below 0.4, but at sample sizes larger than 300, if the overall model fit is good, exclusion is not necessary and should be judged separately for each variable based on sensible background knowledge.[23]

Pairwise residual correlations between observed variables were calculated to identify whether any covariances needed to be added to the model. Of the four specified models, we continued our analysis with the best fit according to CFA, based on the TLI and RMSEA.

TABLE 2 Distribution of explanatory variables by donor status and sex

	New donors		Active donors	
	Women	Men	Women	Men
N	40 172	19 424	39 085	39 233
Age (years)	26 (21–37)	28 (23–37)	47 (31–58)	53 (39–62)
Height (cm)	170 (166–175)	183 (178–188)	170 (166–175)	183 (178–188)
Weight (kg)	68 (62–77)	82 (74–90)	70 (64–80)	85 (78–93)
BMI (kg/m^2)	24 (21–26)	24 (22–27)	24 (22–27)	25 (23–27)
Time since previous donation (days)	NA	NA	154 (132–217)	139 (71–147)
Number of previous donations[a]	NA	NA	3 (2–4)	5 (4–7)
Population density (inhabitants per km^2)	1173 (425–2617)	1246 (477–2936)	827 (322–1855)	814 (320–1824)
Duffy phenotype (proportion)	0.25	0.17	0.28	0.16
Temperature (°C)[b]	11.4 (6.4–16.6)	11.7 (6.6–16.7)	10.4 (6.0–16.0)	10.4 (5.9–16.0)
Socio-economic status	0.04 (−0.21 to 0.22)	0.02 (−0.24 to 0.22)	0.10 (−0.10 to 0.25)	0.12 (−0.07 to 0.26)
Ozone (μg/m^3)	46.9 (45.6–48.8)	46.8 (45.5–48.7)	47.2 (45.9–49.2)	47.2 (45.9–49.1)
PM2.5 (μg/m^3)	10.7 (9.7–11.6)	10.7 (9.8–11.6)	10.5 (9.6–11.5)	10.6 (9.7–11.6)
PM10 (μg/m^3)	18.2 (16.8–19.3)	18.2 (16.9–19.3)	18.0 (16.6–19.0)	18.0 (16.7–19.1)
Soot (μg/m^3)	0.66 (0.54–0.78)	0.66 (0.55–0.78)	0.63 (0.52–0.75)	0.65 (0.54–0.76)
NO_2 (μg/m^3)	17.6 (14.9–21.6)	17.8 (15.1–21.8)	16.8 (14.2–19.7)	16.9 (14.3–19.6)
Ferritin (ng/ml)	47 (28–75)	118 (79–170)	30 (17–47)	34 (20–56)

Note: Data are presented as medians (interquartile range) due to non-normal distributions of the variables.
[a]Within 2 years before the ferritin measurement.
[b]The maximum temperature recorded on the day of donation.

To the model with the best fit, we added the structural component, which contains the relationships between the latent variables and ferritin, the outcome variable. A multiple group SEM was carried out with parameters estimated separately for male and female donors, and for new and active donors. Because the assumption of normality of the explanatory variables does not hold in our data, a different estimator than the default maximum likelihood estimator was used: the 'mean and covariance adjusted weighted least squares estimator', which is robust against violations of the normality assumptions in a multivariate setting.[24]

The same model was fitted in all four groups, although the variables belonging to the *donation history* construct (see Table 1) are not available for new donors, as they do not (yet) have a donation history. The overall fit of the SEM model was assessed using the TLI and RMSEA, as well as the R^2 measure.

All analyses were conducted using *R* programming language and environment for statistical computing version 4.0.3,[25] with package *zoo*[26] for pre-processing environmental data, and *lavaan*[27] for CFA and SEM analyses. Path diagrams were created with yEd Live Graph Editor.[28]

3 | RESULTS

3.1 | Sample composition

Table 2 shows descriptive statistics of the study population by sex and donor status. The size of each of the groups was comparable, except for the group of new male donors, which was only half the size of the other groups. Between new and active donors, age differed considerably, new donors being younger than active donors by 17 years on average ($p < 0.001$ using a two-sample *t*-test). In both new and active donors, men were older (by 6 years on average, $p < 0.001$) and heavier (by 13 kg on average, $p < 0.001$) than women. *p*-values were obtained using two-sample *t*-tests. The time since last donation is higher in women than in men, and the number of prior donations is higher in men than in women. These differences are due to differences in the minimum required donation interval: for women, there must be 122 days between two donations with a maximum of 3 donations per year, while for men, the minimum is 57 days between two donations with a maximum of 5 donations per year. Differences in ferritin levels between the groups are as expected from previous studies: men have higher ferritin levels than women, and repeat donors have lower ferritin levels than new donors.

For pollution and environmental variables, there was little difference between the groups, any differences between new and active donors were most likely due to the different age and geographical distribution of the groups. None of these differences were statistically significant.

We found a weak correlation between the percentage of smokers and SES score (Pearson's $r = -0.4$) and a moderate correlation between the percentage of smokers and population density (Pearson's $r = 0.5$). No correlation was found for any of the other environmental variables.

3.2 | Model selection

CFA did not provide support for the *environment* construct as defined by the three variables *temperature*, *population density* and *socio-economic status*. These variables did not share a high proportion of their variance and consequently there was no convergent validity, effectively ruling out models A and C. In models B and D, variables *Duffy phenotype*, *temperature*, *SES* and *height* were omitted due to very low factor loadings (<0.05). The factor loading for variable *age* was also low (0.35) but this variable was not excluded, as it is expected that this factor loading would be small, considering the other variables in the construct (*weight* and *BMI*) are much more closely related. All other factor loadings were above the suggested threshold of 0.6. All latent constructs (individual characteristics, donation history and environment) showed convergent and discriminant validity in models B and D. Variables *time* and *day of year*, which were added to the model outside the constructs, were also dropped due to very low factor loadings (<0.05).

The presence of a *donation history* construct was the only difference between models B and D, and since new donors do not yet have a donation history, the models only differed for active donors. Model B had a TLI of 0.961 and RMSEA of 0.063, while model D had a TLI of 0.932 and RMSEA of 0.083. Based on these fit measures, model B fit the data best, and was therefore used in the remainder of the analyses.

Based on inspection of the pairwise residual correlations between all observed variables, two covariance terms were added to the model: one for *PM2.5* and *PM10* (residual correlation 0.092–0.102, depending on sex/donor status), and one for *age* and *population density* (residual correlation −0.151 to −0.149, depending on sex/donor status). We also added one covariance term for *weight* and *BMI*, as BMI was calculated using weight and was therefore inherently dependent.

3.3 | Parameter estimates

Figure 1 shows the structure of the final model and the parameter estimates for new donors. Parameter estimates were similar for both sexes, but factor loadings for variables belonging to the *individual characteristics* construct were higher for women than for men, indicating more shared variance. Factor loadings in the *environment* construct did not differ between sexes, showing that the covariance structure of those variables was not dependent on sex. The parameter estimates for the regression coefficients show the relative importance of each latent construct for the outcome variable. Table 3 shows the percentage of variance in ferritin levels that is explained by each construct for each model, adding up to the total percentage of variance explained.

Figure 2 shows the final model for active donors. As in new donors, factor loadings in the *individual characteristics* construct were higher for women than for men, and they were also higher for new donors than for active donors. The relative importance

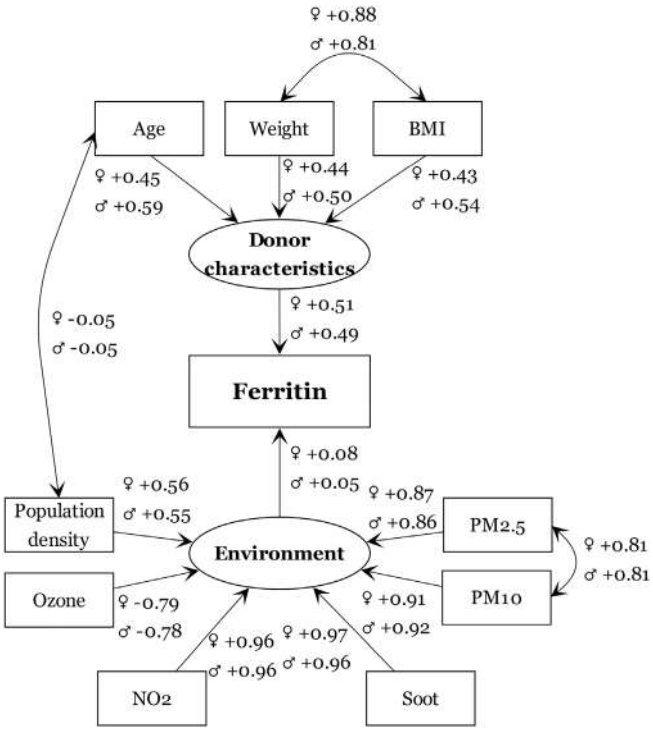

FIGURE 1 Final structural equation model for ferritin determinants in new donors, with parameters estimated separately for men and women. All parameter estimates are standardised so that the variance of each observed variable and latent construct equals 1

TABLE 3 Relative contribution to explanation of variance of ferritin levels per model

	New donors		Active donors	
Construct	**Women**	**Men**	**Women**	**Men**
Individual characteristics	23%	23%	20%	17%
Donation history	NA	NA	14%	25%
Environment	2%	2%	5%	4%
Total % of variance explained	25%	25%	39%	46%

of individual characteristics and donation history was opposite for both sexes: for men, donation history was correlated with ferritin levels more strongly than individual characteristics (0.66 vs. 0.45), while this was reversed for women (0.43 vs. 0.61). The regression coefficient of the *environment* construct is 0.15 for women and 0.10 for men. The *environment* construct explains twice as much variation in ferritin levels in active donors as in new donors.

As for overall model fit, with a TLI of 0.981 and 0.979 and RMSEA of 0.052 and 0.042, for new and active donors respectively, both models fit very well when compared to commonly used thresholds (TLI > 0.95, RMSEA < 0.06).[29] R^2 was calculated separately by sex: for new donors, R^2 was 0.251 for men and 0.252 for women, and for active donors, 0.458 for men and 0.393 for women.

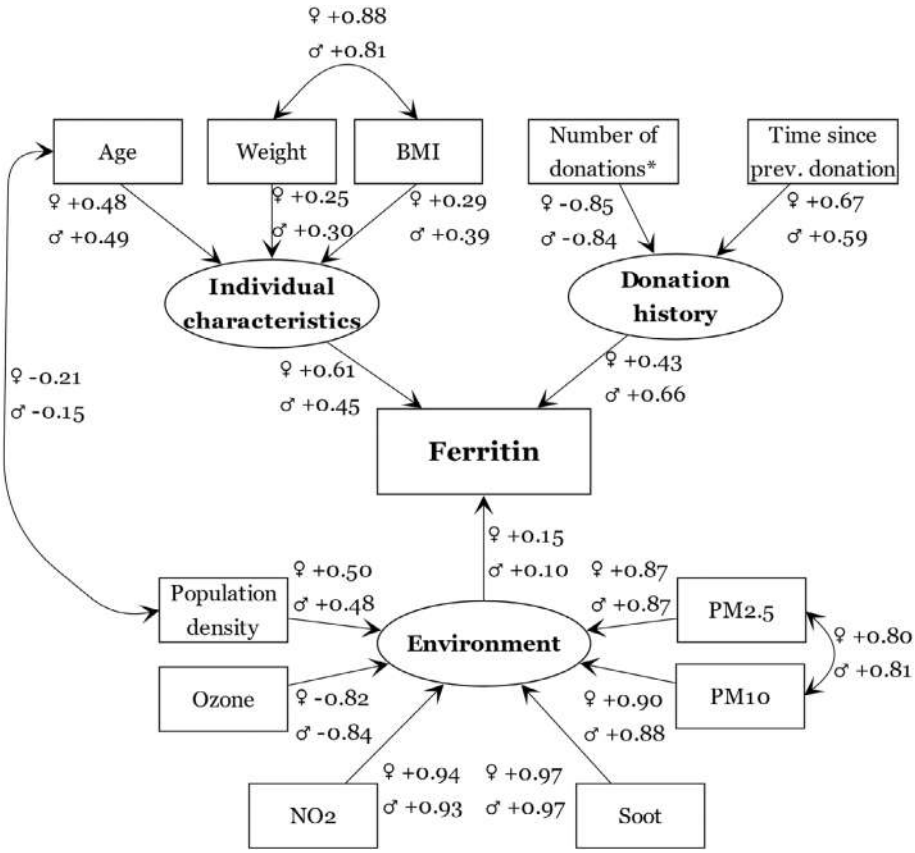

FIGURE 2 Final structural equation model for ferritin determinants in active donors, with parameters estimated separately for men and women. All parameter estimates were standardised so that the variance of each observed variable and latent construct equals 1

4 | DISCUSSION

This study investigated the impact of individual and environmental determinants on ferritin levels in Dutch individuals, using SEM. The model was able to explain 25% of ferritin level variance in new donors for both sexes, and 46% and 39% in active donors for male and female donors, respectively.

We found the construct composed of individual characteristics (age, weight, and BMI) to be the most important determinant of ferritin in female active donors, followed by donation history (time since previous donation, number of donations in the past 2 years). For male active donors, this was the opposite: donation history was a more important determinant than individual characteristics. In both sexes, environmental factors are associated with ferritin levels, albeit to a lesser degree than individual characteristics and donation history.

The relationship between ferritin levels and anthropometric characteristics is well-documented, and the positive correlations we found for ferritin with age, weight and BMI are consistent with those found in other studies.[4,15,30] Men have much higher ferritin levels than women in general and show a larger decrease in ferritin levels after repeated donations. As a result, ferritin levels in active donors are similarly low for women and men.[4] The *donation history* construct explained more variance in ferritin levels in men than in women. Although often not explicitly mentioned, this discrepancy is also found in previous studies, with stronger relationships between variables regarding donation history and ferritin for men than for women.[15] A reasonable explanation for this is that men commonly display more variation in donation history variables due to the possibility of more frequent donations: in many blood services, men are allowed to donate more often than women and are usually less frequently deferred for low haemoglobin levels.[31]

From previous epidemiological studies, we know that environmental factors may play a role in iron metabolism, and that certain pollutants can disrupt iron homeostasis.[32] Our study shows that although environmental factors are less strongly associated with ferritin levels than individual characteristics and donation history, their effects are far from negligible. Because of the wide reach of environmental exposures over geographic areas, even a relatively small influence on individuals can result in a large effect on the population level. As this study includes only data from the Netherlands, which is a relatively small country, associations between environmental variables and ferritin levels were not very strong, as was expected. Repeating this study on a larger, or even global, scale may result in finding a more substantial effect.

Higher values for all but one environmental factor (ozone) were positively correlated with higher ferritin levels. These findings support the hypothesis that air pollution causes higher ferritin levels. The underlying mechanism may be that when certain pollutants enter the lungs, iron is transported away from the lung tissue surface and stored in ferritin complexes to avoid chemical reactions between iron and the pollutant.[18,33] This would imply that using serum ferritin as a proxy for total body iron is less reliable when there is significant air pollution.

The *environment* construct was more strongly associated with ferritin level in active donors than in new donors. In new donors, environmental factors explain 2% of variance in ferritin levels, while in active donors this increases to 4%–5% depending on sex. This indicates that environmental factors are more important for ferritin recovery after blood loss than for naive ferritin level. A plausible explanation for this difference is that since both exposure to air pollution and donating blood causes significant disruptions to iron homeostasis, these disruptions may interact and together have a larger effect than simply additive.

SEM is a technique well-suited to test hypotheses on how different factors interact and correlate with a specific outcome like ferritin levels, especially when there are many factors to consider. Compared to multiple (linear) regression, more complex models can be tested, and for each variable measurement error is taken into account.[34] Moreover, the percentage of variance explained by groups of related variables can be calculated and compared. The stratified approach in this study also adds to the model validity: parameter estimates can be compared across groups, allowing discovery of implausible results. Our analyses show that the convergent validity of the *individual characteristics* construct is lower for active donors than for new donors. This may indicate that new donors are a more homogenous group than active donors, which is likely due to the more narrow age range of new donors. Other strengths of this study are its large sample size and collection of data throughout the country.

Two main limitations of this study should be noted: its generalizability and its restricted scope. One might be tempted to generalise the results of new donors to the general Dutch population, as these donors have never donated blood before. However, even new donors form a very specific, generally healthier subgroup of the general population, which means that selection bias has likely been introduced. We can speculate that less healthy individuals would show a higher rate of inflammation, which may cause higher serum ferritin levels. On the other hand, iron deficient or anaemic individuals are likely underrepresented in our sample. As this selection bias most likely reduced variance in ferritin levels, this may have attenuated our results.

Regarding the scope, data on some other potentially important determinants of ferritin levels were not available in this study, the two most important being genetics and diet.[9,10] Several genetic polymorphisms that have an effect on iron pathways have been identified, and these are likely to play a role in the recovery speed of ferritin levels after blood donation.[12,35–37] Dietary behaviour, and in particular heme iron intake, is also a determinant of iron status in donors.[9,15] Information on iron supplementation was also not available for this study. Sanquin does not prescribe oral supplementation of iron to donors, and only a small minority (8.7%) uses iron supplements.[9] Information on donors' smoking status is also expected to add value to the model. Had these determinants been available for our analysis, the proportion of variance explained in donor ferritin levels would likely have increased.

This study presents a model to explain variance in ferritin levels in individuals with or without donation history, based on three types of determinants. The model explained a relatively large part of the variance, especially in active donors. Individual characteristics and donation history form the most important determinants of ferritin levels. Although environmental factors accounted for less variance than the individual and donation history constructs, their contribution is meaningful and statistically significant. When clinicians or researchers use serum ferritin as a proxy for total body iron, they should be aware of this potentially confounding effect.

For blood services that are considering implementing ferritin testing for their donors, these results are of particular value. The results can be of use while the blood service is deciding on a sensible threshold for donation: rather than implementing a one-size-fits-all threshold, environmental conditions in the country can be taken into account. If there is a high level of air pollution, ferritin levels are likely to be overestimated, and thus a higher threshold for donation may be desired. It could even be taken further to make ferritin thresholds more tailored to a specific donor, by taking into account a donor's individual characteristics.

AUTHOR CONTRIBUTIONS

Rosa de Groot, Katja van den Hurk, and Jeroen Lakerveld conceptualised the study; Mart Janssen and Marieke Vinkenoog designed the methodology; Marieke Vinkenoog, Rosa de Groot, and Jeroen Lakerveld curated data; Marieke Vinkenoog did the formal analysis and wrote the original draft; all authors reviewed and edited the manuscript; Jeroen Lakerveld, Katja van den Hurk, and Mart Janssen supervised the study.

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CONFLICT OF INTEREST

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

Data collected on prospective and active donors by Sanquin Blood Supply Foundation will not be shared due to privacy reasons. The authors are open to research questions from other researchers; proposals for joint research projects may be made to the corresponding author via e-mail. The environmental exposure data provided by the GECCO institute is based on publicly available data, and can be requested via a data access request form available on the website: www.gecco.nl.

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APPENDIX A

A.1 | STRUCTURAL EQUATION MODELLING OVERVIEW

Structural equation modelling (SEM) comprises a set of statistical methods that enables researchers to assess the support for hypothesized relationships between variables of interest. Its purpose is to account for variation and covariation of the variables in the model. Many different techniques are included in SEM, this appendix explains the approach taken in this particular study. In SEM, observed variables and latent constructs are distinguished. Observed variables are variables in the traditional sense, which are observations in the data set that have been

collected by the researcher. Latent constructs are theoretical concepts that cannot be measured, but must be inferred from the observed variables; a well-known example is the latent construct *intelligence* that cannot be measured directly, but can be inferred from observed variables such as scores for an IQ test. Intuitively, observed variables that belong to a latent construct represent the same underlying concept, and latent constructs form in a way a dimensionality reduction of the observed variables. Mathematically, latent constructs represent shared variance of the observed variables related to the construct they belong to.

SEM is composed of two main model components: the measurement model, which shows how observed variables are divided among latent constructs, and the structural model, which shows the relationships between latent constructs and outcome variable(s). First, the measurement model is specified, and test its validity using confirmatory factor analysis (CFA). Often, several measurement models are tested and compared to see which division into latent constructs best fits the data. When the measurement model is considered to have a good fit, the structural part of the model is added, and the model fit is assessed for the full SEM model.

A.1.1. | Measurement model

The validity of the latent constructs must be measured in two ways: each construct must have convergent and discriminant validity. Convergent validity occurs when the observed variables belonging to the latent construct share a high proportion of their variance. This is assessed by the factor loadings of the observed variables onto the latent construct: the higher the (absolute value of the) factor loading, the stronger the indication that this variable belongs to this construct. Very generally speaking, factor loadings greater than 0.4 are acceptable for including a variable within a construct, but this threshold depends greatly on the hypothesized interpretation of the latent variable. Variables with low factor loadings are excluded from the construct.

The discriminant validity of a latent construct is a measure for how well the construct can be distinguished from the other constructs in the model. It is measured by the covariances between latent constructs. A high covariance between two constructs can indicate that these constructs are (partly) overlapping, and thus have no discriminant validity.

If convergent and discriminant validity are satisfactory, model fit indices can be calculated for the measurement model. Commonly used indices are the chi-square test, comparative fit index (CFI), Tucker-Lewis index (TLI) and root mean square error of approximation (RMSEA). The CFI and TLI are both relative measures of fit, and compare the fit of the tested model against a null model, which in CFA means that the means and variances of each variable are freely estimated, but no correlations are included. CFI and TLI are on a scale from 0 to 1, with higher values indicating a better fit of the hypothesized model relative to the null model. The TLI is always more conservative (lower value) than the CFI, because the TLI includes a harsher penalty for the number of parameters estimated. Because the two fit indices are highly correlated, only one should be reported. We chose the TLI because of its more elegant penalty for complexity. Values higher than 0.95 indicate good fit.

The RMSEA is an absolute measure of fit that is not sensitive to large sample sizes, unlike the chi-square test. It uses the covariance matrix of the entire data set and of the fitted hypothesized model, and calculates the differences between these two. This results in a measure between 0 and 1, with lower values indicating smaller differences and better model fit. Cut-offs of 0.08, 0.05, and 0.01 indicate mediocre, good, and excellent fits, respectively.

If multiple measurement models are compared, as in this study, the best fitting model is selected, based on the fit indices described above. If these indicate sufficient model fit, the analysis can be continued with inspection of residual correlation between observed variables. If the pairwise residual correlation between two variables is high (absolute value of 0.1 or higher is a common cut-off), this indicates that these two variables share more variance than is currently captured in the model. If this occurs, the researcher needs to decide whether a covariance term for these two variables should be included in the model. However, this should only be done if there is sufficient theoretical support for an interpretable correlation between these variables. Otherwise there is a risk of overfitting the model to the data; after all, in confirmatory factor analysis we build upon a set of relationships that are hypothesized by the researcher. It is not a data-driven method of finding the best set of relationships. If such an approach is desired, exploratory factor analysis (EFA) can be applied instead of CFA.

A.1.2. | Structural model

The structural component is added to the model once the latent constructs are defined, variables with low factor loadings are removed, and necessary covariance terms are added. The structural component consists of the relationships between latent constructs, or between latent constructs and outcome variable(s). With this, we now have three types of parameters for which an estimate must be calculated:

1. Factor loadings (observed variable → latent construct).
2. Covariances (observed variable ↔ observed variable).
3. Regression coefficients (latent construct → latent construct or outcome variable).

Each parameter adds one degree of freedom to the model, and the number of parameters determines the identifiability of the model. Parameter estimates can only be obtained when the number of free parameters (the number of 'unknowns') is equal to or smaller than the number of independent elements in the covariance matrix of the data (the number of 'knowns'), which is equal to $k(k+1)/2$, where k is the number of observed variables in the model. If there are more unknowns than knowns, the model is under-identified and no solution can be found. If the numbers are the same, the model is just identified, and a unique solution can be obtained. If there are fewer unknowns than knowns, we have an over-identified model, which means that

there is no unique solution but multiple, and we can select the best solution based on fit measures. An over-identified model is desired.

In most software packages parameter estimates are obtained by a maximum likelihood estimator by default, but alternative estimators can be chosen as well. In this study most observed variables did not follow a normal distribution, which violates maximum likelihood estimator assumptions. Therefore, the diagonally weighted least squares (DWLS) method was used instead, which is more robust and provides more accurate parameter estimates in case the normality assumption is violated.

If the model is over-identified, fit measures can be reported along with the parameter estimates. Again, TLI and RMSEA are used to assess model fit, with the same thresholds as seen in the CFA (TLI > 0.9, RMSEA < 0.08). If the model fit is acceptable the parameter estimates can be interpreted. The interpretation of the parameter estimates depends on the specification of the model. By default, one factor loading in each latent construct is set to 1, to fix the scale of the latent construct. However, in order to compare factor loadings across constructs it is useful to consider standardized parameter estimates. The variance of the latent construct is then set to 1 and factor loadings are interpreted in terms of a change in variance. In this study, we look only at the standardized parameter estimates, as we are interested in the relative importance of each observed variable and latent construct.

Factor loadings indicate how much variance of an observed variable is shared with the variance of its latent construct. Higher absolute values indicate more shared variance, and the sign of the factor loading specifies the direction of the association. Covariance terms provide the same information for two observed variables, which can belong to the same construct or to different constructs. If they belong to the same construct, a high covariance term indicates that these two variables share more variance with each other than can be explained by the latent construct. Regression coefficients indicate how much variance of the outcome variable is explained by the variance of the latent construct. To find the relative effect of a single observed variable on the outcome variable, its factor loading must be multiplied by the regression coefficient that connects the latent construct to the outcome.

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ORIGINAL ARTICLES

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The CRYOSTAT2 trial: The rationale and study protocol for a multi-Centre, randomised, controlled trial evaluating the effects of early high-dose cryoprecipitate in adult patients with major trauma haemorrhage requiring major haemorrhage protocol activation

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Abstract

Objectives

To describe the protocol for a multinational randomised, parallel, superiority trial, in which patients were randomised to receive early high-dose cryoprecipitate in addition to standard major haemorrhage protocol (MHP), or Standard MHP alone.

Background

Blood transfusion support for trauma-related major bleeding includes red cells, plasma and platelets. The role of concentrated sources of fibrinogen is less clear and has not been evaluated in large clinical trials. Fibrinogen is a key pro-coagulant factor that is essential for stable clot formation. A pilot trial had demonstrated that it was feasible to deliver cryoprecipitate as a source of fibrinogen within 90 min of admission.

Methods

Randomisation was via opaque sealed envelopes held securely in participating Emergency Departments or transfusion laboratories. Early cryoprecipitate, provided as 3 pools (equivalent to 15 single units of cryoprecipitate or 6 g fibrinogen supplementation), was transfused as rapidly as possible, and started within 90 min of admission. Participants in both arms received standard treatment defined in the receiving hospital MHP. The primary outcome measure was all-cause mortality at 28 days. Symptomatic thrombotic events including venous thromboembolism and arterial thrombotic events (myocardial infarction, stroke) were collected from randomisation up to day 28 or discharge from hospital. EQ5D-5Land Glasgow Outcome Score were completed at discharge and 6 months.

Results

The trial opened for recruitment in June 2017 and the final patient completed follow-up in May 2022.

Discussion

This trial will provide firmer evidence to evaluate the effectiveness and cost-effectiveness of early high-dose cryoprecipitate alongside the standard MHP in major traumatic haemorrhage.

KEYWORDS

MHP, Blood donor, non-blood donors, transfusion

1 | INTRODUCTION

The demand for plasma products is constantly increasing all over the world and the need will rise in the future due to the significant increase of the need of patients with coagulopathy, immunological medicinedocs/en/d/Js21936 en/pdf, 2015).[2] In 2010, over 33 million litres of plasma were fractionated by 78 fractionators.[1] Countries without a domestic fractionation plant can perform contract plasma fractionation with fractionators abroad to decrease the plasma wastage and improve the access of patients to plasma-

factors identified that the most important association of a need for transfusion within the first 48 post-operative hours was a pre-operative Hb <100 g/L (OR 6.64); a nail/canal ratio <70% (OR 3.92), followed by need for open reduction (OR 2.66). Fracture involving the lesser trochanter was also implicated with an increased risk (OR 2.08). Additionally, pre-operative moderate/severe renal impairment (OR 4.56), as well as hypoalbuminaemia on admission (OR 2.10) were biochemical predictors of an increased risk of transfusion. Most importantly, the need for transfusion was associated with an increase in 30-day mortality (OR 12.07).

Conclusion: Several patient, fracture and surgery related factors are implicated with an increased risk for transfusion within the first 48-h post-operatively. Early identification, and where possible correction of these factors can potentially reduce blood loss and risk of transfusion, along with all the associated sequelae and mortality risk.

Level of Evidence: III.

KEYWORDS
blood loss, blood transfusion, complications, open reduction, subtrochanteric

1 | INTRODUCTION

The incidence of hip fractures continues to increase, along with the global expansion of aging population observed secondary to improved healthcare and quality of life.[1] Subtrochanteric fractures are defined as fractures encountered between the inferior border of lesser trochanter and 5 cm distal to it.[2] They represent a complex subset of injuries surrounding the hip, which are most commonly managed with intramedullary (IM) nailing.[3,4] However, their moderate blood supply and being subjected to high concentration of stresses[5–8] meant these injuries are often associated with complications, with re-operation reported to be as high as 4.7%.[4,9]

Blood loss is a common sequelae of trauma and its subsequent surgical management. Bleeding following trauma usually arises from the fractured bony surfaces, its disrupted intramedullary vascular network, and the surrounding soft tissue envelope. With specific reference to the IM nail commonly used to treat subtrochanteric fractures, the reaming of the IM canal risks impairing the local vascularity further and increasing blood loss. Noteworthy, blood transfusion used to address blood loss is known to increase the risk of complications such as adverse transfusion reactions, delayed patient rehabilitation, increased in-hospital stay, the overall cost of treatment, and finally, mortality.[10,11] Hence careful surgical handling with attention paid towards protecting the local blood supply will not only reduce bleeding, but also preserve the vascularity at the fracture site and the chances of successful bone healing.[12] Most crucially, the prompt management of bleeding and appropriate resuscitation will prevent the development of haemorrhagic shock and the lethal triad of coagulopathy, hypothermia, and acidosis.

The aim of our study was to report on the blood loss and incidence of blood transfusion in patients presenting with a subtrochanteric fracture treated with intramedullary nailing. Most importantly, we aim to identify factors associated with the need for transfusion within the first 48 h post-operatively.

2 | METHODS

Following local institutional board approval (LTH#2591), data on eligible patients presenting to our Level 1 Trauma Centre over an 8-year period (2009–2016) were retrospectively collected and analysed. Inclusion criteria of this study included all adult patients presenting with a subtrochanteric fracture managed with an IM nail. Patients sustaining fractures following high energy injuries, presence of polytrauma, pathological fractures, patients receiving prophylactic nailing for bone tumours or incomplete fractures, and primary operation in other institutions were all excluded from the study. In case of bilateral fractures, only the first episode/fracture was considered.

Data on basic demographics, co-morbidities, operation details, complications and outcomes were collected. Russell Taylor classification was used for fracture classification.[13,14] Radiographic features and measurements of each subtrochanteric fracture were analysed and measured independently by MP and JV. Any disagreements were resolved by the senior author (PVG).

All aspects of patient care were managed by the multidisciplinary team, facilitated by a standardised proximal femoral fracture management protocol. Closed reduction of all subtrochanteric fractures was first attempted, with open reduction performed only when closed reduction proved unsuccessful. Following surgery, all patients followed a standardised physiotherapy regime aimed towards early mobilisation. All patients had routine clinical follow up where they were closely monitored for complications. We defined superficial infection as that occurring at the incision site during the early post-operative period, characterised by erythema, warmth, discharge and

raised inflammatory markers, amenable to oral antibiotic treatment.[15] Deep infection was defined as that involving the fascial layers or deeper, often requiring further surgical interventions and prolonged course of intravenous antibiotics.[16] Massive transfusion was defined as: transfusion of ≥10 units of red blood cells (RBC) (equivalent of the total blood volume of an average adult patient) within 24 h; transfusion of >4 units of RBC within 1 h with anticipation of continued need for transfusion; or replacement of >50% of the total blood volume by blood products within 3 h.[17–19]

2.1 | Statistical analysis

Data collected were analysed using the computing environment R (R version 3.6.0).[20] Basic demographic data were presented as count (percentage) or as mean ± SD. Data were tested for normality, with parametric data and non-parametric data analysed using the Pearson's chi square test and Welch unpaired independent *t*-test, respectively. A *p*-value of <0.05 was considered as significant. A simple logistic regression model was used for the initial analysis, to identify potential unadjusted associations with blood transfusion. The revised adjusted model of multiple logistic regression was developed following stepwise removal of covariates based upon their likelihood-ratio and chi-square *p*-values. The reported coefficients and OR from this revised adjusted multiple logistic regression analysis were used to identify associations with blood transfusion.

3 | RESULTS

3.1 | Demographics, mechanism of injury and transfusion requirements

A total of 431 patients (131 males) fulfilled the inclusion criteria; 279 patients (62.3%) required blood transfusion. Majority of blood transfusion were given post-operatively (62.7%, $n = 271$), with only 6.0% ($n = 26$) occurring pre-operatively (Figure 1 and Figure 2). Only 4.2% of patients ($n = 18$) received both pre- and post- operative transfusions (Table 1). Massive transfusion was required in 9 patients

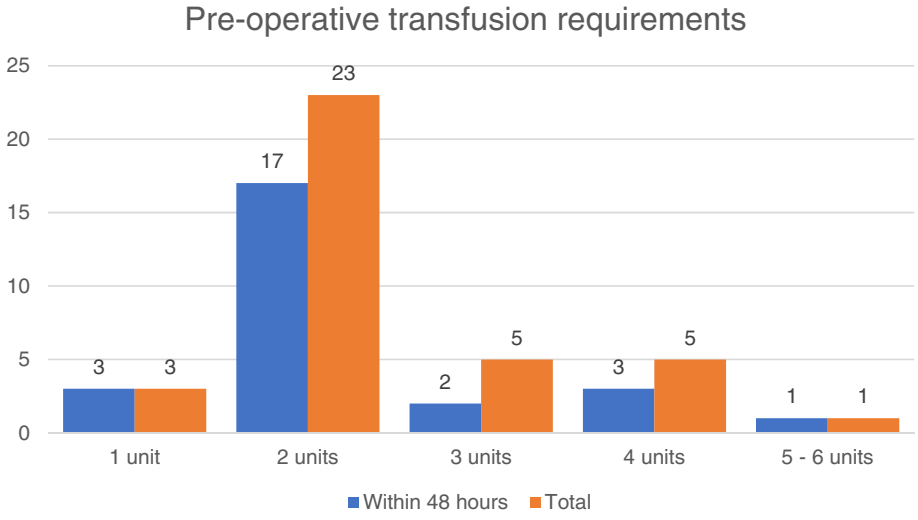

FIGURE 1 Transfusion requirements (within 48 h pre-operatively versus total pre-operative transfusion) of patients presenting to our institution with a subtrochanteric fracture

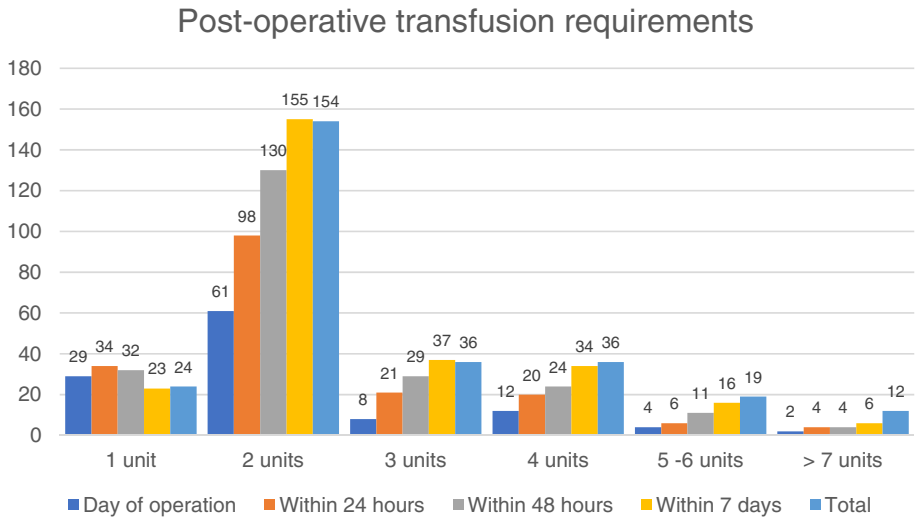

FIGURE 2 Transfusion requirements (within 48 h post-operatively versus total post-operative transfusion) of patients presenting to our institution with a subtrochanteric fracture

(2.1%) (Table 2), whilst transfusion of other blood products was used in 13 patients. Table 1 further illustrates the number and timeframe which these RBC units were transfused. Following transfusion, the mean change in Hb when corrected for units of RBC transfused was 32.5 ± 19.1 g/L (one unit of RBC considered to approximately increase Hb by 10 g/L[21,22]) (Table 3).

TABLE 1 Blood loss and transfusion requirements of patients presenting to our institution with a proximal femur fracture involving the subtrochanteric region

Transfusion rates	
Pre-operative (within 48 h)	26 patients (6.0%) RBC units Tx: 2.3 ± 1.1 (2; 1 to 6)
Pre-operative (at any point)	26 patients (6.0%) RBC units Tx: 2.7 ± 1.0 (2; 1 to 6)
Post-operative (within 48 h)	230 patients (53.2%) RBC units Tx: 2.5 ± 1.4 (2; 1 to 10)
Post-operative (at any point)	271 patients (62.7%) RBC units Tx: 2.7 ± 1.4 (2; 1 to 10)
Both pre- and post-operative	28 patients (6.5%)
Massive transfusion	9 patients (2.1%)
Transfusion of other products	13 patients: • FFP: 11 patients • Platelets: 6 patients • Clotting factors: 1 patient

Abbreviations: FFP, fresh frozen plasma; RBC, red blood cells; Tx, transfusion.

3.2 | Factors associated with risk of transfusion

To identify risk factors that predispose to a greater risk of transfusion, patients who required blood transfusion either intra-operatively or

TABLE 3 Hb values of patients presenting to our institution with a proximal femur fracture involving the subtrochanteric region

Hb values	
Hb value (pre-operatively)	118.8 ± 17.0 g/L (119.5 g/L; 61 to 169 g/L)
Hb value (post-operatively)	91.6 ± 16.2 g/L (92 g/L; 48 to 140 g/L)
Hb change	−27.2 ± 20.2 g/L (−30 g/L; −78 to 38 g/L)
Hb change including Tx[a]	−32.5 ± 19.1 g/L (−33 g/L; −103 to 37 g/L)

Note: Results are presented as: Mean ± SD (Median; Range).
Abbreviations: FFP, fresh frozen plasma; Tx, transfusion.
[a]One unit of RBC was considered to approximately increase Hb by 10 g/L.[21,22]

TABLE 2 Patients presenting to our institution with a proximal femur fracture involving the subtrochanteric region, receiving a massive transfusion peri-operatively (up to 48 h post-operatively)

Patients	Transfusion products	Pre-op Hb (g/L)	ASA	ICU/HDU stay	Open reduction	Surgical time (min)
Patient 1[a]	RBC: 10 units FFP: 8 units Platelets: 3 units	88	4	Yes	Yes	274
Patient 2[b]	RBC: 9 units FFP: 4 units	153	4	Yes	Yes	109
Patient 3	RBC: 8 units FFP: 4 units	96	4	Yes	No	161
Patient 4	RBC: 8 units FFP: 4 units Platelets: 1 unit	124	1	No	Yes	179
Patient 5	RBC: 6 units FFP: 3 units Platelets: 1 unit	131	3	No	Yes	101
Patient 6[c]	RBC: 6 units	94	3	Yes	No	80
Patient 7	RBC: 6 units	126	4	Yes	Yes	202
Patient 8[d]	RBC: 4 units FFP: 4 units	109	4	No	Yes	86
Patient 9	RBC: 4 units FFP: 4 units	92	3	No	No	103

Note: Mortality: Patient 1 and Patient 2 died whilst in-patients. All remaining patients survived at least 1 year after their operations.
Abbreviations: ASA, American Society of Anaesthesiologists Classification; FFP, fresh frozen plasma; RBC, red blood cells.
[a]Patient was found to have an arterial injury intra-operatively (branch of the Profunda femoris), that was controlled with ligation of the branch by the Vascular team.
[b]Patient had significant bleeding pre-operatively; a CT angiogram demonstrated injury to Profunda femoris, which was embolised pre-operatively by the interventional radiologist.
[c]Patient had significant post-operative bleeding; a CT angiogram demonstrated no significant branches to embolise.
[d]Patient has significant peri-operative oozing as was on Clopidogrel.

TABLE 4 Table presenting the demographics/characteristics of patients having a subtrochanteric fracture treated with a long cephalomedullary nail, stratified according to need for blood transfusion within the first 48 h post-operatively

Demographics	All patients	No transfusion	Transfused within 48 h
Total number	431	200	231
Age (years)	79.03 (13.68)	76.08 (14.17)	81.57 (12.77)
Gender			
Male	131 (30.4%)	68 (34.0%)	63 (27.3%)
Female	300 (69.6%)	132 (66.0%)	168 (72.7%)
Injury characteristics	**All patients**	**No transfusion**	**Transfused within 48 h**
Isolated	400 (92.8%)	183 (91.5%)	217 (93.9%)
Side			
Left	235 (54.5%)	112 (56.0%)	123 (53.2%)
Right	196 (45.5%)	88 (44.0%)	108 (46.8%)
Medical comorbidities	**All patients**	**No transfusion**	**Transfused within 48 h**
ASA			
1	9 (2.1%)	5 (2.5%)	4 (1.7%)
2	115 (26.7%)	70 (35.0%)	45 (19.5%)
3	236 (54.8%)	99 (49.5%)	137 (59.3%)
4	71 (16.5%)	26 (13.0%)	45 (19.5%)
Charlson Comorbidity Score	5.84 (2.62)	5.22 (2.55)	6.34 (2.55)
Diabetes	66 (15.3%)	25 (12.5%)	41 (17.7%)
Steroids	17 (3.9%)	10 (5.0%)	7 (3.0%)
Dementia	117 (27.1%)	51 (25.5%)	66 (28.6%)
Osteoporosis	**All patients**	**No transfusion**	**Transfused within 48 h**
Bisphosphonates pre-admission	83 (19.3%)	36 (18.0%)	47 (20.3%)
Bisphosphonates on discharge	127 (31.5%)	63 (32.8%)	64 (30.3%)
Calcium/Vitamin D pre-admission	144 (33.4%)	57 (28.5%)	87 (37.7%)
Calcium/Vitamin D on discharge	235 (58.3%)	105 (54.7%)	130 (61.6%)
Vitamin D loading on admission	84 (20.8%)	37 (19.3%)	47 (22.3%)
Fragility fractures (Before)	112 (26.0%)	39 (19.5%)	73 (31.6%)
Fragility fractures (After)	80 (18.6%)	36 (18.0%)	44 (19.0%)
DEXA result			
Normal	5 (12.5%)	3 (14.3%)	2 (10.5%)
Osteopenia	11 (27.5%)	5 (23.8%)	6 (31.6%)
Osteoporosis	24 (60.0%)	13 (61.9%)	11 (57.9%)
Social history	**All patients**	**No transfusion**	**Transfused within 48 h**
Smoking	67 (15.5%)	42 (21.0%)	25 (10.8%)
Alcohol >10 units/week	77 (17.9%)	48 (24.0%)	29 (12.6%)
Pre-operative mobility			
Independent	186 (43.2%)	110 (55.0%)	76 (32.9%)
Stick(s)/Crutch(es)	132 (30.6%)	57 (28.5%)	75 (32.5%)
Frame	91 (21.1%)	27 (13.5%)	64 (27.7%)
Wheelchair/Hoisted	22 (5.1%)	6 (3.0%)	16 (6.9%)
Frequent falls	145 (33.6%)	53 (26.5%)	92 (39.8%)
Operation characteristics	**All patients**	**No transfusion**	**Transfused within 48 h**
Operation in less than 48 h	345 (80.0%)	157 (78.5%)	188 (81.4%)
Simultaneous procedures	13 (3.0%)	4 (2.0%)	9 (3.9%)
Type of anaesthetic			

(Continues)

TABLE 4 (Continued)

Operation characteristics	All patients	No transfusion	Transfused within 48 h
GA	280 (64.8%)	122 (58.7%)	158 (70.5%)
Spinal	152 (35.2%)	86 (41.3%)	66 (29.5%)
Use of tranexamic acid	103 (23.8%)	45 (22.5%)	58 (25.1%)
Canal reamed	389 (91.3%)	184 (92.5%)	205 (90.3%)
Size of last reamer (mm)			
<12	15 (3.9%)	8 (4.3%)	7 (3.4%)
12–13	83 (21.3%)	38 (20.7%)	45 (22.0%)
13–14	148 (38.0%)	67 (36.4%)	81 (39.5%)
14–15	143 (36.8%)	71 (38.6%)	72 (35.1%)
Nail diameter (mm)			
9	14 (3.3%)	6 (3.0%)	8 (3.5%)
10	6 (1.4%)	5 (2.5%)	1 (0.4%)
11	260 (60.7%)	119 (59.8%)	141 (61.6%)
12	1 (0.2%)	1 (0.5%)	0 (0.0%)
13	147 (34.3%)	68 (34.2%)	79 (34.5%)
Open reduction	191 (44.3%)	69 (34.5%)	122 (52.8%)
Use of cerclage wires	47 (24.6%)	15 (21.7%)	32 (26.2%)
Post-op mobilisation (first 6 weeks)			
FWB	258 (59.9%)	122 (61.0%)	136 (58.9%)
PWB	95 (22.0%)	45 (22.5%)	50 (21.6%)
TTWB	45 (10.4%)	22 (11.0%)	23 (10.0%)
NWB	33 (7.7%)	11 (5.5%)	22 (9.5%)
Surgical time (min)	106.17 (41.10)	100.60 (38.27)	110.97 (42.89)
Anaesthetic time (min)	48.45 (21.56)	48.84 (24.95)	48.12 (18.19)
Time from induction to recovery (min)	172.50 (46.62)	166.39 (43.23)	177.76 (48.84)
Level of first surgeon			
Registrar	272 (63.3%)	123 (61.8%)	149 (64.5%)
Consultant	158 (36.7%)	76 (38.2%)	82 (35.5%)
Level of senior surgeon present			
Registrar	253 (58.8%)	118 (59.3%)	135 (58.4%)
Consultant	177 (42.2%)	81 (40.7%)	96 (41.6%)
Complications	**All patients**	**No transfusion**	**Transfused within 48 h**
Nail related complications*	72 (16.7%)	31 (15.5%)	41 (17.7%)
Failure at lag screw junction	19 (4.4%)	10 (5.0%)	9 (3.9%)
Self-dynamisation	18 (4.2%)	3 (1.5%)	15 (6.5%)
Cut-out	10 (2.3%)	7 (3.5%)	3 (1.3%)
Non-union	59 (13.7%)	28 (14.0%)	31 (13.4%)
Peri-implant fracture	4 (0.9%)	1 (0.5%)	3 (1.3%)
HAP/CAP	93 (21.6%)	37 (18.5%)	56 (24.2%)
UTI	70 (16.2%)	37 (18.5%)	33 (14.3%)
Wound infection			
Superficial	13 (3.0%)	7 (3.5%)	6 (2.6%)
Deep	9 (2.1%)	2 (1.0%)	7 (3.0%)
Renal impairment stage pre-operatively			
Stage I–II	278 (65.6%)	159 (81.5%)	119 (52.0%)

TABLE 4 (Continued)

Complications	All patients	No transfusion	Transfused within 48 h
Stage III–V	146 (34.4%)	36 (18.5%)	110 (48.0%)
Renal impairment stage post-operatively			
Stage I–II	288 (68.1%)	164 (85.0%)	124 (53.9%)
Stage III–V	135 (31.9%)	29 (15.0%)	106 (46.1%)
Acute post-operative renal injury	398 (94.5%) 23 (5.5%)	185 (96.4%) 7 (3.6%)	213 (93.0%) 16 (7.0%)
Pre-operative transfusion	37 (8.6%)	14 (7.0%)	23 (10.0%)
Post-operative transfusion (total)	281 (65.2%)	50 (25.0%)	231 (100%)
Hb drop (g/L)	32.47 (19.11)	3025 (15.41)	34.28 (21.62)
VTE			
No	89 (85.6%)	39 (88.6%)	50 (83.3%)
DVT	9 (8.7%)	5 (11.4%)	4 (6.7%)
PE	6 (5.8%)	0 (0.0%)	6 (10.0%)
Biochemistry	**All patients**	**No transfusion**	**Transfused within 48 h**
Adjusted calcium			
Normal	284 (75.9%)	129 (78.7%)	155 (73.8%)
Low	90 (24.1%)	35 (21.3%)	55 (26.2%)
Albumin			
Normal	117 (29.3%)	72 (40.7%)	45 (20.2%)
Low	283 (70.8%)	105 (59.3%)	178 (79.8%)
Alkaline phosphatase			
High	74 (18.5%)	36 (20.5%)	38 (17.0%)
Normal	289 (72.4%)	121 (68.8%)	168 (75.3%)
Low	36 (9.0%)	19 (10.8%)	17 (7.6%)
Phosphate			
Normal/High	300 (80.0%)	131 (79.4%)	169 (80.5%)
Low	75 (20.0%)	34 (20.6%)	41 (19.5%)
TSH			
High	28 (11.4%)	14 (12.8%)	14 (10.2%)
Normal	215 (87.4%)	94 (86.2%)	121 (88.3%)
Low	3 (1.2%)	1 (0.9%)	2 (1.5%)
Free T4			
High	35 (14.6%)	12 (11.3%)	23 (17.2%)
Normal	199 (82.9%)	93 (87.7%)	106 (79.1%)
Low	6 (2.5%)	1 (0.9%)	5 (3.7%)
PTH			
High	103 (46.4%)	45 (46.9%)	58 (46.0%)
Normal	119 (53.6%)	51 (53.1%)	68 (54.0%)
Total 25OH Vitamin D			
Normal	28 (11.4%)	13 (11.7%)	15 (11.1%)
Low	218 (88.6%)	98 (88.3%)	120 (88.9%)
Radiographic measurements	**All patients**	**No transfusion**	**Transfused within 48 h**
Number of fragments (Comminution)			
Simple	111 (25.8%)	56 (28.0%)	55 (23.8%)
Moderate	237 (55.0%)	111 (55.5%)	126 (54.5%)
Severe	83 (19.3%)	33 (16.5%)	50 (21.6%)

(Continues)

TABLE 4 (Continued)

Radiographic measurements	All patients	No transfusion	Transfused within 48 h
Isolated subtrochanteric extension	62 (14.4%)	33 (16.5%)	29 (12.6%)
Atypical	20 (4.6%)	14 (7.0%)	6 (2.6%)
Distal extension	135 (31.3%)	51 (25.5%)	84 (36.4%)
Lesser trochanter involvement	298 (69.1%)	124 (62.0%)	174 (75.3%)
Medial calcar comminution	24 (5.6%)	13 (6.5%)	11 (4.8%)
Lateral cortex gap size (mm)			
≤4	265 (61.9%)	127 (63.8%)	138 (60.3%)
5–9	109 (25.5%)	48 (24.1%)	61 (26.6%)
≥10	54 (12.6%)	24 (12.1%)	30 (13.1%)
Medial cortex gap size (mm)			
≤4	288 (67.3%)	134 (67.3%)	154 (67.2%)
5–9	98 (22.9%)	47 (23.6%)	51 (22.3%)
≥10	42 (9.8%)	18 (9.0%)	24 (10.5%)
Anterior cortex gap size (mm)			
≤4	287 (66.9%)	137 (68.8%)	150 (65.2%)
5–9	90 (21.0%)	37 (18.6%)	53 (23.0%)
≥10	52 (12.1%)	25 (12.6%)	27 (11.7%)
Posterior cortex gap size (mm)			
≤4	349 (81.4%)	168 (84.4%)	181 (78.7%)
5–9	60 (14.0%)	24 (12.1%)	36 (15.7%)
≥10	20 (4.7%)	7 (3.5%)	13 (5.7%)
Antirotation screw	164 (38.5%)	73 (36.9%)	91 (39.9%)
Distal locking (number of screws)			
1	13 (3.0%)	7 (3.5%)	6 (2.6%)
2	417 (97.0%)	193 (96.5%)	224 (97.4%)
Nail/Canal ratio <0.70	28 (8.2%)	6 (3.4%)	22 (11.4%)
Hospital stay/Mortality	**All patients**	**No transfusion**	**Transfused within 48 h**
HDU/ICU stay	41 (9.5%)	13 (6.5%)	28 (12.1%)
Total length of hospital stay (days)	24.21 (18.75)	23.23 (21.53)	25.06 (16.00)
Weekend admission	137 (31.8%)	58 (29.0%)	79 (34.2%)
Died within 30 days	27 (6.3%)	7 (3.5%)	20 (8.7%)
Died within a year	89 (20.6%)	29 (14.5%)	60 (26.0%)

Note: Dichotomous variables are presented as absolute numbers (percentages) of the positive event. Continuous variables are presented as mean (SD).
*Nail related complications: this included nail failure, peri-implant fracture and peri-implant infection.
Abbreviations: ASA, American Society of Anaesthesiologists Classification; CAP, community acquired pneumonia; DEXA, dual-energy X-ray absorptiometry; DVT, deep vein thrombosis; FWB, full weight bearing; GA, general anaesthetic; HAP, hospital acquired pneumonia; Hb, haemoglobin; HDU, high dependency unit; ICU, intensive care unit; NWB, non-weight bearing; PE, pulmonary embolism; PTH, parathyroid hormone; PWB, partial weight bearing; T4, thyroxine; TSH, thyroid stimulating hormone; TTWB, toe-touch weight bearing; UTI, Urinary tract infection; VTE, venous thromboembolism.

within the first 48 h post-operatively ($n = 200$) were compared against those who did not require any blood transfusion during the same period ($n = 231$) (Table 4). Patient factors associated with the increased need for blood transfusion include age > 75 years ($p < 0.001$), high CCS ($p < 0.001$), smoking ($p = 0.004$), alcohol >10 units/week ($p = 0.002$), reduced mobility ($p < 0.001$), frequent falls ($p = 0.004$), and hypoalbuminaemia on admission ($p < 0.001$) (Table 5). Surgical factors associated with an increased risk include open reduction ($p < 0.001$), prolonged surgical time ($p = 0.010$), prolonged total procedure time (induction to recovery; $p = 0.012$) and a smaller canal/nail ratio ($p = 0.007$) (Table 5). Fracture characteristics found to predispose to a higher risk of transfusion include those with lesser trochanteric involvement ($p = 0.003$), distal extension ($p = 0.016$) and atypical fractures ($p = 0.037$) (Table 5). Impaired

TABLE 5 Unadjusted associations with need for blood transfusion within the first 48 h post-operatively

Demographics	Unadjusted OR (95% CI)	*p*-value
Age >75 year old	2.34 (1.52–3.58)	<0.001
Medical comorbidities	**Unadjusted OR (95% CI)**	***p*-value**
Charlson Comorbidity Score	1.19 (1.10–1.29)	<0.001
Social history	**Unadjusted OR (95% CI)**	***p*-value**
Smoking	0.46 (0.27–1.60)	0.004
Alcohol >10 units/week	0.45 (0.27–0.75)	0.002
Pre-operative mobility		
Stick(s)/Crutch(es)	1.90 (1.21–2.99)	<0.001
Frame	3.43 (2.01–5.87)	<0.001
Wheelchair/Hoisted	3.86 (1.44–10.31)	<0.001
Frequent falls	1.84 (1.22–2.77)	0.004
Operation characteristics	**Unadjusted OR (95% CI)**	***p*-value**
Open reduction	2.12 (1.44–3.15)	<0.001
Surgical time (>120 min)	1.01 (1.00–1.01)	0.010
Time from induction to recovery (min)	1.00 (1.00–1.01)	0.012
Complications	**Unadjusted OR (95% CI)**	***p*-value**
Renal impairment stage pre-operatively		
Stage III–V	4.08 (2.62–6.37)	<0.001
Renal impairment stage post-operatively		
Stage III–V	4.84 (3.01–7.75)	<0.001
Pre-op Hb < 100 g/L	5.45 (2.60–11.43)	<0.001
Hb drop (g/L)	0.99 (0.98–1.00)	0.032
Biochemistry	**Unadjusted OR (95% CI)**	***p*-value**
Albumin		
Low	2.71 (1.74–4.23)	<0.001
Radiographic measurements	**Unadjusted OR (95% CI)**	***p*-value**
Atypical	0.35 (0.13–0.94)	0.037
Lesser trochanter fracture	1.87 (1.23–2.83)	0.003
Distal extension	1.67 (1.10–2.53)	0.016
Nail/Canal ratio <0.70	3.62 (1.43–9.16)	0.007
Hospital stay/Mortality	**Unadjusted OR (95% CI)**	***p*-value**
HDU/ICU stay	1.98 (1.00–3.94)	0.051
Total length of hospital stay ≥21 days	1.62 (1.10–2.38)	0.015
Died within 30 days	2.61 (1.08–6.32)	0.033
Died within a year	2.07 (1.27–3.38)	0.004

kidney function pre- and post- operatively ($p < 0.001$) and pre-operative Hb <100 g/L ($p < 0.001$) were associated with an increased risk for blood transfusion. Finally, patients who required blood transfusion within the first 48 post-operative period were at a greater risk of requiring high dependency/intensive care unit care ($p = 0.050$), prolonged LOS ($p = 0.015$), 30-day mortality ($p = 0.033$) and one-year mortality ($p = 0.004$) (Table 5).

Having adjusted for the different variables associated with blood transfusion, subsequent regression analysis identified the most important associations for transfusion within the first 48 h post-operative period following subtrochanteric fractures. These were pre-operative Hb of <100 g/L (OR 6.64; 95% CI 2.54–17.37), followed by nail/canal ratio of <0.70 (OR 3.92; 95% CI 1.34–11.46) and the need for open reduction (OR 2.66; 95% CI 1.60–4.42) (Table 6). Fracture involving the lesser trochanter was also a significant risk factor for blood transfusion (OR 2.08; 95% CI 1.20–3.61). Pre-operative moderate/severe renal impairment (OR 4.56; 95% CI 2.61–7.97) and hypoalbuminaemia on admission (OR 2.66; 95% CI 1.60–4.42) were biochemical predictors of increased blood transfusion risk. Most importantly, need for transfusion was associated with an increase in 30-day mortality (OR 12.07; 95% CI 1.20–121.44).

4 | DISCUSSION

The early identification of clinical signs and sites/sources of blood loss following trauma forms a crucial part of the initial patient assessment. This needs to be followed by the prompt management and resuscitation aimed towards haemorrhage control and ultimately, preventing haemorrhagic shock and the lethal triad of coagulopathy, hypothermia, and acidosis.[23] Femoral shaft fractures have a well-described association with substantial blood loss requiring blood transfusion, with an estimated average blood loss of 1200 ml being reported in the literature.[24,25] However, with specific reference to subtrochanteric fractures, there remains very little evidence to date on the estimated volume of blood loss and risk factors for blood transfusion. Thus, our study aims to report on the blood loss and blood transfusion needs in patients with subtrochanteric femur fractures, and to identify factors associated with the need for transfusion within the first 48 h post-operatively.

The incidence of blood transfusion in our patient cohort was 6.0% during the pre-operative period (mean: 2.7 units RBC transfused) and 62.7% post-operatively (mean: 2.7 units RBC transfused), with the mean estimated Hb drop being 32.5 g/L. Findings from our study were therefore similar to Shukla et al.'s study: 54% and 69% of patients with subtrochanteric fractures required blood transfusion following closed and open reduction respectively. Interestingly, Shukla et al. found that the mean Hb drop and number of units transfused were similar between the closed and open reduction groups (mean Hb drop: closed reduction 30 g/L, open reduction 32 g/L; mean RBC units transfused: closed reduction 3.0 units; open reduction 3.1 units).[26]

Massive transfusion was identified in nine of our patients (2.1%). This was generally associated with the presence of significant comorbidities (ASA 3 and ASA 4) and need for open reduction (six patients). Only two patients had an arterial injury requiring intervention (in one patient the responsible branch was ligated intra-operatively and in the other embolised pre-operatively), whilst the remaining patients had

	OR	95% CI	*p*-value
30-day mortality	12.07	1.20–121.44	0.034
Pre-operative Hb <100 g/L	6.64	2.54–17.37	<0.001
Pre-operative renal impairment (Moderate/Severe)	4.56	2.61–7.97	<0.001
Nail/Canal ratio <0.70	3.92	1.34–11.46	0.012
Open reduction	2.66	1.60–4.42	<0.001
Albumin (Low)	2.10	1.22–3.60	0.007
Lesser trochanter involvement	2.08	1.20–3.61	0.009

Abbreviations: CI, confidence interval; OR, odds ratio.

TABLE 6 Multivariate models demonstrating associations of need for blood transfusion within 48 h post-operatively following a subtrochanteric fracture

significant 'oozing' from the fracture/surgical wound(s). There is a paucity of evidence in the literature regarding the need for massive transfusion following low energy subtrochanteric fractures, not only because of the low incidence of significant bleeding in this group of patients, but also because of incontinences in the definition of 'massive' transfusion and the retrospective nature of most of these studies.

Comparing the blood loss between femoral diaphyseal fractures and that of 'extremity' fractures (70.3% being subtrochanteric fractures), Wertheimer et al. reported a higher incidence of blood transfusion (within first 48 h and overall total transfusion requirement) in patients with 'extremity fractures'. Interestingly, when compared against intertrochanteric femoral fractures (95.8%), findings from both our study (overall: 62.7%) and Shukla et al. (closed reduction group: 54%; open reduction group: 69%) revealed a smaller proportion of subtrochanteric femur fractures requiring blood transfusion.[26,27] Noteworthy, the number of units RBC required (mean: 1.4–1.7 units dependant on implant choice) by patients with intertrochanteric fractures was lesser than those with subtrochanteric fractures, as observed by both our study and Shukla et al.[26,27] Taken altogether, the higher incidence for blood transfusion as observed in subtrochanteric fractures could be explained by the greater proportion of these patients being elderly fragility fractures, often with a lower biological reserve of baseline Hb.[23]

In their regression analysis, Wertheimer et al. examined the risk factors for blood transfusion in the first 48 h.[23] They identified admission Hb as the only statistically risk factor determining the need for blood transfusion ($p < 0.01$), with only a trend observed for male gender ($p = 0.08$).[23] However, multivariate subsequent regression analysis from our study identified that in addition to a pre-operative Hb of less than 100 g/L (OR 6.64), there were several other important factors associated with the risk of requiring blood transfusion within the first 48 post-operative hours.

One of the factors associated with transfusion is a nail/canal ratio of <0.70 (OR 3.92). Inasmuch as reaming the intramedullary canal guarantees greater definitive nail diameter and construct stiffness used to treat the fracture; reaming the IM canal has however been associated with adverse effects such as additional blood loss.[28,29] As alluded by our findings, improving the nail diameter and therefore the nail/canal ratio could therefore reduce the risk of blood loss associated with intramedullary reaming. We therefore advise using the largest possible nail diameter following reaming, to achieve a 'tamponade effect' in the medullary canal.

The need for open reduction during surgery (OR 2.66) was another risk factor identified by our study as significantly associated with blood transfusion. Although Codesido et al. reported no increase in transfusion requirements following open reduction,[30] our findings do support the higher transfusion requirements following open reduction as reported by Shukla et al.' study.[26] Our study also confirmed cerclage wiring not to be an independent risk factor for the need of blood transfusion.

Additionally, our study identified subtrochanteric fractures with lesser trochanteric involvement (OR 2.08) as an important association with blood transfusion. This may suggest that more complex fracture patterns correlate to higher severity of soft tissue injury, often require more extensive tissue dissection, and are undoubtedly at an increased risk of bleeding and therefore blood transfusion. Furthermore, we have also identified pre- and post-operative moderate/severe renal impairment (OR 4.56) as important risk factors associated with blood transfusion. The lower glomerular filtration (below 60 ml/minute) and decline in endogenous erythropoietin production observed in patients with chronic kidney disease explains the higher risk of blood transfusion observed in elderly patients and those with renal impairment.[31–33] Finally, hypoalbuminaemia on admission (OR 2.10) was another important risk factor linked to the need for transfusion. Aldebeyan et al. reported similar findings in patients undergoing surgery for hip fractures,[34] as well as other studies investigating the effect of hypoalbuminaemia in joint replacement surgery.[35–37] Taken altogether, our group advocate meticulous soft tissue dissection and peri-operative optimisation focused on improving patient's physiology and biology which provide them with a greater prospect of a successful recovery and fracture healing.

Interestingly, in our study the need for post-operative transfusion was associated with an increase in 30-day mortality (OR 12.07), but not one-year mortality. Similar to our findings, Arshi et al. identified increased risk of 30-day mortality,[38] in contrast to a meta-analysis by Oberle et al., where patients undergoing major orthopaedic surgery did not present with a higher risk.[39] Regarding one-year mortality, Huette et al. and Smeets et al. reported no association,[40,41] whereas Greenhalgh et al. reported an almost two and a half times increased.[42]

To the best of our knowledge, this study is the largest cohort series in the literature to date reporting on blood loss and the risk factors associated with the need for blood transfusion in subtrochanteric

femur fractures treated with IM nailing. With no exclusion criteria imposed upon age or comorbidity, this study provides a better epidemiological overview of adult subtrochanteric fractures encountered in a Level 1 Trauma Centre serving a metropolitan population. By performing a multivariate subsequent regression analysis adjusted for confounding factors, we have reduced the bias that could result from baseline differences observed between the two populations. However, the retrospective nature of this study meant that data collected may still be subjected to bias. An example of this would be the classification and radiological assessment of the fracture which is subject to intra- and inter-observer reliability, which we overcome by having two independent assessors for the analysis.

5 | CONCLUSION

We have identified patient, fracture and surgical factors that were associated with an increased risk of transfusion need in the first 48 post-operative hour. These were pre-operative Hb <100 g/L, nail/canal ratio of <0.70, need for open reduction, subtrochanteric fractures involving the lesser trochanter, hypoalbuminaemia on admission, and pre-operative moderate/severe renal impairment. Most importantly, 30-day mortality seems to be increased in this group. Early identification, and where possible correction of these factors can potentially reduce blood loss and risk of transfusion, along with all the associated sequelae.

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No funding was received for the completion of this project.

CONFLICT OF INTEREST

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author, M.P., upon reasonable request.

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ORIGINAL ARTICLES

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Serological and molecular characterisation of the most prevalent weak D variants in Croatian population

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Abstract

Objectives

To describe the protocol for a multinational randomised, parallel, superiority trial, in which patients were randomised to receive early high-dose cryoprecipitate in addition to standard major haemorrhage protocol (MHP), or Standard MHP alone.

Background

Blood transfusion support for trauma-related major bleeding includes red cells, plasma and platelets. The role of concentrated sources of fibrinogen is less clear and has not been evaluated in large clinical trials. Fibrinogen is a key pro-coagulant factor that is essential for stable clot formation. A pilot trial had demonstrated that it was feasible to deliver cryoprecipitate as a source of fibrinogen within 90 min of admission.

Methods

Randomisation was via opaque sealed envelopes held securely in participating Emergency Departments or transfusion laboratories. Early cryoprecipitate, provided as 3 pools (equivalent to 15 single units of cryoprecipitate or 6 g fibrinogen supplementation), was transfused as rapidly as possible, and started within 90 min of admission. Participants in both arms received standard treatment defined in the receiving hospital MHP. The primary outcome measure was all-cause mortality at 28 days. Symptomatic thrombotic events including venous thromboembolism and arterial thrombotic events (myocardial infarction, stroke) were collected from randomisation up to day 28 or discharge from hospital. EQ5D-5Land Glasgow Outcome Score were completed at discharge and 6 months.

Results

The trial opened for recruitment in June 2017 and the final patient completed follow-up in May 2022.

Discussion

This trial will provide firmer evidence to evaluate the effectiveness and cost-effectiveness of early high-dose cryoprecipitate alongside the standard MHP in major traumatic haemorrhage.

KEYWORDS

MHP, Blood donor, non-blood donors, transfusion

1 | INTRODUCTION

The demand for plasma products is constantly increasing all over the world and the need will rise in the future due to the significant increase of the need of patients with coagulopathy, immunological

medicinedocs/en/d/Js21936 en/pdf, 2015).[2] In 2010, over 33 million litres of plasma were fractionated by 78 fractionators.[1] Countries without a domestic fractionation plant can perform contract plasma fractionation with fractionators abroad to decrease the plasma wastage and improve the access of patients to plasma-

1 | INTRODUCTION

Human neutrophil antigen 2 (HNA-2) is encoded by *CD177* gene (Figure 1),[1–3] which is also known as PRV-1 for the reason that *CD177* gene is over-expressed in polycythemia rubra vera patients.[4] HNA-2 expression on neutrophils is very heterogeneous among individuals with the percentage of HNA-2-positive (HNA-2^+) neutrophils ranging from 0% to 100%. The mean percentage of NHA-2^+ neutrophil subpopulation is between 45% and 65% in human populations. Approximately 3%–5% Caucasian Americans do not express HNA-2 and are referred as HNA-2 null subjects.[5] Immune system of individuals who do not express HNA-2 endogenously would recognise HNA-2 as a foreign antigen. Transfusion, pregnancy, and bone marrow transplantation could introduce the foreign HNA-2 antigen into bodies of HNA-2 null individuals whose immune responses to HNA-2 lead to the production of isoantibodies (or alloantibodies) by B cells. Accordingly, HNA-2 null human subjects are prone to produce anti HNA-2 isoantibodies. HNA-2 isoantibodies are involved in a number of disorders such as neonatal alloimmune neutropenia, autoimmune neutropenia, drug-induced immune neutropenia, and graft failure following marrow transplantation.[6–10] Additionally, HNA-2 isoantibodies cause transfusion related acute lung injury (TRALI) and various pulmonary disorders.[11–14] Consequently, HNA-2 is considered as one of the most important neutrophil antigens in human medicine.[5,15]

CD177 gene located at chromosome 19q13.31 region contains nine exons (Figure 1). Recently, we and others unravelled the primary genetic mechanism of HNA-2 deficiency and expression variations, which is caused by a nonsense single nucleotide polymorphism (SNP c.787A > T) within the CD177 coding region.[16–18] The HNA-2 null allele (STP allele) with the nonsense c.787T substitution likely originated from ectopic allelic conversion of the *CD177* pseudogene.[17] *CD177* pseudogene is highly homologous to *CD177* and hinders the genetic analysis of *CD177*.[19,20] Accurate and easy genetic assays for the identification of HNA-2 null individuals are not available up to now. In the current study, we designed and tested an easy polymerase chain reaction (PCR) assay to determine genotypes of the SNP c.787A > T responsible for HNA-2 expression deficiency. Our assay will aid clinical laboratories in diagnosis and prognosis of disorders implicated in transfusion and bone marrow transplantation.

2 | MATERIALS AND METHODS

2.1 | Study subjects

Healthy blood donors were recruited at the Memorial Blood Center in St. Paul, Minnesota. The age of healthy control donors ranged from 19 to 84 years old as described previously.[16] The human study has been approved by the Institutional Review Board for Human Use at the University of Minnesota.

2.2 | Nucleic acid isolation

Human genomic DNA was isolated from EDTA anti-coagulated peripheral blood using the Wizard Genomic DNA Purification kit (Promega, Madison, WI) following the vendor's instruction.

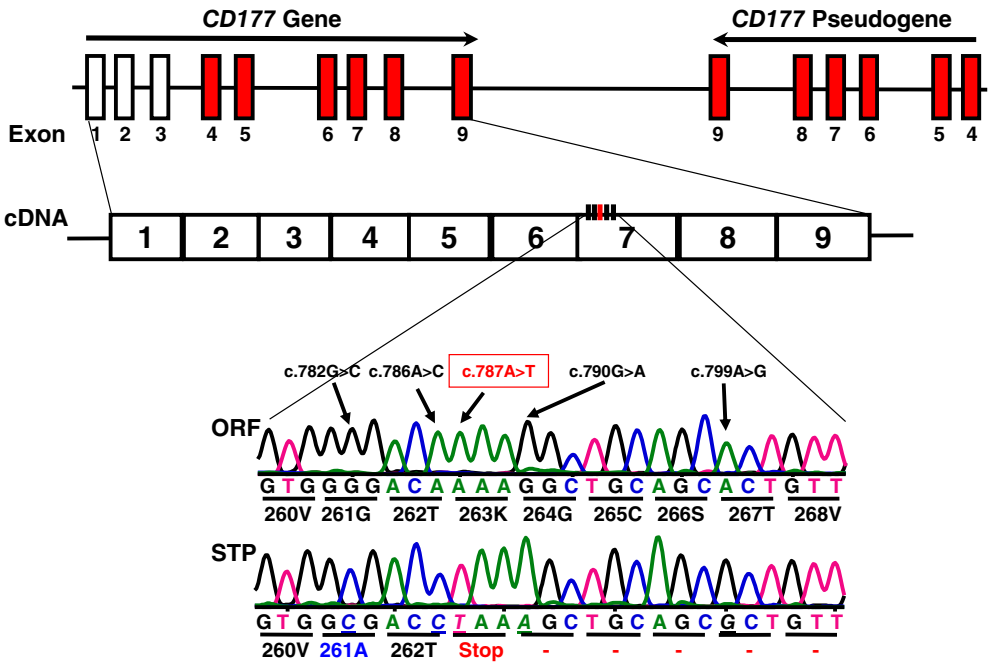

FIGURE 1 *CD177* SNPs responsible for HNA-2 expression deficiency. *CD177* gene contains nine exons and the *CD177* exon 4, 5, 6, 7, 8, and 9 are highly homologous to those of *CD177* pseudogene (upper panel). Locations of *CD177* SNPs responsible for HNA-2 expression deficiency are marked as vertical bars on cDNA in the middle panel. *CD177* ORF/STP haplotypes are formed by five cSNPs (c.782G > C, c.786A > C, c.787A > T, c.790G > A, and c.799A > G) (lower panel)

2.3 | Assessment of HNA-2 expressions on neutrophil

The expression of HNA-2 and the percentage of HNA-2^{+} neutrophils in healthy blood donors were determined as described.[16] Briefly, fresh whole blood samples were stained with FITC-conjugated mouse anti-human CD177 (HNA-2) mAb MEM-166 or FITC-conjugated mIgG1 isotype control (ThermoFisher Scientific). Blood samples were subsequently treated with 1× FACS Lysing Solution (BD Biosciences) before being analysed on a FACS Canto flow cytometer (BD Biosciences). The flow cytometry data were analysed using FlowJo software (Tree Star Inc., http://www.flowjo.com/). The same criteria were used to identify HNA-2 null individuals.[21]

2.4 | PCR-based *CD177* SNP genotyping assays

A PCR assay was designed to genotype *CD177* SNP c.787A > T (Figure 2). A single PCR reaction was carried out with two primers for the amplification of *CD177* c.787A (ORF) allele, two primers for *CD177* c.787T (STP) allele, and two primers for the amplification of human growth hormone gene (as the internal control) (Table 1). The PCR was performed with 20 ng DNA, 200 nM of each primer, 200 μM of dNTPs, 1.5 mM of $MgCl_2$, and 1 U of *Taq* DNA polymerase in a 25-μl reaction volume. The ABI Veriti 96-well Thermal Cycler was used for the PCR reaction starting with 95°C for 3 min; 35 cycles of denaturing at 95°C for 15 s, annealing at 60°C for 30 s, extension at

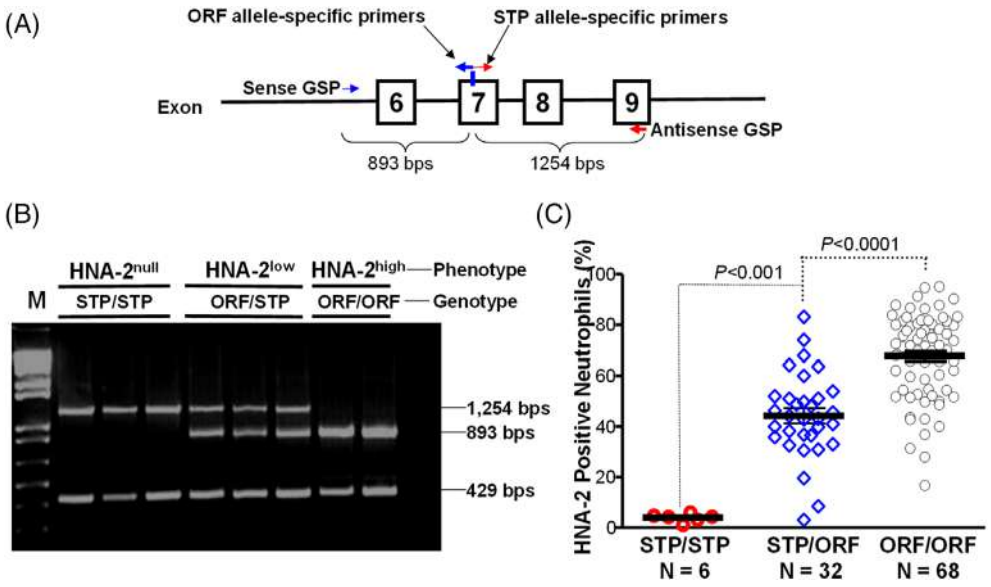

FIGURE 2 Polymerase chain reaction (PCR)-based assay to determine ORF/STP genotypes. (A) Primer locations of PCR-based assay. A sense *CD177* gene-specific primer (Sense GSP) was paired with an antisense *CD177* ORF allele-specific primer and a sense *CD177* STP allele-specific primer was paired with an antisense *CD177* gene-specific primer (Antisense GSP) to yield allele-specific DNA fragments of 893 and 1254 bps, respectively. (B) The PCR products were separated on an agarose gel. The DNA fragment size of internal control (human growth hormone gene) is 429 bps. The *CD177* genotypes were determined by the sizes and species of the DNA fragments in a single reaction. ORF-allele produces a DNA fragment of 893 bps. STP-allele produces a DNA fragment of 1254 bps. (C) *CD177* ORF/STP genotypes were determined with the PCR-based genotyping assay on a cohort of human subject whose HNA-2 expression was examined with flow cytometry analysis using HNA-2 positive plasma. All STP homozygous donors ($N = 6$) were HNA-2 null. The percentages of HNA-2 positive neutrophils from ORF/STP heterozygous donors ($N = 32$) were significantly ($p < 0.0001$) lower than those from ORF homozygous donors ($N = 68$)

TABLE 1 List of *CD177* allele-specific analysis primers

Genotype	Primer sequences (5′ → 3′)[a]	DNA Fragment size
CD177-ORF	F: ^{6609}ATTATGACACACGGAAACTTGGCTC^{6,633}	893 bps
	R: ^{7501}AACAGTGCTGCAGCCTTTTGTCC^{7,479}	
CD177-STP	F: ^{7465}ATCAACCCTGGTGGCGACCTAAA^{7,486}	1254 bps
	R: ^{8718}GTCCAAGGCCATTAGGTTATGAGGTCAGA^{8,690}	
hGH Internal controls	F: GCCTTCCCAACCATTCCC	429 bps
	R:TCACGGATTTCTGTTGTGTTTC	

[a]Nucleotide positions of primers on the CD177 genomic sequence (NC_000019.10 Reference GRCh38.p14 Primary Assembly Range: 43 353 686–43 366 081) are indicated as superscript numbers.

TABLE 2 Primers and probes of TaqMan assays to determine *CD177* ORF>STP genotypes

Primers and probes	DNA Sequences (5′ to 3′)[a]	DNA Fragment size
Gene-specific PCR	^{6609}ATTATGACACACGGAAACTTGGCTC^{6,633}	2110 bps
	^{8718}GTCCAAGGCCATTAGGTTATGAGGTCAGA^{8,690}	
TaqMan primers	^{7449}CACCCTCAGGACTCACATCAAC^{7,470}	81 bps
	^{7529}TGGTGGTCTTCTGGGAATTTTG^{7,508}	
TaqMan probes[b]		Not applicable
ORF allele	Vic-^{7481}AC***AA***AA***G***GCTGCAGC***A***C^{7,497}-MGB-NFQ	
STP allele	Fam-^{7476}TGG***C***GAC***CT***AA***A***G^{7,487}-MGB-NFQ	

[a]Nucleotide positions of primers on the CD177 genomic sequence (NC_000019.10 Reference GRCh38.p14 Primary Assembly Range: 43 353 686–43 366 081) are indicated as superscript numbers.
[b]TaqMan probes were labelled with fluorescent dye (Vic or Fam-6) at the 5′ end and with minor groove binding molecule (MGB) plus non-fluorescent quencher (NFQ) at the 3′-end. Bold, italic and underlined nucleotides are haplotype SNPs in respective haplotypes.

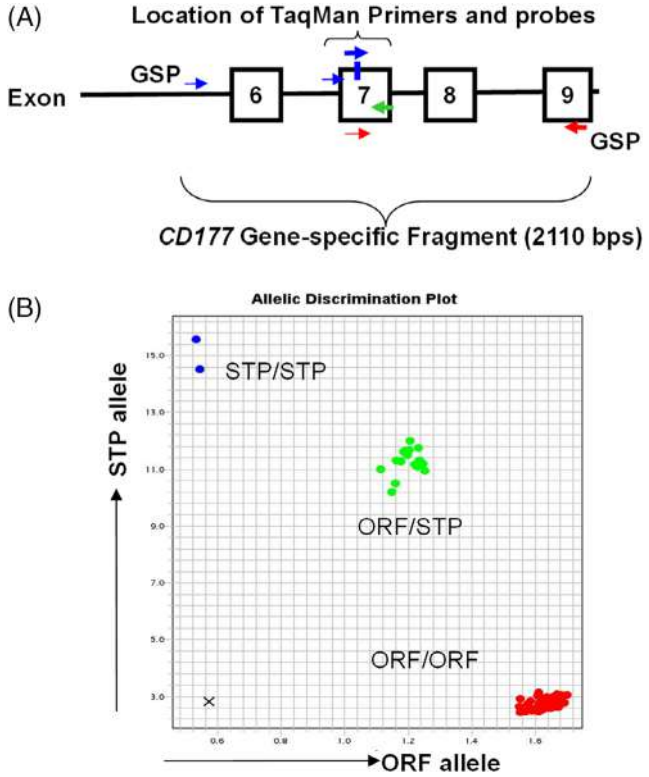

FIGURE 3 High-throughput *CD177* ORF/STP TaqMan genotyping assay. (A) Primer locations of TaqMan assays to determine the genotypes of the *CD177* SNP ORF or STP haplotypes. The *CD177* gene-specific PCR products (2110 bps) were amplified with gene-specific primers (GSP) and subsequently used as TaqMan assay template. (B) Plot of *CD177* TaqMan genotyping assay. *CD177* genotypes of the STP/ORF variant are clustered in three populations. The STP homozygous genotype is on the upper left corner, the ORF/STP heterozygous genotype in the middle, and the ORF/ORF homozygous on the lower right corner

72°C for 1 min and 20 s; with a final extension at 72°C for 7 min. Agarose gels (1.5%) were used to visualise and estimate the sizes of DNA fragments.

2.5 | *CD177* TaqMan SNP assay

CD177 gene and *CD177* pseudogene have identical nucleotide sequences at the region containing the SNP c.787A > T.[17] TaqMan genotyping assay could not directly be used to determine *CD177* SNP c.787A > T genotypes as the TaqMan assay primers could amplify both *CD177* and the *CD177* pseudogene. To avoid the interference of the *CD177* pseudogene, a *CD177* gene-specific PCR fragment containing the *CD177* SNP c.787A > T was used as the template for TaqMan genotyping assay. The gene-specific sense primer (5′-ATT ATG ACA CAC GGA AAC TTG GCT C-3′) and antisense primer (5′-GTC CAA GGC CAT TAG GTT ATG AGG TCA GA-3′) were used in PCR to amplify the *CD177*-specific genomic DNA fragment (2110 bps) containing the SNP c.787A > T. (Table 2 and Figure 3). The PCR reaction of 25-μl volume contained 50 ng DNA, 240 nM of each primer, 200 μM of dNTPs, 1.5 mM of $MgCl_2$, and 1 U of Taq DNA polymerase. The PCR was carried out with a denaturation step at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, extension at 72°C for 2 min with a final extension step at 72°C for 7 min. Agarose gel electrophoresis was used to confirm the production *CD177*-specific DNA fragments. A fraction (0.5 μl) of PCR products was subsequently used as the template for TaqMan assay to determine the genotypes of *CD177* variants. TaqMan genotyping assay was carried out according to the standard protocol on an ABI 7500 Real-Time PCR System using Genotyping Master Mix (Applied Biosystems) with the sense primer (5′-CAC CCT CAG GAC TCA CAT CAA C-3′), the antisense primer (5′-TGG TGG TCT TCT GGG AAT TTT G-3′), the FAM-6 labelled c.787 T (STP) allele probe (5′-FAM-TGG CGA CC***T*** AAA G-MGBNFQ-3′), and the VIC-labelled c.787A (ORF) allele probe (5′-VIC-ACA ***A***AA GGC TGC AGC AC-MGBNFQ-3′) (Table 2).

2.6 | Statistical analysis

The nonparametric *t*-test (Mann–Whitney test) was used to determine whether HNA-2 positive cell population sizes and the HNA-2 null are statistically associated with the *CD177* ORF/STP genotypes.

TABLE 3 Population allele frequencies of *CD177* SNPs associated with HNA-2 deficiency

Exon	dbSNP#	Nucleotide change[a]	Residue change[b]	Population minor allele frequencies[c]				
				EAS $n = 1008$	EUR $n = 1006$	AFR $n = 1322$	AMR $n = 694$	SAS $n = 978$
7	rs200660811	c.782G > C	261Gly > Ala	0.2371	0.3668	0.1929	03156	0.3640
7	rs587670082	c.786A > C	262Thr > Thr	0.2371	0.3668	0.1929	03156	0.3640
7	rs201821720	c.787A > T	263Lys > Ter	0.2371	0.3668	0.1929	03156	0.3640
7	rs200145410	c.790G > A	264Gly > Ser	0.2371	0.3668	0.1929	03156	0.3640
7	rs12978146	c.799A > G	267Thr > Ala	0.2371	0.3668	0.1929	03156	0.3640
8	rs556762097	c.950G > delG	319 V>fsm[d]	0.0000	0.0030	0.0000	0.0000	0.0031
9	rs772586043[e]	c.1254G > A	418Trp > Ter	0.0000	0.0000	0.0000	0.0000	0.0000
9	rs78718189	c.1291G > A	431Gly > Arg	0.0000	0.1083	0.0053	0.0560	0.0170

[a]Nucleotide position is counted from the ATG start codon.
[b]Residue position is numbered by starting from the methionine coded by the ATG start codon.
[c]SNP minor allele frequencies of EAS (East Asia), EUR (Europe), AFR (Africa), AME (America), and SAS (South Asia) populations were obtained from the 1000 Genomes Project data set deposited in dbSNP database. The letter "*n*" represents chromosome sample counts in each population.
[d]fsm represents frame-shift mutation.
[e]This rare SNP was found recently in Thai individuals by Siriphanthong et al.[23] and the minor allele frequency is 0.00003 in global population based on the Reference SNP (rs) report.

3 | RESULTS

3.1 | PCR-based ORF/STP genotyping assay

We previously demonstrated that five coding SNPs (c.782G > C, c.786A > C, c.787A > T, c.790G > A, and c.799A > G) within the *CD177* exon 7 are in complete linkage disequilibrium and form two haplotypes (Figure 1).[16] The open reading frame (ORF) haplotype contains c.782G, c.786A, c.787A, c.790G, and c.799A while the stop codon (STP) haplotype has c.782C, c.786C, c.787T, c.790A, and c.799G (Figure 1). To determine *CD177* ORF/STP genotypes, we developed a single-tube PCR assay. As shown in Figure 2A, a sense *CD177* gene-specific primer was paired with an antisense *CD177* ORF allele-specific primer while a sense *CD177* STP allele-specific primer was paired with an antisense *CD177* gene-specific primer in PCR to yield allele-specific DNA fragments of different sizes. Human growth hormone gene product (DNA fragment of 429 bps) served as an internal control in the allele-specific PCR reaction. The ORF allele-specific PCR generated a DNA fragment of 893 bps while the STP allele-specific PCR produced a DNA fragment of 1254 bps (Figure 2B). Genotypes of 396 human subjects determined by the PCR-based ORF/STP genotyping assay were completely (100%) matched with the genotypes previously determined using Sanger DNA sequencing method in same human subjects,[16] confirming the specificity and accuracy of the assay. Additionally, using the PCR-based ORF/STP genotyping assay, we genotyped another cohort of 106 normal healthy blood donors whose HNA-2 expressions were determined by flow cytometry analysis with HNA-2 positive antiserum. Figure 2C shows that all six (5.7%) homozygous STP genotype donors manifested as the HNA-2 null phenotype. In addition, 32 (30.2%) subjects with ORF/STP heterozygous genotype had significant lower percentages of HNA-2 positive subpopulation of neutrophils (mean percentage = 45.6%) than 68 (64.1%) subjects of homozygous ORF donors (mean percentage = 67.7%). Our data demonstrate that the PCR-based ORF/STP genotyping assay accurately identified HNA-2 deficient subjects carrying STP homozygous genotype.

3.2 | TaqMan *CD177* ORF/STP genotyping assay

High-throughput genotyping assay is needed in determining genotypes of large numbers of clinical samples. We have previously used gene-specific PCR products as templates to successfully genotype several FcγR genes with TaqMan analysis.[22] For *CD177* TaqMan assay, we used two gene-specific primers to generate *CD177* gene-specific PCR products (2110 bps) (Figure 3A), which was subsequently used in the TaqMan reactions. As shown in Figure 3B, the *CD177* genotypes of ORF/STP variants are clustered into three populations as analysed in Applied Biosystem 7500 Software. The STP homozygous genotype is on the upper left corner, the ORF/STP heterozygous genotype in the middle, and the ORF homozygous on the lower right corner. The genotypes obtained by TaqMan assay were subsequently compared to those determined by Sanger sequencing methodology. A perfect (100%) concordance of genotypes between TaqMan assay and direct sequencing analysis was achieved in all 396 human subjects, confirming the specificity and accuracy of the TaqMan *CD177* ORF/STP assay.

4 | DISCUSSION

Two haplotypes (ORF/STP) containing five *CD177* coding SNPs (c.782G > C, c.786A > C, c.787A > T, c.790G > A, and c.799A > G) determine HNA-2 deficiency and expression variations.[16] The SNP c.787A > T is a nonsense SNP that terminates the protein translation and causes the HNA-2 expression defect. Our previous study revealed

that c.787T homozygosity is the primary genetic determinant for HNA-2 deficiency,[16] which was confirmed by independent studies from other groups.[17,18,23,24]

Flesch et al. recently carried out a multicentre study on the molecular mechanisms of HNA-2-phenotypes using samples from regular blood donors, HNA-2-deficient individuals, mothers, and the respective children with neonatal immune neutropenia.[25] They found that 43 out of 54 HNA-2 null individuals were homozygous for the *CD177* c.787T (or STP) allele. This comprehensive study clearly demonstrates the impact of the *CD177* SNP c.787A > T on the expression of HNA-2 on the neutrophil surface. The SNP c.787A > T with the c.787T allele frequencies ranging from 0.1929 to 0.3668 in world populations is the most important genetic determinant for HNA-2 null phenotype based on genomic data from dbSNP database (Table 3). In the present study, we have developed a simple PCR-based assay to genotype CD177 for the determination of HNA-2 deficiency in human population. The accuracy of our assay was confirmed by genetic analysis of 396 human subjects, in which obtained 100% concordance with the results obtained from the Sanger DNA sequencing method. Our user-friendly and cost-effective PCR-based genotyping assay will provide a rapid method to identify HNA-2 deficient individuals in clinical laboratories. Furthermore, the high-throughput TaqMan assay we have developed can be used to determine *CD177* ORF/STP genotypes with precision and cost-effectiveness, which will be particularly valuable for genetic screen of large number of human subjects.

In the previous report, we also identified a rare SNP c.950G > ΔG contributing to HNA-2 deficiency in combination with c.787A > T.[16] However, the *CD177* c.950G > ΔG mutation is extremely rare as the mutant allele frequency was 0.0034 in our study cohort and ranges from 0.0000 to 0.0031 in 2504 aggregated world populations (Table 3), indicating a negligible impact of c.950G > ΔG on overall HNA-2 deficiency. Interestingly, another rare nonsense mutation (c.1254G > A) (Table 3) was also recently found to associate with HNA-2 null phenotype in Thai individuals[23] but the same mutation was absent in American and European populations.[16,26] The SNP c.1254G > A has not been identified in the 1000 Genomes Project and the minor allele frequency estimated by multiple SNP datasets is 0.00003 in global population based on the Reference SNP (rs) report. Therefore, the *CD177* SNP c.1254G > A may be a Thai population-specific mutation/SNP that contribute to HNA-2 deficiency. Thus, the combination of c.1254G > A and c.787A > T genotyping assays may be needed to effectively determine HNA-2 null phenotype in Thai populations. The *CD177* SNP c.1291G > A is associated with HNA-2 low expression and null phenotypes in *CD177* c.787A > T heterozygous donors.[25,26] Based on data from the 1000 Genomes Project, the minor allele (c.1291A) responsible for low HNA-2 expression or HNA-2 null is absent in East Asians (allele frequency = 0.0000) while the c.1921A allele frequencies are very low in Africans (allele frequency = 0.0053) and South Asians (allele frequency = 0.0170) (Table 3). However, the c.1291A allele is frequent in Europeans (allele frequency = 0.1083) and Americans (allele frequency = 0.0560). Future studies are needed to investigate whether the SNP c.1291G > A directly lead to the absence of HNA-2 expression. A genotyping assay for the SNP c.1291G > A may be needed to identify HNA-2 null individuals in Europeans and Americans if the SNP c.1291G > A is truly a causative polymorphism for the HNA-2 null phenotype.

Sanger sequencing analysis could be used to determine CD177 genotypes. However, *CD177*-specific PCR-amplified DNA fragment needs to be processed with either exonuclease plus phosphatase or gel purification for BigDye sequencing analysis. It typically takes 2 days and about 10 dollars to obtain DNA sequence result for one sample starting from setting up PCR to analysing DNA sequence data for genotype determination. Our PCR-based assay typically takes three to 4 h from setting up PCR to imaging the DNA agarose gel for genotype determination and costs less than a dollar for each sample. In conclusion, we have successfully developed accurate, cost-effective, and high-throughput genetic assays to identify HNA-2 null individuals. Our newly developed genetic assays will enable the reliable prediction of risks for HNA-2-related diseases in humans and will facilitate the diagnosis and prognosis of HNA-2-associated human disorders. Most importantly, our assays will significantly reduce the requirement for labour-intensive laboratory tests and the associated inconvenience of patients.[18]

AUTHOR CONTRIBUTIONS

Jianming Wu conceived and designed research. Yunfang Li, Randy M. Schuller and Jianming Wu performed research and analysed data. Jianming Wu wrote the manuscript.

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CONFLICT OF INTEREST

The authors have no competing interests.

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ORIGINAL ARTICLE

Efficacy and safety of BT595 (10% human intravenous immunoglobulin) in adult patients with chronic immune thrombocytopenia

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Biotest AG (Dreieich, Germany) manufacturer of BT595

Abstract

Purpose: This trial investigated the efficacy and safety of the new 10% human intravenous immunoglobulin (IVIg) BT595 (Yimmugo®).

Methods: Adult patients with chronic immune thrombocytopenia (ITP) received a total dose of 2 g/kg body weight (bw) IVIg either over 2 or 5 days.

Results: Response as defined by the European Medicines Agency (EMA) was achieved in 18 of 34 patients (52.9%) in the full analysis set (FAS), with a complete response in 11 patients (32.4%). The median time to response was 1.0 days (range 1–4); the median duration was 28.0 days. In a subgroup with a baseline platelet count <20*10^9/L evaluated according to FDA criteria, a platelet response ≥50*10^9/L was achieved in 18 of 19 patients at day 8. No fatal case occured. One serious treatment-emergent adverse event (TEAE) (anaemia, not related) was reported (2.9%). The most frequent infusional adverse drug reaction (ADR) was headache, which was reported for 14.7% of all patients. All other infusional ADRs (pyrexia, [intravascular] haemolysis, skin reaction, tinnitus, and Coombs test positive) occurred in only one patient (2.9%). Premedication was administered only once. The 5-day schedule showed less side effects with similar efficacy.

Conclusion: The benefit-risk profile of BT595 is favourable.

Trial Registration Number: Eudra CT Number 2015-003653-17, ClinicalTrials.gov NCT02859909.

KEYWORDS
BT595, chronic immune thrombocytopenia, ITP, phase III trial, pivotal

1 | INTRODUCTION

Primary immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterised by isolated thrombocytopenia, defined as a peripheral blood platelet count less than 100*10^9/L, and the absence of any other underlying cause. The estimated incidence of ITP is 100 cases per 1 million persons per year, and about half of these cases occur in adults.[1] Criteria for the diagnosis of chronic ITP have been published by the International Working Group.[2]

The successful administration of human intravenous immunoglobulins (IVIgs) in patients with acute and chronic ITP was first reported by Imbach et al.[3] Since then the infusion of IVIgs has become a well-established therapy in patients with ITP, especially to raise the platelet count before surgery to reduce the risk of bleeding. This, if effective, avoids or defers the risk of more toxic treatments (immunosuppressants) and reduces corticosteroid exposure.[4] In acute ITP, a permanent increase of platelet count may be achieved; whereas, in chronic ITP, response to IVIg therapy usually diminishes after several weeks.[5,6]

According to the European Medicines Agency (EMA) guideline on the clinical investigation of human normal immunoglobulins for intravenous administration,[7] there are no data to support the equivalence of different IVIg preparations, especially with regard to immunomodulatory activities; therefore, a clinical trial is required to establish the efficacy of a new product in this indication. BT595 (Yimmugo®, a registered trademark in the EU and Switzerland, in the USA the trademark application is pending, Biotest AG, Dreieich, Germany) is a new 10% normal intravenous immunoglobulin G preparation, manufactured by using plasma from healthy donors. The extraction of the IgG from the pooled plasma was performed by fractionated cold/ethanol precipitation, a method that was originally developed by Cohn, followed by ion exchange chromatography and by other downstream purification steps including the removal of potentially contaminating viruses, and achieving high purity and a low content of polymers and dimers. The final sugar-free, glycine-stabilised product of BT595 contains 100 mg/ml human plasma protein (i.e., 10% solution). The distribution of IgG subclasses is approx. 62% IgG1, 32% IgG2, 4% IgG3, and 2% IgG4. The IgA content is limited to ≤0.5 mg/ml. In a clinical trial in primary immunodeficiency disease, the steady state total IgG pharmacokinetic profile after BT595 administration as determined by non-compartmental analysis resulted in a long half-life of >20 days (mean $t_{1/2}$ of 24.2 and 31.1 days in the 3-week and 4-week schedule group, respectively) and a low clearance (mean clearance of 0.09 and 0.08 L/day).[8]

1.1 | Design

This open-label, prospective, randomised, multicenter phase III trial was designed following the revised EMA guidelines 'Guideline on the clinical development of medicinal products intended for the treatment of chronic primary immune thrombocytopenia'[9] and 'Guideline on the clinical investigation of human normal immunoglobulin for intravenous administration (IVIg)'.[10] The latter was revised.[7] However, the design of this trial is still in line with the latest recommendations given.

The trial period consisted of up to a 14-day screening period and 2 or 5 days of treatment per patients. According to clinical experience in chronic ITP,[11] the platelet count generally returns to pretreatment levels within 3 to 4 weeks after IVIg treatment; therefore, an observation period of approximately 28 days was implemented. In addition, all patients attended a closing (follow-up) visit approximately 36 days after the patient's first BT595 treatment. The patients were randomised in a 1:1 ratio to receive BT595 either 1 g/kg body weight (bw) per day for 2 consecutive days or 0.4 g/kg bw per day for 5 consecutive days, resulting in a total dose of 2 g/kg bw.

The primary objective was to determine the rate of patients with a response (R), defined as a platelet count of ≥30*10^9/L and at least a 2-fold increase of the baseline count, confirmed on at least 2 separate occasions at least 7 days apart, and the absence of bleeding. The occurrence and severity of bleeding symptoms was measured according to the primary immune thrombocytopenia–specific bleeding assessment tool (ITP-BAT) and the WHO bleeding scale.

In addition to further efficacy assessments, the response rates according to FDA criteria (increasing platelet count to ≥50*10^9/L within 7 days of the first infusion) in the full analysis set (FAS), and stratified by baseline platelet count <20*10^9/L were evaluated as secondary parameters. The safety was also evaluated.

The results of this trial (platelet response, occurrence of bleeding, and selective secondary efficacy data) were compared with available data from literature. This included three studies using the criteria identical to this trial according to the revised EMA guideline.[10,12–14]

Male or female patients aged 18 through 75 years were eligible for participation if they had a diagnosis of chronic ITP (>12 months' duration), including a diagnosis of refractory ITP, and as defined by the International Working Group.[2] Further main inclusion criteria were a mean screening platelet count of <30*10^9/L from three qualifying platelet counts performed within approximately 7 to 14 days before the start of treatment, with no individual platelet count above 35*10^9/L and an indication of treatment because of a high risk of bleeding or a need to raise the platelet count.

Exclusion criteria included secondary thrombocytopenia or acquired medical conditions known to be associated with secondary thrombocytopenia (e.g., chronic lymphocytic leukaemia, lymphoma, multiple myeloma, thyroid disease, drug-induced thrombocytopenia, cirrhotic liver diseases), and severe concomitant diseases that in the judgement of the investigator would interfere with the trial (e.g., autoimmune hemolytic anaemia, acute renal failure, noncontrolled arterial hypertension). Patients with a history of thrombotic events (including myocardial infarction, cerebral vascular accident, pulmonary embolism, and deep vein thrombosis) in the past 6 months before treatment start or those with the presence of significant risk factors for thrombotic events were excluded. Further, patients with additional therapy with high-dose corticosteroids (equivalent to >30 mg prednisone/day), thrombopoietin receptor agonists, and/or immunosuppressives and/or other therapies (e.g., infusion of platelets) within 1 month before the start of the trial were excluded. Patients on stable doses of ITP active treatment were not allowed to modify the dose in the 2 weeks prior the trial and had to maintain their pre-study dose during the trial. Rescue therapy with short courses, that is, 1–4 days, of high-dose steroids and IVIgs were allowed up to 2 weeks before trial inclusion.

BT595 was administered as an intravenous infusion at an initial infusion rate of 0.3 ml/kg/h for 30 min, to be increased to 1.4 ml/kg/h

for a further 30 min. If well tolerated, the infusion rate could then be gradually increased to a maximum of 2.0 ml/kg/h for the remainder of the infusion. From the third infusion (in the 5-day treatment schedule only), patients' infusion rates could be gradually increased to maximum of 6 ml/kg/h at the investigator's discretion. For treatment visits, patients remained at the site for at least 1 h after the end of infusion and any new adverse events were reported to the investigator at the site.

Enrollment of approximately 40 adults was planned to ensure at least 30 evaluable patients (with at least one postbaseline platelet count). The statistical analysis was performed using SAS® Software version 9.4. The safety set comprised all patients who received at least 1 dose of study medication and was used for safety evaluation.

A Data and Safety Monitoring Board regularly reviewed the safety data.

2 | RESULTS

2.1 | Demographics

Of the 62 patients screened, 34 were randomised and received at least 1 dose of study medication and had at least 1 post baseline platelet count value (Figure 1). Thus, the full analysis set (FAS) used for efficacy evaluation was identical to the safety set. The 34 eligible patients were randomised at 12 sites in 5 countries in Europe (Spain, Bulgaria, Hungary, Serbia, Germany). 18 patients were randomly assigned to the 2-day treatment schedule and 16 to the 5-day treatment schedule.

The mean (standard deviation, SD) age was 45.7 (16.86) years and the median age was 48.0 years, ranging from 19 to 74 years. 20 of the 34 patients (58.8%) were female. All patients were white. Seven (20.6%) had refractory ITP. Further baseline characteristics are given in Table 1.

2.2 | Efficacy

The primary efficacy endpoint (rate of response [R]) was achieved in 18 of 34 patients, corresponding to 52.9% of patients in the FAS (95% CI: 35.1 to 70.2). In the FAS, there were no relevant differences in R rates between the 2 treatment schedules. In the per-protocol set (PPS), 17 of 28 patients (60.7%; 95% CI: 40.6 to 78.5) achieved R, whereby the R rate was higher for the 2-day treatment schedule (10 of 14 patients [71.4%]; 95% CI: 41.9 to 91.6) than for the 5-day treatment schedule (7 of 14 patients [50.0%]; 95% CI: 23.0 to 77.0). The median time to R in the FAS was 1.0 day (range: 1 to 4 days). The median duration of R was 28.0 days (range: 18 to 36 days). Of the 18 patients in the FAS who achieved R at least once during the trial, 12 (66.7%; 95% CI: 41.0 to 86.7) had a subsequent loss of R after day 15. There were minor differences in number and percentage of patients with loss of R between the 2 treatment schedules (60.0% in the 2-day treatment schedule versus 75.0% in the 5-day treatment schedule).

Complete response (CR) was achieved in 11 patients, corresponding to 32.4% in the FAS (95% CI: 17.4 to 50.5) and there were no relevant differences the 2 treatment schedules. In the PPS, 10 of 28 patients (35.7%; 95% CI: 18.6 to 55.9) achieved CR,

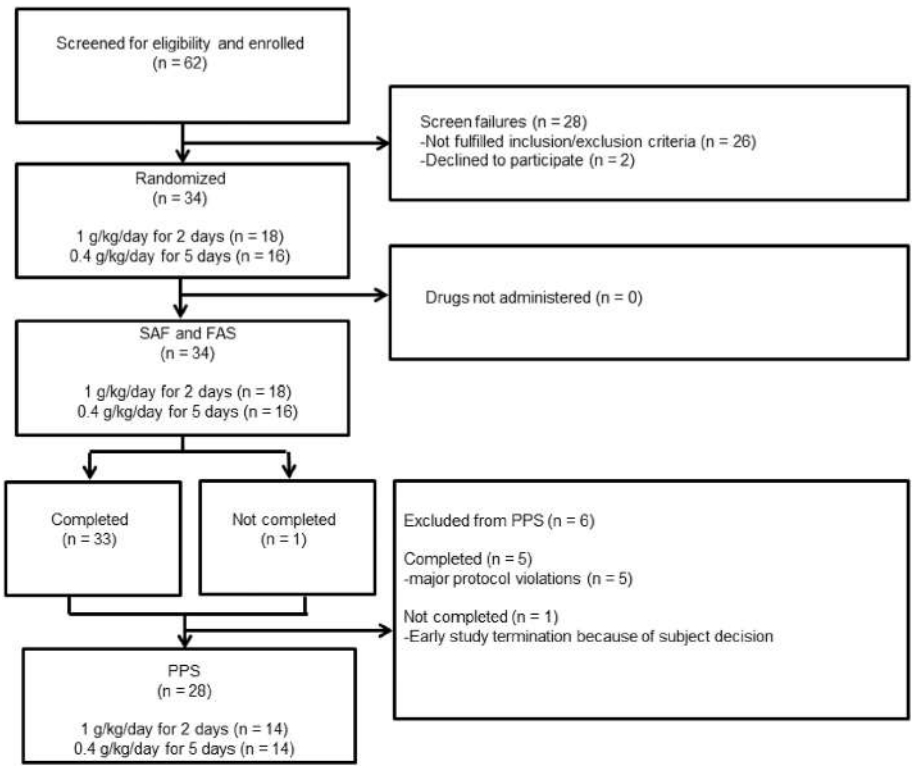

FIGURE 1 Subject disposition. FAS, full analysis set; PPS, per-protocol set; SAF, safety set.

whereby the CR rate was higher for the 2-day treatment schedule (6 of 14 patients [42.9%]; 95% CI: 17.7 to 71.1) than for the 5-day treatment schedule (4 of 14 patients [28.6%]; 95% CI: 8.4 to 58.1). The median time to CR in the FAS was 2.0 days (range: 2 to 3 days). The median duration of CR was 20.0 days (range: 12 to 34 days). Of the 11 patients who achieved CR at least once during the trial, 10 (90.9%; 95% CI: 58.7 to 99.8) had a subsequent loss of CR. There were minor differences in number and percentage of patients with loss of CR between the 2 treatment schedules (83.3% in the 2-day treatment schedule versus 100% in the 5-day treatment schedule).

Rate of R appeared to be dependent on the type of ITP, although data need to be interpreted with caution because of the low number of patients with refractory ITP. In the FAS, only 2 of 7 patients with refractory ITP (28.6%) (95% CI: 3.7 to 71.0) achieved R, while 16 of 27 patients with nonrefractory ITP (59.3%) (95% CI: 38.8 to 77.6) achieved R (note: the 95% CIs overlapped). However, the rate of CR appeared to be independent of the type of ITP. In the FAS, 2 of 7 patients with refractory ITP (28.6%) (95% CI: 3.7 to 71.0) achieved CR and 9 of 27 patients with nonrefractory ITP (33.3%) (95% CI: 16.5 to 54.0) achieved CR. In the current trial with BT595 it was observed that patients with refractory ITP more often required rescue medication (3 of 7 patients; 42.9%) than with nonrefractory ITP (6 of 27 patients; 22.2%).

Further, the rate of R appeared to be independent of the use of ITP medication at baseline. In the FAS, 6 of 12 patients who took ITP medication at baseline (50.0%) (95% CI: 21.1 to 78.9) achieved R and 12 of 22 patients without ITP medication at baseline (54.5%) (95% CI: 32.2 to 75.6) achieved R.

Among the 19 patients in the FAS with a baseline platelet count <20*10^9/L (FDA criteria), 18 patients (94.7%; 95% CI: 74.0 to 99.9) achieved a platelet response ≥50*10^9/L on day 8, that is, within 7 days of the first BT595 infusion. The median time to a platelet count increase to ≥50*10^9/L in this subgroup of patients was 2.0 days (range: 1 to 7 days). Further, the median duration of a platelet count increase to ≥50*10^9/L was 17.0 days (range: 4 to 35 days), with a median duration for the 2-day treatment schedule of 20.0 days (range: 6 to 35 days) and for the 5-day treatment schedule of 14.0 days (range: 4 to 28 days).

In the FAS, all 34 patients (100.0%) (95% CI: 89.7 to 100.0) had a platelet count increase to ≥30*10^9/L until day 5. Furthermore, 30 of 34 patients (88.2%; 95% CI: 72.5 to 96.7) showed platelet counts ≥30*10^9/L 8 days after the first BT595 infusion, 23 of 34 patients (67.6%; 95% CI: 49.5 to 82.6) 15 days, 13 of 34 patients (38.2%; 95% CI: 22.2 to 56.4) 22 days, and 11 of 34 patients (32.4%; 95% CI: 17.4 to 50.5) 29 days after the first BT595 infusion, respectively.

After BT595 administration, the mean platelet count over time showed a pattern typical for treatment with IVIg. Platelet count showed continuous increase from baseline through day 8 and then decreased. In the FAS, the median maximum platelet count achieved was 205.50*10^9/L (normal platelet count), ranging from 32.0*10^9/L to 597.0*10^9/L. The median time to maximum platelet count was 7.0 days, ranging from 3 to 21 days and mean platelet count peak values were most frequently observed on day 8.

In general, signs of bleeding assessment tools (ITP-BAT) showed an improvement over the course of the trial. There were 6 of 34 patients (17.6%) with occurrence of bleeding according to ITP-BAT at baseline. All these patients reported regression of bleeding according to ITP-BAT on day 2. Assessment of bleedings using the WHO bleeding scale confirmed these results. Improvement in platelet counts (percentage of patients with Grade 0 platelets) correlates with a decreased proportion of haemorrhages over time (Figure 2).

TABLE 1 Baseline patient characteristics, ($N = 34$)

Parameter	Value
Age (years)	
Mean (SD)	45.7 (16.86)
Median	48.0
Min – Max	19–74
Gender, *n* (%)	
Male	14 (41.2)
Female	20 (58.8)
Height (cm), Mean (SD)	168.6 (8.64)
Weight (kg), Mean (SD)	78.61 (17.636)
Platelet count at baseline (10^9/L), Mean (SD)	18.52 (12.515)
Baseline platelet count, *n* (%)	
<20*10^9/L	19 (55.9)
≥20*10^9/L	15 (44.1)
ITP medication at baseline, *n* (%)	
Yes	12 (35.3)
No	22 (64.7)
Previous ITP medication	
Patients with at least 1 prior ITP medication	32 (94.1)
Corticosteroids for systemic use	30 (88.2)
Antihemorrhagics	10 (29.4)
Immunosuppressants	10 (29.4)

Abbreviations: ITP, primary immune thrombocytopenia; *N*, number of subjects; *n*, number of subjects in a specified category; SD, standard deviation.

2.3 | Safety

A total of 84 treatment-emergent adverse events (TEAEs) were reported in 34 patients. These TEAEs were defined as either infusional or noninfusional. One of these TEAEs in 1 subject (2.9%) was considered serious. It was defined as an infusional TEAE of anaemia as it occurred within 72 h after infusion 5 in a patient of the 5-day treatment schedule. This serious TEAE was of mild severity and assessed as not related by the investigator and sponsor. There was no fatal case in this trial.

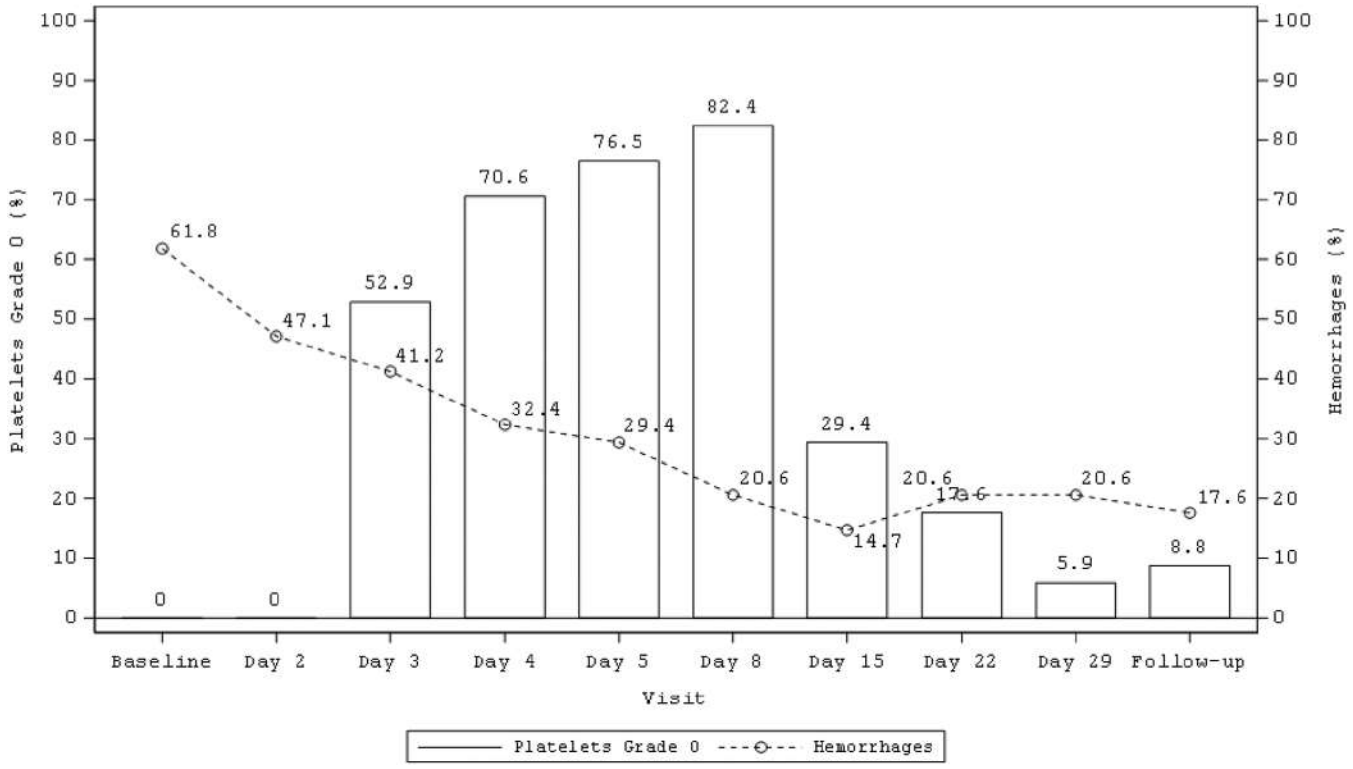

FIGURE 2 Change in Platelet Count (Grade 0 according to WHO Bleeding Scale) Versus All Haemorrhages Over Time – Full analysis set. Platelet Grade 0 was a value ≥100*10^9/L.

Thromboembolic events and (intravascular) haemolysis were both defined as adverse events of special interest prior to the conduct of the trial. No case of thromboembolic events was reported. Five cases (14.7%) of (intravascular) hemolysis were reported. All of them were nonserious TEAEs and assessed as related by the investigator and sponsor.

There was 1 nonserious TEAE (asthma) leading to investigational medicinal product discontinuation reported for 1 of 34 patients (2.9%; in the 5-day treatment schedule). It was defined as an infusional TEAE as it occurred within 24 h after infusion 4. This TEAE was of mild severity and assessed as not related by the investigator and the sponsor.

11 of 34 patients (32.4%) had at least 1 related TEAE; a total of 17 related TEAEs were reported. Related TEAEs observed in more than 1 patient included headache (5 patients; 14.7%), (intravascular) hemolysis (5 patients; 14.7%), and direct antiglobulin/Coombs test positive (2 patients; 5.9%). Twelve of these 17 related TEAEs were infusional and were reported in 8 of 34 patients (23.5%). The only infusional related TEAE observed in more than 1 patient was headache (5 patients; 14.7%). All other infusional adverse drug reactions (ADRs) such as pyrexia, (intravascular) hemolysis, skin reaction, tinnitus, and Coombs test positive each occurred in 1 patient (2.9%). Headache was reported more frequent in the 2-day treatment schedule than in the 5-day treatment schedule (22.2% versus 6.3%, respectively). Noninfusional ADRs occurred in 14.7% of patients: hemolysis (8.8%), intravascular hemolysis (2.9%), and Coombs test positive (2.9%).

Patients received a total of 115 infusions during this trial: 36 in the 2-day treatment schedule and 79 in the 5-day treatment schedule. Of the 36 infusions in the 2-day treatment schedule, 12 (33.3%; upper 95% CI: 51.0) were associated with a TEAE. Of the 79 infusions in the 5-day treatment schedule, 13 (16.5%; upper 95% CI: 26.5) were associated with a TEAE. Therefore, there were fewer infusions associated with TEAEs with the 5-day treatment schedule where the daily dose of BT595 was lower.

In general, BT595 was very well tolerated; even in patients with a higher infusion rate (4 of 16 patients in the 5-day treatment schedule had a maximum infusion rate of ≥4 ml/kg/h). The maximal administered infusion rate in this trial was ≥2 to <4 ml/kg/h for all 18 patients in the 2-day treatment schedule according to Clinical Trial Protocol. In the 5-day treatment schedule, the maximal administered infusion rate was ≥2 to <4 ml/kg/h for 12 of 16 patients (75.0%), ≥4 to <6 ml/kg/h for 3 of 16 patients (18.8%), and ≥6 ml/kg/h for 1 of 16 patients (6.3%).

3 | DISCUSSION

There is heterogeneity in defining response in previous studies of IVIg preparations. The majority of studies used a cut-off platelet

TABLE 2 Comparison with other studies following revised European Medicines Agency (EMA) guidelines

	Arbach et al. (2019)[12a]	Rodeghiero et al. (2018)[14]	Hong et al. (2018)[13]	BT595
Baseline characteristics				
n	36 in FAS (total 40 enrolled)	38	81 (43 with chronic ITP)	34
Mean age (SD), years	36.7 (15.3)	37.2 (11.8)	53.9 (17.5)	45.7 (16.9)
Dosage 2 g/kg (bw)	2 days	2 days	2 days	2 or 5 days
Subjects with platelet count ≤20*10^9/L, *n* (%)	36 (100)	23 (60.5)	81 (100)	19 (55.9)
Refractory ITP and/or splenectomy, *n* (%)	10/40 (25.0)	6/38 (15.8)	6/81 (7.4)	7/34 (20.6)
Median or mean (SD) time since diagnosis, months	Median: 47.2 (range 13–317)[b]	Median: 43.8 (range 12–488.4)	Mean: 55.0 (7.83)	Median: 81.5 (range 12–420) Mean: 116.2 (105.17)
Efficacy variables				
Response (R), *n*/*N* (%); [95% CI]	24/36 (66.7); [unknown][c]	24/38 (63.2); [46.0–78.2][d]	37.7[d]/81 (46.5); [35.6–57.5][c]	18/34 (52.9); [35.1–70.2][d]
Median or mean (SD) duration of R, days	Unknown	Median: 13.5 (95% CI: 10.0–20.0)	Mean: 17.2 (7.5)	Median: 28.0 (range: 18–36) Mean: 26.8 (6.95)
Platelet response ≥50*10^9/L (FDA), *n*/*N* (%); [95% CI]	29/36 (80.6); [63.98–91.81]	19/23 (82.6) [61.2–95.0] (previous EMA guideline)	61.3[e]/81 (75.7) [1-sided 97.5% lower CI: −3.90]	18/19 (94.7) [74.0–99.9]
Complete Response (CR), *n*/*N* (%); [95% CI]	18/36 (50.0); [unknown]	11/38 (28.9); [15.4–45.9]	13.9/81 (17.2); [8.8–25.5]	11/34 (32.4) [17.4–50.5]
Mean platelet count over time – peak value (*10^9/L)[e]	Unknown	139.4 (day 7)	113.14 (day 8)	186.25 (day 8)
Median or mean (SD) duration of platelet response ≥50*10^9/L (FDA), days	Median: 14.0 (95% CI: 10.00 to 17.00) Mean: 12.4 (95% CI: 10.17 to 14.59)	unknown	Mean: 9.13 (8.40)	Median: 17.0 (range: 4–35) Mean: 17.2 (8.67)
Mean maximum platelet count (*10^9/L)	237	165	unknown	224.81
n/*N*, Proportion (percentage) of subjects with bleedings at baseline that completely resolved	18/23 (78.3) by day 8	16/29 (55.2) by unknown day	unknown	6/6 (100) by day 2
Earliest confirmation of R (at least 7 days apart)	Individually determined	Visits on days 9, 11, 14, 21, and 30	Visits on days 8, 11, 15, 22, 29, and 85	Visits on days 8, 15, 22, 29, and approx. 36

Abbreviations: bw, body weight; CR, complete response; EMA, European Medicines Agency; FAS, full analysis set; FDA, Food and Drug Administration; ITP, primary immune thrombocytopenia/idiopathic thrombocytopenic purpura; IVIg, intravenous immunoglobulin; R, response; SD, standard deviation.

[a]Food and Drug Administration Clinical Review 2015 – PANZYGA®. https://www.fda.gov/media/116097/download.

[b]In total of 40 enrolled subjects.

[c]Secondary endpoint.

[d]Primary endpoint.

[e]Subject number was calculated with multiple imputations for missing values and the first digit after the decimal point is reported.

count of < (or ≤) 20*10^9/L at baseline and response was defined as an increase in platelet count to ≥50*10^9/L within 7 days after administration (FDA criteria). These criteria are almost identical to the criteria described in the previous EMA guideline[15] in which R was defined as subjects with a baseline platelet count of about 20*10^9/L who achieved a platelet count of ≥50*10^9/L on at least 1 evaluation during the trial.

This open-label, prospective, randomised, multicenter phase III study is 1 out of 4 clinical trials using the latest, more stringent definition of response from the revised EMA guidelines[7,10] to evaluate the efficacy of an IVIg in patients with chronic ITP. Although these guidelines have a less stringent platelet count cutoff for defining response (≥30*10^9/L vs. ≥50*10^9/L previously), additional criteria need now to be met (≥2-fold increase from baseline; confirmed at 2 visits at least 7 days apart; absence of new bleeding) making R more difficult to achieve. However, these response criteria better reflect the sustainability of platelet count increase and additionally focus on stopping or successfully preventing bleedings (bleeding control). In

this trial, careful attention was also paid to the absence of prohibited drugs that could lead to treatment failure.

The R rate in the current trial with BT595 was 52.9%. As expected, the more stringent endpoint according to the revised EMA guidelines resulted in a lower response rate than the response rate in other studies using FDA or previous EMA criteria (response rate in these studies was reported to be in between 70% and 91.7%). In comparison with the three other studies that followed revised EMA guidelines to determine R, the efficacy from a clinical point of view was comparable, as response rates were between 46.5% and 66.7% (Table 2).

The effect of IVIg in ITP is rapid but usually transient and platelet count drift back to near pretreatment levels 3 to 4 weeks following IVIg treatment.[16] The highest estimated platelet count is reached around days 6 to 8 after start of treatment.[12–14,17] Thus, a transient response because of a deteriorating platelet count on day 15 (compared to earlier timepoints like day 9 or 11) is expected and consistent with response patterns generally observed after treatment with IVIgs.

It should be noted that those other three studies confirmed response at an earlier point in time (e.g., day 9 or 11) than in the current trial (day 15) (Table 2). However, since not only a 2-day treatment schedule was evaluated in the current trial, but also a 5-day treatment schedule, confirmation of R after treatment with BT595 was fixed for all patients on day 15 (7 days apart from day 8, which was considered as latest time point achieving response in the 5-day treatment arm). Response rates within the different trials are not determined at the same time point and thus results are varying.

The median time to R in the current trial with BT595 was fast (median 1.0 day) and similar to the studies of Rodeghiero et al.[14] and Arbach et al.[12] Nonetheless, the median/mean duration of R (median 28 days, mean 26.8 days) was better in this trial than in the studies of Rodeghiero et al.[14] (median 13.5 days) and Hong et al.[13] (mean 17.2 days).

The secondary endpoint analyses supported the efficacy of BT595. Especially, the response rate of 94.7% in the subgroup of 19 patients with a baseline platelet count <20*10^9/L who achieved a platelet count increase to ≥50*10^9/L on day 8 (FDA criteria) compares favourably with response rates reported for approved IVIg products.

The median time to platelet response ≥50*10^9/L in the current trial with BT595 (median 2.0 days) was similar to the studies of Arbach et al.[12] and Hong et al.[13] The median/mean duration of platelet response ≥50*10^9/L (median 17.0 days, mean 17.2 days) was better than in these two studies (median 14.0 days and mean 9.13 days, respectively).

The CR rate in the current trial with BT595 was 32.4%. This is comparable to the rate observed in the studies of Rodeghiero et al.[14] (28.9%) and Hong et al.[13] (17.2%) but lower than in the study of Arbach et al.[12] (50.0%). The median/mean duration of CR (median 20 days, mean 21.1 days) was better in this study than in the studies of Rodeghiero et al.[14] (median 12.0 days) and Hong et al.[13] (mean 12.1 days). The mean platelet count over time-peak value was higher in the current trial with BT595 (186.25*10^9/L) than in the studies of Rodeghiero et al.[14] (139.4*10^9/L) and Hong et al.[13] (113.14*10^9/L).

Due to the quite rapid increase of platelets, it has been discussed whether a single dose of 1 g/kg bw might be sufficient to treat this indication. However, in accordance with the current guidelines[7] this dosage was not tested in the trial.

TABLE 3 Subjects with drug related TEAEs (>5%)—Comparison with other studies following revised European Medicines Agency (EMA) guidelines

	Arbach et al. (2019)[12] (*n* [%]) *N* = 40	Rodeghiero et al. (2018)[14] (*n* [%]) *N* = 38	Hong et al. (2018)[13] (*n* [%]) *N* = 81	BT595 (*n* [%]) *N* = 34
Overall	23 (57.5)	25 (65.8)	46 (56.8)	11 (32.4)
Headache	13 (32.5)	13 (34.2)	33 (40.7)	5 (14.7)
Pyrexia	8 (20.0)	6 (15.8)	5 (6.2)	0
Nausea	5 (12.5)	2 (5.3)	10 (12.4)	0
Vomiting	4 (10.0)	3 (7.9)	6 (7.4)	0
Creatinine renal clearance decrease or GFR decrease	0	4 (10.5)	0	0
Systolic blood pressure increase	0	3 (7.9)	0	0
Body temperature increase	0	2 (5.3)	0	0
Influenza like illness	0	2 (5.3)	0	0
Arthralgia	0	2 (5.3)	0	0
Chills	0	0	7 (8.6)	0
Hemolysis	0	0	0	3 (8.8)
Intravascular hemolysis	0	0	0	2 (5.9)
Coombs test positive	0	0	0	2 (5.9)

Abbreviations: GFR, glomerular filtration rate; *N*, number of subjects; *n*, number of subjects in a specified category; TEAE, treatment-emergent adverse event.

Improvement of bleeding status also provided evidence of the therapeutic effect of BT595. Bleedings present at baseline resolved under therapy; new bleeding events reported in this trial were representative of the disease and occurred later during the trial period (≥day 15), when platelet response decreased in some patients. In the current trial, the bleeding assessment tool (ITP-BAT) according to the standardisation defined by Rodeghiero et al.[18] was used. Different assessment tools have been used in other studies.

In the current trial with BT595, the mean time (SD) since diagnosis was 116.2 (105.17) months and the median time since diagnosis was 81.5 months (Table 2). This is longer than the time since diagnosis in the other three studies, indicating a sicker population (Arbach et al.[12]: median of 47.2 months; Rodeghiero et al.[14]: median of 43.8 months; Hong et al.[13]: mean of 55.0 months). The population (mean age, Table 2) in two of these studies was also younger. In summary, BT595 seems to be as effective as the preparations used in the three other studies.

BT595 has been shown to have a favourable benefit/risk ratio and was well tolerated. Safety evaluations, including adverse events (AEs), laboratory values, physical examinations, and vital signs did not uncover any unexpected safety issues. Most TEAEs were mild or moderate in severity and were not considered product-related. No fatal case and no related serious TEAE occurred during the trial.

Headache was observed in the three other studies in 32.5%, 34.2%, and 40.7% of patients,[12–14] respectively (Table 3). Thus, in this trial the rate of headache was less than half of the rate observed in the three competitor products. In addition, more patients experienced pyrexia (20.0%, 15.8%, 6.2%), nausea (12.5%, 5.3%, 12.4%), and vomiting (10.0%, 7.9%, 7.4%) than in this trial (<5%).

Of the 115 infusions of BT595 administered during the trial, most (90 infusions [78.3%]) were not temporally associated with a TEAE. Of the 36 infusions in the 2-day treatment schedule, 12 (33.3%) (upper one-sided 95% CI: 51.0) were associated with a TEAE. Of the 79 infusions in the 5-day treatment schedule, 13 (16.5%) (upper one-sided 95% CI: 26.5) were associated with a TEAE. This was slightly lower than in the study of Rodeghiero et al.[14] where 36 of 73 infusions (49.3%) were associated with at least 1 TEAE. The studies of Arbach et al.[12] and Hong et al.[13] did not report the amount of infusions associated with adverse events. During our trial, only one patient received premedication.

A comparison between patients with drug related TEAEs (>5%) of the three other studies that followed revised EMA guidelines and the current trial showed that BT595, with the exception of patients with (intravascular) hemolysis and Coombs test positive, has a lower occurrence of patients with related TEAEs than competitor products (Table 3). In comparison, obviously more patients experienced headache, pyrexia, nausea, and vomiting than in this trial and thus it can be concluded that BT595 is better tolerated than the three competitor products.[12–14] While these three most relevant competitor studies investigated only a 2-day treatment schedule; this trial investigated also a 5-day treatment schedule. For sensitive patients, the 5-day treatment schedule could be a good option for therapy as it showed less side effects with similar efficacy.

Regarding patients experiencing (intravascular) hemolysis and Coombs test positive, Arbach et al.[12] reported laboratory abnormalities consistent with hemolysis in 6 of 40 patients (15·0%). In the study of Rodeghiero et al.[14] positive direct antiglobulin tests without biological evidence of hemolysis were observed in 2 of 38 patients (5.3%). Furthermore, it should be noted that it is very difficult to compare a condition that is mostly a transient laboratory finding without clinical symptoms. Respective changes of laboratory values used for diagnosis of (intravascular) hemolysis and Coombs test positive are only detectable during a short time frame after administration of IVIgs. It is known that a positive Coombs test reaction is strongly related to autoimmune hemolysis, but because of rapid removal of sensitised red blood cells it will be positive for the first few days only,[19–21] respectively 4 to 5 days after IVIg administration.[22]

As a summary, BT595 was effective in rapidly increasing platelet counts in patients with ITP. Following administration of 2 g/kg bw BT595, the response rate (defined by revised EMA criteria), and time to and duration of response were similar to other IVIg 10% preparations. Assessment of response by using FDA criteria additionally demonstrated not only the efficacy of BT595, but also underlined the differences in response rates seen by using the two different criteria for defining response.

AUTHOR CONTRIBUTIONS

Judit Demeter was Coordinating Investigator and edited the manuscript, Aryan Hamed, Szerafin László and Nada Suvajdzic were investigators and reviewed the manuscript. Christiane Staiger, Silke Aigner and Birgit Börner were involved in the management and reporting of the study. The first draft of the manuscript was written by Christiane Staiger and all authors read and approved the final manuscript.

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CONFLICT OF INTEREST

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CASE STUDY

Acute haemolytic transfusion reaction after transfusion of fresh frozen plasma in a neonate—Preventable by using solvent/detergent-treated pooled plasma?

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Abstract

Background: Plasma is a commonly used blood product and is available in the form of fresh frozen plasma (FFP) or pooled solvent/detergent-treated plasma. In the Netherlands, solvent/detergent-treated plasma has become the standard product in the adult population since several years, but for neonatal use, FFP remains the product of preference.

Description: A preterm neonate developed lung bleeding at day 8 postpartum, for which intubation and mechanical ventilation was required and transfusions with packed red blood cells and plasma, in the form of FFP, were given. Five hours after transfusion, a red discoloration of the urine occurred. An acute haemolytic transfusion was suspected, confirmed by laboratory investigations (fast decrease in haemoglobin, increased free haemoglobin, decreased haptoglobin, increased lactate dehydrogenase and a positive direct antiglobulin test [IgG 2+]). Additional research showed that the FFP product contained nonspecific auto-antibodies that reacted with the transfused erythrocytes, most test erythrocytes and the donor's own erythrocytes.

Conclusion: A neonate experienced an acute haemolytic reaction, most probably caused by administrating a FFP product containing auto-antibodies. If transfused with solvent/detergent-treated plasma, such antibodies would have been diluted or captured. This case adds a new argument to the discussion on expanding the use of solvent/detergent-treated plasma to the paediatric population.

KEYWORDS
fresh frozen plasma, neonatal transfusion, solvent/detergent-treated plasma, transfusion reaction

1 | INTRODUCTION

Plasma is a commonly used blood product, mainly for treatment and prevention of bleeding in patients with coagulopathy and as replacement fluid in plasma exchange.[1] In the Netherlands, around 60 000 units of plasma are transfused annually.[2] Plasma products include fresh frozen plasma (FFP) and pooled solvent/detergent-treated plasma (SD plasma). FFP is a product derived from a single donor, while SD plasma is a product made by pooling several hundreds of plasma units.[3] In the Netherlands, SD plasma (Omniplasma®, made from Dutch donor plasma, manufacturer: Octapharma, Austria) has become the standard product in plasma transfusion and plasma exchange for adults since 2014. However, for neonatal use FFP is still the product of first choice. This is mainly caused by two reasons. First, there is limited evidence of SD plasma in neonatal use. There are no large high-quality randomised controlled trial available comparing efficacy and safety of FFP to SD plasma products, specifically in children. The evidence available consists of smaller or observational studies investigating SD plasma in paediatric use.[4–8] Due to this limited evidence, the product information of SD plasma states that experience with use in children is limited.[9] However, it should be noted that high-quality large trials for FFP in paediatric or neonatal use are lacking as well, but the main difference is that FFP is not registered as drug, and therefore stricter regulations apply for SD plasma. The second reason for preference of FFP over SD plasma is related to the costs and volume of the plasma products. FFP is available in both 150 and 300 mL, and preterm neonates generally need a volume lower than 150 mL, so the smaller (and cheaper) product is sufficient. SD plasma is available in only 200 mL, which has the same price as 300 mL FFP. Therefore use of SD plasma for neonatal use would result in higher costs. A Canadian study investigating cost-effectiveness of SD plasma and FFP, also showed that replacement of all FFP by SD plasma would increase costs with $16.5 million per year.[10]

However, we question if the preference of FFP over SD plasma is correct. We describe a case in which FFP was administered to a neonate, resulting in a transfusion reaction. In our opinion, this transfusion reaction would most likely not have occurred if SD plasma had been given to this patient, therefore we advocate for considering expanding the indication of SD plasma and include paediatric and neonatal use.

1.1 | Patient and clinical information

A preterm neonate, born after 28 + 5 weeks of pregnancy and part of a dichorionic twin pair, had a uncomplicated clinical course during the first week after birth. However, at day 8 postpartum lung bleeding of unknown aetiology occurred, for which intubation and mechanical ventilation was required and transfusion with packed red blood cells (pRBCs) (15 mL kg^{-1}) was given. Given the acute bleeding and abnormal coagulation parameters, vitamin K and plasma, in the form of FFP, (15 mL kg^{-1}), were given. The activated partial thromboplastin time and haemoglobin value improved after the transfusion of pRBCs and plasma (Table 1). However, 5 h after transfusion, discoloration of the urine into red/reddish brown urine started, which lasted for 1 day. The patient also experienced a mild rise in body temperature (38.2°C). Based on these symptoms, the occurrence of an acute haemolytic transfusion reaction was suspected, and additional laboratory measurements were performed (Table 1). The decreased haptoglobin, increased free haemoglobin, the increase in lactate dehydrogenase and the fast decrease in haemoglobin from 7.7 mmol L^{-1} (12.4 g dL^{-1}) just after the transfusion to 6.6 mmol L^{-1} (10.6 g dL^{-1}) the day

TABLE 1 Laboratory values of patient around time of lung bleeding and transfusion

Laboratory value	Day 8 postpartum (in evening lung bleeding and transfusion)	Day 9 postpartum	Reference value
Haemoglobin[a]	4.6 → 7.7[b] [7.4 → 12.4[b]]	6.6 [10.6]	7.5–10.0 mmol L^{-1} [12.1–16.1 g dL^{-1}]
LDH	415[e]	1286	<600 U L^{-1}
Haptoglobin	–	<0.1	Unknown in children <1 year 0.3–2.0 g L^{-1} for age > 1 year
Free haemoglobin	–	28	<2 μmol L^{-1}
Total bilirubin[a]	86 → 100[b]	160	<17 μmol L^{-1}
APTT	88.0	45.2	26.9–74.1 s[d]
PT	18.9	21.6	10.0–15.3 s[d]
Fibrinogen	2.6	3.9	1.6–4.2 g L^{-1}[d]
Direct antiglobulin test	Negative[c]	Positive (IgG 2+)	Negative

Abbreviations: APTT, activated partial thromboplastin time; LDH, lactate dehydrogenase; PT, prothrombin time.

[a]Measured in an arterial blood gas syringe.

[b]First value is before transfusion of packed red blood cells, second value after transfusion.

[c]Reference values for healthy preterm neonates at day 5. These reference values are used at the neonatology department and are derived from Andrew et al.[11]

[d]Result of this direct antiglobulin test is before transfusion of blood products.

[e]Measured at day 7 postpartum. No measurement from day 8 postpartum.

after transfusion were suggestive for a haemolytic reaction. In the urine sediment, no intact erythrocytes were seen, ruling out a nephrological or urological cause for the discoloured urine. Since the raised body temperature could possibly be attributable to a bacterial infection, broad-spectrum antibiotic treatment was started and stopped after 3 days when bacterial cultures turned out negative.

The patient recovered after the lung bleeding and haemolytic reaction, however at day 15 postpartum another lung bleeding occurred. At this time no deviations in coagulation parameters were apparent, but a repeated plasma transfusion was given, together with an pRBC transfusion. Haemoglobin concentration increased from 5.1 mmol L^{-1} (8.2 g dL^{-1}) to 7.4 mmol L^{-1} (11.9 g dL^{-1}) without any clinical signs of a subsequent transfusion reaction.

The aetiology of the lung bleeding was unknown and was considered to be most likely related to the prematurity of the patient or the patent ductus arteriosus that appeared to be present at day 8 after birth. A CT scan of the lungs did not show any abnormalities. The twin sibling also experienced a bleeding (stomach bleeding), therefore a hereditary coagulation disorder was considered as well. However, as coagulation parameters were not aberrant during the second bleeding, and there was no family history of coagulation disorders, this was considered unlikely. At the age of 7 months, Von Willebrand disease was investigated for both siblings, but results showed no abnormalities. The twins did not experience an increased bleeding tendency anymore and were in good health.

1.2 | Laboratory investigations of transfusion reaction

After suspicion of the acute haemolytic transfusion reaction, additional laboratory investigation with a blood sample of the patient, drawn after the reaction, and material of the transfused blood products was performed. The direct antiglobulin test (DAT) of the neonate was positive for IgG 2+. In comparison, a DAT performed on the day of birth was negative. Although the DAT was positive, the eluate by both freeze and acid elution methods did not show any reactions against reagent red cells. AB0-antagonism was ruled out (the mother was A positive, neonate AB positive, transfused pRBCs 0 negative and transfused FFP AB positive). No anti-A or anti-B IgG antibodies were shown for the mother. Additional research with a blood sample from the mother of the neonate was performed to investigate if maternal antibodies transferred during pregnancy could have caused a transfusion reaction. However, a screening panel of the mother was negative and cross-matching of the transfused pRBCs with plasma of the mother did not produce a positive reaction.

As the laboratory tests in the hospital laboratory did not result in an explanation for the transfusion reaction, additional investigation on the transfused products was performed at the national immunohematology reference laboratory of the Netherlands (Sanquin Diagnostic Services, Amsterdam, the Netherlands). Here, serologic analysis was performed with samples of the same FFP-donor and fresh blood samples of the pRBC donor. A blood sample of the patient was not available anymore. The tests exposed that there were antibodies present in plasma of the FFP donor. The antibodies reacted with the erythrocytes of the pRBC donor, but also with most test erythrocytes (reagent cells) in LISS column agglutination techniques. The DAT of the erythrocytes from the FFP-donor was positive with anti-IgG (2+) while acid elution resulted in weak reactions with reagent cells in LISS column agglutination techniques. The antibodies of the FFP donor were considered to be nonspecific auto-antibodies. Other FFP units from this donor were recalled by Sanquin Blood Bank, and samples from these products showed the same reactivity. No transfusion reactions were reported for the already transfused FFP units. The FFP donor had no signs of haemolysis. The screening for RBC antibodies at the time of the first donation was negative. According to the Dutch guidelines there was no screening for RBC antibodies performed on the current donation.

2 | DISCUSSION AND CONCLUSION

In this report we describe a neonate that developed an acute haemolytic reaction, with red discoloured urine, which was possibly caused by transfusion of a FFP product containing auto-antibodies. These weak autoantibodies of the FFP donor caused acute haemolysis presumably of the transfused donor erythrocytes after both products were transfused.

Published literature reviews and guidelines show that studies to guide plasma use in neonates are limited. There is no clear evidence for prophylactic plasma transfusions to neonates with abnormal coagulation values.[12–14] In the neonatal intensive care unit where the patient in this case report was treated, the use of plasma transfusions decreased significantly over the years (analysed between 2004 and 2019).[15]

FFP is in the Netherlands a product derived from a single donation of male donors. The FFP product is frozen within 24 h after collection and then stored frozen for a period of at least 6 months after which it can be released if tests for blood transmittable infectious diseases for the donor are negative after the second donation. Male-only plasma is used since 2007 to reduce the risk of transfusion-related acute lung injury caused by anti-human leukocyte antigen and/or neutrophil antibodies which are more prevalent in the female population.[16]

SD plasma is a product in which plasma from 600 to 1200 donors is pooled. The pooling of hundreds to thousands of single-donor plasma units, reduces neutralising antibodies present in the plasma pool through dilution, lowers the antibody titres against blood cells and plasma proteins, resulting in an improved safety profile.[3,4] The manufacturing process includes several steps to remove pathogens: by a solvent/detergent treatment enveloped viruses are eliminated, and by filtration with nanofilters the non-enveloped blood transmittable infectious diseases including prions are removed, except for very small non-enveloped viruses such as Parvo B19. This is monitored by PCR-testing of batches.[3] Due to this pooling, SD plasma has a standardised content of plasma proteins, in contrary to FFP plasma, which is prone to interindividual differences between donors. A drawback is that SD plasma had reduced protein S concentrations compared with

FFP, which can contribute to risk of thrombosis in patients receiving large volumes for plasma exchange.[17]

Arguments against the use of SD plasma in neonatal or paediatric care is due to limited literature evidence for the use of SD plasma in children, and therefore is not included for paediatric indications in the drug label. In addition, due to the pooling, multiple donor exposure in neonates can result in increased risk of adverse effects like transfusion transmitted infections and immunomodulation.[18]

Although literature evidence of SD plasma in children is limited, there are several published studies. In a large prospective observational study the use of FFP and SD plasma were compared in critically ill children in 101 paediatric intensive care units in 21 countries.[4] 419 paediatric patients (median age 1 year, interquartile range 0.2–6.4 years) were included, of which 357 received FFP and 62 SD plasma. ICU mortality was lower in the patient group treated with SD plasma (14.5%) compared with the FFP group (29.1%, $P = 0.02$). The effect on the coagulation parameter international normalised ratio (INR) was comparable for both groups (in both groups a reduction of 0.2 of the INR, $P = 0.80$).[4]

A retrospective study investigated paediatric cardiac surgical patients that were aged below 2 years and less than 10 kg (undergoing complete tetralogy of Fallot repair), that received either FFP or SD plasma (OctaplasLG) during surgery.[7] The study included 105 patients over a 10-year period, of which 5 years of FFP use and 5 years of SD plasma use. The study showed that SD plasma was as effective in achieving haemostasis as FFP.[7]

In a recently published phase IV study in which the safety, tolerability and efficacy of SD plasma (Octaplas) was investigated, 50 patients aged <16 years were included, of which 37 were 0–2 years old.[5] No hyperfibrinolytic or thromboembolic events or adverse drug reactions were reported after transfusion of SD plasma. Although a relatively small study population, this study suggests that SD plasma can be used safely in this paediatric population.[5]

The patient described in this report experienced an acute haemolytic reaction, possibly related to FFP. In general, an acute haemolytic reaction on FFP is very rare. In 2019, 17 events of an acute haemolytic transfusion reaction were reported in the Netherlands, but non-related to a plasma product (16 related to a red cell product, 1 to a platelet transfusion).[2]

For neonatal transfusions, it can also be considered to perform minor cross-matching (i.e. combining donor serum and recipient red blood cells), which can prevent incompatibilities and transfusion reactions such as described in this case. Minor cross-match is however not standard practice in the Netherlands for (neonatal) plasma transfusions. As long as the unit of plasma is ABO compatible with the recipient and plasma of the donor is screened for unexpected non-ABO antibodies a minor cross-match is not required. We did consider a retrospective minor cross-match with the FFP donor in this case, but unfortunately there was no blood sample left of the recipient. More regular antibody screening of the FFP donor could perhaps also have prevented this problem, but currently, according to Dutch guidelines, only first donations are screened for RBC antibodies.

In the Netherlands, SD plasma is generally not used in a paediatric population. Therefore, the neonate in this case was given a plasma transfusion with FFP, according to standard of care. Also in guidelines for neonatal transfusion in the United Kingdom, only use of FFP is recommended.[19] We believe that the transfusion reaction most likely would not have happened if SD plasma would have been given to this patient, as antibodies such as the ones present in the FFP donor in this case are diluted in pooled SD plasma. This case adds a new argument to the discussion on expanding the use of solvent/detergent-treated plasma to the paediatric population.

AUTHOR CONTRIBUTION

None.

CONFLICT OF INTEREST

The authors have no competing interests.

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