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

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

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

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REVIEW

Filariasis and transfusion-associated risk: a literature review

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Abstract

Background and objectives Filariae are parasitic worms that include the pathogens *Loa loa*, *Onchocerca volvulus, Wuchereria bancrofti*, *Brugia* spp. and *Mansonella* spp. which are endemic in parts of Africa, Asia, Asia-Pacific, South and Central America. Filariae have a wide clinical spectrum spanning asymptomatic infection to chronic debilitating disease including blindness and lymphedema. Despite successful eradication programmes, filarial infections remain an important –albeit neglected – source of morbidity. We sought to characterize the risk of transfusion transmission of microfilaria with a view to guide mitigation practices in both endemic and non-endemic countries.

Materials and methods A scoping review of scientific publications as well as grey literature was carried out by a group of domain experts in microbiology, transfusion medicine and infectious diseases, representing the parasite subgroup of the International Society of Blood Transfusion.

Results Cases of transfusion-transmitted filariasis are rare and confined to case reports of variable quality. Transfusion-associated adverse events related to microfilariae are confined to isolated reports of transfusion reactions. Serious outcomes have not been reported. No known strategies have been implemented, specifically, to mitigate transfusion-transmitted filariasis yet routine blood donor screening for other transfusion-transmissible infections (e.g. hepatitis B, malaria) may indirectly defer donors with microfilaremia in endemic areas.

Conclusion Rare examples of transfusion-transmitted filariasis, without serious clinical effect, suggest that filariasis poses low transfusion risk. Dedicated mitigation strategies against filarial transfusion transmission are not recommended. Given endemicity in low-resource regions, priority should be on the control of filariasis with public health measures.

Key words: blood transfusion, public health, epidemiology, filariasis.

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Introduction

Filariae belong to the phylum *Nematoda*. These parasitic worms require an arthropod vector to establish a reproductive infection in humans whereby an infectious larval form enters the arthropod bite wound, develops into an adult worm that releases microfilariae into the blood [1–3]. Filariae that are pathogenic to humans include *Loa loa*, *Onchocerca volvulus, Wuchereria bancrofti*, *Brugia* spp. and *Mansonella* spp. These pathogens are broadly distributed across Africa, Asia, Asia-Pacific, South and Central America [4–13]. There is a wide

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clinical spectrum spanning asymptomatic infection [14] to chronic, and often debilitating, disease including blindness [15] and lymphedema (i.e. elephantiasis) [16–19].

Concerted eradication efforts have been underway to address filariasis. For example, lymphatic filariasis (LF) has been the target of mass drug administration (MDA) programmes over a 20-year period [6, 7, 20–22]. As a result, the number of people infected with the nematode causing LF has decreased (i.e. from 199 million in 2000 to 51 million in 2018). However, focal areas of endemicity remain in Africa and South-East Asia [23].

The overarching question is whether there is transfusion-associated risk either from donors living in filariasis endemic countries or from donors who have travelled to those countries. We reviewed the literature to characterize the risk of transfusion-transmitted filaria to inform related recommendations and blood transfusion policy.

Materials and methods

A scoping literature review was undertaken that focused on the following elements: (1) the epidemiology and clinical burden of filariasis in both general and blood donor populations, (2) available diagnostic testing for filariasis and surveillance strategies (e.g. in donors), and (3) estimation of the risk of transfusion-transmitted filariasis along with extant mitigations strategies.

In this review, the terms "microfilaria", "filaria" (singular) and "filariae" (plural) were used to generally describe the nematode pathogens. The term "filarial infection" was used to generally describe infections with filariae. The term "filariasis" were used to generally describe the diseases caused by this nematode pathogen. The term "microfilaremia" was used to describe the blood phase of the "microfilaria".

In order to capture the broadest information about filariae, two broad search approaches were used for this review. This first approach was a scoping literature review that was conducted using two searches engines: PubMed (The United States National Library of Medicine at the National Institutes of Health, Bethesda, MD, USA) and Google (Google, Menlo Park, CA, USA). Both search engines were used to query individual terms, phrases, and additive combinations including "filaria", "filariasis", "vectors", "entry phase", "anatomic location of adults", "anatomic location of microfilaria", "pre-patent period", "disease severity in naturally infected individuals", "human vaccine present", "epidemiology" and "diagnostic testing available".

For the second approach, a review of the literature was undertaken primarily using search tools with medical subject headings that are listed in Table S1. Dates for searches were as follows: Embase (Elsevier, Amsterdam, Netherlands):1974–2020, Scopus (Elsevier, Amsterdam, Netherlands): No time limit until 2020, Web of Science (Clarivate Analytics, Philadelphia, PA, USA):1900–2020. In general, these reviews included published papers in English and published papers in other languages with English abstracts. If abstracts were not available, then the manuscripts were accessed, if available to SJD's academic institution, to determine relevance. Articles were not considered relevant if they did not directly discuss human filariasis or did not address human health issues (e.g. animal models, veterinary medicine). We did not include meeting abstracts in published proceedings.

A detailed description of members of the genus *Dirofilaria* which cause dog, racoon and felid heartworm has not been included.

Results

The life cycle of filarial nematodes in potential blood donors

Several genera and species of filarial nematodes can infect humans, with a varied clinical spectrum spanning asymptomatic infection to severe, chronic and debilitating disease (Tables 1 and 2). Infected arthropod vectors introduce L3 larva (third larval stage of nematode, infective to humans) via bites into humans. Adult worms develop, mate and produce microfilariae that circulate in the blood. Those circulating microfilaria may be transmissible by blood transfusion, although this is regarded as a dead-end route of transmission, thus precluding further reproduction [24]. The variability in timing or presence of periodicity in the blood between different genera of microfilariae suggests that the risk of collecting a positive blood donation would not be uniformly distributed across all genera (Tables 3 and 4). Not all filarial nematodes involve blood, for example, microfilariae from the species *M. streptocerca* primarily reside in the dermis and subcutaneous tissue and rarely reach the peripheral blood [13, 25] (Table 2).

Epidemiology

Factors that affect the prevalence of filariae in human populations include the environment and its impact on human and vector behaviour, the potential for human–vector interaction, and public health use of anti-filarial agents to treat large populations. Human filarial infections can be categorized by vector genus and/or species, genus and/or species of nematode, and the associated disease process (Tables 1 and 2). For example, similar clinical manifestations such as elephantiasis can be caused by different nematodes (e.g. *W. bancrofti* and *Brugia* spp.).

Table 1 Epidemiology

	Loa loa	***Onchocerca volvulus***	***Wuchereria bancrofti***	***Brugia malayi***	***Brugia timori***	***Mansonella perstans***	***Mansonella ozzardi***	***Mansonella streptocerca***
Vector	Tabanid flies; *Chrysops silacea*, *C. dimidiate*	Blackfly; *Simulium*	Mosquitos; Genera and species vary geographically; *Culex, Anopheles* [67], *Coquillettidia* The major vectors of *W. bancrofti* are mosquitoes of the genus *Culex* (in urban and semi-urban areas), *Anopheles* (in rural areas of Africa and elsewhere) and *Aedes* (in islands of the Pacific	Mosquitos; *Mansonia*, *Aedes*, *Anopheles*, *Coquillettidia* [68, 69]	Mosquitos: *Aedes* [70], *Anopheles* [71]	Biting midges *Culicoides* [30]	*Black flies*, *Simulium or* biting midges *Culicoides*	Biting midges *Culicoides* [13]
Presence in blood donors	Described in Nigeria [52] Can survive in stored blood > 21 days [72]	Possible	Serological evidence in Australian blood donors [73]; Can survive in stored blood 12–14 days [72]	Possible	Possible	Described in Nigeria [52]	Described in regular US blood donor with travel to Caribbean [12]; Described in Brazilian blood donors [51]	Possible; microfilaria primarily in skin, rarely reaching peripheral blood [13, 25]
Human vaccine present	Not developed	No; mouse model [74], gerbil model [75, 76]	No, Jird model [77]	No; macaque model for *B. malayi* [78, 79] work done in mouse models [80]	No; macaque model for *B. malayi*; work done in mouse model for *B malayi* [80]	None described; immunity of infection under study [14]	None described; infection still poorly understood; immune response still being studied [81]	None described

Table 1 (Continued)

	Loa loa	*Onchocerca volvulus*	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Mansonella perstans*	*Mansonella ozzardi*	*Mansonella streptocerca*
Epidemiology	Central and Western African rainforests; prevalence varies depending on location with 20–40% of people in endemic areas indicate a history of infection [4]	99% infected people in 31 African countries, also Latin American (Central and South America)and Yemen; 20·9 million; infections; 14·6 skin disease, 1·15 million vision loss [5]	Causes 90% of lymphatic filariasis[6], Pockets in sub-Saharan Africa (east, central and southern Africa), widespread transmission West Africa, contemporary transmission South and South-East Asia and the Pacific (worldwide: tropics and subtropics) [7]	South-East Asia, South Asia, Indian subcontinent, East Asia [29]; along with *B. timori* probably makes up 10% of cases of LF globally [6]	No detailed estimations about the prevalence or distribution of *B. timori*. *B. timori* limited to some islands of the lesser Sunda archipelago; Detailed knowledge of the distribution of *B. timori* is scarce. East Timor, Sumba Island, Alor island and Flores [8]	Sub-Saharan Africa [31], Caribbean, Latin America [9–11]	Identified in South America, Central America and West Indies [12]	Described in Africa; Western and Central [13]

Table 2 Clinical characteristics of filariasis

	Loa loa	*Onchocerca volvulus*	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Mansonella perstans*	*Mansonella ozzardi*	*Mansonella streptocerca*
	Loiasis	Onchocerciasis; River blindness	Bancroftian LF	Brugian LF [1]	Brugian LF	Mansonellosis	Mansonellosis	Mansonellosis
Entry phase	L3 larva[a] enters bite wound	L3 larvae enters bite wound	L3 larvae enter skin at site of blood meal	L3 larvae enter skin at site of blood meal	L3 larvae enter skin at site of blood meal	L3 larvae enters host at bite site [82]	L3 larvae enters host at bite site [83]	L3 larvae enters skin at bite site [13]
Location of adults (filaria)	Subcutaneous tissues, conjunctivae	Subcutaneous nodules, deeper tissue [2]	Lymphatics	Lymphatics	Lymphatics [70]	body cavities, connective tissue (pericardium, mesentery, perirenal and retroperitoneal connective tissues), mesenteries [25]	Unclear; *Erythrocebus patas* (Patas monkey) model suggests subcutaneous [83, 84]	Dermis of trunk and upper shoulder girdle
Location of microfilaria	Sheathed microfilaria in **blood (day)**, urine, lungs (non-circulation), sputum, cerebral spinal fluid [3]	Unsheathed microfilaria mostly in connective tissue lymphatics; **sometimes in blood**; sometimes in urine and sputum;	Sheathed microfilaria in lymph channels and blood [85, 86]	Sheathed microfilaria in lymphatics and blood [13]	Sheathed microfilaria in lymphatics and blood [87]	Unsheathed microfilaria in blood [13]	Unsheathed microfilaria in blood [83, 88, 89]	Unsheathed microfilaria in skin, rarely in peripheral blood [13, 25]
Disease severity in naturally infected individuals	Calabar swelling, 'eye worm'[90], renal, cardiac, neurological [91, 92]	Ocular and skin infection; conjunctival infections, corneal neovascularization, blindness [15]	Chronic evolving lymphedema common, acute attacks of cute attacks of dermato-lymphangio-adenitis (ADLA) mostly in limbs/ scrotum; entire affected limb, the genitals or breasts [16–19]	Chronic evolving lymphedema common, acute attacks of cute attacks of dermato-lymphangio-adenitis (ADLA) mostly in limbs/ scrotum; lymphedema in legs below the knee, upper limbs below elbow; not involve genitals/ breast [16–18]	Similar to B. malayi [8, 70]	Infection appears to be silent but may modulate hosts immune system [14]; Some non-specific symptoms described [13]	Asymptomatic	Asymptomatic

[a] L3 larva – third larval stage of nematode, infective to humans.

Table 3 Transfusion risk and mitigation strategies (e.g. efficacy of pathogen reduction, leucoreduction, include plausibility of success and impact on blood supply)

	Loa loa	***Onchocerca volvulus***	***Wuchereria bancrofti***	***Brugia malayi***	***Brugia timori***	***Mansonella perstans***	***Mansonella ozzardi***	***Mansonella streptocerca***
Pre-patent period for microfilaria in blood (blood smear)	*Mandrillus sphinx* model (Mandrills) 147 days; none in *Macaca fascicularis* (cynomolgus monkeys) [93]	23–29 months in a Chimpanzee model [94]	82–111 days [56]	120–142 days [56]	Unknown	Unknown	Patas monkeys developed patent infections 149–186 days post-inoculation; three chimpanzees, four squirrel monkeys, the capuchin, and five rhesus monkeys failed to develop patent infections with M. ozzardi [84]	Possible: identified in skin snips; not identified in blood smears but may rarely reach peripheral blood [13, 25]
Transfusion transmitted	Experimental survived < 1 week in blood [56]	None identified	Experimental ; survived 1–2 weeks in blood [56]	Not identified; B. *malayi* can bind to theoretical endothelial cells *in vitro* [95, 96]	Not identified; theoretical	Yes [39]; survived > 2 years in blood [56]	Experimental ; survived 2–3 years in blood [56]	None identified
Disease severity in naturally infected individuals	See Table 2							
Presence in blood donors	See Table 1							
Consequences of transfusion-transmitted infection	*Macaca fascicularis* (cynomolgus monkeys) showed no sustained microfilaria; possible strong immune response [93]	Not identifying blood transfusion/injection models; ocular models published [97]	Transfused blood into human volunteers led to variable responses; no clinical response to aches, pains and increased temperature [98]	Not identified	Not identified	Not identified	No reactions described in small number of human subjects transfused [57]	Not identified

Table 3 (Continued)

	Loa loa	*Onchocerca volvulus*	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Mansonella perstans*	*Mansonella ozzardi*	*Mansonella streptocerca*
Low-risk donors?	No history of filariasis [99]	No history of filariasis	No history of filariasis	No history of filariasis	No history of filariasis	No history of filariasis	No history of filariasis	No history of filariasis
Blood donor screening assays	Not described							
Leucodepletion/ pathogen inactivation effective	Unknown [24]	Unknown/not applicable	Unknown					Possibly not applicable as sheathed microfilaria in skin and rarely in peripheral blood [13, 25]
Pathogen Reduction Efficacy for Plasma Derivatives	Unknown							Possibly not applicable as sheathed microfilaria in skin and rarely in peripheral blood [13, 25]

Table 4 Diagnostic testing

	Loa loa	*Onchocerca volvulus*	*Wuchereria bancrofti*	*Brugia malayi*	*Brugia timori*	*Mansonella perstans*	*Mansonella ozzardi*	*Mansonella streptocerca*
Periodicity of microfilaria in blood	Diurnal	None/not applicable [100]	Nocturnal	Nocturnal	Nocturnal [8, 100]	None [100, 101]	None [89, 100]	Possibly not applicable as sheathed microfilaria in skin and rarely in peripheral blood [13, 25]
Diagnostic testing available	Thick and thin smear ICT, serology, NAT [25]	Skin snip NAT, serum antigen immunoblot, urine antigen dipstick, serum enzyme immunoassay, skin snip microscopy [102]	Serology [103], blood smear, ICT, NAT [25]	Genus level serology [103, 104], blood smear, ICT, NAT [25]	Blood smear, NAT [105] genus level serology [104]	Thick and thin smear [42] NAT [42, 106], serology [25]	Thick and thin smears, NAT [33, 51], serology [25]	Skin snips NAT, serology [25]

Insect vectors for these pathogens also vary; for example, Tabanid flies (*Chrysops spp.*) transmit *L. loa* and black-flies (*Simulium*) transmit *O. volvulus*. Vectors may also vary for an individual genus and/or species depending on the geographic location [26–28].

LF impacts over 70 countries, in tropical and subtropical regions, spanning Africa, Asia, the Caribbean, South and Central America, and the Western pacific. In 2000, the World Health Organization (WHO) estimated that over 120 million people were infected with nematodes that cause LF [22]. The overwhelming majority (~90%) of LF is caused by *W. bancrofti* [6, 7, 23] with *B. malayi* accounting for most of the remaining cases [6, 29]. Some rare cases of LF may be caused by *B. timori* [8]. The different genera and species of filariae responsible for LF vary widely with respect to disease burden and geographic distribution (Table 1).

All three *Mansonella* species, *M. perstans, M. ozzardi* and *M. streptocerc*a, are transmitted via biting midges of the genus *Culicoides* [13, 30]; *M. ozzardi* may also be transmitted by black flies of the genus *Similium*. Both *M. perstans* and *M. ozzardi* may overlap in the Caribbean and Latin America [9–12, 31] (Table 1), whereby morphological approaches to diagnosis (i.e. microscopy) may occasionally lead to misclassification of these two species [32]. Infection rates of *Mansonella* species in human populations may vary widely both within a particular geographic region and in a particular area over time [13, 33–35].The natural environment (e.g. temperature, humidity and elevation) also plays an important role in the epidemiology of filariasis, through its impact on vector distribution. For example, adult *Culicidae* are more abundant at lower altitudes and in regions with higher humidity even within the same geographic region or country [36]. In central Nepal, the abundance of female *A. aegypti* increased with rising temperatures, yet decreased with greater rainfall and humidity [37].

Clinical presentation/pathology of transfusion-transmitted filariasis

Cases of transfusion-transmitted filariasis are rarely reported (Table 3). There is a suggested, albeit unconfirmed, association between filarial infections and allergic transfusion reactions [38]. In one study in India, patients who had transfusion reactions were investigated. A total of 47 of 11 752 (0·4%) transfused patients had reported transfusion reactions, 29 (61·7%) of which were assessed as being allergic reactions. Fourteen (29·8%) of the 47 patients who had reactions had been transfused with blood from donors who were positive for microfilariae, filarial antigen and/or antibodies. Notable limitations of this study included the absence of a control group, and

lack of information on the timing of testing relative to transfusion.

A case of transfusion-associated *M. perstans* was reported in Chad [37]; this was deemed an incidental finding; that is, the patient, a child, was undergoing investigation for malaria and microfilariae were identified on a peripheral blood smear. There was no adverse event attributed to the transfusion. The authors postulated that only the adult worms result in clinical penetrance; by contrast, rapid clearance of transfused *M. perstans* microfilariae may not produce clinical manifestations [39].

In the United States, a regular blood donor, who had travelled to the Caribbean, was found to have *M. ozzardi* microfilariae on blood smear at time of donation [12]. The donor was also seropositive (titre of 1:512) by indirect hemagglutination antibody testing (IHA). None of the recipients of blood that had been collected from the index donor had sustained transfusion related adverse events. Four of five recipients of the donor's blood had died from their underlying diseases. While microfilariae were not detected in the fifth recipient's blood, the recipient was seroreactive (indirect hemagglutination, 1:512; bentonite flocculation, 1:5). Of note, the recipient had not undergone pre-transfusion testing. The index donor was treated [12].

Diagnostic laboratory testing

Historically diagnosis of filariasis involved direct detection of microfilariae through examination of skin snips or blood smears of capillary blood (Table 4). Although feasible (i.e. given low cost and relative simplicity) in remote locations, specimen collection may require sampling at specific times depending on whether there is a periodicity of the microfilaremia (Table 4).

Elimination campaigns targeting onchocerciasis and LF have led to a number of diagnostic advances [25] including development of antibody and antigen detection assays, as well as nucleic acid tests. Immunoassays requiring laboratory facilities as well as point-of-care (POC) tests have been widely used for surveillance and clinical diagnosis of Bancroftian filariasis. Detection of circulating filarial antigen (CFA) has now replaced direct detection of Bancroftian filariasis given its robust performance characteristics, including improved sensitivity in individuals without circulating microfilariae and the ability to collect blood samples independent of time of day. The immunochromatographic card test (ICT), which was introduced in 1997 has been replaced by the Filariasis Test Strip (FTS) for POC detection of CFA. These antigen detection platforms have been invaluable to the WHO's elimination programme for LF [40]. Although deployed specifically for the diagnosis of *W. bancrofti*, the ICT appears to cross-react with other filariae species, thus necessitating revision of transmission maps and programmatic operations [41, 42].

In addition to supporting patient care, the ICT and rapid POC serology for Brugian filariasis have advanced the MDA activities of the LF elimination programme by helping to map transmission risk, thus guiding scale-up (or discontinuation of MDA in the case of waning risk), and post-MDA surveillance [43]. Advances in molecular diagnostic techniques have also assisted with post-MDA surveillance of vector mosquito populations for LF and for mapping risk from the less-resourced *Mansonella* spp. [44, 45].

Donor surveillance

At least five donor surveillance studies have been conducted in Nigeria. In one, microfilaria of *L. loa* and *M. perstans* were detected in 1·3% and 15·6% of those evaluated, respectively; co-infection was detected in 0·2% [46]. In another (n = 115), *L. loa* microfilaria were observed in 3·5% of donors [47].

In Lagos, 300 donors were screened by microscopy of modified thick smear stained with Giemsa and Haematoxylin stains [48]. One donor (0·33%) was positive for *W. bancrofti*, one (0·33%) had *M. perstans*, and four (1·33%) had *L. loa*.

In a cross-sectional study in Ido Ekiti, Nigeria, 863 blood donors were screened for parasites by microscopy of Giemsa stained thick smears [49]. Twenty-two (2·5%) donors were positive for microfilaria. The identities of the infecting microfilaria were *L. loa* (n = 9), *O. volvulus* (n = 6), *M. perstans* (n = 5) and *W. bancrofti* (n = 2).

In a study in Ile-Ife, South-west Nigeria, 250 blood donors were evaluated for parasites using thick and thin smears; 23(9·2%) were positive for filarial worms, all of which were identified as *M. perstans* [50].

In a cross-sectional donor surveillance study of *M. ozzardi* in the Brazilian Amazon, 28/356 (7·9%) were smear positive and 54/227 (23·8%) were positive by a qualitative nested *M. ozzardi* NAT [51]. Of the 30 donations, 27 were ultimately deemed to be unusable given seroreactivity for other transfusion-transmitted infections (TTIs) (mostly HBV, e.g. anti-HBc). Of note, the odds of seroreactivity were 15-fold higher in *M. ozzardi* positive vs negative individuals. There was no follow-up on recipients of the three *M. ozzardi* contaminated units that were transfused [51].

Transfusion risk and mitigation strategies

Cases of transfusion-transmitted filariasis are rare and confined to case reports of variable quality (Table 3).

Much of the data pertaining to transfusion-transmitted filariasis have been summarized in a letter to the editor in which the author concluded with a recommendation to screen for filariasis [52]. Filarial persistence in blood donors, tolerance of blood storage and processing, and transfusion practices may also reduce the risk of transfusion-transmitted filariasis. The persistence in blood depends on the causative species: *L. loa* (21 days) [53]; *W. bancrofti* (<5 weeks) [54]; *M. ozzardi* (2 years); and *M. perstans* (3 years) [55–57]. The limited available literature suggests variable survival in refridgerated stored blood from 12 days (*W. bancrofti*) to >21 days (*L. loa*). Microaggregate filters, which have a pore size of ~40 μm, have been shown to reduce at least 90% of surviving microfilariae in blood [53, 58]. The smaller pore size of modern leucoreduction filters (~4μm), would further minimize transmission of microfilariae in settings where leucoreduction filters are used. Nonetheless, leucoreduction is not routinely undertaken in low- and middle-income countries, where filariasis is endemic [59].

There are no known mitigation strategies that have been implemented specifically for filariasis, and as described, there is a paucity of data both on donor surveillance for filariasis and on transfusion-transmitted cases (Table 3). There is much greater risk to public health posed by the major TTIs (e.g. HIV, HBV and HCV), particularly in low-resource settings where filariasis is endemic and blood safety is suboptimal. Coupled with generally mild clinical effect of transfusion transmission of filaria (e.g. allergic reactions), mitigation strategies against transfusion-transmitted filariasis are not recommended by the World Health Organization for both endemic and non-endemic (i.e. following traveller exposure) settings [60].

If donor screening for filariasis were to be undertaken, the individual strengths and limitations of available laboratory methods would need to be considered. Some species require sampling at specific times of day to coincide with periodicity of microfilaremia. In the aforementioned study of transfusion reactions in India, the investigators recommended deferral of individuals with active filariasis and suggested that antigen testing, which correlated with microfilariasis, could be used for screening [38]. Point-of-care antigen or serologic testing may be easier to operationalize in resource limited settings. However, antigen tests in general may be less sensitive than antibody tests during early infection in Bancroftian filariasis [61]. Antigen detection assays may not be readily available for the detection of other nematodes (e.g. *O. volvulus*) [62]. By contrast, molecular testing is both sensitive and specific and may have a role in testing suspected travel-related cases where resources are available; however, it is challenging in many of the areas where filariasis is endemic given resource constraints spanning high costs, equipment, reagents and availability of skilled personnel [63].

In regions with high prevalence of co-infection of malaria, filariasis and HBV has been described, indirect deferrals for filariasis may be achieved by screening donors for malaria or testing donations for HBV (e.g. anti-HBc). For example, in 2019 Abrahim et al. reported a 90% interdiction of *M. ozzardi* positive donors by the concomitant presence of other infectious markers currently used as screening tests; OR = 15·8; CI95%: 4·5–56·1, $P < 0·0001$] [51].

Discussion

A review of the literature suggests that there is a paucity of data both on filarial infection in blood donors and on transfusion-transmitted filariasis [51, 52]. When cases have been described in donors, they have originated in rural, endemic areas predominantly in low-resource settings where haemovigilance (i.e. donor screening, post-transfusion surveillance and laboratory testing for higher-priority TTIs) is low [48, 50, 51, 64]. No serious adverse events have been ascribed to transfusion-transmitted filariasis; rather, there is a suggested (i.e. unconfirmed) association between filarial infections and allergic transfusion reactions [38].

The prevalence of filariasis in donors is variable even in endemic countries; the rate of smear-positive donors ranges from less than one per cent to greater than >10 per cent, depending on the geographic location, donor population and species [46, 47, 51]. The reported prevalence also depends on the method of ascertainment, for example serology [43] vs NAT [51]. Although blood donors in non-endemic countries may become infected during travel [12], they may be deferred for other travel- or long-term residence-related deferrals (e.g. malaria) [65].

Even where resources may be available, such as in high-income countries, testing options are limited. Nonetheless, several elements during blood collection and processing may serve to mitigate risk of transfusion-transmitted filariasis. For one, the nocturnal periodicity of *W. bancrofti* and *Brugia* spp. microfilaria and the rarity of *M. streptocerca* microfilaria within the peripheral blood limit the opportunity for collecting microfilaria-positive donations (Table 4). Blood processing steps such as pre-storage leucoreduction (i.e. in routine use in many high-income countries) would reduce the numbers of microfilaria that are ultimately in a transfused product [53, 58]. Possible short survival times for *L. loa* and *W. bancrofti* (e.g. less than two weeks) during storage may also reduce the possibility of transmitting viable microfilariae to recipients during prolonged storage (Table 3) [56].

This review has limitations. First, the search strategy was restricted to English language communications, at least to the abstract level; grey literature and peer-reviewed publications in other languages were not included. Second, as the search strategy focused on publicly accessible information on filariasis, the authors did not have access to less publicly available information such as public health or health administrative databases. Third, a lack of open access opportunities for publication of transfusion-transmitted cases in endemic regions prior to the 1990s may have biased the searchable literature from those regions.

Conclusion

Rare cases of transfusion-transmitted filariasis suggest that filariasis does not pose significant risk to the blood supply. Many of the countries that are impacted by filariasis are the among the World's poorest [66], whereby resources are better expended on prevention of the major TTIs. In short, filariasis is a potential distraction for blood safety and the absence of dedicated transfusion strategies is appropriate.

Conflict of interest

EMB reports personal fees and non-financial support from Terumo BCT, Grifols Diagnostics Solutions and Abbott Laboratories, outside of the submitted work; EMB is a member of the United States Food and Drug Administration (FDA) Blood Products Advisory Committee. Any views or opinions that are expressed in this manuscript are that of the author's, based on his own scientific expertise and professional judgement; they do not necessarily represent the views of either the Blood Products Advisory Committee or the formal position of FDA, and also do not bind or otherwise obligate or commit either Advisory Committee or the Agency to the views expressed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Table S1 Supplemental Search terms and strategy

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Genetic determinants of ferritin, haemoglobin levels and haemoglobin trajectories: results from Donor InSight

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Vox Sanguinis

Background and objectives Blood donors might develop iron deficiency as approximately 250 mg of iron is lost with every donation. Susceptibility to iron deficiency and low haemoglobin levels differs between individuals, which might be due to genetic variation. Therefore, the aim of this study was to investigate associations between single nucleotide polymorphisms (SNPs) and haemoglobin trajectories, haemoglobin levels and ferritin levels in blood donors.

Materials and methods In 2655 donors participating in the observational cohort study Donor InSight-III (2015–2017), haemoglobin and ferritin levels were measured in venous EDTA whole blood and plasma samples, respectively. Haemoglobin trajectories (stable/declining) were determined by fitting growth-mixture models on repeated pre-donation capillary haemoglobin measurements. Genotyping was done using the UK Biobank – version 2 Axiom Array. Single SNP analyses adopting an additive genetic model on imputed genetic variants were performed for haemoglobin trajectories, haemoglobin levels and ferritin levels. Conditional analyses identified independent SNPs.

Results Twelve, twenty and twenty-four independent SNPs were associated with haemoglobin trajectories, haemoglobin levels and ferritin levels respectively ($P < 1 \times 10^{-5}$). Rs112016443 reached genome-wide significance for ferritin levels, which influences *WDSUB1* expression.

Conclusion Rs112016443 was genome-wide significantly associated with ferritin levels in Dutch donors. Further validation studies are needed, as well as studies towards underlying mechanisms and predicting iron deficiency using SNPs.

Key words: ferritin, GWAS, haemoglobin, haemoglobin trajectories, SNPs.

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Introduction

Blood donors are prone to iron deficiency as they lose significant numbers of erythrocytes with every whole blood donation, resulting in a temporary decline in haemoglobin (Hb) levels [1]. Hb levels are restored by an increased erythropoiesis supported by an increase in the absorption of dietary iron and the release of iron from iron stores, the latter indicated by ferritin levels [1–3]. Frequent blood donations, however, can result in iron deficiency and a subsequent decline in Hb levels. To ensure sufficient blood product Hb content, and to protect donor health, the council of Europe advises as criteria: (1)

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pre-donation Hb levels of at least 7·8 mmol/l for women and 8·4 mmol/l for men and (2) a minimum donation interval of 56 days [4]. Additionally, ferritin measurements are recommended for iron status monitoring.

Susceptibility to low Hb levels seems to vary between individual donors, as some donors often get temporarily deferred for donation because of low Hb levels, while others do not. A previous study by Nasserinejad *et al.* (2015) on 5388 blood donors showed that indeed different subgroups of donors could be distinguished based on their Hb trajectories [5]. A stable Hb trajectory was found in 29% and a declining Hb trajectory in 71% of the donors, suggesting that some donors may be better able to maintain Hb levels with repeated donations than others [5–9].

Genetic variation may explain part of the differences in Hb trajectories, Hb levels and ferritin levels between blood donors [10,11]. In our recently performed systematic review of population-based studies, we found 1237 single nucleotide polymorphisms (SNPs) to be associated with erythrocyte parameters, including Hb levels, of which only ten SNPs were repeatedly found in multiple studies [12]. Most of the included studies had been performed in the general population, hence mostly reflecting genetic determinants of erythrocyte parameters under steady-state conditions. More recently, a genome-wide association study (GWAS) among 173 480 participants from the UK Biobank and INTERVAL studies, testing 29·5 million genetic variants, found 905 independent SNPs to be associated with red blood cell traits, among which Hb levels [13]. Only a few genetic association studies have been performed in blood donors, but these have all been restricted to only a limited number (two to three) of SNPs tested [14–16]. In these studies among blood donors in the United States and Germany, *HFE* mutations were associated with higher initial Hb levels [14,15], but not with the rate of Hb level decline with repeated blood donations [14]. The Danish Blood Donor Study found associations for three of six studied SNPs (rs1800562 in *HFE*, rs1799945 in *HFE* and rs855791 in *TMPRSS6*) with Hb levels [17]. With regard to ferritin levels, GWASs are performed under steady-state conditions in general populations [18–22]. In these GWASs 10 different SNPs located in or near ten genes (i.e. *HFE, WDR75* and *SLC40A1, ABO, TEX14, TMPRSS6, ALDH2, BRAP, PMS1* and *ORMDL1* and *GAB3*) were found to be associated with ferritin levels [18–22]. A limited number of studies investigated a small number (four to eleven) of SNPs for associations with ferritin levels specifically among blood donors, of which seven SNPs in or near four genes (i.e. *BTBD9, HFE, TMPRSS6* and *BMP2*) were reported to be associated with ferritin levels.[23–26]

The second follow-up of Donor InSight (DIS-III) was set-up to study genetic markers that predict whether a blood donor can donate frequently without a risk for developing iron deficiency or anaemia. DIS-III is the first and only GWAS among blood donors, in which not only Hb levels of blood donors were measured, but also Hb trajectories based on repeated Hb measurements, and ferritin levels [27]. This combination of phenotypes provides the unique opportunity to investigate associations between SNPs and Hb trajectories, Hb levels and ferritin levels in Dutch blood donors.

Materials and methods

Study population

Data for this study were collected as part of the second follow-up (2015–2016) of DIS-III, an observational cohort study conducted among blood and plasma donors in the Netherlands aimed to gain insight into characteristics, health and behaviour of donors [27]. See also 'Supplementary methods in Appendix S1' for more detailed information on the study population, phenotypes, genotyping, quality control and imputation, covariates, statistical analyses and power.

Phenotypes

DIS-III blood samples were collected from the sampling pouch if the DIS-III visit was combined with a blood donation, and otherwise through venepuncture. Collected DIS-III blood samples were used to (1) perform a full blood count, (2) measure ferritin levels and (3) isolate DNA. Detailed in 'Supplementary methods in Appendix S1'.

Genotyping, quality control and imputation

DNA was genotyped in one batch using the UK Biobank – version 2 Axiom Array (Thermo Fisher Scientific, San Jose, CA, USA). Sample quality control (QC) was done by Thermo Fisher [28–30], and further QC was performed according to the UK Biobank protocol [31]. Imputation of SNPs was based on the Wellcome Sanger Institute imputation pipeline (Eagle phasing and BWT imputation using the HRC v1.1 panel) [32–34]. See 'Supplementary methods in Appendix S1'.

Statistical analyses

Analyses were performed on imputed genetic variants available for GWAS analyses using PLINK2 (www.cog-genomics.org/plink/2.0/) [35]. Single SNP analyses were

performed for each phenotype, in which an additive genetic model was adopted. Ferritin levels showed a skewed distribution and were therefore log-transformed. Logistic and linear regression modelling was performed for dichotomous and continuous outcomes, respectively. Detailed in 'Supplementary methods in Appendix S1'.

Results

Table 1 shows the characteristics of all 2649 participants as included in the GWAS for Hb and ferritin levels (n = 10 missing). Hb trajectories had been established for 1827 donors. Analyses were performed on 7 435 577 imputed SNPs and genomic-control inflation factors were 1·019 for Hb trajectories, 1·004 for ferritin levels and 1·012 for Hb levels. See 'Supplementary results in Appendix S1' for more results on Hb trajectories/levels, ferritin levels, SNP based heritability and gene expression.

Hb trajectories and levels

The Manhattan plots for Hb trajectories and levels show that although some signals reached the suggestive threshold, none reached GW significance (Fig 1a,b, Fig S1A-B).

Table 1 Characteristics of the study population

	Donor InSight-III population (n = 2655)
Female sex	1465 (55·2)
Age, years[a]	48·3 ± 13·3
Hb level, mmol/l	8·8 ± 0·7
Hb trajectory (if known[b])	
Stable	783 (29·5)
Declining	1044 (39·3)
Ferritin level, µg/l	44·2 (24·4–75·6)
Haem iron intake, mg/day	1·0 (0·7–1·3)
Non-haem iron intake, mg/day	8·7 (6·9–10·6)
Use of iron supplements/medication, yes	209 (7·9)
Current smoker, yes[c]	189 (7·1)
Alcohol consumption, yes[d]	1916 (72·2)
Menstruation, yes[e]	688 (25·9)
Donation interval, days[f]	245 (119–910)
Donations in 2 years before DIS-III, number	3 (0–5)

Continuous variables: mean ± SD or median (interquartile range) if skewed; Dichotomous variables: n (%).

[a]Age at DIS-III donation.

[b]Hb trajectories could only be fitted if Hb levels of the first donation were available in the blood bank information system.

[c]Current smoker n = 472 (17·7%) missing values.

[d]Alcohol consumption n = 491 (18·4%) missing values.

[e]Menstruation not applicable in men n = 1190 (44·8%).

[f]Donation interval is time between DIS-III donation and previous visit in days.

Trails were mainly seen on chromosomes 3, 8, 16, 17 and 20 for Hb trajectories and on chromosomes 1, 4, 5, 6, 17, 18 and 22 for Hb levels. In total, eighteen and 50 SNPs reached the suggestive threshold for an association with Hb trajectories and levels, respectively, of which twelve and twenty were independent (Tables 2 and 3). An effect allele frequency above 0·2 was seen in four and ten of the independent SNPs for Hb trajectories and Hb levels, respectively.

The strongest association with Hb trajectory was found for SNP rs7214973 on chromosome 17, showing an odds ratio (OR) of 1·43 (1·24 to 1·66) for rs7214973-*A* with a corresponding *P*-value of 9·76E-7. This SNP is located in the *FLOT2* gene.

Regarding Hb levels, the strongest association was found for SNP rs2492935 on chromosome 6 near *TAF8*. The *A* allele of this SNP was associated with a 0·10 (0·06 to 0·14) mmol/l increase in Hb levels (P = 1·11E-7). The second strongest and independent signal was also found on chromosome 6 at rs79045090 (in *KIF25*). The *T* allele decreases Hb level with 0·14 (–0·19 to –0·08) mmol/l (P = 4·87E-7). Three other independent signals were found on chromosome 6 for SNPs rs12205562 (near *MTRNR2L9*), rs9487381 (in *METTL24*) and rs74928432 (in *KIF25*). In contrast to the two other independent SNPs on chromosome 6, these SNPs are relatively rare with effect allele frequencies between 0·025–0·028. See 'Supplementary results in Appendix S1'.

Ferritin levels

The Manhattan plot for log-transformed ferritin levels shows that several SNPs on chromosome 2 reached GW significance (Fig 1c and Fig S1C). In total, 40 SNPs reached the suggestive threshold on chromosome 2, of which twelve reached GW significance for an association with ferritin levels. Seven SNPs on chromosome 2 showed independent effects, of which one was GW significant. Also, several ′trails′ reaching the suggestive threshold were seen on chromosomes 1, 7, 9, 14, 15 and 18. In total, 104 SNPs reached the suggestive threshold for an association with ferritin levels, of which 24 were independent (Table 4). An effect allele frequency above 0·2 was seen in three of the independent SNPs for ferritin levels.

The most significant – and also the only independent SNP reaching GW significance – was rs112016443 on chromosome 2. The *A* allele of this SNP was associated with 1·52 (1·32 to 1·75) µg/l higher geometric mean ferritin levels (P = 2·2E-9). The frequency of this effect allele is 2·5 per cent, resulting in one participant with *AA* and 130 participants with *AG* in our study population. As shown in Fig 2, ferritin levels were higher in participants with an *A* allele versus participants with two *G* alleles. The LocusZoom plot in Fig 3 demonstrates that the

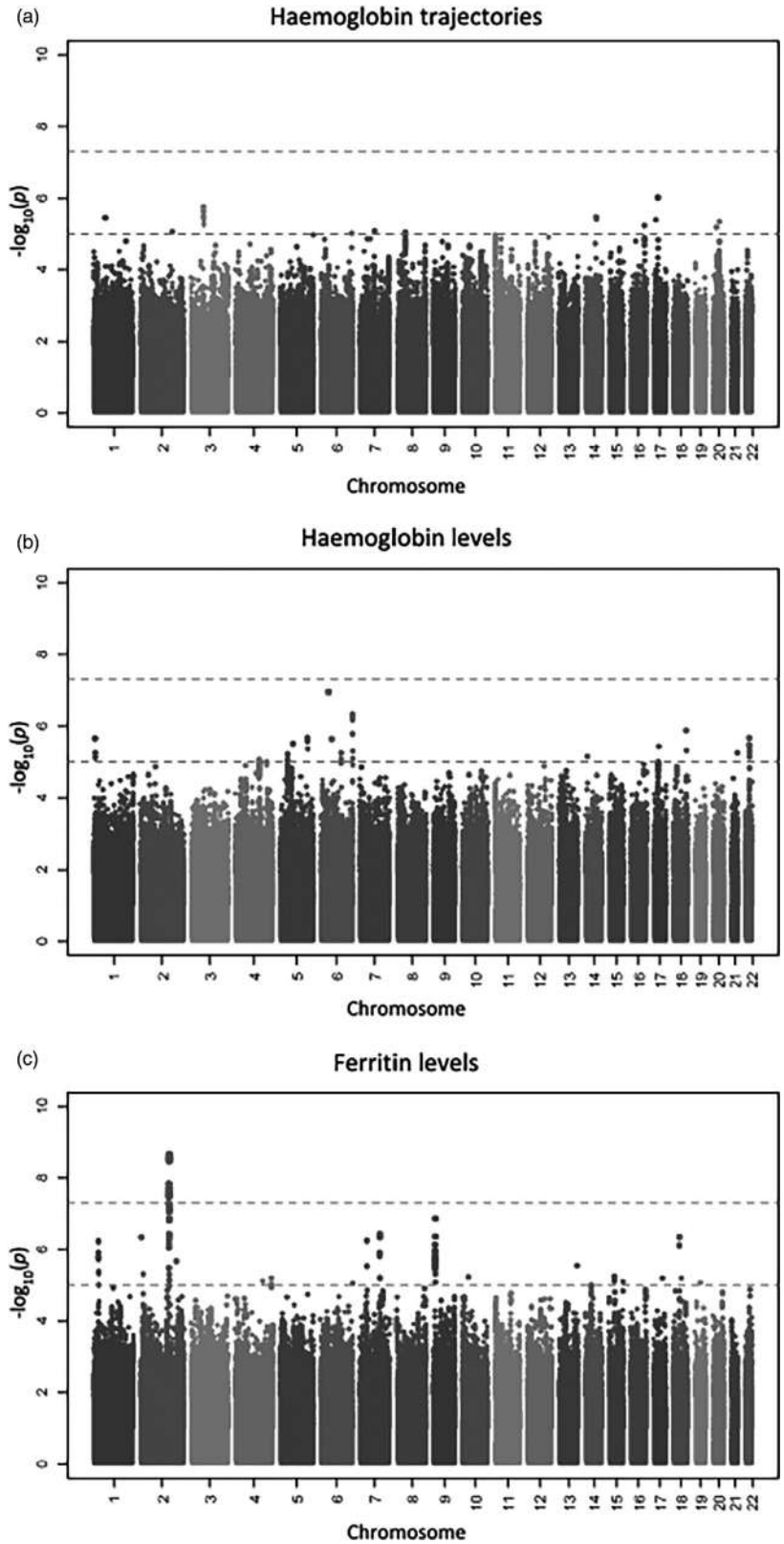

Fig 1 Manhattan plot for haemoglobin trajectories, haemoglobin levels and ferritin levels. All *P*-values are negative log10 transformed. Line at 1 x 10^{-5} represents the suggestive threshold; line at 5 $\times$ 10^{-8} represents the genome-wide significance threshold.

Table 2 Genomic loci independently associated with haemoglobin trajectories at $P < 1 \times 10^{-5}$

CHR	POS	SNP	REF	EA	EAF	OR (95% CI)	*P*-value	Nearest gene
1	70873758	rs140293087	*C*	*T*	0·020	0·25 (0·14 to 0·45)	3·59E-06	*CTH*
2	177351938	rs79533233	*G*	*T*	0·796	1·45 (1·23 to 1·71)	8·45E-06	*NA*
3	65692063	rs1499508	*G*	*A*	0·179	1·60 (1·32 to 1·94)	1·85E-06	*MAGI1*
6	165457847	rs35833993	*G*	*T*	0·182	0·67 (0·56 to 0·80)	9·58E-06	*C6orf118*
7	68515041	rs74467603	*C*	*T*	0·057	2·10 (1·51 to 2·90)	8·17E-06	*AUTS2, LOC100507468*
8	26098158	rs12548903	*G*	*C*	0·565	0·74 (0·64 to 0·84)	8·72E-06	*PPP2R2A*
14	76585871	rs11624297	*A*	*G*	0·428	0·72 (0·63 to 0·83)	3·46E-06	*GPATCH2L*
16	82995774	rs12324975	*C*	*T*	0·178	0·67 (0·56 to 0·80)	5·87E-06	*CDH13, LOC101928417*
17	10085997	rs149828063	*A*	*C*	0·021	0·25 (0·14 to 0·45)	4·04E-06	*GAS7*
17	27222745	rs7214973	*G*	*A*	0·625	1·43 (1·24 to 1·66)	9·76E-07	*FLOT2*
20	17486785	rs7268734	*A*	*G*	0·037	1·53 (1·27 to 1·84)	6·49E-06	*BFSP1*
20	38201675	rs13044261	*A*	*G*	0·199	0·41 (0·28 to 0·60)	4·45E-06	*LOC339568*

CHR, chromosome; 95% CI, 95% confidence interval; EA, effect allele; EAF, effect allele frequency; NA, information not available; OR, odds ratio; POS, position; SNP, single nucleotide polymorphism; REF, reference allele.
Adjusted for age, sex (men, premenopausal women, and postmenopausal women), first 10 principal components.

Table 3 Genomic loci independently associated with haemoglobin levels at $P < 1 \times 10^{-5}$

CHR	POS	SNP	REF	EA	EAF	β (95% CI)	*P*-value	Nearest gene
1	10154460	rs113304383	*C*	*T*	0·019	−0·28 (−0·40 to −0·17)	2·27E-06	*UBE4B*
4	121772135	rs72680432	*T*	*G*	0·053	0·16 (0·09 to 0·23)	8·46E-06	*PRDM5*
4	121855571	rs1114026	*G*	*T*	0·864	−0·11 (−0·15 to −0·06)	9·14E-06	*PRDM5*
5	35710906	rs6875940	*T*	*G*	0·637	0·08 (0·04 to 0·11)	6·16E-06	*SPEF2*
5	72587455	rs4422515	*G*	*T*	0·600	−0·08 (−0·11 to −0·04)	3·18E-06	*TMEM174*
5	149442779	rs11738211	*A*	*C*	0·073	−0·15 (−0·21 to −0·09)	2·25E-06	*CSF1R*
6	42053778	rs2492935	*G*	*A*	0·248	0·10 (0·06 to 0·14)	1·11E-07	*TAF8*
6	62206677	rs12205562	*A*	*G*	0·026	−0·26 (−0·37 to −0·15)	2·40E-06	*MTRNR2L9*
6	110654066	rs9487381	*T*	*C*	0·028	0·23 (0·13 to 0·32)	5·63E-06	*METTL24*
6	168411160	rs74928432	*C*	*T*	0·025	−0·24 (−0·34 to −0·13)	4·97E-06	*KIF25*
6	168420387	rs79045090	*C*	*T*	0·101	−0·14 (−0·19 to −0·08)	4·87E-07	*KIF25*
14	30041273	rs78788422	*T*	*A*	0·014	0·32 (0·18 to 0·46)	7·05E-06	*MIR548AI*
17	27426153	rs12453624	*G*	*A*	0·332	−0·07 (−0·11 to −0·04)	1·00E-05	*MYO18A*
17	32903538	rs4795936	*A*	*G*	0·094	−0·13 (−0·19 to −0·08)	3·71E-06	*C17orf102*
18	70981472	rs9966816	*T*	*C*	0·484	−0·07 (−0·10 to −0·04)	4·82E-06	*LOC100505817*
18	70987438	rs1943819	*C*	*A*	0·294	0·09 (0·05 to 0·12)	1·35E-06	*LOC100505817*
21	43024340	rs9975090	*A*	*G*	0·437	−0·07 (−0·11 to −0·04)	5·57E-06	*LINC00111*
22	37453256	rs2235320	*G*	*T*	0·123	−0·11 (−0·16 to −0·07)	4·18E-06	*KCTD17*
22	37470224	rs2413450	*T*	*C*	0·543	0·08 (0·04 to 0·11)	2·25E-06	*TMPRSS6*
22	37495051	rs733655	*T*	*C*	0·251	−0·08 (−0·12 to −0·05)	7·38E-06	*TMPRSS6*

CHR, chromosome; 95% CI, 95% confidence interval; EA, effect allele; EAF, effect allele frequency; POS, position; REF, reference allele; β, regression coefficient; SNP, single nucleotide polymorphism.
Adjusted for age, sex (men, premenopausal women, and postmenopausal women), first 10 principal components.

nearest gene was shown to be *WDSUB1*. See 'Supplementary results in Appendix S1'.

Discussion

In this genome-wide association study among Dutch blood donors, we found a total of twelve, twenty and twenty-four independent SNPs reaching the suggestive threshold for an association with Hb trajectories, Hb levels and ferritin levels, respectively. Rs112016443 on chromosome 2 reached GW significance, and the *A* allele of this SNP was associated with 0·42 μg/l higher log ferritin levels. This SNP is associated with the gene expression of *WDSUB1* in several tissues.

Five SNPs associated with Hb levels in this GWAS were also previously identified in our systematic review either

once or repeatedly, namely rs9610638 (near *KCTD17*), rs4820268 (in *TMPRSS6*), rs2076085 (in *TMPRSS6*), rs2413450 (in *TMPRSS6*) and rs2072860 (in *TMPRSS6*). *TMPRSS6* is involved in iron homeostasis as it encodes for a protein matriptase-2 which is involved in the regulation of hepcidin levels [36]. Hepcidin is considered the key regulator of iron homeostasis, as it can restrain iron absorption by blocking ferroportin [37,38]. Comparing results of four SNPs previously repeatedly associated with Hb levels showed similar (direction of) effects and p-values below 0·001 in the current GWAS. So, with this GWAS the role of these SNPs in Hb levels has been replicated. Three of these SNPs are known to be involved in iron homeostasis, rs5756504 and rs4820268 are located in the earlier mentioned *TMPRSS6* gene and rs1800562 is located in the *HFE* gene. The HFE protein binds the transferrin receptor, an iron transport receptor, and thereby regulates the absorption of iron [39]. To our knowledge, we were the first to determine Hb trajectories, therefore results from this GWAS cannot be compared with results from other GWASs on this phenotype. Comparing effects of top SNPs (i.e. SNPs reaching the suggestive threshold) for the three phenotypes showed that there seems to be some correlation in the effects of the top SNPs between the phenotypes (Fig S4). There was no overlap in top SNPs for Hb trajectories and top SNPs for Hb or ferritin levels. For Hb levels, this is on the one hand surprising as in our study population Hb trajectories are associated with Hb levels (Fig S5C). On the other hand, Hb levels and Hb trajectories are different phenotypes, with Hb levels representing the current Hb level and Hb trajectories representing Hb level changes over the course of repeated blood donations.

Despite our relatively small sample size, we were able to identify one GW-significant hit – rs112016443 – for ferritin levels, which has not yet been described in existing literature. This SNP on chromosome 2 was found to be associated with ferritin levels and twenty-three independent SNPs reached the suggestive threshold for an association with ferritin levels. In the literature, a meta-analysis of eleven European-population studies identified six GW-significant SNPs (rs744653 near *WDR75* and *SLC40A1* [18], rs1800562 and rs1799945 in *HFE*, rs651007 near *ABO*, rs411988 in *TEX14*, rs855791 in

Table 4 Genomic loci independently associated with log-transformed ferritin levels at $P < 1 \times 10^{-5}$ and at $P < 5 \times 10^{-8}$ indicated in bold

CHR	POS	SNP	REF	EA	EAF	β (95% CI)	*P*-value	Nearest gene
1	27278521	rs3010110	*G*	*A*	0·261	–0·12 (–0·17 to –0·07)	5·92E-07	*KDF1*
2	5676661	rs138521957	*C*	*G*	0·020	–0·40 (–0·56 to –0·25)	4·69E-07	*LINC01248*
2	13528298	rs13000558	*A*	*T*	0·158	0·14 (0·08 to 0·20)	4·90E-06	*LOC100506474*
2	160141997	rs78712300	*A*	*T*	0·044	0·26 (0·16 to 0·36)	9·03E-07	*WDSUB1*
2	**160146025**	**rs112016443**	***G***	***A***	**0·025**	**0·42 (0·28 to 0·56)**	**2·22E-09**	***WDSUB1***
2	160159642	rs73006707	*C*	*G*	0·039	0·29 (0·18 to 0·40)	4·23E-07	CNTNAP2
2	160170899	rs141083117	*G*	*C*	0·023	0·37 (0·23 to 0·52)	7·39E-07	*BAZ2B*
2	202351922	rs77580183	*C*	*A*	0·016	–0·40 (–0·56 to –0·23)	2·10E-06	*ALS2CR11*
4	140503723	rs148722960	*T*	*G*	0·016	0·40 (0·23 to 0·57)	7·49E-06	*SETD7*
4	183581152	rs4386638	*G*	*A*	0·186	–0·12 (–0·18 to –0·07)	6·27E-06	*TENM3*
6	168702763	rs73034306	*G*	*A*	0·117	0·15 (0·09 to 0·22)	8·83E-06	*DACT2*
7	28422134	rs887623	*T*	*C*	0·194	–0·14 (–0·19 to –0·08)	5·82E-07	*CREB5*
7	103972084	rs2188490	*A*	*C*	0·111	–0·17 (–0·24 to –0·11)	3·69E-07	*LHFPL3*
9	7938795	rs10758915	*G*	*T*	0·092	0·20 (0·13 to 0·27)	1·35E-07	*TMEM261*
9	7953253	rs10815728	*C*	*T*	0·041	0·27 (0·16 to 0·37)	7·63E-07	*TMEM261*
10	26135839	rs35450555	*C*	*A*	0·022	0·35 (0·20 to 0·50)	5·84E-06	*LOC101929073*
13	109624455	rs4771622	*T*	*C*	0·812	–0·13 (–0·18 to –0·08)	2·84E-06	*MYO16*
14	50385982	rs58936650	*T*	*A*	0·094	-0·16 (–0·24 to –0·09)	9·73E-06	*ARF6*
15	51665817	rs2459400	*C*	*T*	0·023	0·33 (0·19 to 0·47)	5·65E-06	*GLDN*
15	97394826	rs6496190	*A*	*G*	0·568	–0·10 (–0·14 to –0·05)	7·92E-06	*SPATA8*
17	55922063	rs140127928	*G*	*A*	0·023	–0·35 (–0·49 to –0·20)	6·37E-06	*MRPS23*
18	34624603	rs147813292	*A*	*G*	0·036	–0·32 (–0·44 to –0·19)	4·55E-07	*KIAA1328*
18	45865998	rs11663923	*A*	*G*	0·182	0·13 (0·07 to 0·18)	6·32E-06	*ZBTB7C*
19	29934907	rs79005430	*G*	*A*	0·011	0·45 (0·25 to 0·65)	8·53E-06	*LOC284395*

CHR, chromosome; 95% CI, 95% confidence interval; EA, effect allele; EAF, effect allele frequency; POS, position; REF, reference allele; β, regression coefficient; SNP, single nucleotide polymorphism.

Adjusted for age, sex (men, premenopausal women, and postmenopausal women), first 10 principal components.

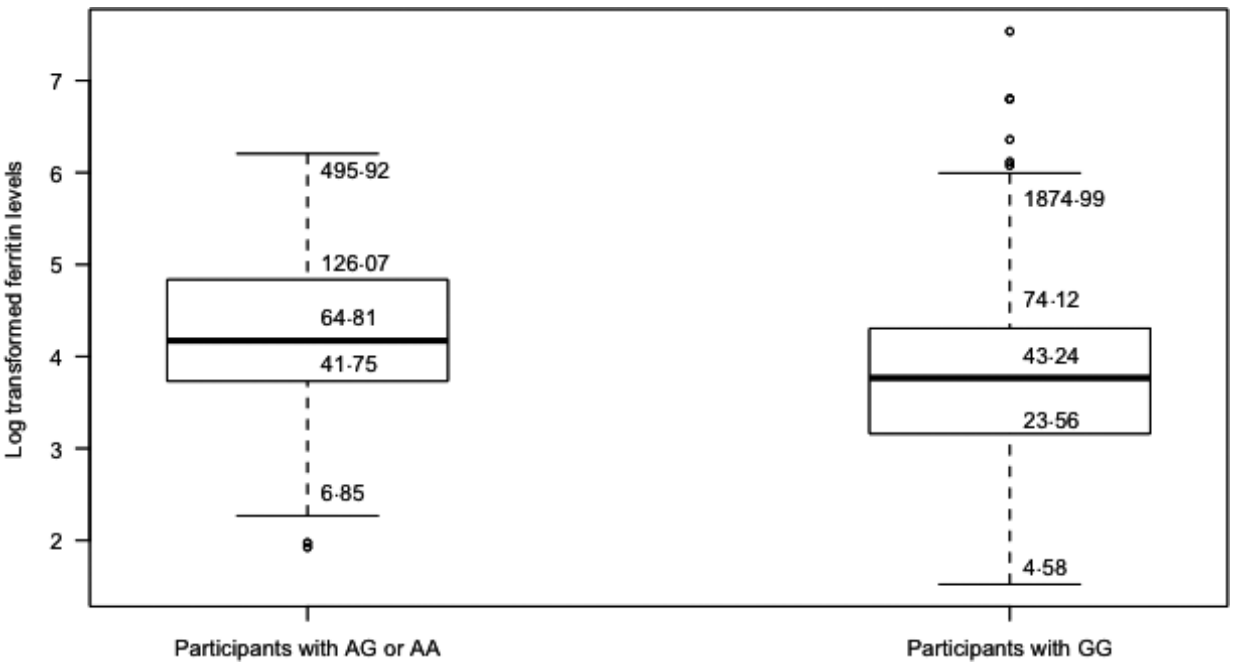

Fig 2 Ferritin concentrations (μg/l) in different genotypes of rs112016443. Numbers inside the plot represent untransformed ferritin levels.

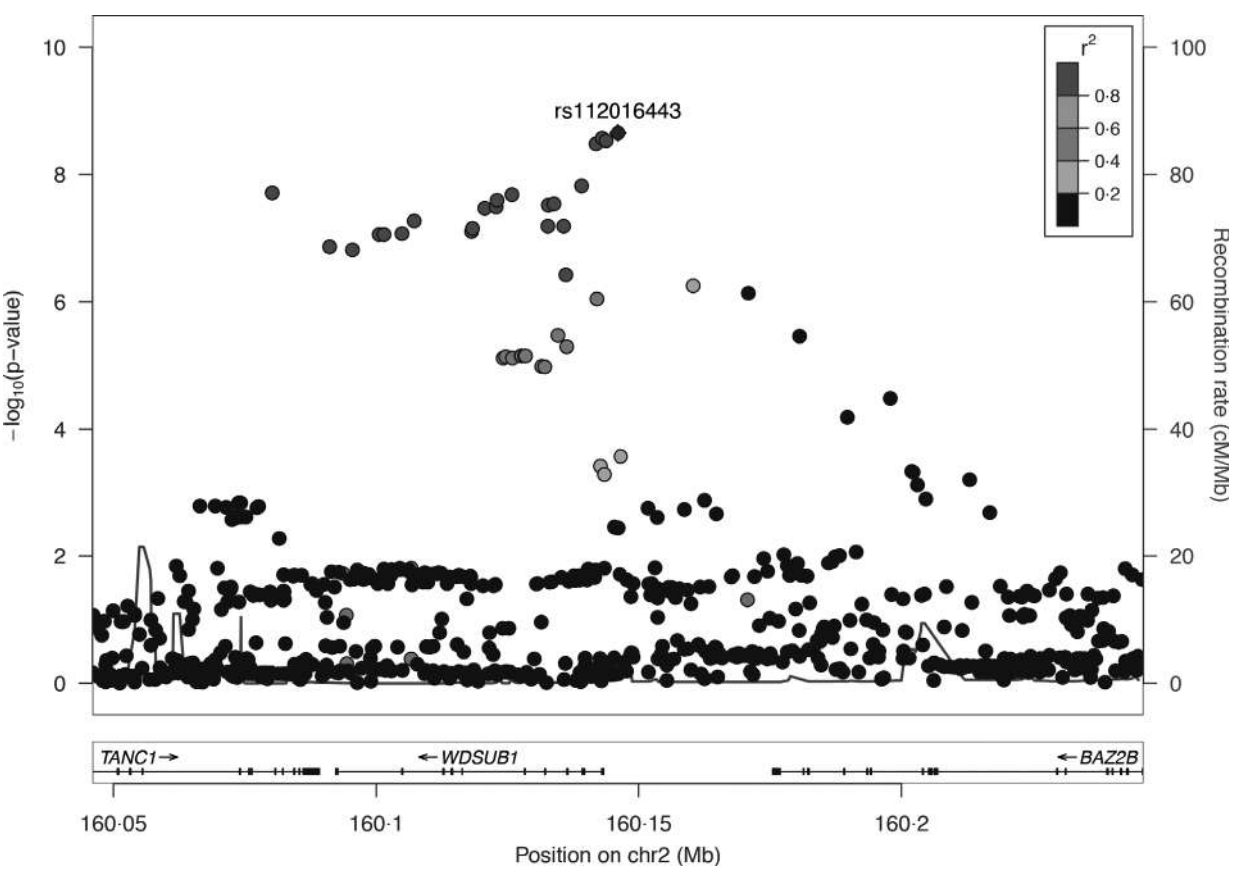

Fig 3 LocusZoom plot for SNPs rs112016443 reaching $P < 1 \times 10^{-8}$ for ferritin levels.

TMPRSS6) to be associated with ferritin levels [18], three SNPs (rs671 in *ALDH2*, rs3782886 in *BRAP* and rs5742933 in *PMS1* and *ORMDL1*) were identified in Chinese populations [19,21], one SNP (rs1799945 in *HFE*) in a Hispanic/Latino population [20] and one SNP (rs141555380 in *GAB3*) in an African American population [22]. However, all of these SNPs were located a large distance (>2 000 000 base pairs) away from rs112016443 and did not reach suggestive significance in our study (Table S3). Not finding rs112016443 in Asian populations is not surprising, as this SNP is virtually non-existing in this population [40]. Among blood donors, limited numbers of SNPs were studied, and reported SNPs (rs9296249 and rs3923809 in *BTBD9* [23,26], rs1800562 and rs1799945 in *HFE* [24], rs855791 and rs4820268 in *TMPRSS6* [24,26] and rs235756 near *BMP2* [26]) did also not reach suggestive significance in our study population (Table S4) [23–26]. Rs112016443 is known to influence expression levels of *WDSUB1*, a protein-coding gene. *WDSUB1* is known to be part of the main U-box Ubiquitin Ligase-encoding genes found in humans, and is involved in sequence-specific DNA binding and ubiquitin-protein transferase activity [41–43]. The exact underlying mechanism connecting *WDSUB1* to ferritin levels in blood donors remains unclear and needs further investigation.

Differences in results between this GWAS and previous studies could have several reasons. First, differences could be due to differences in study populations. Our study population consisted of Europeans, while most of the studies investigating ferritin levels were performed among non-European populations [44]. Furthermore, our study population consisted of blood donors, while previous studies regarding erythrocyte parameters and ferritin levels were not specifically performed in blood donors. Reduced ferritin levels due to blood donations may not be directly comparable with ferritin levels in the general population and might be more dependent on lifestyle behaviours or a person's ability to recover quickly from a donation (e.g. to quickly absorb and store iron). Last, differences could be due to heterogeneity in Hb and ferritin measurements and genotyping platforms used.

A major strength of our study is the fact that by fitting growth-mixture models, we were the first to study genetic determinants of trajectories of repeated Hb measurements in blood donors, alongside ferritin and Hb levels based on single measurements [7,16,45]. As this is the first and only GWAS investigating associations between SNPs and Hb trajectories/levels and ferritin levels in blood donors, this study was not reflecting genetic determinants of erythrocyte parameters under steady-state conditions as is the case in previous studies conducted among general populations. Furthermore, this study was not restricted by a limited number of SNPs tested and studied, as in previous studies among blood donors (Table S4). Another strength of this study is the use of an extensive SNP array, namely the UK Biobank – version 2 Axiom Array (Thermo Fisher). In addition to the genome-wide 800K SNPs this array is extended with SNPs that might be relevant for blood transfusion, such as all known SNPs in genes encoding blood group antigens, but also SNPs which have previously been found to be associated with red cell traits [13,46,47]. A limitation of this study is the relatively small sample size, which results in limited power to detect smaller effect sizes, especially for less frequent variants. Furthermore, our study population also included inactive donors, who most likely had higher Hb and ferritin levels than they would have had as active blood donors. However, we took this into account by correcting for time since last donation. After adjusting for number of donations in the past two years 'trails' were still seen on the same chromosomes (Fig S6). This suggests that the extra variation in Hb and ferritin level caused by differences in donation history did not influence the outcome of our analysis. Additionally, there was no interaction between rs112016443 and number of donations in the past two years with ferritin levels (results not shown). This indicates that the association of rs112016443 with ferritin levels is similar for active and inactive donors.

Our study provides new possibly relevant SNPs – one reaching GW significance – for Hb trajectories, Hb levels and ferritin levels in blood donors. We showed that the *A* allele of SNP rs112016443 on chromosome 2 was associated with higher ferritin levels. Further studies are needed to replicate our findings as well as to unravel possible underlying mechanisms. Furthermore, future research – for example to identify SNPs that could potentially be associated with low iron – should indicate whether or not it is recommendable to implement genetic screening to gain insight into which donors are prone to iron deficiency in order to personalize donation intervals.

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Conflict of interests

The authors have no conflict of interest.

Author contributions

W.L.A.M.d.K., C.E.v.d.S. and K.v.d.H. designed the project. T.C.T. performed the research and took the lead in writing the manuscript. M.W.T.T. performed the GWAS. C.J.P. performed the quality control and imputation of the GWAS. N.S.G. designed the array and some of the software involved in analysis. K.E.S. ensured that DNA samples met the requirements and organized the shipment, storage and laboratory analysis of the DNA samples. M.W.T.T., K.v.d.H., W.L.A.M.d.K. and C.E.v.d.S. supervised the analyses and interpretation of results of this work. All authors contributed to the interpretation of the results, provided feedback and helped shape the research, analyses and manuscript.

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Supporting Information

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

Appendix S1. Supplementary methods, results and references.
Figure S1. Q-Q plots for independent SNPs reaching$p < 1 \times 10^{-5}$ for haemoglobin trajectories, haemoglobin levels and ferritin levels.
Figure S2. Manhattan plots of the gene-based test as computed by MAGMA in FUMA. Dashed line represents the genome-wide significance threshold ($P < 2{\cdot}7 \times 10^{-6}$) for gene-based test.
Figure S3. Q-Q plots for (A) SNPs previously found to be associated with erythrocyte parameters, and (B) SNPs previously found to be associated with ferritin levels.
Figure S4. Scatterplot of effects of top SNPs found for haemoglobin (Hb) trajectories (A1 and A2), haemoglobin levels (B1 and B2), and ferritin levels (C1 and C2).
Figure S5. Associations between (A) haemoglobin levels (mmol/l) and log transformed ferritin levels (μg/l), (B) log transformed ferritin levels and haemoglobin trajectories (stable/declining), and (C) haemoglobin levels and haemoglobin trajectories. P = *P*-value; *r* = correlation coefficient.
Figure S6. Manhattan plot for haemoglobin trajectories, haemoglobin levels and ferritin levels adjusted for age, sex (men, premenopausal women, and postmenopausal women), first ten principal components, number of donations in two years prior to DIS-III and donation interval (i.e. time between DIS-III donation and previous visit). All *P*-values are negative log10 transformed. Line at 1×10^{-5} represents the suggestive threshold; line at 5×10^{-8} represents the genome-wide significance threshold.

Table S1. Further adjustments for covariates of genomic loci independently associated with Hb trajectories, Hb and ferritin levels.
Table S2. Significant single tissue expression quantitative trait loci (eQTLs) of independent SNPs for Hb trajectories, Hb levels and ferritin levels with whole blood indicated in bold.
Table S3. Effects and *P*-values of SNPs found to be associated with ferritin levels in previous GWASs and corresponding *P*-values for ferritin levels (μg/l) in DIS-III.
Table S4. Effects and *P*-values of SNPs found to be associated with haemoglobin and ferritin levels in previous studies among blood donors and corresponding effects and *P*-values for haemoglobin and ferritin levels in DIS-III.
Table S5. Effects and *P*-values of SNPs found to be associated with erythrocyte parameters in our previous systematic review and corresponding *P*-values for Hb levels (mmol/l) and Hb trajectories (stable/declining) in DIS-III.

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ABO blood group and SARS-CoV-2 antibody response in a convalescent donor population

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Background and Objectives ABO blood group may affect risk of SARS-CoV-2 infection and/or severity of COVID-19. We sought to determine whether IgG, IgA and neutralizing antibody (nAb) to SARS-CoV-2 vary by ABO blood group.

Materials and Methods Among eligible convalescent plasma donors, ABO blood group was determined via agglutination of reagent A1 and B cells, IgA and IgG were quantified using the Euroimmun anti-SARS-CoV-2 ELISA, and nAb titres were quantified using a microneutralization assay. Differences in titre distribution were examined by ABO blood group using non-parametric Kruskal–Wallis tests. Adjusted prevalence ratios (aPR) of high nAb titre (≥1:160) were estimated by blood group using multivariable modified Poisson regression models that adjusted for age, sex, hospitalization status and time since SARS-CoV-2 diagnosis.

Results Of the 202 potential donors, 65 (32%) were blood group A, 39 (19%) were group B, 13 (6%) were group AB, and 85 (42%) were group O. Distribution of nAb titres significantly differed by ABO blood group, whereas there were no significant differences in anti-spike IgA or anti-spike IgG titres by ABO blood group. There were significantly more individuals with high nAb titre (≥1:160) among those with blood group B, compared with group O (aPR = 1·9 [95% CI = 1·1–3·3], P = 0·029). Fewer individuals had a high nAb titre among those with blood group A, compared with group B (aPR = 0·6 [95%CI = 0·4-1·0], P = 0·053).

Conclusion Eligible CCP donors with blood group B may have relatively higher neutralizing antibody titres. Additional studies evaluating ABO blood groups and antibody titres that incorporate COVID-19 severity are needed.

Key words: ABO group, convalescent plasma, COVID-19, SARS-CoV-2, titre.

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Introduction

Since its first description in China in December 2019, over 66 million cases of severe acute respiratory

syndrome-associated coronavirus 2 (SARS-CoV-2) infection – the cause of coronavirus-19 disease (COVID-19) – have been reported, spanning 191 countries or territories and accounting for over 1·5 million deaths [1]. Over a fifth (>14 million) of those cases have been reported in the United States.

Amid established sociodemographic (e.g. older age, male sex, racial and ethnic minorities) and clinical (diabetes, hypertension, smoking) risk factors for COVID-19 severity, the possibility of an association with ABO blood group was raised early in the pandemic. Specifically, blood group A individuals were suggested to be at greater risk of infection, while blood group O was observed to be protective [2–5]. Nonetheless, while overrepresentation of group A (and under-representation of group O) has been observed among COVID-19 cases, the findings have been mixed with respect to clinical outcomes [6–9].

Blood groups have long been suspected as playing a part in the pathogenesis of infectious diseases, spanning malaria to parvovirus B19 [10]. An association between SARS-CoV-2 risk (i.e. susceptibility and/or disease severity) and ABO blood group is plausible, yet remains uncertain. Prior to the COVID-19 pandemic, a retrospective study of healthcare workers in Hong Kong reported group O participants to be less likely to become infected with SARS-CoV – a closely related virus to SARS-CoV-2 – than non-O participants [11]. A subsequent study showed that anti-A isoagglutinins were capable of inhibiting the S protein/angiotensin-converting enzyme 2 interaction suggesting a role blocking the virus from its receptor [12]. It has also been suggested that antibody subclass may be important with protection ascribed to anti-A IgG rather than IgM [13]. Of note, anti-A IgG is more common in group O individuals as compared to those of other non-A groups. In short, anti-A – rather than blood group itself – may be the central factor [14].

Given the plausibility of a role in immunopathogenesis of COVID-19, we sought to assess the association between ABO blood group and SARS-CoV-2 antibody titres (IgA, IgG and neutralizing antibodies [nAbs]) in eligible COVID-19 convalescent plasma (CCP) donors. Of note, transfusion of plasma from convalescent individuals (i.e. 'CCP') has emerged as a leading therapy for COVID-19 [15–20]. If there is a difference in titre by ABO type, it could potentially be exploited through preferential recruitment of CCP donors of certain types and/or selective use for manufacture of hyperimmune globulin. This offered another rationale for pursuing this study.

Materials and methods

Study participants

The study population comprised individuals who were deemed to be eligible to donate COVID-19 convalescent plasma at the beginning of the pandemic in the United States (i.e. prior to FDA requirements for specific titres). Individuals aged at least 18 years who had a history of COVID-19 as confirmed by a positive molecular test for SARS-CoV-2 were eligible to participate in the study. Recruitment was undertaken using a combination of self-identification (in response to advertising or media postings) and referral from healthcare providers. Initial screening was conducted telephonically: individuals were informed that they needed to satisfy standard eligibility criteria for blood donation. Notable exclusion criteria included pregnancy in the preceding six weeks and/or an established diagnosis or risk factors for transfusion-transmitted infections (notably HIV, hepatitis B virus or hepatitis C virus). Those who passed the initial telephonic screening were invited to participate in the study. Basic demographic information (age, sex, hospitalization with COVID-19) was collected, and the original diagnosis of SARS-CoV-2 infection was confirmed by medical chart review or sharing of source documentation. Enrolment was performed under full informed consent after which ~25 ml of whole blood was collected in ACD tubes. The samples were separated into plasma and peripheral blood mononuclear cells within 12 h of collection. The plasma samples were immediately frozen at −80°C. The study was approved by the Johns Hopkins University School of Medicine Institutional Review Board prior to initiation.

ABO testing

Manual reverse group testing (i.e. determination of group A and group B antibodies) of the subjects' samples was performed in accordance with the AABB (formerly American Association of Blood Banks) procedure for manual, tube-based testing with A1 and B cells [21]. Agglutination reactivity was graded from 0 to 4+. Agglutination reactions of weak to 2 + were verified by repeat testing, of which all samples confirmed the same ABO blood group.

IgG, IgA and nAb titres

IgA, IgG and nAbs were quantified as previously described [22]. Briefly, Euroimmun anti-SARS-CoV-2 IgG and IgA ELISAs (Mountain Lakes, NJ) for the S1 domain of spike protein were utilized per the manufacturer's

instructions. The optical density (OD) of the sample divided by calibrator provided arbitrary unit ratio (A.U.) that ≥1·1 were considered positive and ≥0·8 to <1·1 were considered indeterminate. Continuous AU values were interpreted as anti-SARS-CoV-2 IgG and IgA titre levels. Quantification of nAb titres against 100 fifty per cent tissue culture infectious doses (TCID50) was performed using a microneutralization (NT) assay. nAb area under the curve (AUC) values were estimated using the exact number of wells protected from infection at every plasma dilution; samples that had no NT activity were assigned an arbitrary value of one-half of the lowest nAb AUC. The overall distribution of nAb AUC values in this sample was a median of 60 (interquartile range [IQR]: 10, 150). For analytic purposes and consistency with our previous analyses using early recommendations by the FDA, nAb AUC values ≥160 were considered to indicate high neutralization potency [23,24].

Statistical analysis

Characteristics of the study population were examined overall and stratified by ABO blood group using descriptive statistics. The primary study outcome was nAb titres to SARS-CoV-2 (AUC value); IgA and IgG antibody levels to the spike-1 protein of SARS-CoV-2 (A.U.) were examined as secondary outcomes. Continuous titre outcome measures were $\log_2$-transformed to approximate normal distributions. Differences in the distribution of titre outcome measures were examined by ABO blood group using global non-parametric Kruskal–Wallis tests.

The association of ABO blood group with nAb titres was further examined using univariable and multivariable ordinary least-squares linear regression. To assess whether ABO blood group is an independent correlate of nAb titres, the primary multivariable model included all covariates that have previously been shown to be determinants of SARS-CoV-2 antibody responses: age, sex, hospitalization status and time since first PCR-positive test date for SARS-CoV-2 [22]. To further assess the primary outcome, the association between blood type and a high neutralizing antibody titre AUC value (≥160) was also examined, similar to previous investigations [24].For this analysis, prevalence ratios and adjusted prevalence ratios (aPR) were estimated by univariable and multivariable Poisson regression models with robust variance. The multivariable Poisson model included all covariates previously described.

In our conceptual framework, race/ethnicity was not considered as a potential covariate since it is a social construct and we are unaware of existing evidence that indicates it is a determinant of SARS-CoV-2 antibody responses. However, since blood type is linked to race/ethnicity, a sensitivity analysis was performed that included adjustment for race/ethnicity (White vs. all other races due to sparse data). A likelihood-ratio test was used to assess whether inclusion of this race/ethnicity variable improved model fit to the data as compared to the multivariable linear regression model used in the primary analysis. The Akaike's information criterion (AIC) was also compared between the multivariable models. Since the majority of the sample population was White, a separate sensitivity analysis was also performed restricted to White donors. Finally, since this sample is selected on those who recovered from COVID-19 and hospitalization status may potentially be influenced by ABO type, a sensitivity analysis was conducted restricted to donors who were known to not have a history of hospitalization due to COVID-19 (i.e. mild/moderate cases).

All *P* values are two-sided. Analyses were performed in Stata/MP, version 15.1 (StataCorp, College Station, TX, USA).

Results

A total of 202 unique study participants were evaluated (Table 1). Overall, at the time of sample collection, the median number of days since PCR + nasal pharyngeal swab was 46 days (interquartile range [IQR], 39–56 days). The median age was 43 years (IQR: 32–56), and 53% were male; 76% were White. A total of 15 (7%) reported prior hospitalization for COVID-19 (Table 1). Of the study population, 85 (42%) were blood group O, 65 (32%) were blood group A, 39 (19%) were blood group B, and 13 (6%) were blood group AB. Table 1 also provides sociodemographic and clinical characteristics of the study population stratified by ABO blood group. Of note, the median age was 48 (IQR: 34–57) years among donors with blood group A, 47 (IQR: 36–63) years among donors with blood group B, 43 (32–58) years among donors with blood group AB and 39 (IQR = 30–50) years among donors with blood group O.

There were no significant differences in the distribution of anti-SARS-CoV-2 IgG and IgA levels by ABO blood groups (Fig. 1a,b). In contrast, the distribution of nAb titre AUC values varied significantly by ABO blood group (Kruskal–Wallis, $P = 0{\cdot}018$; Fig. 1c). The mean nAb $\log_2$(AUC) value was 5·5 (standard deviation [SD] = 2·4) among donors with blood group A, 6.4 (SD = 2·4) among donors with blood group B, 4·4 (SD = 2·1) among donors with blood group AB and 5·3 (SD = 2·2) among donors with blood group O. In multivariable linear regression analysis, donors with blood group B had significantly higher nAb titres ($\log_2$[AUC]) than donors with blood group O (adjusted $\beta = 0{\cdot}9$ [95% CI: 0·1, 1·8], $P = 0{\cdot}026$) (Table 2). Donors with blood group A (adjusted $\beta = -0{\cdot}9$

Table 1 Characteristics of the study population overall and stratified by ABO blood group

Characteristic	Overall (n = 202)	Blood group			
		A (n = 65)	B (n = 39)	AB (n = 13)	O (n = 85)
Median age (IQR), years	43 (32–56)	48 (34–57)	47 (36–63)	43 (32–58)	39 (30–50)
Age group, years					
18–29	40 (20%)	11 (17%)	5 (13%)	3 (23%)	21 (25%)
30–39	42 (21%)	10 (15%)	7 (18%)	3 (23%)	22 (26%)
40–49	43 (21%)	13 (20%)	11 (28%)	1 (8%)	18 (21%)
50–59	40 (20%)	17 (26%)	4 (10%)	4 (31%)	15 (18%)
≥60	37 (18%)	14 (22%)	12 (31%)	2 (15%)	9 (11%)
Sex					
Female	94 (47%)	28 (43%)	19 (49%)	6 (46%)	41 (48%)
Male	108 (53%)	37 (57%)	20 (51%)	7 (54%)	44 (52%)
Race/ethnicity					
White	154 (76%)	52 (80%)	26 (67%)	11 (85%)	65 (76%)
Black	9 (4%)	0 (0%)	3 (8%)	0 (0%)	6 (7%)
Hispanic	8 (4%)	2 (3%)	0 (0%)	0 (0%)	6 (7%)
Asian	22 (11%)	8 (12%)	7 (18%)	1 (8%)	6 (7%)
Mixed/Other/Unknown	9 (4%)	3 (5%)	3 (8%)	1 (8%)	2 (2%)
Hospitalized (severity)					
No	185 (92%)	60 (92%)	37 (95%)	12 (92%)	76 (89%)
Yes	15 (7%)	4 (6%)	2 (5%)	0 (0%)	9 (11%)
Unknown	2 (1%)	1 (2%)	0 (0%)	1 (8%)	0 (0%)
Median days since PCR + (IQR)	46 (39–56)	43 (39–51)	43 (35–56)	46 (39–55)	49 (42–59)

Abbreviations: IQR, interquartile range; PCR, polymerase chain reaction.

[95% CI: −1·8, −0·1], P = 0·031) and blood group AB (adjusted β = −2·0 [95% CI: −3·4, −0·6], P = 0·005) had significantly lower nAb titres than donors with blood group B.

The prevalence of high nAb titres (AUC ≥ 160) was 25% (16/65) among donors with blood group A, 44% (17/39) among donors with blood group B, 0% (0/13) among donors with blood group AB and 20% (17/85) among donors with blood group O. Notably, donors with blood group B were significantly more likely to have high nAb titres than donors with blood group O (aPR = 1·9 [95% CI = 1·1, 3·3], P = 0·029) (Table 2). In addition, fewer donors had a high nAb titre among those with blood group A, compared to those with group B (aPR = 0·6 [95%CI = 0·4, 1·0], P = 0·053).

Effect estimates for other covariates in the primary linear regression and modified Poisson regression models are shown in Table S1. Multivariable regression results were insensitive to adjustment for race/ethnicity (Table S2). Inclusion of race/ethnicity in the multivariable linear regression model did not significantly improve model fit to the data based on a likelihood-ratio test (likelihood-ratio χ^2 = 2·47, P = 0·116). This was further supported by minimal change in the AIC value between the linear regression models (primary analysis multivariable model: AIC = 874·6 vs. sensitivity analysis multivariable model with race/ethnicity: AIC = 874·2). When the analysis was restricted to White donors, the comparison of nAb levels between donors with blood group A vs. B was significantly attenuated (Table S3). However, all other results in the White donor sample remained in the same direction of association as the primary analysis. Associations observed in the analysis restricted to donors who did not have a history of hospitalization were consistent with those observed in the primary analysis (**Table S4**).

Discussion

The ABO blood group of an individual has been suggested to impact the risk of susceptibility to SARS-CoV-2 infection and/or disease severity in COVID-19. Using a sampling of convalescent individuals, we sought to determine whether there were differences in antibodies against SARS-CoV-2 by ABO type. Although significant differences in anti-spike IgA or anti-spike IgG by ABO blood group were not detected, those individuals of blood group B had higher nAb titres, especially when compared to those who were group AB and group O. There were also significantly more individuals with blood group B than blood group O with high nAb titres (≥1:160).

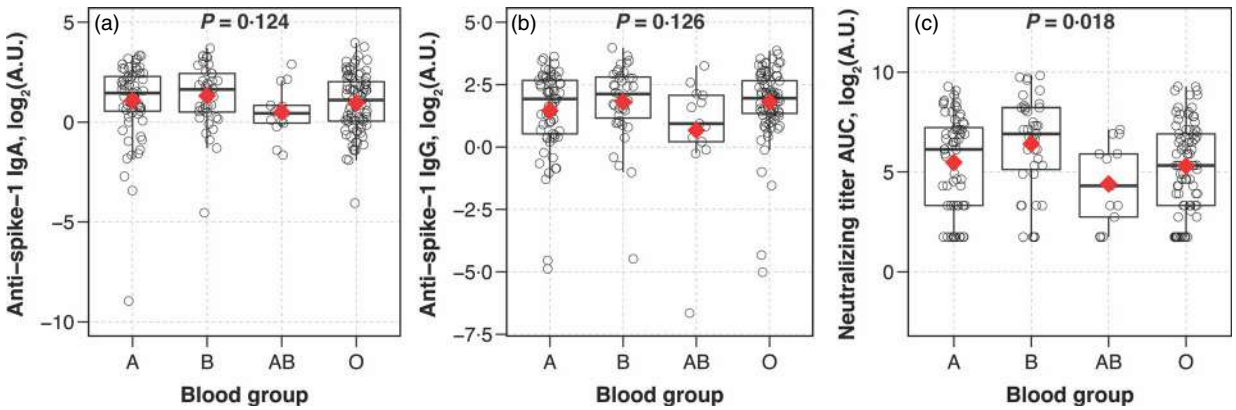

Fig. 1 Distribution of IgA, IgG and neutralizing antibody titres to SARS-CoV-2 by ABO blood group in eligible convalescent plasma donors. Box-and-whisker plots were used to depict the median (thick horizontal line), interquartile ranges and upper/lower extreme limits. The red diamond depicts the arithmetic mean. Circles depict the individual data points. *P* values were determined from non-parametric Kruskal–Wallis tests.

Table 2 Association of ABO blood group with neutralizing antibody titres to SARS-CoV-2 in eligible convalescent plasma donors

	SARS-CoV-2 neutralizing titre AUC, log2(arbitrary units)			
	Univariable		**Multivariable**	
Blood group (vs. reference blood group)	**β (95% CI)[a]**	***P* value**	**β (95% CI)[b]**	***P* value**
A vs. O	0·2 (−0·6, 0·9)	0·637	0·0 (−0·7, 0·7)	0·994
B vs. O	**1·1 (0·2, 2·0)**	**0·014**	**0·9 (0·1, 1·8)**	**0·026**
AB vs. O	-0·9 (−2·2, 0·5)	0·201	-1·0 (−2·3, 0·3)	0·116
A vs. B	**-0·9 (−1·9, −0·0)**	**0·048**	**-0·9 (−1·8, −0·1)**	**0·031**
AB vs. B	**-2·0 (−3·5, −0·5)**	**0·008**	**-2·0 (−3·4, −0·6)**	**0·005**
A vs. AB	1·1 (−0·3, 2·5)	0·131	1·0 (−0·3, 2·4)	0·120
	SARS-CoV-2 neutralizing titre AUC ≥ 160 arbitrary units			
	Univariable		**Multivariable**	
Blood group (vs. reference blood group)	**Crude PR (95% CI)[c]**	***P* value**	**Adjusted PR (95% CI)[d]**	***P* value**
A vs. O	1·2 (0·7, 2·2)	0·500	1·1 (0·6, 2·0)	0·684
B vs. O	**2·2 (1·2, 3·8)**	**0·006**	**1·9 (1·1, 3·3)**	**0·029**
AB vs. O[e]	-	-	-	-
A vs. B	**0·6 (0·3, 1·0)**	**0·044**	0·6 (0·4, 1·0)	0·053
AB vs. B[e]	-	-	-	-
A vs. AB[e]	-	-	-	-

Abbreviations: AUC, area under the curve; CI, confidence interval; PR, prevalence ratio.

Bold values correspond to statistically significant findings.

[a]β represents the absolute difference in $\log_2$ SARS-CoV-2 nAb AUC value with the reference group as estimated by univariable linear regression.

[b]β represents the absolute difference in $\log_2$ SARS-CoV-2 nAb AUC value with the reference group after adjusting for blood group, age, sex, hospitalization status and time since first PCR + test for SARS-CoV-2 infection, as estimated by multivariable linear regression.

[c]Crude prevalence ratios for SARS-CoV-2 nAb AUC ≥ 160 were estimated from univariable modified Poisson regression models with robust variance.

[d]Adjusted prevalence ratios for SARS-CoV-2 nAb AUC ≥ 160 were estimated from multivariable Poisson regression models with robust variance. The multivariable model included adjustment for age, sex, hospitalization status and time since first PCR + test for SARS-CoV-2 infection.

[e]Group AB had no observations with SARS-CoV-2 nAb AUC ≥ 160; thus, estimates were not calculated.

A growing number of studies suggest that ABO type plays a role in the pathogenesis of COVID-19 [2,4–6,25,26]. A large genome-wide association analysis of patients with COVID-19-induced respiratory failure identified genetic susceptibility at the ABO blood group locus, offering a biological basis for an association with ABO type [27]. In the same study, group A was associated with a significantly higher risk of COVID-19, while group O was observed to have lower risk. This finding has been reported in other studies [2,4,6,27]. Further, a lower prevalence of nAbs against SARS-CoV-2 was reported among group O individuals (compared with donors of other types) in a large cross-sectional sample of asymptomatic French blood donors [9]. This suggested an effect on susceptibility to infection (i.e. relative protection in the case of group O individuals), and not only clinical outcomes.

Why blood group B individuals should have higher SARS-CoV-2 titres is unknown. While speculative, one possibility is cross-reactivity between the virus and the B antigen, thus stimulating antibody production. Alternatively, the viral antigen may appear more foreign to individuals who are blood group B compared with blood group A or O. Further research is needed in this regard. Nonetheless, blood groups have long been recognized to interact with diverse viruses, parasites and bacteria, playing a role in both susceptibility to infection and severity of resulting disease [10].

While an association between ABO type and nAb titres was demonstrated in our study, this was not the case for ABO type and IgG or IgA against spike protein. The titres of nAbs have been shown to correlate well – albeit not perfectly – with those of antibodies against spike protein or receptor binding domain, as determined using clinical assays (i.e. ELISAs) [28–32]. Other factors (e.g. advanced age, male sex and hospitalization status) that are known to impact the antibody response to SARS-CoV-2 were controlled for in the study [22].

The kinetics of the humoral response to SARS-CoV-2 are still being learned [24,33–35]. While speculative, there are many factors that could account for the conflicting finding across studies investigating the relationship between ABO group and COVID-19. For one, there are differences in the populations that are being studied. While the racial distribution in our study population was representative of the U.S. blood donor population (i.e. over-representation of White patients), the ABO blood group distribution was disproportionately skewed towards group B and AB individuals. Specifically, the expected frequencies by ABO type in a US donor population for groups O, A, B and AB are 45%, 40%, 11% and 4%, respectively [21]; by contrast, the observed frequencies in our study were 42%, 32%, 19% and 6%, respectively. Of note, a previous study reported over-representation of group A and under-representation of group O in White patients with COVID-19, yet did not observe a difference in Black or Hispanic patients [6].

This study has limitations. For one, it was confined to a cross-sectional convenience sample of eligible donors from the Baltimore/Washington DC metropolitan areas. The small sample size particularly for some blood types (e.g. AB) may have resulted in sparse data bias and prevented us from conducting additional stratified analyses, including by titre. Second, selection of prospective blood donors may also limit generalizability, as these donors are not representative of a general population, with respect to both race/ethnicity and health status [36]. Selection is also skewed towards COVID-19 survivors who were sufficiently healthy to be recruited as convalescent plasma donors. By extension, the low proportion of subjects who had been hospitalized reflects individuals with a lower index of disease severity. In short, the study sample is unlikely to be representative of all patients with COVID-19 and additional studies that address disease severity by ABO type are needed. Third, only plasma was available for testing; this precluded forward typing of the red cells; that is, the ABO group was presumed based on the reverse typing. Fourth, this study population had a higher-than-expected proportion of group B and AB patients. However, sensitivity analyses confirm the findings of the higher titres are not due to the racial/ethnic composition of the study population. Fifth, titering of ABO isoagglutinins was not undertaken. That could have provided further insight. This study also did not evaluate IgM; while one cannot rule out the possibility that IgM accounts for the observed differences with the neutralization assay, it is was deemed unlikely given the time since test positivity (i.e. a surrogate of the initial infection). When combined with incubation and duration of symptomatic disease, IgM was considered to be an unreliable marker whereby the majority of individuals were at least six weeks from initial diagnosis. Finally, there may be residual and unmeasured confounding from variables that have not been considered.

In conclusion, individuals with blood group B who survive COVID-19 may potentially have higher nAb titres against SARS-CoV-2 compared with survivors of other blood groups, particularly blood group O. Further work is warranted in different populations to test generalizability of these results.

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Conflict of interests

EMB reports personal fees and non-financial support from Terumo BCT, Grifols Diagnostics Solutions and Abbott Laboratories outside of the submitted work; EMB is a member of the United States Food and Drug Administration (FDA) Blood Products Advisory Committee. Any views or opinions that are expressed in this manuscript are those of the authors, based on his own scientific expertise and professional judgement; they do not necessarily represent the views of either the Blood Products Advisory Committee or the formal position of FDA, and also do not bind or otherwise obligate or commit either advisory committee or the agency to the views expressed.

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Supporting Information

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

Table S1. Full multivariable model results from primary analysis of associations with neutralizing antibody titers to SARS-CoV-2 in eligible convalescent plasma donors.
Table S2. Sensitivity analysis: Association of ABO blood group with neutralizing antibody titers to SARS-CoV-2 in eligible convalescent plasma donors adjusting for race/ethnicity.
Table S3. Sensitivity analysis: Association of ABO blood group with neutralizing antibody titers to SARS-CoV-2 in eligible convalescent plasma donors who identified as white.
Table S4. Sensitivity analysis: Association of ABO blood group with neutralizing antibody titers to SARS-CoV-2 in eligible convalescent plasma donors without a history of hospitalization due to COVID-19.

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Impact of the COVID-19 pandemic on blood supply and demand in the WHO African Region

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Background and Objectives The coronavirus disease 2019 (COVID-19) affected millions of people worldwide and caused disruptions at the global level including in healthcare provision. Countries of the WHO African region have put in place measures for the COVID-19 pandemic containment that may adversely affect blood system activities and subsequently reduce the supply and demand of blood and blood components. This study aims to assess the impact of the COVID-19 pandemic on blood supply and demand in the WHO African Region and propose measures to address the challenges faced by countries.

Materials and Methods A survey questionnaire was sent to all 47 countries in the WHO African Region to collect information on blood supply and demand for the first 5 months of 2019 and 2020, respectively, and on COVID-19 Convalescent Plasma therapy in September 2020.

Results Thirty-seven countries provided responses. The total number of blood donations dropped in 32 countries while it increased in five countries. The proportion of blood drives also decreased in 21 countries and increased in nine countries. The blood requested and issued for transfusion decreased for blood demand and for blood issued for transfusion in 30 countries. Ten countries reported some activities of convalescent plasma. However, very few units of this product collected have been transfused to COVID-19 patients.

Conclusion The COVID-19 pandemic has led to a reduction of blood related activities in the region, including the supply and demand. Countries preparedness plans for health emergencies need more emphasis to maintaining blood stock.

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Key words: blood demand, blood donation, blood supply, convalescent Plasma, COVID-19 pandemic, WHO African Region.

Introduction

The coronavirus disease (COVID-19) pandemic is caused by the coronavirus 2 (SARS-CoV-2) primarily transmitted by the respiratory route. The characteristics of SARS-CoV-2 infection have been described by multiple reports [1,2]. The pandemic has caused significant socio-economic disruptions at the global level with an impact on healthcare provision. The COVID-19 outbreak continues to evolve in the WHO African Region (AFRO) since it was first detected in Algeria on 25 February 2020 with 8 confirmed cases. As of 23 June 2020, the 47 countries in AFRO are affected and have reported 236 909 cumulative confirmed cases of COVID-19 with 5257 deaths [3]. For blood transfusion services, experience with outbreaks of other coronaviruses suggested that there will be significant impact on blood supply due to reduced blood donation [4–6]. The COVID-19 pandemic has the potential to reduce the supply of blood and blood components and adversely affect blood system activities.

The World Health Organization (WHO) has developed and disseminated an interim guidance on maintaining a safe and adequate blood supply during the COVID-19

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pandemic [7]. This guidance recommends (1) mitigating potential risk of transmission through blood transfusion, staff risk and donor exposure to COVID-19, as well as risk of reduced availability of blood donors; (2) managing blood demand; (3) ensuring undisrupted supply of critical materials and equipment; (4) communicating to ensure that donors, recipients, all staff, relevant stakeholders and the population are properly informed; and (5) collection of convalescent plasma from patients who have recovered from COVID-19.

WHO estimated that the COVID-19 pandemic caused 20% to 30% reduction of blood supply in all its six regions, and it was noted that the donor attendance rate has fallen by 10–30% in the state of Washington in the United States of America (USA) and by 30% at Canadian Blood Services. However, in the early stages of the pandemic, this trend was compensated by a reduction in demand for blood because of a decrease in elective surgery and medical treatment [8,9]. The USA blood centres reported their lowest blood supply levels since the beginning of the pandemic, and blood drives continued to be cancelled since many businesses, schools and other organizations remained closed [10].

After imported cases of COVID-19 were reported in Saudi Arabia, donor attendance and blood supply at blood bank-based collection centres showed a drop of 39·5% and, on the other hand, blood demand during the same period was reduced by 21·7% [11]. In Malaysia, despite various promotional activities, the status of blood collection had not been satisfactory reaching only 57% of the target, and during the movement control order (MCO) enforcement, blood supply at the national blood centre and other blood centres throughout the country had decreased by 40% compared with previous years [12]. Due to the COVID-19 pandemic, the number of whole blood donors also dropped by 67% and the success rate of recruitment for donations dropped by 60% in Zhejiang province in China [13].

Regarding the COVID-19 convalescent plasma (CCP), clinical trials have been conducted in USA and other clinical trials are underway around the world to evaluate the effectiveness of using plasma derived from the blood of recovered COVID-19 patients to reduce the severity of illness among people infected with COVID-19 [14]. Furthermore, medical researchers in the Netherlands had recruited up 1500 people recovered from the new coronavirus to donate blood as part of an international push to develop a treatment for the virus from their plasma [15]. However, in Malaysia the Ministry of Health had 22 blood plasma packs donated by former COVID-19 patients for treatment and further research [16]. In the African Region, this approach was used by Mauritius in the current epidemic, in line with the national decision to use serum plasma therapy for critically ill COVID-19 patients [17].

Before the current COVID-19 pandemic, results of surveys on availability and access to safe blood and blood products showed that some improved functionality metrics for the national blood transfusion systems in most countries in the African Region. However, significant challenges still remain, the biggest one being the insufficient and unsustainable resources at the disposal of the national blood transfusion services (NBTS). This seriously compromises timely availability and access to safe blood for all patients who need blood in the region [18–20]. During the current COVID-19 pandemic, it was therefore necessary to assess the level of preparedness of countries in the region to maintain blood supply and demand and to respond appropriately to the emerging challenges.

The purpose of this paper is to outline key findings of the survey, discuss the impact of the COVID-19 pandemic on blood supply and demand in the African Region and propose measures to address challenges countries encountered as a result of the pandemic.

Materials and methods

We conducted a quick survey on the impact of COVID-19 on blood supply and demand in the WHO African Region. All 47 countries in the Region were invited to complete a structured questionnaire and send in their response from 21 May to 14 June 2020. For comparison purpose, countries were requested to provide data for the period from 1 January to 31 May 2019 and 1 January to 31 May 2020. A second round of the survey was conducted during the first two weeks of September 2020 especially focused on the COVID-19 convalescent plasma (CCP).

The survey questionnaire covered selected blood supply and demand indicators across key transfusion system metrics, namely blood donors and blood collection, blood demand and blood issued for transfusion and some key managerial aspects relevant for blood services including funding. With regard to CCP, the questionnaire covered the mapping of available study protocols on its use and the institutions that are performing the CCP collection; the study setting such as randomized controlled trial (RCT), observational study (OS), compassionate use (CU) or other; as well as the appreciation whether the protocols had regulatory authorization or not. The CCP donors who had consented to donate were recruited according to the guidelines from the organizing committee of the ISBT Working Party on Global Blood Safety [21].

All data provided were verified with countries during a virtual meeting for completeness and accuracy. Variables reported were expressed in number and or percentage of increase or decrease for blood donations, blood drives,

blood demand, blood use and CCP. The difference between the high and low figures and averages with ranges of key indicators were calculated for each set of data, where applicable. Data entry and analysis were performed using the Microsoft Office 365 Pro Plus Excel and presented in the form of tables. The figures with minus signs mean decrease and those without an increase.

The limitation of the study is that although countries put in place appropriate measures around end of June 2020 to encourage blood donation, we were not able to continue with the survey because the blood supply started to stabilize.

Results

Out of the 47 countries in the WHO African Region, 37 (78·7%) provided responses to the questionnaire as of 14 June 2020 of which two provided partial data limited to a single centre. The ten countries that did not provide any data were left out of the analysis. Figure 1 shows country responses to the survey on COVID-19 and blood supply and demand in the WHO African Region, over the period.

Overall, while blood donation rate reduction varied in 32 countries from 0·07% to 44·2%, five countries reported an increase ranging from 0·4% to 14% during the reported period. Out of 19 countries that had reached the regional target of 80–100% VNRBD in 2019, two countries failed to meet this target in the first half of 2020. One country reported 8343 paid blood donations in 2020 representing 5·7% of the total number of blood donations collected in that country.

In 21 countries, the number of blood drives also decreased from 24 467 in 2019 to 18 509 in 2020. This represents a proportion decrease ranging from 12·1% to 100%. Regular blood donation drives were drastically reduced as secondary schools, universities and most of the non-essential sectors were closed; thus, it was not possible to recruit blood donors in these institutions. However, nine countries reported an increase in the proportion of mobile drive ranging from 0·8% to 388·9%. In these countries, the number of blood donations remained on the pre-COVID marks or even increased. Satisfactory volunteer recruitment rates were reported to have been associated with the following considerations: late entry into countrywide lockdown, collaboration with national blood donor associations, civil society organizations, armed and security forces and good coordination with the national COVID-19 task force. In one country, there was no change in the number of blood drives, while 6 countries did not report any data on such collections.

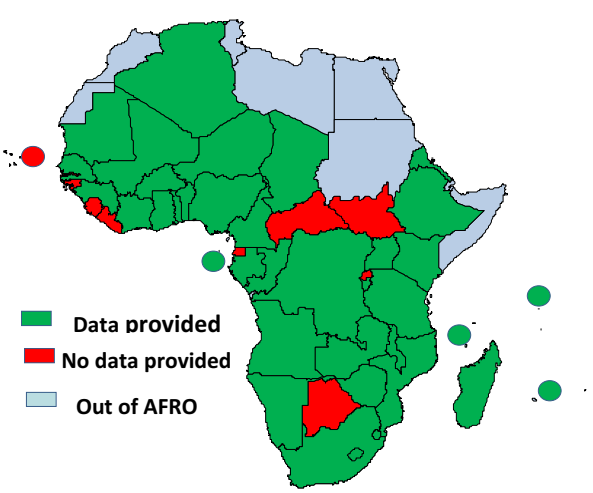

Fig. 1 Country responses to the survey on COVID-19 and blood supply and demand in the WHO African Region.

In 26 countries, the number of deferred donors dropped from 151 459 in 2019 to 105 755 in 2020 representing a percentage of decrease range from 1·6% to 100%. Two countries reported an increased deferred donor of 73·7% and 88·4%, respectively, while nine countries did not provide any data on donor deferred over the reported period.

The proportional drops ranged from 0·1% to 44% for blood demand and from 0·5% to 46·6% for blood issued for transfusion in 30 countries. However, seven countries reported an increase of blood demand ranging from 0·7% to 13·7%, while nine countries did so for the blood issued for transfusion ranging from 1·6% to 39%. The detailed results for each country for 2019 and 2020 related to the number of blood donations, blood drives, blood demand and blood issued are reported in Table 1 (Gap between blood donations in 2019 and 2020 in the African Region), in Table 2 (Gap between blood drives in 2019 and 2020 in the African Region) and in Table 3 (Gap between blood demand and blood issued for transfusion in 2019 and 2020 in the African Region). For most countries, blood issued was lower than blood demand and this could be explained by non-availability of sufficient blood to meet the overall requests/demands. In DRC, Senegal and South Africa the demand numbers and issue numbers were exactly the same because no complete data was given for blood demand or blood issued in these three countries.

Apart from platelet concentrates (PLT), for which the demand was slightly up, by 0·6%, demands for other blood components dropped by 21·1% for whole blood (WB), 10·8% for red blood cell (RBC), 4·0% for fresh frozen plasma (FPP) and 9·0% for cryoprecipitates. As for blood demand, blood issued for transfusion also dropped by 22% for WB, 8·3% for RBC, 14·9% for PLT, 3·5% for FFP and 23·4% for cryoprecipitate. Blood demanded and blood issued for transfusion decreased in healthcare facilities in most countries were generally due to reasons such

Table 1 Gap between blood donations in 2019 and 2020 in the African Region

	Number of blood donations			
Countries	**2019**	**2020**	**Difference**	**Proportion of decrease or increase (%)**
Algeria[a]	6025	4470	−1555	−25·8
Angola	68 965	49 353	−19 612	−28·4
Benin	29 636	25 352	−4284	−14·5
Burkina Faso	45 229	29 391	−15 838	−35·0
Burundi	34 237	39 041	4804	14·0
Cameroon	47 275	32 328	−14 947	−31·6
Chad	9860	9903	43	0·4
Comoros	1 106	848	−258	−23·3
Congo	25 998	26 098	100	0·4
Côte d'Ivoire	60 201	51 374	−8 827	−14·7
Democratic Republic of Congo	255 460	146 693	−108 767	−42·6
Eritrea	4536	4593	57	1·3
Eswatini	7185	5174	−2011	−28·0
Ethiopia	98 340	106 582	8242	8·4
Gabon	9175	6999	−2176	−23·7
Ghana	73 063	57 269	−15 794	−21·6
Guinea	8543	6806	−1737	−20·3
Kenya	59 858	33 419	−26 439	−44·2
Lesotho	2765	2550	−215	−7·8
Madagascar	21 868	16 733	−5135	−23·5
Malawi	22 560	21 210	−1 350	−6·0
Mali	22 747	16 765	−5 982	−26·3
Mauritania	8017	7968	−49	−0·6
Mauritius	19 685	13 727	−5958	−30·3
Mozambique	54 811	49 207	−5604	−10·2
Namibia	15 204	14 900	−304	−2·0
Niger	9619	8560	−1059	−11·0
Nigeria[a]	9450	5879	−3571	−37·8
Sao Tome and Principe	499	364	−135	−27·1
Senegal	16 442	13 345	−3097	−18·8
Seychelles	675	594	−81	−12·0
South Africa	385 983	384 263	−1720	−0·4
Togo	16 054	16 043	−11	−0·1
Uganda	122 598	116 856	−5742	−4·7
United Republic of Tanzania	130 038	100 764	−29 274	−22·5
Zambia	55 085	46 453	−8632	−15·7
Zimbabwe	41 444	26 899	−14 545	−35·1
Total	1 800 236	1 498 773	−301 463	−16·7

[a]Data from BTS of Blida for Algeria and NBTS of Lagos State for Nigeria.

as (1) decrease in requests for blood from the prescribers that has contributed to making the shortage of blood less pronounced; (2) outpatient department shut down; (3) routine surgery suspension; (4) difficulty in transporting blood and blood components from a blood bank to another; (5) management of the blood request by a good communication between health professionals working in health facilities to give priority to emergency situations; (6) most of NBTS do not have records to capture all blood requested and only the blood units issued get recorded or health facilities report only the quantity of units transfused without giving a break-down by components; and (g) non-availability of data on blood demanded and blood issued in some countries.

Even though countries that reported an increase in blood demanded and blood issued did not provide a specific reason, this might be linked to the maintaining normal clinical routine activities. Whatever, the situation

Table 2 Gap between blood drives in 2019 and 2020 in the African Region

Countries	Number of blood drives			
	2019	2020	Difference	Proportion of decrease or increase (%)
Algeria[a]	17	19	2	11·8
Angola	-	-	-	-
Benin	553	386	−167	−30·2
Burkina Faso	994	450	−544	−54·7
Burundi	638	643	5	0·8
Cameroon	-	-	-	-
Chad	9	44	35	388·9
Comoros	-	-	-	-
Congo	70	71	1	1·4
Côte d'Ivoire	155	87	−68	−43·9
Democratic Republic of Congo	6387	3667	−2720	−42·6
Eritrea	53	46	−7	−13·2
Eswatini	-	-	-	-
Ethiopia	889	1094	205	23·1
Gabon	18	19	1	5·6
Ghana	506	240	−266	−52·6
Guinea	25	11	−14	−56·0
Kenya	1613	378	−1235	−76·6
Lesotho	56	9	−47	−83·9
Madagascar	46	8	−38	−82·6
Malawi	893	629	−264	−29·6
Mali	57	43	−14	−24·6
Mauritania	67	56	−11	−16·4
Mauritius	925	759	−166	−17·9
Mozambique	-	-	-	-
Namibia	1410	1159	−251	−17·8
Niger	-	-	-	-
Nigeria[a]	96	99	3	3·1
Sao Tome and Principe	3	3	0	0·0
Senegal	129	72	−57	−44·2
Seychelles	4	1	−3	−75·0
South Africa	4867	4279	−588	−12·1
Togo	44	24	−20	−45·5
Uganda	2667	2201	−466	−17·5
United Republic of Tanzania	1	0	−1	−100·0
Zambia	918	1548	630	68·6
Zimbabwe	657	464	−193	−29·4
Total	24 467	18 509	−6258	−25·3

[a]Data from BTS of Blida for Algeria and NBTS of Lagos State for Nigeria.

might be with routine clinical practices, and in preparation for the resumption of clinical activities after the COVID-19 pandemic, the NBTS should reach out to healthcare professionals responsible for transfusion activities. The NBTS should advise on the use of blood and components only when clinically appropriate.

With regard to the CCP survey, out of 47 countries in the region, 29 provided information of which ten showed some CCP activities. The mapping of the CCP therapy is indicated in Table 4 on COVID-19 convalescent plasma therapy in the WHO African Region.

The developed protocols varied from the institutional level to the national level. The use of CCP was also varied from RCT, OS or CU.

The risk of stock-out of reagents and consumables used along the blood transfusion chain, from the blood collection to transfusion to patients, increased from eleven (29·7%) countries in 2019 to 22 (59·5%) in 2020. Despite

Table 3 Gap between blood demand and issued for transfusion in 2019 and 2020 in the African Region

	Number of blood units demand				Number of blood units issued/transfused			
Countries	**2019**	**2020**	**Difference**	**%**	**2019**	**2020**		
Difference		**%**						
Algeria*	18 434	15 647	−2787	−15·1	10 337	8 186	−2151	−20·8
Angola	129 796		99 616		−30 180	−23·3	-	- - -
Benin	42 418	43 520	1102	2·6	31 769	36 926	5157	16·2
Burkina Faso	47 571	30 473	−17 098	−35·9	38 019	37 501	−518	−1·4
Burundi	-	-	-	-	30 836	33 123	2287	7·4
Cameroon	-	-	-	-	43 128	27 692	−15436	−35·8
Chad	5350	5755	405	7·6	6832	9500	2668	39·1
Comoros	-	-	-	-	1010	805	−205	−20·3
Congo	-	-	-	-	20 330	26 264	5934	29·2
Côte d'Ivoire	-	-	-	-	73 262	58 123	−15139	−20·7
Democratic Republic of Congo	237 218	141 677	−95 541	−40·3	237 218			
141 677		−95541		−40·3				
Eritrea	5740	6526	786	13·7	4511	4490	−21	−0·5
Eswatini	6343	3543	−2800	−44·1	5887	3543	−2344	−39·8
Ethiopia	-	-	-	-	123 581			
109 773		−13808		−11·2				
Gabon	9483	10 483	1000	10·5	9194	7795	−1399	−15·2
Ghana	28 698	27 014	−1684	−5·9	30 480	23 276	−7204	−23·6
Guinea	5 145	4 617	−528	−10·3	5120	5379	259	5·1
Kenya	127 182	93 637	−33 545	−26·4	57 936	30 943	−26993	−46·6
Lesotho	3924	3457	−467	−11·9	2784	2244	−540	−19·4
Madagascar	-	-	-	-	24 395	17 988	−6407	−26·3
Malawi	42 207	40 762	−1 445	−3·4	28 079	23 276	−4803	−17·1
Mali	-	-	-	-	13 011	12 177	−834	−6·4
Mauritania	13 552	14 108	556	4·1	9 130	9 560	430	4·7
Mauritius	50 943	46 052	−4891	−9·6	38 384	33 856	−4528	−11·8
Mozambique	63 301	63 768	467	0·7	60 309	61 330	1021	1·7
Namibia	–	–	–	–	15 657	15 354	−303	−1·9
Niger	16 578	15 110	−1468	−8·9	7266	6948	−318	−4·4
Nigeria[a]	6230	3610	−2620	−42·1	6230	3610	−2620	−42·1
Sao Tome and Principe	517	440	−77	−14·9	514	439	−75	−14·6
Senegal	17 659	13 606	−4053	−23·0	17 659	13 606	−4053	−23·0
Seychelles	2770	894	−1876	−67·7	–	–	–	–
South Africa	448 529	438 985	−9544	−2·1	448 529			
438 985		−9544		−2·1				
Togo	26 070	24 331	−1739	−6·7	20 046	20 360	314	1·6
Uganda	164 365	164 230	−135	−0·1	106 809			
105 841		−968		−0·9				
United Republic of Tanzania	–	–	–	–	105 953	78 012	−27941	−26·4
Zambia	116 452	120 270	3818	3·3	47 631	53 499	5868	12·3
Zimbabwe	46 870	38 957	−7913	−16·9	36 758	27 630	−9128	−24·8
Total	1 683 345	1 471 088	−212 257	−12·6	1 718 594			
1 489 711		−228883		−13·3				

[a]Data from BTS of Blida for Algeria and NBTS of Lagos State for Nigeria.

the COVID-19 pandemic, no country in the region has reported any specific budget allocation from the government related to the pandemic to strengthen national blood supply system. However, in 18 countries efforts have been undertaken by the NBTS to mobilize resources from other sources, mainly through (1) awareness raising; (2) advocacy with health authorities and partners; (3) risk mitigation to ensure business continuity; (4) development

Table 4 COVID-19 Convalescent plasma therapy in the WHO African Region

Country	National protocol for collection and use of CCP and regulatory authorization	Institutions that collect CCP	Use of CCP	CCP collected and patients treated	Comments and any additional information
Algeria	No				A protocol has been developed by the University Hospital of Blida, but still waiting for approval
Ethiopia	No: The NBTS however has a protocol for the selection of donor and collection of plasma from recovered patients. There were efforts by the national public health institute to get approval of CCP trial by the regulatory authority	NBTS	Compassionate Use	12 units (using routine whole blood collection and processing techniques)	The activity was halted as the Apheresis techniques was deemed more convenient and the process of procuring consumables for Apheresis collection was initiated.
Ghana	Yes: the protocol has received regulatory approval from the Ghana Foods and Drugs Authority	NBTS (Southern and Central Zonal Blood Centres)	Observational Study planned, but not commenced.	Collection has not started due to challenges with implementation	
Guinea	Yes: not yet approved	NBTS	Compassionate Use	15 units were collected, and 8 ICU COVID-19 patients treated.	
Mauritius	Yes	NBTS	Compassionate Use	3 ICU ventilated patients received CCP, 2 recovered well and the third patient passed away due to the development of sepsis and renal failure	150 recovered donors have consented to donate CCP Country does not have any community transmission of COVID 19 presently
Namibia	No				The country has not started collecting CCP, however, such an activity could be integrated in the plasmapheresis programme introduced in February 2020
Nigeria	Yes: There is a protocol for collection and use. This yet to be captured on the NBTS data.	Nigeria Institute for Medical Research (NIMR) and for now, Lagos State Blood Transfusion Services			Regulatory authority was sought and received by the NIMR. Country is working on harmonizing details through the Federal Ministry of Health
South Africa	Yes: received regulatory authorization	SANBS and WCBS	Randomized Controlled Trial	More than 50% of the required CCP for the RCT have been collected	SANBS and WCBS have collaborated on two studies with regard to CCP under the title of PROTECT trials. No data have been published

Table 4 (Continued)

Country	National protocol for collection and use of CCP and regulatory authorization	Institutions that collect CCP	Use of CCP	CCP collected and patients treated	Comments and any additional information
Uganda	Yes	Uganda blood transfusion service	Randomized Controlled Trial	The trial regulatory authorization certificate indicates a sample size of 136 patients. The projected need for the study period is 300 units	The trial is registered under clinicaltrials.gov.
Zimbabwe	Yes: the protocol has neither been shared publicly nor sent for regulatory approval	NBSZ	Compassionate Use	There has not been any collection done and no requests received	

and submission of proposals to potential donors for funding; (5) creating partnerships; and (6) cost recovery initiatives of blood issued in government and private hospitals. In four countries, 15 staff were reported to have been infected with COVID-19.

Discussions

Like all regions in the world, the African Region is facing socio-economic disruption due to the COVID-19 pandemic. It makes a significant impact on health service delivery. Overall, it was revealed through the survey that the safe blood supply and demand were at risk, since there was a decrease of these activities in most of respondent countries in the region, especially in the beginning of the COVID-19 pandemic. Most NBTS conducted a risk assessment, which focused on blood collection, laboratory testing and workplace transmission to adapt their response to the pandemic. The authors are cognizant of the fact that many African countries only started to experience the early infections of the pandemic in mid to late March 2020, with lockdown being implemented. However, this was a quick survey carried out over the considered period in order to monitor the impact of the COVID-19 pandemic on blood supply and demand.

The proportion of the reduction in the number of blood donations both at the regional and country levels were also reported by other countries such as Saudi Arabia, Canada, USA, Malaysia and in Zhejiang province in China [8–13]. In the countries with reduced blood donations, lockdown orders, donor anxiety and fear of COVID-19 infection during blood donation, which often stems from popular misconceptions and misinformation, have hindered blood donors from accessing blood transfusion services. Unfortunately, it was not possible to correlate the blood collection and usage to the time period where the countries enforced the social distancing measures and when infections started rising due to the declining of the COVID-19 cases in the countries of the region.

To improve blood donations and mitigate potential risks for blood donors, countries implemented measures such as (1) public awareness campaigns through local radio and TV, newspapers, social media platforms, bulk text messaging and direct call donors; (2) transporting donors to and from their homes with authorization from relevant national authorities; (3) providing facemasks, hand washing equipment and sanitizers; (4) modifying donor screening questionnaire for exposure to COVID-19; and (5) temperature measurement. Comparable measures have also been implemented by blood banks in China during the COVID-19 pandemic to reduce panic among potential blood donors [22].

However, after the strategies put in place during the lockdown, countries in the region need to think forward

and identify appropriate ways and methods of encouraging and increasing blood donations after the lifting of lockdown because blood donors may always be reluctant to come and donate, whereas needs of blood will expectedly increase again, especially in countries where routine clinical activities have been reduced, postponed or even suspended. Special attention needs to be given to the most affected countries in the region.

The CCP collected and transfused in the region remain very weak. The main issue for the use of this therapy in most countries in the region was the insufficient capacity of NBTS to safely collect, process and store it in a quality-assured manner. The challenges raised by some countries included the approval still awaited from their National Regulatory Authorities to conduct clinical trials and the lack of test kits for determination of anti-COVID-19 titres in the CCP due to lack of resources. However, some countries planned to establish partnerships with institutions taking part in ongoing RCT or OS or CU to collect CCP from recovered patients for the management of critically ill COVID-19 patients. Considering the trends of the COVID-19 pandemic in the African Region, whose cases are decreasing in most countries, it was not possible to collect statistically enough numbers of CCP.

The main reasons observed in 2019 regarding the stock-outs were related to delays in release and allocation of budget, whereas in 2020, the additional reasons were (1) border control measures; (2) transport and movement restrictions that led to the shortages of critical supplies and equipment needed for blood donation, processing, testing and transfusion to patients. Furthermore, the capacities of the central medical stores in most countries were exceeded because of the urgent need for procurement and supply of other commodities used for the COVID-19 pandemic response. As part of its working procedures, South Africa reported that it had put in place a risk mitigation measure through the procurement of additional stock items prior to the pandemic taking off to ensure business continuity. The supply chain systems with the national procurement agencies were facing some challenges due to COVID-19 pandemic such as borders closure, transport restrictions in Algeria, Benin, Burundi, Cameroon, Cote d'Ivoire, Ethiopia, Eritrea, Gabon, Mauritania, Mozambique, Niger, Tanzania and Zambia; inadequate funding from national governments to procure sufficient blood reagents and consumables in Burkina Faso, Eswatini and Lesotho as well as donor funding withdrawal in Kenya.

The biggest challenge with blood supply in many African countries is the insufficient resources allocated to NBTS by governments and drastic cut in funding from partners to make available safe and quality-assured blood and blood components for all patients who need blood. Countries also reported that there was no specific blood transfusion budget related to COVID-19 pandemic. Regarding staff, the challenges were the shortage of human resources due to travel restrictions within the same country, which prevented many employees from reaching places of work because of lockdown; staff safety especially the provision of personal protection equipment (PPE) for every employee; training for COVID-19 exclusion; social distancing during donor sessions and temperature monitoring.

To address the resource challenges faced by countries, most NBTS have undertaken initiatives such as (1) advocating for more allocation from the central government and other non-traditional organizations to support the service; (2) drawing up costed work plans to share with the MOH and the partners; (3) using available funds to implement workplace related measures to prevent COVID-19 as alternative means of mobilizing blood donors will in the long run affect ability to sustain operations if no replacement funding is available.

Conclusion

The COVID-19 pandemic has led to an overall reduction of blood transfusion activities in most countries in the region, in particular blood donations, blood demands and use in health facilities. However, the experience and measures implemented by countries to overcome the reduced activities and gain confidence of donors in safe blood donation yielded results. These experiences and measures should find an appropriate place in preparedness plans for future similar shocks to the blood transfusion system.

There is need to continue supporting countries through strengthening capacity of national blood transfusion systems, enhancing risk mitigation to ensure business continuity, developing a preparedness plan for future similar shocks to the blood transfusion system, raising awareness through advocacy with national health authorities and partners for sustainable funding of blood supply and demand in the region.

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Conflicts of interests

The authors declared no conflict of interest.

Authorship

André Loua: participated in the development of the assessment tool, the study design and supervision, data analysis and interpretation, and wrote the draft of the manuscript. Other co-authors: contributed to the revision of the draft of the manuscript and approved the final version submitted for publication.

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Transfusion-associated circulatory overload and high blood pressure: A multicentre retrospective study in Japan

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Abstract

Background Transfusion-associated circulatory overload (TACO) is an adverse reaction associated with a high risk of mortality. The actual incidence of TACO and hypertension associated with transfusion in Japan is unknown.

Methods A multicentre retrospective observational study was conducted across 23 institutions during the 1-year period of 2016. Patients were included if they developed TACO or their blood pressure (either systolic or diastolic) increased by at least 30 mmHg during the transfusion. TACO was confirmed by the primary physicians and transfusion medicine teams and recorded in the data on passive surveillance, and additional data were extracted from electronic medical records.

Results In our patient cohort of 31 384 patients who underwent transfusion, the incidence of TACO and hypertension was 0·03% and 0·2%, respectively. However, 43% of the participating institutions didn't report any cases. When comparing risk factors between the TACO and hypertension groups, there were significant differences in comorbidities, such as abnormal findings on chest x-ray. Significant differences between the two groups were observed post-transfusion pulse rate, body temperature and oxygen saturation ($P < 0·01$). In the group of patients with hypertension, the level of BNP increased significantly after transfusion in

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45% (5/11) of the patients. We identified 4 patients in the hypertension group who met the new ISBT's TACO criteria.

Conclusion Our study suggests that more attention should be given to TACO in Japan, particularly in terms of improving surveillance systems. For the early diagnosis of TACO, it is crucial to carefully monitor vital signs including blood pressure.

Key words: TACO, hypertension, haemovigilance, BNP, vital signs.

Introduction

Transfusion-associated circulatory overload (TACO) is an adverse reaction and a common cause of transfusion-associated mortality in the United States [1], United Kingdom [2] and France [3]. It also comprises a large proportion of severe transfusion-associated adverse reactions, and results in prolonged hospital stays [4]. TACO is caused by heart failure owing to circulatory system overload. It is frequently associated with a combination of comorbidities, such as heart and kidney dysfunction, excessive fluid volume and old age [5]. In addition, several lines of evidence have suggested that inflammatory factors are involved in TACO. For example, previous studies demonstrated that some patients with TACO present with fever, and that leucocyte depletion and washing prior to transfusion reduce the risk of TACO [6].

In Japan, the incidence of TACO has been tracked by the Japanese Red Cross Society (JRCS) in their haemovigilance system, which was established in 1993. It has shown an increase in the reported incidence of TACO since 2012, when it was included as part of the haemovigilance system, and the annual number of cases reached 45 in 2016 [7]. JRCS's TACO criteria include the following essential items: (1) acute respiratory failure, (2) pulmonary congestion on chest radiography and (3) evidence of transfusion and/or fluid overload. In addition, these criteria exclude patients with complications that often lead to circulatory overload, such as heart failure, haemodialysis, etc., to identify patients with circulatory overload associated with transfusion. The Japanese Ministry of Health, Labour and Welfare Research Group guidelines for TACO include items (2) and (3) of the above, and newly developed hypoxaemia during or up to 6 h after transfusion.

However, the cases identified by this system are primarily categorized as severe adverse reactions (all cases of TACO), and the information is based on voluntary reporting of the hospitals. Therefore, the actual incidence of TACO in Japan remains unclear. To obtain more comprehensive data about adverse reactions associated with transfusion, the Hemovigilance Committee of the Japan Society of Transfusion Medicine and Cell Therapy created a web-based platform named 'Information System for Blood Product-related Adverse Reactions' in 2007 [8]. In 2016, a total of 44 hospitals representing the use of approximately 7% of all red blood cell (RBC) products supplied in Japan submitted their report. During the previous 5 years, there has not been any notable changes in the incidence of TACO, with 2–9 cases annually.

High blood pressure (hBP) is an important indicator that helps to detect TACO at an early stage [9]. However, the clinical significance of hBP itself as a transfusion-associated adverse reaction remains to be elucidated.

As the ageing population in Japan is growing faster than that of any other country, the number of patients at risk of TACO is expected to increase. As healthcare costs increase, there is an urgent need to reform the medical delivery system and improve the efficiency of medical services. This need also applies to transfusion medicine, and medical teams involved in transfusion are focusing their efforts to improve safety control measures. However, little is known about how these measures are actually applied in clinical practice. Therefore, in the present study, we performed a multicentre retrospective review to analyse the current status of TACO and cases of transfusion-associated increases in blood pressure in Japan.

Methods

Study design

A retrospective observational study was conducted using the data of patients with TACO/hypertension who were treated between January and December 2016 at teaching hospitals that are affiliated with the Japan Society of Transfusion Medicine and Cell Therapy. TACO and hBP were identified by passive surveillance based on each institution's reporting system of all inpatients and outpatients who received blood transfusion.

A questionnaire was distributed in August 2017 and data were collected between December 2017 and February 2019 by mail. The primary endpoint was the incidence of reported TACO/hBP. The secondary endpoint was a

comparison of risk factors and clinical characteristics between the TACO and hBP groups.

The questionnaire asked basic information about the hospital and transfusion-associated information about the patients. Specifically, the following information about the hospitals was collected: the number of beds, annual number of patients requiring transfusion, the amount of packed RBC, platelet concentrates (PC) and fresh frozen plasma (FFP) used per year, the annual number of patients with TACO, number of patients with a transfusion-associated increase in blood pressure, and number of patients with 1-year survival among the reported cases with transfusion reactions, the timing at which transfusion-associated adverse events are evaluated, whether the development of adverse events is evaluated following transfusion, whether and how the information about TACO patients and surveillance measures is communicated among staff, and whether there were patients suspected to have both TACO and transfusion-associated acute lung injury (TRALI).

Similarly, the following information about the patients was collected: age group, weight, brain natriuretic peptide (BNP) level and estimated glomerular filtration rate as surrogate markers of congestive heart failure (CHF) and chronic kidney disease (CKD), respectively, comorbidities, complications, outcomes of reactions (1-year survival and comorbidities), symptoms prior to transfusion, duration from the start of transfusion to the onset of initial symptoms, symptoms or physical findings at the onset of a reaction and during the course of the treatment, amount and type of blood products used, rate of transfusion, findings on chest x-ray, BNP level following the onset of symptoms, efficacy of treatments, and changes in vital signs, including blood pressure, pulse rate, body temperature, and oxygen saturation, prior to transfusion and up to 12 h after transfusion. The diagnosis of TACO was made by primary physicians and transfusion medicine teams based on both the Japanese Ministry of Health, Labour and Welfare Research Group guidelines [10], and the International Society of Blood Transfusion Working Party July 2011 diagnostic criteria [11]. Furthermore, a revised criteria established in 2019 was applied to subsequent analysis [12].The above information was also collected from patients whose blood pressure (either systolic or diastolic) increased by at least 30 mmHg following transfusion.

Statistical analysis

The Mann–Whitney *U* test and Kruskal–Wallis test were used to compare continuous variables, and the Fisher exact test was used to compare categorical variables to identify factors that are associated with TACO. A *P*-value of less than 0·05 was considered to indicate a statistically significant difference between two groups. SPSS Statistics23 software (IBM Corporation) were used for all analyses.

Results

Characteristics of the hospitals

Among the 23 participating hospitals, a total of 210 683 units of RBC (1 unit was derived from 200 ml of whole blood) were used during 2016, representing approximately 3% of all units used in Japan. A total of 476 207 units of PC, and 106 516 units of FFP were also used. The total number of patients receiving a transfusion was 31 384. Among these, 10 patients developed TACO (TACO group) and 71 patients developed hBP (hBP group). Based on the total number of transfusions performed at the participating hospitals, the incidence of TACO and transfusion-associated increase in blood pressure were 0·03% (10/31 384) and 0·2%, (71/31 384) of patients transfused, respectively. Neither of these conditions were reported from 10 of the participating hospitals.

Most hospitals reported that they examine patients for possible adverse reactions at 5 and 15 min from the start of the transfusion, and at the end of the transfusion. Only five hospitals reported that they monitor patients more than three times. Approximately 80% of the hospitals were equipped with a system to report adverse reactions following transfusion. However, only six hospitals had a specific indicator for 'post-transfusion' events in their systems. Other hospitals used different methods for reporting, such as documentation in the nursing notes of electronic patient records and by phone calls to the division responsible for performing the transfusion. Approximately 70% of the hospitals shared information about TACO to their healthcare workers.

However, only 20% of the hospitals actively discussed about TACO in conferences or meetings, and most hospitals only provided information pamphlets and explanations about TACO. There were no significant differences in terms of characteristics of the hospitals (e.g. timing and methods of reporting adverse events, and opportunities for information sharing) between those with and without cases of TACO and/or hBP (data not shown).

Clinical course of the patients

Information were obtained for nine patients in the TACO group and 71 patients in the hBP group. Although there were no deaths owing to transfusion-associated adverse reactions, two patients in the TACO group and 20 patients in the hBP group died from their primary disease within

1 year after the transfusion. None of the patients in both groups developed any comorbidities owing to their reaction to the transfusion. Furthermore, none of the patients in the hBP group were subsequently diagnosed as having TACO during their hospitalization, although blood pressure increased again in approximately 20% of the patients.

Background and characteristics of the patients

The background and characteristics of the patients who received blood products are summarized in Table 1. Between the TACO and hBP groups, there were no significant differences in the age of the patients, the proportion of inpatients, duration between the start of transfusion and the onset of symptoms, or the type of blood products used. Patients in the TACO group were significantly heavier and received a significantly fewer number of transfusions. In terms of the type of blood products used in the TACO group, packed RBC were most common (n = 6), followed by a mixture of packed RBC and FFP (n = 1) and autologous whole blood (n = 1). Except for one patient, the onset of TACO was noted relatively soon after the beginning of transfusion (median: 95 min; range: 28–720 min; ≤60 min, n = 4; 61–120 min, n = 2; 121–180 min, n = 2; >180 min, n = 1).

Comorbidities and symptoms

Common comorbidities included haematological malignancies (n = 4) and other malignancies (n = 2) in the TACO group, and advanced CKD (n = 33), including end-stage kidney disease (n = 21), haematological malignancies (n = 15), haematological benign diseases (n = 13) and other malignancies (n = 12) in the hBP group. Common initial symptoms in the TACO group included shortness of breath (n = 5), reduced oxygen saturation (n = 5) and fever (n = 4). In the hBP group, 51 patients did not develop symptoms other than hBP, but four patients developed other symptoms, including a 3% to 4% reduction in oxygen saturation (n = 2) and fever (n = 2) during the follow-up period. Among them, two patients were diagnosed as having TACO according to the new criteria [12]. Furthermore, we reviewed the original data of all hBP patients, and identified two additional TACO patients, leading to a total of four additional TACO patients.

Table 1 Patient characteristics.

Variable	TACO (n = 9)	hBP (n = 71)	P-value
Age (years)	63·3	65·6	0·65
Hospitalization	9/9 (100%)	54/72 (75%)	0·19
Body weight (kg)	65·8 ± 10·9	52·1 ± 12·2	0·003
Onset of symptoms (min)	162·6 ± 214·2	128·6 ± 58·9	0·34
Transfusion volume (ml)	140 (100–3160)	280 (200–960)	0·01
Component type			
RBC only	6	55	0·32
PC only	1	13	
FFP only	0	3	
Autologous blood only	1	0	
Mixed component	1	0	

Data are reported as the mean ± SD or number (%).

Risk factors and clinical findings

A comparison of the risk factors and clinical findings in the TACO group demonstrated that a history of CHF and abnormal findings on chest x-ray (e.g. cardiac dilation, pleural effusion and congestion) prior to transfusion were often observed (Table 2). Diuretics were administered to four out of nine patients in the TACO group, whereas treatments were not administered to most of the patients in the hBP group (P < 0·001). There was no significant difference in BNP level both before and after transfusion between the two groups. Analysis of BNP is usually ordered only for some patients when it is judged to be necessary. The level of BNP increased significantly by an average of 2·1 times after transfusion in 5 out of the 11 patients in the hBP group in whom BNP was measured both before and after transfusion (Fig. 1).

Vital signs

Among the vital signs that were compared, there were significant differences in the pulse rate before and after transfusion, and in the post-transfusion body temperature and oxygen saturation level between the two groups (Table 3). In the TACO group, four patients were febrile with body temperatures higher than 37·2°C before transfusion, and seven patients had a fever after transfusion. There were no significant differences in systolic or diastolic blood pressure between the two groups. However, although blood pressure was high before or at the time of the onset of symptoms in the TACO group, it was high after transfusion in most of the patients in the hBP group.

Discussion

The present challenge regarding TACO is the lack of an international consensus for its diagnostic criteria, although a revised surveillance case definition has recently been validated [12]. However, there are many challenges regarding TACO, such as the lack of a definitive diagnostic test(s), overlap with positive fluid balance symptoms caused by non transfusion-associated therapies (saline, liquid

Table 2 Risk factors and clinical findings.

Variable	TACO (n = 9)	hBP (n = 71)	P-value
History of CHF	3/9	2/71	0·02
Coronary artery disease	2/9	5/71	0·18
CRF	2/9	33/71	0·29
eGFR	31·8 ± 24·3	30·3 ± 30·5	0·62
Abnormal findings on chest x-ray before transfusion	4/9	10/71	0·04
BNP (pre-transfusion)	257·9 ± 276·0 (n = 3)	208·7 ± 230·8 (n = 19)	0·77
BNP (at onset)	1122·7 ± 392·1 (n = 3)	473·2 ± 406·3 (n = 14)	0·23
Transfusion rate (ml/kg/h)	1·77 ± 0·73	3·65 ± 2·66	0·003
Excess of fluid balance	1/9	0/71	0·11
Use of diuretics	4/9	0/71	<0·001
No treatment	0/9	67/71	<0·001

Data are reported as the mean ± SD or number.

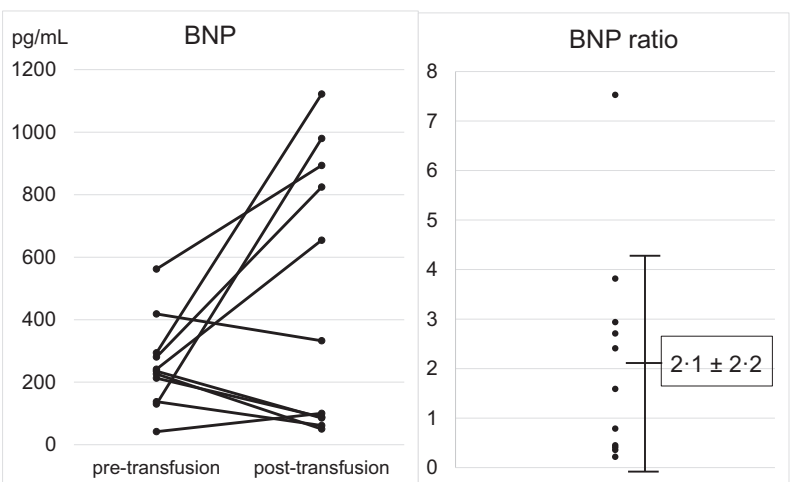

Fig. 1 Changes in BNP levels in the hBP group. The levels (left) and ratios (right) of BNP in 11 patients in the hBP group whose BNP was measured both before and after transfusion are shown. The level of BNP increased significantly by an average of 2·1 times after transfusion in 5 of the 11 patients.

antibiotics, etc.) resulting in the under-reporting of potential cases of TACO, and overlap/confusion at times with TRALI. The most crucial problem is the lack of a clear understanding of the pathophysiology of TACO [13,14].

In the present study, we performed a retrospective review of patients with TACO who were diagnosed based on several guidelines. The incidence of TACO in our patient cohort was 0·03%, which is lower than that previously reported in similar retrospective studies [15,16]. The low incidence of TACO in Japan may be owing to differences in blood products used in Japan; specifically, the amount of RBC products used per population is lower in Japan than in other countries. In addition, 1 unit of packed RBC is derived from 200 ml of whole blood which is the same for FFP and PC. However, a prospective study reported that 7 out of 82 surgical patients (8·5%) developed TACO [17]. This suggests that there is a large number of patients who are not properly diagnosed as having TACO. As heart failure is well known to be caused by transfusion and/or excessive fluid, TACO may be categorized as a complication of the primary disease. As such, physicians may not necessarily report it to the division responsible for performing the transfusion. The reasons why a limited number of cases of TACO are reported in Japan may be insufficient knowledge, lack of adequate judgement, or a culture that does not generally report incidences.

Our study demonstrated that hospitals use different systems to report adverse reactions following transfusion, suggesting that the incidence of post-transfusion TACO is under-reported. Indeed, there was only 1 case of post-transfusion TACO reported in our patient cohort. As only 5% of heart failure cases during transfusion are considered medical accidents caused by inappropriate transfusion [18], incidences of TACO should be more actively reported

Table 3 Vital sign values.

Variable	TACO (n = 9)	hBP (n = 71)	P-value
Systolic blood pressure (mmHg)			
Pre-transfusion	118·3 ± 21·5	125·3 ± 20·7	0·52
After 15 min	123·3 ± 35·9	139·6 ± 24·6	0·54
At the end	160·2 ± 24·2	165·2 ± 22·7	0·51
Diastolic blood pressure (mmHg)			
Pre-transfusion	66·6 ± 15·4	64·6 ± 15·1	0·90
After 15 min	63·3 ± 9·2	70·6 ± 16·0	0·37
At the end	88·3 ± 15·9	77·3 ± 15·3	0·08
Pulse rate (/min)			
Pre-transfusion	94·3 ± 13·6	81·8 ± 15·8	0·03
After 15 min	89·6 ± 12·0	81·1 ± 17·8	0·11
At the end	115·3 ± 28·2	81·2 ± 18·6	0·002
Body temperature (°C)			
Pre-transfusion	37·2 ± 0·6	36·7 ± 0·9	0·08
After 15 min	36·8 ± 0·3	36·7 ± 0·9	0·36
At the end	38·3 ± 1·0	36·9 ± 0·7	<0·001
Oxygen saturation (%)			
Pre-transfusion	96·9 ± 1·2	96·6 ± 3·1	0·76
After 15 min	96·0 ± 1·0	96·1 ± 3·2	0·50
At the end	86·9 ± 11·1	96·9 ± 1·6	0·002

Data are reported as the mean ± SD.

to the transfusion department. We also found that 10 hospitals reported no cases of TACO or hBP during the 1-year period. This suggests the need to revisit these hospitals to assess whether there is a proper system in place to report transfusion-associated adverse reactions. Moreover, a potential cause for the under-reporting of TACO is that an overall positive fluid balance may be missed for patients who have been in hospital for several days [5].

Our study included a small number of cases of TACO that were relatively easy to diagnose. In the reported cases, TACO developed from an expected to relatively fast rate (0·8–3·3 ml/kg/h) with a small volume of transfusion (100–3160 ml, median: 140 ml), suggesting that patients at risk of TACO should be carefully monitored from an early stage [19]. The difference in weight and the rate of transfusion between the TACO and hBP groups may have been because the number of patients in the TACO group was small.

Recent studies suggested that an increased heart rate increases the risk of mortality and progression of heart failure in patients with heart failure and sinus rhythm [20,21]. As the pretransfusion pulse rate was relatively high in our patients, they may have been at a higher risk of developing TACO than previously reported patients. Fever was also more common in our patients than in those previously reported, with 44% and 77% of patients having fever pre-transfusion and post-transfusion, respectively [9,22]. A recent study proposed a 2-hit model, in which the first hit occurs because patients are not tolerant to volume overload, and the second hit occurs as some factors in blood products elicit an immune response [23]. The 2-hit model is based on observational studies, and the levels of cytokines, specifically post-transfusion IL-6 and pre-transfusion and post-transfusion IL-10 levels, were high in TACO patients [24,25].

Fever was common in our TACO patients, but rare in the hBP cohort, even in patients diagnosed as TACO using the new criteria. In a study investigating the association between fever and TACO, TACO with fever was not associated with patient age or product age, nor reflected severity. The odds for a pre-existing fever in TACO vs. in an allergic reaction was 15, whereas the odds for newly developing a fever in TACO was 5 [22]. Whereas the pathophysiology of fever in TACO remains unclear, it appears to be a useful marker for risk assessment and follow-up of patients with some risk factors of TACO.

High blood pressure during transfusion is an important indicator of volume overload in the diagnosis of TACO [9]. However, its clinical significance is unknown regarding patients without TACO. In terms of haemodynamics, blood pressure is determined by the product of cardiac output and peripheral vascular resistance. Thus, transfusion may increase blood pressure as the volume of circulating blood, and consequently cardiac output, increase. However, a study using a swine model of transfusion, reported that transfusion of blood at a volume equivalent to that of circulating blood did not cause a significant increase in the volume of circulating blood, cardiac output or blood pressure [26]. The authors suggested that the compensatory mechanism of the heart functions to cause leakage of plasma components outside of the vessels. Although it is unclear whether the same mechanism applies in humans, transfusion rarely results in increased blood pressure, and it is unlikely that the transfusion of 10%–20% of the volume of circulating blood increases cardiac output and blood pressure. Nevertheless, most patients receive therapeutic fluid and salts that affect the overall fluid balance. Therefore, it is possible that a small transfusion volume may cause volume overload in such situations, and vital signs should be carefully monitored from an early stage.

Previous studies also suggested that RBC-derived microparticles reduce the bioavailability of nitric oxide, which leads to vasoconstriction and a subsequent increase in blood pressure [27,28]. Thus, increased peripheral vascular resistance may increase blood pressure. Moreover, hypertension was the most frequently observed adverse event associated with infusion of haematopoietic stem cell products [29], and overall, patients with high blood pressure and those who were underweight had a similar rate of complications [30]. The authors of these studies suggest the possibility that a reaction to alloantigens in the infused products contributes to hypertension.

In the hBP group, the level of BNP increased significantly in 5 of the 11 patients in whom BNP level was measured both before and after transfusion. This suggested that the ventricles were affected by the overload. Furthermore, two of the five patients were determined as having TACO according to the new criteria. Because the remaining three patients did not exhibit acute or worsening respiratory compromise at least in their medical records, they were not diagnosed as having TACO.

Although BNP (NT-proBNP) is a well-investigated biomarker of TACO, study results are inconsistent and there is at present no consensus on the cut-off level of BNP for the diagnosis of TACO [31–33]. However, a recent systematic review suggested that the risk of TACO is low when the post-transfusion BNP level is less than 300 pg/ml, and that TACO should be suspected when the ratio of the pre-transfusion and post-transfusion NT-proBNP level is more than 1·5 [34]. In our five patients in whom BNP levels were measured both before and after transfusion, BNP level increased by more than 2·1 times and exceeded 600 pg/ml following transfusion, suggesting that there was significant cardiac overload that may lead to TACO. Nevertheless, BNP measurement was only ordered in some patients in whom it was thought to be necessary. There hence may be a considerable number of undiagnosed TACO cases owing to the lack of oxygen saturation data (61/71 patients) and lack of chest x-ray findings (25/71 patients). Five out of the eight patients who showed a high post-transfusion BNP level (>600 pg/ml) died from their primary disease within 1 year in the hBP group, whereas one out of the seven patients who did not show a high BNP level (<600 pg/ml) died during the same period. These findings may indicate that patients with a high BNP have severe primary diseases and/or comorbidities, and possibility to develop TACO subsequently.

Conclusion

In this study, we demonstrated the lack of an appropriate system to report transfusion-associated adverse reactions, suggesting the need for improving surveillance systems of TACO in clinical settings. To enable the early diagnosis of TACO, it is crucial to collect information about risk factors of the patient prior to transfusion, and carefully monitor the patient's blood pressure, pulse rate, oxygen saturation and body temperature during the course of and after transfusion.

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Conflicts of interest

The authors declare that they have no conflicts of interest associated with this study.

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Deferasirox-associated Fanconi syndrome in adult patients with transfusional iron overload

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Vox Sanguinis

Background and Objectives Deferasirox is an oral chelator approved for iron overload, which has a potential side effect of renal Fanconi syndrome, with proximal tubular dysfunction and tubular acidosis. Monitoring of renal function is recommended, though no standard for monitoring exists. We aim to describe cases of deferasirox-associated Fanconi syndrome in adults and the renal monitoring required to detect these findings.

Materials and Methods We present a review of the literature and six cases from our institution of deferasirox-associated partial Fanconi syndrome in adult patients with transfusional iron overload secondary to β-thalassemia or Diamond Blackfan Anaemia.

Results While prior cases in the literature occurred at high doses of deferasirox, our series included patients on doses as low as deferasirox 10 mg/kg who had been aggressively chelated. All patients had resolution of laboratory abnormalities with drug interruption.

Conclusion Rather than chelating to normal iron levels, this series supports prior suggestions that deferasirox dose be reduced if ferritin <500–1000 ng/ml, and also supports dose reduction if liver iron content <3 mg iron per g dry weight or for those undergoing aggressive chelation with rapid decrease in iron. Monitoring with metabolic panel and urinalysis were sufficient to detect clinically significant proximal tubular dysfunction, but should be followed up with additional studies to confirm the diagnosis while deferasirox dose is decreased or held.

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Key words: chelation, iron overload, side effects, transfusions.

Introduction

For transfusion-dependent patients, such as those with β-thalassemia major, complications of iron overload have historically been the leading cause of morbidity and mortality. Since the advent of modern chelators, this morbidity and mortality has decreased significantly, but chelation can be very cumbersome including daily or continuous subcutaneous injections, which can lead to noncompliance.

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Deferasirox is unique as the only once-daily oral chelator currently approved for iron overload. Deferasirox was approved initially in 2005 as dispersible tablets (Exjade, Novartis, East Hanover, NJ, USA) and reformulated and approved in 2015 as film-coated tablets (Jadenu, Novartis). Dosing is based on weight and the degree of chelation desired. While generally well-tolerated, nephrotoxicity is common, with 10% of patients experiencing a 33% increase from their baseline creatinine [1]. This nephrotoxicity may manifest as decreases in glomerular filtration rate or proximal tubular dysfunction, which can progress to Fanconi syndrome, generalized proximal tubular dysfunction with typical laboratory findings including normal anion gap metabolic acidosis, hypokalaemia, hypophosphatemia, hypouricemia,

glucosuria, phosphaturia and aminoaciduria [2]. Proposed mechanisms include local iron deficiency in the renal cortex or potential intrarenal haemodynamic changes [3].

We present a review of the literature and describe our single institution experience including six adult patients who developed partial Fanconi syndrome during treatment with deferasirox.

Materials and methods

We identified English-language reports of deferasirox-related nephrotoxicity and Fanconi syndrome. Related articles and citation were reviewed as well.

A retrospective chart review was performed to identify cases of nephrotoxicity in patients who were treated with deferasirox. Partial Fanconi syndrome was diagnosed based on proximal tubular defects with new onset of proteinuria, glucosuria or metabolic acidosis detected on screening metabolic panel and urinalysis. Cases were included even if they did not meet full criteria for Fanconi syndrome, as routine monitoring of renal function did not include phosphate, uric acid, urine electrolytes, urine amino acids or urine β2-microglobulin. Liver iron content (LIC) was determined via Ferriscan, which is performed every 6 to 12 months.

Results

We present the cases of six adult patients with β-thalassemia or Diamond Blackfan Anaemia (DBA) who developed partial Fanconi syndrome while being treated with deferasirox (all patients were receiving film-coated tablets). Four patients (66%) were female (Table 1), and the median age was 41·5 years (range 19–66 years). Median ferritin was 1032 ng/ml, and median LIC (based on most recent Ferriscan, all within the prior 12 months) was 3·75 mg iron per gram dry weight (range 1·8–7·0 mg/g). The average decrease in ferritin in the three months leading up to diagnosis of Fanconi syndrome was 436·5 ng/mL. Three patients had acute increases in creatinine of at least 33%, three had a decreased serum bicarbonate, and two had hypokalaemia. All patients had glucosuria. Phosphate, uric acid and urine electrolytes and amino acids were not obtained in any patient. No patients were taking other nephrotoxic medication at the time of diagnosis.

Illustrative cases

Patient 1 was a 19-year-old woman with β-thalassemia major who was being aggressively chelated with deferasirox 25 mg/kg/day and deferiprone 40 mg/kg/day. In

Table 1 Patient characteristics and laboratory parameters at diagnosis of partial Fanconi syndrome

Age (years)	Sex	Diagnosis	Dose (mg/kg/day)	LIC (mg iron/g dry weight)	Ferritin (ng/ml)	Ferritin 3 months prior (ng/ml)	Cr (mg/dl)	Baseline Cr (mg/dl)	K (mEq/l)	HCO3 (mEq/l)	Urine glucose (mg/dl)	Urine protein (mg/dl)
19	F	β-thal	25	7·0	1618	3724	0·35	0·38	3·3	21	>1000	30
66	M	β-thal	10	1·8	395	942	0·68	0·55	3·5	26	>500	30
45	F	β-thal	15	4·8	1202	1822	1·34	1·05	4·0	21	200	100
48	F	β-thal	21	2·2	1391	1284	0·90	0·67	4·2	20	100	negative
38	F	β-thal	29	5·4	815	1395	1·14	0·66	3·7	22	Trace	1+
35	M	DBA	28	2·7	862	1542	1·20	1·02	4·2	22	1000	30

β-thal, β-thalassemia; Cr, creatinine; DBA, Diamond Blackfan Anaemia; F, female; HCO3, bicarbonate; K, potassium; LIC, liver iron content; M, male.

Table 2 Summary of cases in the literature

Report	Age/Sex	Diagnosis	Dose	Ferritin (ng/ml)	AKI	HypoK	HypoP	HypoUr	Acidosis	Glucosuria	Urine B2M
Rafat, *et al.* 2009 [11]	78 M	CLL	24 mg/kg/day	1150	+	−	+	−	+	+	+
Even-Or, *et al.* 2010 [6]	18 M	DBA	20 mg/kg/day	1200	+	+	+	+	−	+	+
	11 F	β-thal	20 mg/kg/day	313	−	+	+	+	−	+	+
Grange, *et al.* 2010 [7]	77 M	NHH	1500 mg/day	376	+	+	+	−	+	+	
Yacobovich, *et al.* 2010 [14]	8 F	β-thal	33 mg/kg/day	1360	−	+	+			−	
	11 F	β-thal	30 mg/kg/day	575	−	+	+	+	+	+	
	8 M	β-thal	30 mg/kg/day	480	+	+	+	+	+	+	
	32 F	β-thal	38 mg/kg/day	1335	−	+	+	+	+	+	
Wei, *et al.* 2011 [13]	18 M	β-thal	1375 mg/day	183	+	+	+	+	+	+	+
Rheault, *et al.* 2011 [12]	7 M	β-thal	500 mg/day	688	−	+	+			+	+
	8 F	β-thal	500 mg/day	1 536	−	−	−			−	+
Milat, *et al.* 2012 [8]	28 F	β-thal	1500 mg/day	475	−		+			+	+
Murphy, *et al.* 2013 [9]	21 M	Ewing Sarcoma	1125 mg/day	0	+	+	+		+	+	
Dee, *et al.* 2014 [5]	2 F	β-thal	20–30 mg/kg/day	6243	−	−	−		−		+
	4 M	β-thal		564	−	+	+		+		+
	4 M	β-thal		1199	−	+	+		−		+
	5 M	β-thal		955	−	−	+		+		+
	6 F	β-thal		1419	−	+	+		+		+
	18 M	β-thal		3718	−	−	−				+
	20 M	β-thal		1065	−	−	+				+
	20 F	β-thal		829	−	+	−				+
	22 F	β-thal		4700	−	−	−		−		+
	23 M	β-thal		2130	−	−	−		−		+
Chuang, *et al.* 2015 [4]	3 F	β-thal	25·5 ± 4·9 mg/kg/day	4977 ± 3218		−	+		+	+	
	6 F	β-thal				−	+		+	+	
	8M	β-thal				−	+		+	+	
	7 F	β-thal				+	+		+	+	
	1 M	β-thal				−	+		+	+	
Papneja, *et al.* 2016 [10]	16 M	DBA	41·5 mg/kg/day	628	−	+	+		+	+	
	14 M	DBA	36 mg/kg/day	512	+	+	+		+	+	
Khan *et al.*, 2019 [15]	20 M	DBA	24 mg/kg/day*	--	+	+	+		+	+	
Fraser *et al.*, 2020 [19]	33 M	β-thal	28 mg/kg/day*	981	+	+	+		+	+	

AKI, acute kidney injury; CLL, chronic lymphocytic leukaemia; DBA, Diamond Blackfan Anaemia; F, female; HypoK, hypokalaemia; HypoP, hypophosphatemia; HypoUr, hypouricemia; β-thal, β-thalassemia; M, male. NHH, nonhereditary hemochromatosis.

*Film-coated tablets.

one year, her LIC decreased from >43 to 7·0 mg iron per g dry weight. In the preceding three months, ferritin had improved from 3724 to 1618 ng/ml. Laboratory abnormalities resolved after holding the medication, and deferasirox was later restarted at a lower dose without recurrence of proximal tubular dysfunction.

Patient 2 was a 66-year-old gentleman with transfusion-dependent β-thalassemia intermedia who was on a low dose of deferasirox with low ferritin and LIC <2 mg iron per g dry weight, when partial Fanconi syndrome was diagnosed. Renal function normalized while holding deferasirox, and it has not yet been restarted given his low levels of iron.

Patient 4 was a 48-year-old woman with β-thalassemia major who had been noted previously to have acute kidney injury while taking deferasirox dispersible tablets. This kidney injury was presumed to be acute tubular injury, and resolved with interruption of deferasirox. She was transitioned to film-coated tablets, which she initially tolerated well, but upon dose increase, she had recurrent tubular injury and partial Fanconi syndrome. The tubular dysfunction resolved after drug interruption and has not recurred at a lower dose.

Review of the literature reveals thirteen case reports/series describing 32 unique patients who developed at least partial Fanconi Syndrome attributed to deferasirox use (Table 2) [4–15]. The majority of cases describe transfusion-dependent paediatric patients with diagnosis of β-thalassemia major or DBA, although there are also adult cases of transfusional iron overload. The formulation of deferasirox is not clearly stated in most of the cases, but given the timing of publication, we assume most cases were associated with dispersible tablets rather than film-coated tablets. There is insufficient data to any relationship between deferasirox formulation and incidence of Fanconi Syndrome. The average dose was 20·8 mg/kg/day for film-coated tablets (n = 16), equivalent to 29·6 mg/kg/day for dispersible tablets. Hypophosphatemia was the most common laboratory abnormality. Glucosuria and elevated urine β2-microglobulin were nearly uniformly present when assessed. The majority of cases did not report urine electrolytes or urine amino acids. In all cases, when deferasirox was discontinued, the renal dysfunction improved. However, there were three cases where patients had ongoing evidence of tubular dysfunction and required ongoing electrolyte repletion. Four patients had recurrent Fanconi syndrome after restarting deferasirox, even at lower doses.

Discussion

We present here six cases of deferasirox-associated partial Fanconi syndrome in adult patients receiving deferasirox.

Given the known effects on proximal tubular function, the manufacturer recommends monitoring renal function at least monthly, but no standards are provided for monitoring, and practices vary widely from one institution to another. Our series suggests that a basic metabolic panel and urinalysis are sufficient to detect clinically significant Fanconi syndrome, although it is unclear whether diagnosis could have been made earlier with the availability of phosphate, uric acid or additional urine studies such as urine β2-microglobulin. Proteinuria may be one of the earlier signs of tubular injury but is nonspecific, and a high index of suspicion must be maintained for Fanconi Syndrome, with any abnormalities followed up with testing of phosphate, uric acid and additional urine studies [16]. Elevated urine β2-microglobulin or hyperaminoaciduria are the most specific findings for Fanconi Syndrome [16].

The average LIC in our patients was 3·75 mg iron per g dry weight, lending further support that deferasirox-associated Fanconi syndrome is most likely to occur in well-chelated patients with low iron stores, or in those patients undergoing aggressive chelation with rapid decrease in iron. Recommendations have been made for dose reduction of deferasirox for ferritin <1000 ng/ml, and cessation if ferritin <500 ng/ml [17] or if tubular dysfunction is detected [18]. This data supports these recommendations and also supports dose-reducing or holding deferasirox if LIC <3 mg iron per g dry weight. Development of nephrotoxicity was not strictly dose-related, as deferasirox dose varied, and was as low as 10 mg/kg/day.

Conclusions

The series presented suggests an interaction between deferasirox dose and iron stores, with Fanconi syndrome more likely to occur even at low/moderate doses of deferasirox when iron stores are low or patients have had rapid decreases in iron. These data suggest that to avoid toxicity, chelation in transfusion-dependent patients should not target normal iron levels. Our recommendation is for ongoing monitoring of renal function with metabolic panel and urinalysis in all patients on deferasirox. With any concern for deferasirox-associated Fanconi syndrome, deferasirox dose should be decreased or interrupted while confirmatory testing of phosphate, urine electrolytes, urine amino acids and urine β2-microglobulin is performed. Tubular dysfunction is expected to resolve with drug cessation, and often deferasirox can safely be reintroduced at a lower dose.

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None.

Conflict of interests

All authors declare no conflict of interest.

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Understanding the role of therapeutic plasma exchange in COVID-19: preliminary guidance and practices

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Vox Sanguinis

Abstract

Background and objectives Cytokine release syndrome in COVID-19 is due to a pathological inflammatory response of raised cytokines. Removal of these cytokines by therapeutic plasma exchange (TPE) prior to end-organ damage may improve clinical outcomes. This manuscript is intended to serve as a preliminary guidance document for application of TPE in patients with severe COVID-19.

Material and methods The available literature pertaining to the role of TPE for treatment of COVID-19 patients was reviewed to guide optimal management. It included indication, contraindication, optimal timing of initiation and termination of TPE, vascular access and anticoagulants, numbers and mode of procedures, outcome measures and adverse events.

Results Out of a total of 78 articles, only 65 were directly related to the topic. From these 65, only 32 were acceptable as primary source, while 33 were used as supporting references. TPE in critically ill COVID-19 patients may be classified under ASFA category III grade 2B. The early initiation of TPE for 1–1·5 patient's plasma volume with fresh frozen plasma, or 4–5% albumin or COVID-19 convalescent plasma as replacement fluids before multiorgan failure, has better chances of recovery. The number of procedures can vary from three to nine depending on patient response.

Conclusion TPE in COVID-19 patients may help by removing toxic cytokines, viral particles and/or by correcting coagulopathy or restoring endothelial membrane. Severity score (SOFA & APACHE II) and cytokine levels (IL-6, C-reactive protein) can be used to execute TPE therapy and to monitor response in COVID-19 patients.

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Key words: COVID-19, cytokine release syndrome, therapeutic plasma exchange, preliminary guidance.

Introduction

Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) was first reported to the World Health Organization (WHO) on 31 December 2019 and was subsequently declared as a pandemic on 11 March 2020. This causative SARS-CoV-2 virus is classified under Family *Coronaviridae* which is enveloped, spherical shaped virion with 80–220 nm diameter, containing positive-sense single-stranded RNA genome. The disease caused by this virus is collectively termed as coronavirus disease 2019 (COVID-19), and the viral RNA genome has >90% nucleotide identity with pangolin and bat coronaviruses (CoV), but only 50% identity to the common cold CoV [1]. Although it may produce less frequent respiratory symptoms in infected patients, it can also cause acute respiratory distress syndrome (ARDS), multiorgan failure (MOF), significant coagulopathy and substantial mortality [2,3].

As in severe sepsis, critically ill COVID-19 patients often die from the host's maladaptive response rather that the primary infection. Cytokine release syndrome (CRS) or cytokine storm, a pathological inflammatory response at local and systemic levels following infection, is thought to influence disease severity and mortality [4,5]. CRS was previously described during the severe acute respiratory syndrome coronavirus type 1 (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS) epidemics, when significantly higher levels of serum cytokines were observed, which correlated with pulmonary pathology [6–11]. During SARS-CoV-2 infection, similar phenomena were observed [12,13] with increased levels of inflammatory cytokines activating T helper type 1 (Th1) and Th2 responses [5]. Although cytotoxic T cells generally assist in attenuating viral infections, they also accelerate progression of the systematic inflammatory response in COVID-19 by increasing circulating $CD14^+$ $CD16^+$ monocytes and interleukin (IL)-6 levels [13].

Critically ill COVID-19 patients have elevated levels of multiple inflammatory cytokines, including IL-1β, IL-2, IL-6, IL-7, IL-8, IL-10, granulocyte colony-stimulating factor, interferon γ-induced protein 10, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α, tumour necrosis factor-α, and chemokines (e.g. C-C motif chemokine ligand (CCL)-2, CCL-3, CCL-5) [13]. Lung epithelial cells produce IL-8, a neutrophil and T-cell chemoattractant, which encourages pulmonary infiltration of inflammatory cells [14]. In COVID-19, cytokine levels (e.g. IL-2R, IL-6) positively correlate with disease severity, progression, ARDS and mortality [15,16].

Therapeutic plasma exchange (TPE), where patient plasma is replaced by an iso-oncotic fluid (e.g. donor plasma, colloid [frequently 4–5% albumin], crystalloid), was proposed as a potential treatment for CRS. TPE was previously used in patients with septic shock and MOF [17,18], and it was also applied as an optional treatment during SARS-CoV-1, H1N1 influenza and MERS epidemics. For example, three cycles of TPE on consecutive days were associated with improved outcomes in three critically ill paediatric H1N1 influenza patients with ARDS and hemodynamic instability [19].

Removing cytokines and blocking the CRS, as early as possible, before end-organ damage or substantial endothelial damage has occurred, are currently thought to provide the best chance for therapeutic benefit [1]. To date, very few high-quality studies have evaluated TPE in COVID-19 [20,21]. Considering the lack of standardized guidelines, the current manuscript can serve as a guidance document for clinicians and transfusion apheresis professionals regarding using TPE to treat COVID-19 patients especially those with evidence of CRS.

Materials and methods

This document was prepared by a subgroup of the convalescent plasma working group of the International Society of Blood Transfusion (ISBT), consisting of physicians from different parts of the world with extensive expertise in clinical apheresis and critical care. The subgroup developed and addressed a series of questions related to this topic using current American Society for Apheresis (ASFA) guidelines and evidence from peer-reviewed publications; the latter were identified using PubMed, Science Direct and Google Scholar. The search terms were "COVID-19," "SARS-CoV-2," "Cytokine Storm," "Cytokine Release syndrome," "Apheresis," "Plasma Exchange," and "Therapeutic Plasma Exchange."

All identified publications such as case series, case reports or original studies were included. The inclusion criteria of publications were directly related to COVID-19 and TPE therapy, and they included all outcome of interest such as mortality, length of hospital or ICU stay, or requirement of mechanical ventilation after treatment. Literature search on other infectious diseases or previous viral outbreaks in which TPE had partial or proven

beneficial effect was performed. Data collection table was designed according to the purpose of the study. It included (1) study characteristics (year and country of publication, type of publication, patient population, number of TPE performed, type of replacement fluid and anticoagulant used), (2) quality assessment and (3) outcome (mortality, detachment from mechanical ventilation, length of hospital and ICU stay). One author extracted data from all searched manuscripts to data collection table and assessed the quality of each study. Remaining group of authors evaluated the searched publications and validated the extracted data.

Discussion points were cross-referenced based on available data to prepare the objective variables of this study. These variables of TPE demarcated from data extractions were indication and contraindication, optimal time to start and stop the procedure, choice of vascular access and anticoagulants, mode of therapy, outcome measure(s) to determine the number of procedures, and possible adverse events.

Results

The literature search identified 78 potential articles. Of these, only 65 were directly related to COVID-19, CRS or TPE, and the remaining 13 were excluded because they were not directly related to the topic. Of the 65 selected manuscripts, 32 were considered acceptable as primary source material, while the remaining 33 were considered supporting references because they related to other septic diseases, reply letters to previous articles, and pre-trial protocols (Fig. 1).

From all, selected manuscripts were summarized:

(1) **American Society for Apheresis (ASFA) Guidelines:** As per ASFA-2019 guidelines [22], TPE for patients with infections and MOF due to various causes falls under Category III Grade 2B, meaning that the optimum role of apheresis therapy in treating these patients is not established, and that the current moderate quality of peer-reviewed evidence only supports an overall weak recommendation for this approach. Therefore, using TPE for COVID-19 patients with MOF may also be considered under this category.

(2) **Indications:** Studies of TPE in septic patients with MOF, due to pathogens other than SARS-CoV-2, suggested that TPE can help to restore hemodynamic stability [23,24]. It has been applied in a small case series of 3 severe to critically ill COVID-19 patients when they begin to exhibit thrombocytopenia, elevated levels of C-reactive protein and lactate dehydrogenase, and an increased neutrophil/lymphocyte ratio, along with deteriorating oxygenation that might require high flow nasal oxygen or mechanical ventilation and/or exhibiting initial signs of shock or MOF [12]. For example, an expert panel from the National Clinical Research Centre for Infectious Diseases in China suggests that blood purification therapy, artificial liver support system (ALSS), may be indicated for COVID-19 patients when pro-inflammatory cytokines (e.g. IL-6) are more than 5 times the normal value or have doubled in 24 h along with >10% lung involvement by pulmonary imaging [24].

(3) **Contraindications:** Although there are no absolute contraindications for TPE in COVID-19, patients who may be at higher risk for complications include those who are coagulopathic (e.g. with disseminated intravascular coagulation or receiving anticoagulants), severely haemodynamically unstable (or unable to tolerate significant fluid shifts), unable to tolerate central line placement, allergic to donor plasma or albumin, severely hypocalcaemic, or who received angiotensin-converting enzyme inhibitors within the last 24 h.

(4) **Optimal Time to Initiate Therapy:** Owing to the short half-life of pro-inflammatory cytokines, early initiation of TPE was suggested for patients with severe COVID-19, especially for those exhibiting symptoms of MOF and ARDS. However, waiting until specific markers of MOF and ARDS are present may limit efficacy [18]. Nonetheless, some case reports and case series reported that TPE at 2–20 days after PCR positivity for SARS-CoV-2 improved clinical parameters, including PaO2/FiO2, extubation rates, C-reactive protein levels, neutrophil/lymphocyte ratios, and all-cause mortality at 14 and 28 days [12,25–29]. Various healthcare scoring systems (e.g. Acute Physiology and Chronic Health Evaluation II (APACHE), Age, PaO2/FIO2, and Plateau Pressure Score (APPS), Sequential Organ Failure Assessment (SOFA), Paediatric Logistic Organ Dysfunction (PELOD), Organ Failure Index (OFI)) were devised to help predict patient outcomes and drive clinical decisions; they have been variably used in assessing COVID-19 patients [30–32]. For example, in one study, eight patients with maximum SOFA scores were treated with TPE, and changes in the SOFA score correlated well with CRS patient outcomes, suggesting that changes in SOFA scores, and potentially other scoring systems, could help predict outcomes in COVID-19 patients [33].

(5) **Mode of Therapy:** TPE can be performed alone or in conjunction with other blood purification modalities, including column immunoadsorption, double volume plasma filtrations, continuous plasma filtration and adsorption, multi-filtration systems, continuous veno-venous haemofiltration, slow continuous

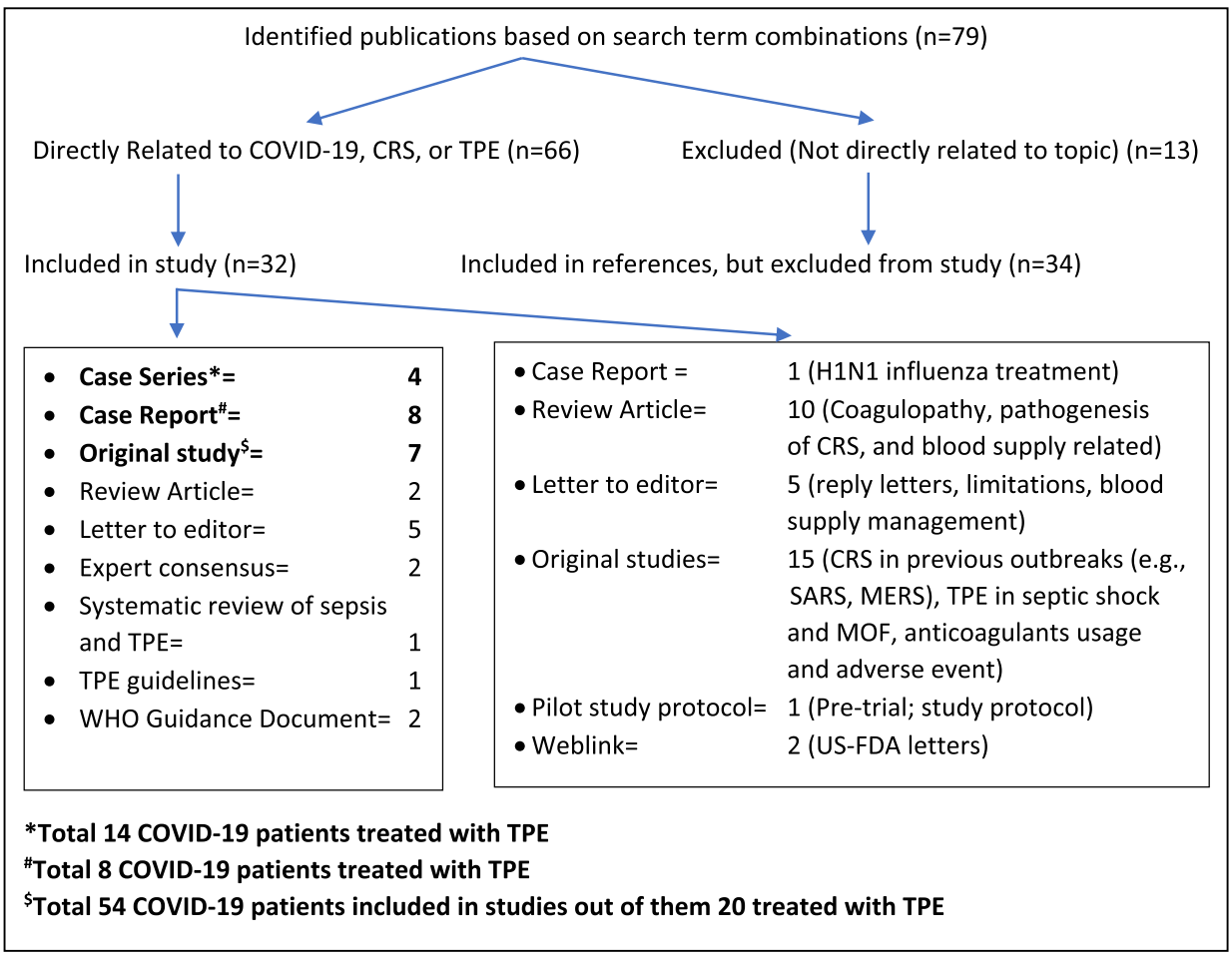

Fig. 1 Literature search strategy. CRS, Cytokine Release Syndrome; TPE, Therapeutic Plasma Exchange; WHO, World Health Organization; SARS, Severe Acute Respiratory Syndrome; MERS, Middle East Respiratory Syndrome; MOF, Multiorgan Failure; US-FDA, United States Food and Drug Administration.

ultrafiltration, artificial liver plasma exchange, adsorption, and perfusion [12,34]. Additional treatment options (e.g. immunoglobulins [35], corticosteroids [36], IL-6 receptor inhibitor tocilizumab [37], alpha adrenergic receptor antagonists [38]) have also been considered with variable success to date. As such, this article focuses mainly on the use of TPE in isolation.

(6) **Vascular access:** Although vascular access for TPE can be peripheral or central, central venous access is preferred, especially for patients with severe disease admitted to an intensive care unit and receiving multiple procedures. Ultrasound-guided insertion, preferably of a double lumen dialysis central venous femoral or jugular catheter, is recommended to reduce the risk of vascular damage and infection risk to medical staff by limiting the number of insertion attempts [34].

(7) **Anticoagulation:** Due to its safety and efficacy, Acid Citrate Dextrose formula-A (ACD-A) is the recommended anticoagulant for TPE [39]. Citrate anticoagulation has also been suggested in septic patients with MOF due to its potential immunosuppressive role [40]; however, it can induce hypocalcaemia, as 1-1·8 mg/kg/min of a citrate infusion reduces ionized calcium by 25–35% [41]. Because infused citrate is mainly metabolized by the liver and kidneys, hypocalcaemia occurs more frequently in patients with liver or renal dysfunction. To prevent severe hypocalcaemia during TPE, oral or intravenous calcium supplementation, or combined citrate-heparin anticoagulation are recommended. Despite undesirable side-effects (e.g. bleeding, thrombocytopenia, osteoporosis), heparin alone may be considered in selected patients with contraindications to citrate infusion, including those with hypoxemia, insufficient

tissue perfusion, severe liver or renal dysfunction, or receiving long duration TPE or LDL apheresi s [42].

(8) **Replacement Fluid:** Due to the haemodynamic fragility of COVID-19 patients, the use of iso-oncotic replacement solutions is recommended (e.g. fresh frozen plasma (FFP), normal saline, or 4–5% albumin; separately or in combination) [43]. Although albumin and saline avoid donor exposure risks, their excessive use can increase risks of bleeding or thrombosis by depleting coagulation proteins [44]. Due to the thrombotic diathesis in COVID-19 [43], FFP may be preferred as it has been shown to replenish levels of antithrombin III, protein C, protein S, and tissue plasminogen inhibitor [44]. When all three types of replacement fluid are used together, it is preferable to end the TPE procedure with FFP to help restore pretreatment levels of fibrinogen and other coagulation factors. Also, COVID-19 convalescent plasma (CCP) as a replacement fluid has been considered for critically ill patients because it can possibly add (neutralizing) anti-SARS-CoV-2 antibodies in addition to restore the dysregulated coagulation [45,46].

(9) **Exchange Volume:** Exchanging 1–1·5 of the patient's plasma volume is recommended, as ~65% of an intravascularly present toxic substance (i.e. an "ideal solute") is removed in this way. Plasma volume can be calculated by the patient's total blood volume (TBV) and haematocrit (Hct) using formula: $TBV = (\sim 70\ ml/kg) \times (1 - Hct/100)$ [47]. In one case series of three severe COVID-19 patients, all 3 patients improved in oxygenation, CRP and IL6 levels following a single TPE procedure using 3L of FFP [12]; however, when FFP is scarce, a minimum of 2L is suggested [34].

(10) **Using Outcome Measures to Determine the Total Number of TPE Procedures:** The expected outcome from TPE is improvement in those parameters that led to the initiation of TPE, including amelioration of CRS. In some studies, patient outcomes improved after only one TPE procedure [12,27,29]; however, others used three to nine procedures to yield the desired effects [25–29,35,48,49].

(11) **Adverse events:** In COVID-19 patients, all commonly known adverse events (AEs) of TPE can occur, including allergic reactions, hypocalcaemia and hypomagnesemia due to citrate, hypotension, arrhythmias, sensations of cold with temporarily elevated temperature, and vasovagal reactions [22]. Most AEs are mild and transient, although severe and potentially life-threatening symptoms can occur, especially in critically ill patients (e.g. shock, severe hypotension requiring pressor drugs, persistent arrhythmias, haemolysis) [50]. In a pilot study, acute kidney injury and pulmonary embolism occurred in 10% and 20% of critically ill COVID-19 patients, respectively [51]. In addition to pro-inflammatory cytokines, TPE also removes other plasma proteins, such as coagulation factors and immunoglobulins. Although a short course of TPE is unlikely to produce immunodeficiency, there is evidence that TPE might, at least temporarily, negatively affect the adaptive immune system (especially T-cell) homeostasis [50,52]. Monitoring the patient's haemostasis parameters especially if FFP and/or CCP are not used as replacement fluids can mitigate additional haemorrhagic and thrombotic risks. Finally, TPE influences coagulation parameters in patients receiving therapeutic anticoagulation; thus, anti-Xa levels may decrease while partial thromboplastin and prothrombin times may increase, which might lead to mild risks of bleeding or thrombosis [53].

Discussion

In view of the global increase in the number of critically ill COVID-19 patients, clinicians have tried many experimental therapies. ASFA guidelines suggest TPE as a supportive therapy in patients with sepsis and MOF. Therefore, based on its pathophysiology and disease course, using TPE for COVID-19 patients may be considered; nonetheless, high-level evidence is still needed. Four mechanisms were proposed supporting the role of TPE to help modify the course of COVID-19 and ameliorate end-organ damage. The first is the removal of toxic substances with suppression of CRS [22]; the second suggests that double filtration TPE removes 60–140 nm viral particles [54]; the third proposes that TPE can correct the coagulopathy in COVID-19 patients when using FFP as a replacement fluid [55]; and the fourth hypothesizes that TPE provides factors that stabilize and restore endothelial membranes [1].

The current contribution, based on available peer-reviewed publications, is intended to guide clinicians and transfusion apheresis professionals on using TPE in COVID-19. Because high-level evidence is still lacking, we strongly support monitoring these patients within the context of clinical studies, ideally prospective randomized controlled trials, whenever possible. Suggested initiation and target levels of severity scores and cytokines for these studies are outlined in Table 1, based on severity criteria for CRS and other previous case reports and case series [56]. Assessing all parameters may not be necessary to start or stop the TPE procedures; single or multiple combinations of these parameters can be used to assess eligibility for TPE and subsequent responses. At this time, we cannot endorse one parameter over another; however,

we do encourage using these tools to help identify and report on COVID-19 patients who would benefit most from TPE.

The number of procedures for COVID-19 patients can be decided based on the reduction or maintenance of pro-inflammatory cytokine levels [57], organ severity scores, and/or improvement in respiratory function [12] following TPE. For example, a greater than 2-fold reduction in IL-6 levels, as compared to pre-treatment values, or normal IL-6 levels for 3 consecutive days, or blood lactate levels below 2 mmol/L for 3 days, or CRP levels <50 mg/L [57]. Improved respiratory function can be assessed by a decrease or cessation of the need for supplementary oxygen or mechanical ventilation, and/or pulmonary imaging demonstrating reduction of pulmonary lesion area by >30% as compared to earlier imaging [34]. Others also suggested normalization of heart rate and/or body temperature as potential endpoints [21,34]. The number of procedures can also be determined on a case-by-case basis using at least one parameter in Table 1 as a key indicator to guide therapy.

Multiple pros and cons should be considered regarding using TPE in COVID-19 patients (Table 2). One critical consideration is the high cost, which limits using TPE as a primary treatment, particularly in low- and middle-income countries (LMIC), especially when more affordable therapies (e.g. dexamethasone) might be readily available. Another concern is that the efficacy of TPE for CRS is questionable because of the short half-life and rapid activity of cytokines [58]; thus, therapies blocking cytokine action (e.g. receptor antagonists) might be more effective than intermittent cytokine removal by TPE [59]. A third consideration is decontamination and local restrictions after use of apheresis equipment after apheresis procedures in highly contagious patients. Also, the personal safety of the apheresis operator is of importance [60].

The choice of replacement fluid is also another important consideration for TPE. For example, albumin and/or saline alone can worsen the coagulopathy. However, using FFP alone is also challenging due to its geographically variable risk of transfusion-transmitted infections and transfusion-related acute lung injury (TRALI) dependent on the country's TRALI mitigation strategies and a relatively limited, type-specific blood supply, particularly because the pandemic has affected blood centres' collection abilities worldwide [61,62]. Solvent-detergent-treated plasma or pathogen-inactivated plasma could overcome these challenges, but this method may not be available in all countries. However, using albumin at the beginning of TPE, followed by FFP towards the end of the procedure, can mitigate these issues. The exact volume of FFP to use can be determined based on the patient's coagulation parameters. Although in limited supply, CCP, which contains neutralizing antibodies, can also be used as a replacement fluid, again preferably at the end of procedure, because it can supply both coagulation factors and virus-neutralizing antibodies [63]. Another option is to use albumin and FFP in a 1:1 ratio, substituting CCP towards the end of last TPE procedure, if available.

Recently, the United States' FDA provided emergency use authorization (EUA) for TPE therapy with the Spectra Optia Apheresis system, along with the Marker Therapeutics' Depuro D2000 adsorption cartridge, for treating adult COVID-19 patients admitted to an ICU with confirmed or imminent respiratory failure [64]. According to the manufacturers' press release, the disposable column is intended

Table 1 Critical levels and proposed target levels of various parameters for TPE in COVID-19.

Parameters	Levels to initiate TPE	Target levels after TPE
SOFA score [31]	≥3	≤2
APACHE II score [30]	≥17	<17
PiO2/FiO2 [56]	<150	≥150
Oxygen saturation [12]	≤93%	≥98%
Respiratory rate [12]	>30/Min	<20/min
Lymphocyte Count (1·1–3·2 × 10^9/L) [56]	≤0·6	>1·1
Neutrophil–lymphocyte ratio (NLR) [66]	≥3·3	<3·3
C-reactive protein (10–50 mg/L) [56,57]	≥100 at presentation, or ≥50 and doubled in past 48 h	<50
Lactate dehydrogenase (100–190 U/L) [56]	≥250	<250
Ferritin (23–336 μg/ml) [56]	≥600 at presentation ≥300 and doubled in past 24 h	<300
D-dimer (<1 μg/ml) [56]	≥1	<1
IL-6 (1–7 pg/ml) [56]	≥30	<30

Table 2 Pros and Cons of TPE procedure in COVID-19.

Pros	Cons
Studies from other causes of CRS suggesting a potential role of TPE in sepsis	Current level of moderate–high quality of peer-reviewed evidence only supports a weak recommendation for TPE in COVID-19
Likely effective in early stage of illness to reduce severity or prevent MODS progression	Expensive, time- and resource-intensive procedure not readily available everywhere, especially in rural settings and LMIC. Patients must be triaged to determine who will most likely benefit
No absolute contraindications for usage, procedure can be ended successfully at any time, if needed	Adverse events of TPE (e.g. citrate toxicity, hypotension) may contribute to haemodynamic instability
Can be used as an adjunctive therapy with other drugs	Removing patient's neutralizing antibodies or therapeutic drugs during TPE can possibly delay recovery or reduce therapeutic benefit
Can directly remove 60-140nm viral particles	This is only beneficial for removing intravascular free viral particles, although viraemia is minimal-absent in COVID-19
FFP as a replacement fluid is helpful in coagulopathy. Pathogen-inactivated FFP may provide additional safety.	Using albumin or saline as a replacement fluid can increase risk of coagulopathy.
	The use of FFP alone may increase risk of complications, including transfusion reactions
Using CCP towards the end of the TPE procedure can provide patients with neutralizing antibodies	May affect FFP and CCP inventory, based on local supply and volume used.
	Operators need appropriate training for personal protective equipment (PPE) donning and doffing.
	Instruments require decontamination if used for COVID patients or requirement of dedicated apheresis instrument for treatment of COVID-19 patients.

to reduce inflammatory cytokines and other inflammatory mediators [65]. It has shown some initial promise in treating patients with ARDS, pneumonia, liver failure and sepsis; in addition, at least one clinical trial is underway (ClinicalTrials.gov Identifier: NCT04358003). Nonetheless, the clinical efficacy of this adsorption cartridge for treating COVID-19 patients is still unknown.

Limitations

This guidance document has several limitations, the primary one being the lack of high-level evidence based on prospective randomized controlled trials. Presently, no such trials of TPE in COVID-19 have been published. In contrast, most of the cited publications were individual case reports, or small case series, or were derived from related, but non-COVID-19 sources. This preliminary guidance document also includes reference statements from other diseases or previous viral outbreaks in which the optimum role of apheresis therapy is not established, like SARS-CoV-1, H1N1 infection and MERS infection. It is worthwhile to note that this guidance provided herein comprised only 42 COVID-19 patients in total, which is far from ideal (Fig. 1). Fortunately, this research field is very active, and additional studies are underway. This preliminary guidance should be reviewed and updated based on future accumulating evidence.

Conclusions

Several encouraging experimental therapies are emerging for COVID-19, but there is currently no definitive cure. Based on general apheresis practice and results of TPE in patients with COVID-19 compatible disease, it is reasonable to consider TPE as a potential option to treat critically ill COVID-19 patients with CRS and it may help in reducing mortality. Therapy can be initiated, and outcome determined using the critical and target levels of multiple parameters described herein. Because the safety and efficacy of TPE are uncertain in haemodynamically unstable and critically ill COVID-19 patients, the use of this therapy should be considered on an individual basis. Nonetheless, it would be best to evaluate the use of TPE alone, or in combination with other therapies, for COVID-19 patients within the context of prospective, randomized controlled clinical trials. This approach may yield fruitful results by saving lives and paving the way for future consideration of TPE in similar diseases.

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Conflict of interest

The authors have no conflict of interest.

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Is there an optimal trade-off between anaemia and red blood cell transfusion in surgical critically ill patients after oncologic surgery?

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Vox Sanguinis

Abstract

Background and objectives Equipoise remains on the optimal transfusion strategy in surgical oncologic patients. The primary objective of our study was to determine the impact of anaemia and red blood cells (RBCs) transfusion on severe postoperative complications in surgical oncologic critically ill patients.

Materials and methods Retrospective single-centre study. Adults admitted to intensive care unit after major oncologic surgery were eligible. Analyses to determine the independent risk factors, including anaemia or RBC transfusion, for postoperative complications and/or hospital mortality were performed.

Results Of the 283 patients included, 246 patients (86.9%) had anaemia. Fifty-five patients (19·4%) were transfused. Patients exposed to moderate-to-severe anaemia or RBC transfusion had more often severe complications, especially acute kidney injury and infectious complications. Multivariate analysis found an independent association between moderate and severe anaemia and severe postoperative complications (moderate anaemia: OR 14·02 [2·52–264]; severe anaemia: OR 16·25 [2·62–318·5]; $P < 0·05$). Elderly, obese patients and patients operated from abdominal surgery appeared to be more vulnerable to anaemia than other patients. Transfusion was also an independent risk factor for postoperative complications (OR 4·19 [2·12–8·39]; $P < 0·001$). When considering moderate-to-severe anaemic patients, RBC transfusion was no longer associated with postoperative complications.

Conclusions Anaemia was associated with severe postoperative complications, and this association was stronger in elderly, obese patients and after abdominal surgery. RBC transfusion also negatively impacts on patients' prognosis. However, this association was not found in case of moderate-to-severe anaemia exposure (haemoglobin < 10 g/dl).

Key words: red blood cell transfusion, anaemia, critical care, oncologic surgery, peri-operative care.

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Introduction

Up to 90% of oncology patients develop anaemia [1]. Prevalence of anaemia varies with anaemia definition, disease stage and tumour location [2]. Lung,

gynaecological, ear-nose and throat cancers are more frequently associated with anaemia [2,3]. Several mechanisms lead to anaemia in oncology patients, including tumour-related bleeding, impairment of erythropoietin synthesis secondary to cytokine secretion, haemolysis, cancer treatments' adverse effects and nutritional deficiencies [4].

Treatment of anaemia is crucial in oncology patients, as anaemia is associated with worse patient outcomes through several mechanisms. Anaemia is responsible for tumour hypoxia that might stimulate angiogenesis, leading to chemotherapy and radiotherapy resistance and increasing morbidity and mortality [1,4]. Anaemia has also been associated with poor quality of life in oncology patients [1,2,5–8]. In the postoperative setting, red blood cell (RBC) transfusion is the main treatment to promptly correct anaemia. As a consequence, 13–40% of oncologic surgical patients receive RBC transfusion [9–12]. However, RBC transfusion has also been associated with adverse events and increased morbidity and mortality in oncology patients [10,13]. In patients with cancer, it remains uncertain whether RBC transfusion, through transfusion-related immunomodulation (TRIM), contributes to tumour relapse and postoperative infection [14–16].

The European Society of Medical Oncology (ESMO) recommends RBC transfusion for symptomatic anaemia (irrespective of haemoglobin [Hb] level) and/or severe anaemia (defined as haemoglobin level < 7–8 g/dl) [17]. In non-oncologic surgery, safety and potential benefit of a restrictive transfusion strategy is supported by several large randomized trials [18–20]. At the opposite, De Almeida et al. reported a benefit of a « liberal » transfusion strategy (Hb level < 9 g/dl) in oncologic surgical patients [21]. Thirty-day (8·2% vs. 22·8%; $P = 0{\cdot}005$) and 60-day mortality (11·3% vs. 23·8%; $P = 0{\cdot}022$) were lower in the « liberal » transfusion strategy group. However, this is a single-centre study limiting its external validity, and the small difference between the median number of administered RBC units between groups might not be clinically meaningful (2·67 RBC units vs. 2·85 RBC units) [22,23]. There is currently no well-designed multicentre clinical trial to guide transfusion practice in the oncologic surgical setting. Determining the optimal RBC transfusion strategy to limit adverse effects from anaemia without adding RBC transfusion complications has been identified as a research priority [24]. Observational studies are necessary to accumulate data that would help to design such trials and insure their feasibility.

Our study aims to analyse the impact of peri-operative anaemia and transfusion on postoperative complications and/or hospital mortality in oncologic surgical critically ill patients.

Materials and methods

Study design and participants

This is a retrospective single-centre study conducted in the university-affiliated hospital of Brest, France. Eligible patients were identified through the hospital diagnostic code system with the primary or secondary diagnosis of 'oncologic surgery' or 'cancer surgery'. Eligible patients were patients admitted to the 21-bed surgical intensive care unit (ICU), including 6 beds of intermediate intensive care, between January 2017 and December 2018 after oncologic surgery. Admission to ICU was commonly decided during the preoperative anaesthesia consultation based on the type of surgery and patients' comorbidities. Intra-operative complications could also be a reason for ICU admission. We focus on critically ill patients because there are the sickest and the most likely to require RBC transfusion. Based on the French national guidelines for RBC transfusion, surgical patients received RBC when the haemoglobin was below 7 g/dl in the absence of acute or chronic cardiovascular disease, or when the haemoglobin was between 8 and 9 g/dl or below 10 g/dl, in case of past medical history of cardiovascular disease or in case of acute coronary disease, respectively [25]. Exclusion criteria included: age less than 18-years, palliative surgery and the absence of consent. Cerebral biopsy, meningioma, hemangioblastoma, astrocytoma, urinary tract surgery by vesico-ureteroscopy, endoscopic gastro intestinal biopsy and surrenalectomy were excluded because of their very low risk of anaemia and/or blood transfusion exposure. Patient outcomes were collected up to hospital discharge. The study was approved by the Research Ethics Committee of Brest University Hospital and registered in clinical trial.gov (NTC04101240). According to the Ethics Committee recommendations, an information letter was sent to all eligible patients. Patients who did not want to participate had to mention it to the investigators.

Data collected

Data were collected from the patients' medical records. They included gender, age, body mass index (BMI), comorbidities with arrhythmia, chronic obstructive pulmonary disease, cirrhosis, chronic renal failure, diabetes and coronary artery disease. Any of the following therapeutics within the month before surgery were recorded: chemotherapy, radiotherapy, erythropoietin, iron supplementation, beta-blockers, anticoagulant and antiplatelet agents. The following data related to surgery were collected: the American Society of Anesthesiologist score,

the Preoperative Score to Predict Postoperative Mortality (POSPOM) [26], type of surgery, length of surgery, surrogate of intra-operative complications, and estimated blood loss during surgery, that included unexpected blood loss. The lowest daily haemoglobin (Hb) concentration from the day prior to surgery to the fifth day after surgery (day 5) and at hospital discharge (when patients were still hospitalized after day 5) were recorded. The number of RBC units transfused at any time during the hospital stay was collected, and Hb concentrations prior to transfusion were collected when available for the RBC units administered in the operating room and up to the 2 first RBC units transfused.

Based on the definition of the World Human Organization (WHO), patients were classified as anaemic when their Hb level was less than 13 g/dl for males and less than 12 g/dl for females [27]. Anaemia severity was further categorized according to international guidelines in oncology as mild when the Hb level was equal or higher than 10 g/dl (from 10 to 12·9 g/dl for males; from 10 to 11·9 g/dl for females), as moderate when Hb level was equal or higher than 8 and less than 10 g/dl (from 8 to 9·9 g/dl) and as severe when Hb level was less than 8 g/dl [28]. RBC exposure was defined as the need of at least one RBC unit during hospital stay regardless of the administration time (i.e. during surgery, in ICU or after ICU discharge).

Outcomes

The primary outcome was a composite outcome including any of the following severe postoperative complications: acute respiratory failure requiring invasive or non-invasive ventilation; acute kidney injury (AKI) defined according to KDIGO criteria [29]; cardiogenic pulmonary oedema; ST elevation myocardial infarction (STEMI) or non-ST elevation myocardial infarction (nSTEMI); symptomatic thromboembolic events (venous thromboembolism confirmed by duplex ultrasound and/or pulmonary embolism confirmed by CT scan); ischaemic or haemorrhagic stroke confirmed by CT scan; revision surgery for surgical site infection; revision surgery for haemorrhagic complications; sepsis or septic shock (defined as per the Sepsis-3 definition [30]); pneumonia and/or hospital mortality. Other outcomes included ICU length of stay and hospital length of stay.

Statistical analysis

Variables were described with mean (±standard deviation, SD) for normally distributed continuous variables or median (interquartile range, IQR) for other continuous variables and with percentage for categorical variables. For any continuous variables with less than 20% of missing data, we used multiple imputations with five iterations as recommended elsewhere [31].

Descriptive analysis was used to explore variables associated with anaemia (no anaemia, mild, moderate, or severe anaemia), RBC transfusion (at least 1 RBC unit during hospital stay) and postoperative complications. Bivariate analysis was performed with the Student t-test or Kruskal–Wallis for continuous variables and chi-square or Fisher's exact test for categorical variables. Variables with between-group differences ($P < 0{\cdot}10$) were considered for multivariate analysis.

We hypothesized that anaemic patients and/or transfused patients had more postoperative complications and/or a higher mortality than patients never exposed to these events. Multivariate logistic regressions were performed to determine the impact of anaemia (mild, moderate or severe) and/or RBC transfusion on the primary outcome. The results were presented with odds ratio (OR), 95% confidence interval (CI) and P value. For multivariate analyses, we considered two-tailed P values of less than 0·05 as significant. All statistical analyses were performed with R statistical software (version 3.3.2).

Results

Study population

From the 1st January 2017 to the 31th December 2018, 3 494 patients had surgery for cancer. Of those, 323 patients (9·2%) were admitted to ICU after surgery. Twenty-one patients (6·4%) were excluded as they had undergone palliative surgery and 15 patients (4·5%) were excluded because their surgery was minor. Three patients (0·9%) withdrew their consent and one patient was excluded as they had no data available on RBC transfusion and Hb concentrations. Finally, 283 patients were included in the study (Fig. S1).

Patient characteristics are displayed in Table 1. Surgery was mostly elective surgery (92·5%) and was for abdominal malignant tumour in approximately half of the cases (51·2%), and neurological tumour in one third of the cases (34·3%) (Table 1 and Table S1). The mean POSPOM was 28·5 (±4), related to a predictive hospital mortality of 4% (Table 1) [26].

Sixty-seven patients (23·7%) had at least one postoperative complication: infection in 53 patients (18·7%), acute kidney injury in 26 patients (9·2%) with 13 patients requiring renal replacement therapy (RRT) and thromboembolic events in 10 patients (3·5%). Hospital mortality was 4·9% (Table 1).

Table 1 Characteristics in overall population and comparison between patients with and without severe postoperative complication and/or hospital mortality

	Overall *n* = 283	Alive and No severe complication *n* = 213	Severe complication or death *n* = 70	*P*
Age, years (mean, SD)	62·7 (12·2)	61·4 (12·8)	66·7 (9·1)	0·001
BMI, kg/m^2 (mean, DS)	25·2 (5·6)	25·7 (5·2)	27·9 (6·4)	0·004
Comorbidities (*n*, %)				
Arterial hypertension	148 (52·1)	105 (49·3)	43 (61·4)	0·104
Coronary artery disease	53 (18·7)	41 (19·2)	12 (17·1)	0·78
Valvulopathy	13 (4·6)	8 (3·8)	5 (7·1)	0·4
Arteritis	36 (12·5)	24 (11·3)	12 (17·1)	0·28
Arrhythmia	10 (3·5)	8 (3·8)	2 (2·9)	1
COPD	47 (16·5)	35 (16·4)	12 (17·1)	1
Cirrhosis	13 (4·6)	10 (4·7)	3 (4·3)	1
Chronic Kidney Disease	33 (11·6)	20 (9·4)	13 (18·6)	0·06
Diabetes	53 (18·7)	31 (14·6)	22 (31·4)	0·003
Current smoker	93 (32·9)	73 (34·3)	20 (28·6)	0·46
Preoperative treatment (*n*, %)				
Chemotherapy	34 (12)	24 (11·3)	10 (14·3)	0·64
Radiotherapy	5 (1·8)	2 (0·9)	3 (4·3)	0·19
Antiplatelet agents	65 (22·9)	42 (19·7)	23 (32·9)	0·04
DOA	9 (3·2)	9 (4·2)	0	0·18
VKA	8 (2·8)	4 (1·9)	4 (5·7)	0·21
Beta blockade agents	50 (17·6)	36 (16·9)	14 (20)	0·68
Surgery (*n*, %)				
Abdominal	145 (51·2)	93 (43·7)	52 (74·3)	<0·001
Neurosurgery	97 (34·2)	91 (42·7)	6 (8·6)	<0·001
Thoracic	37 (13·1)	25 (11·7)	12 (17·1)	0·34
Length of surgery, min (mean, SD)	236 (125)	214 (113)	297 (138)	<0·001
Estimated blood loss, ml (mean, SD)[§]	587 (1,335)	501 (718)	828 (2,316)	0·13
POSPOM (mean, SD)	28·5 (4)	27·9 (3·8)	30 (4·2)	<0·001
Haemoglobin level (Nadir), g/dl (mean, SD)	10·5 (2)	10·9 (1·9)	9·5 (1·8)	<0·001
Anaemia during hospital stay (*n*, %)				< 0·001
No anaemia	36 (12·7)	35 (16·4)	1 (1·4)	
Mild (according to WHO definition)	146 (51·6)	118 (55·4)	28 (40)	
Moderate (Hb: 8–9·9 g/dl)	68 (24)	41 (19·2)	27 (38·6)	
Severe (Hb < 8 g/dl)	32 (11·3)	18 (8·5)	14 (20)	
RBC transfusion (*n*, %)	55 (19·4)	26 (12·2)	29 (41·4)	< 0·001
Amount of RBC unit transfused (mean, SD)	4·8 (6)	3·2 (2·9)	6·2 (7·7)	0·07
Length of stay (mean, SD)				
In ICU	3·8 (7·6)	2 (2·8)	9·4 (13·1)	<0·001
In hospital	16·5 (17·6)	11·5 (9·1)	31·7 (26·3)	<0·001

BMI, Body Mass Index; COPD, Chronic Obstructive Pulmonary Disease; DOA, Direct Oral Anticoagulant; Hb, Haemoglobin; ICU, Intensive Care Unit; POSPOM, Preoperative Score to Predict Postoperative Mortality; SD, Standard Deviation; VKA, Vitamin K Antagonist; WHO, World Health Organization.
[§] Indicated variables with more than 20% of missing data.

Peri-operative anaemia

We compared patients without anaemia, patients with mild, moderate or severe anaemia. We found differences in types of surgery, surgical blood loss and postoperative complications between these groups (Table 2). Two hundred and fifty patients (86·9%) were anaemic between the day prior to surgery and the fifth day (Day 5) after surgery. One hundred and forty-six patients (51·6%) experienced mild anaemia; 68 (24%) moderate anaemia and 32 (11·3%) severe anaemia. Ninety patients (31·8%) were already anaemic before surgery, and anaemia was mild, moderate and severe in 79 (27·9%), 9 (3·2%) and 2 patients (0·7%), respectively. Patients exposed to

preoperative anaemia were older (65 years vs. 61·7, $P = 0·03$) and had more often abdominal surgery (66·7% vs. 44%, $P = 0·001$) than patients without preoperative anaemia. They also received more often RBC transfusion during and after surgery (38·9% vs. 10·4%, $P < 0·001$). Preoperative anaemia was significantly associated with hospital mortality (10% vs. 2·6, $P = 0·017$) and the occurrence of postoperative complications (33·3% vs. 19·2, $P = 0·014$) (Table S3). Ninety patients had both preoperative anaemia and postoperative anaemia, those patients were also more frequently transfused (38·9% vs. 12·7%, $P < 0·001$) than patients with postoperative anaemia only.

In the univariate analysis comparing patients without anaemia, patients with mild anaemia and patients with moderate-to-severe anaemia at any time, the following variables were associated with moderate-to-severe anaemia: age (61·5 vs. 65·1 years; $P = 0·02$), abdominal surgery (38·8% vs. 74%; $P < 0·001$), estimated blood loss (375 ml vs. 893 ml; $P = 0·006$) and length of surgery (205 min vs. 290 min; $P < 0·001$). Moderate-to-severe anaemic patients had more severe postoperative complications than non-anaemic or mildly anaemic patients (15·8% vs. 41%; $P < 0·001$), including infections (12·6% vs. 30%; $P = 0·001$) and renal complications (3·3% vs. 20%; $P < 0·001$). Hospital mortality was also significantly higher in patients with moderate-to-severe anaemia (1·6% vs. 11%; $P = 0·001$). The rate of postoperative complications was similar in patients exposed to severe anaemia or to moderate anaemia (Table S4).

Interventions for anaemia – including RBC transfusion

No patients were transfused within the preoperative period. Three patients (1·1%) received preoperative intravenous iron administration and no patients received preoperative erythropoietin. No patients received tranexamic acid before or during surgery.

Fifty-five patients of the 283 (19·4%) received one or more RBC unit within their hospital stay. First RBC transfusion happens in the operating room, between day 1 and day 5, and after day 5 for 21 patients (38·2%), 22 patients (40%) and 12 patients (21·8%), respectively. Patients first transfused after day 5 developed significantly more complications than patients first transfused in the operating room or between day 1 and day 5 (Table S2). A total of 304 RBC units were administered. The mean Hb level prior to transfusion collected for 71 RBC transfusion was 7·8 g/dl (±1·4). Haemoglobin threshold was higher in patients first transfused in the operating room (9·25 g/dl) compared to patients first transfused after surgery (from 6·95 g/dl between day 1 and day 5 and 8 g/dl after day 5) (Table S2). Rate of transfusion was more important in case of abdominal (28·3%) and thoracic (24·3%) surgery compared to neurosurgery (4·1%) (Table S5).

The median number of RBC units transfused per patient was 3 [2–5]. Transfused patients were older (62·1 vs. 65·5 years; $P = 0·066$), had more often abdominal surgery (45·6% vs. 74·5%; $P < 0·001$), had a lower preoperative Hb (12·1 vs. 14·2 g/dl; $P = 0·05$) and higher estimated blood loss (1 430 vs. 340 ml; $P < 0·001$) than non-transfused patients (Table 3). Trends in Hb level from day 0 to day 5 and on the day of hospital discharge according to transfusion status is displayed on Fig. S2. Transfused patients had worse outcomes than non-transfused patients, with more postoperative complications (18% vs. 52·7%; $P < 0·001$) and higher hospital mortality (1·3% vs. 20%; $P < 0·001$) (Table 3 and Fig. S3).

Patient outcomes

The variables associated with postoperative complications and/or hospital mortality in univariate analysis are displayed in Table 1 and included RBC transfusion (12·2% vs. 41·4%; $P < 0·001$) and mean Hb concentration from day 1 to day 5 (11·2 vs. 10·4 g/dl; $P < 0·05$). Figure 1 illustrates the differences in Hb concentrations between patients with and without severe complications.

To identify potentially more vulnerable patients to the adverse effects of anaemia and/or transfusion, we performed subgroup analyses. Six subgroups of patients were identified based on their higher risk of complications identified by the univariate analysis (Table 1). These 6 groups included patients older than 65 years old, patients who received an antiplatelet agent, abdominal surgery, chronic kidney disease (CKD), diabetes and obese patients. In each subgroup, there was a stepwise increase in severe postoperative complications and/or hospital mortality when patients had one risk factor and/or was moderate-to-severe anaemic compared to patients with no risk factor and no moderate-to-severe anaemia (Fig. 2). Elderly (age > 65 years old), obese patients (BMI > 30 kg/m^2) and patients who had abdominal surgery were more vulnerable to adverse effects of anaemia.

Multivariate analysis

In the multivariate analysis, moderate anaemia (OR 14·02 [2·52–264], $P = 0·014$) and severe anaemia (OR 16·25 [2·62–318·5], $P = 0·012$) remained independently associated with the primary outcome (Fig. 3). Mild anaemia was not associated with severe postoperative complication (OR 6·25 [1·18–116], $P = 0·083$). Preoperative anaemia (based on the WHO definition) was not associated with the primary study outcome in the multivariate analysis

Table 2 Comparisons of patient's characteristics and outcomes between anaemic and non-anaemic patients based on anaemia severity: mild anaemia (defined as Hb level: Female 10–11·9 g/dl; Male 10–12·9 g/dl); moderate anaemia (defined as Hb level: 8–9·9 g/dl) and severe anaemia (defined as Hb level < 8 g/dl)

	No anaemia N = 37	Mild anaemia N = 146	Moderate anaemia N = 68	Severe anaemia N = 32	*P*
Age, years (median, IQR)	61 [52–67]	64 [57–71]	68 [62–71]	68 [56–72]	0·05
Surgery (*n*, %)					
Abdominal	4 (11·1)	67 (45·9)	49 (72·1)	25 (78·1)	<0·001
Neurosurgery	28 (77·8)	54 (37)	13 (19·1)	1 (3·1)	<0·001
Thoracic	4 (11·1)	23 (15·8)	5 (7·4)	5 (15·6)	0·44
Comorbidities (*n*, %)					
Arterial hypertension	16 (44·4)	78 (53·4)	36 (52·9)	18 (56·2)	0·74
Coronary artery disease	7 (19·4)	28 (19·2)	12 (17·6)	6 (18·8)	0·99
Arteritis	2 (5·6)	20 (13·7)	11 (16·2)	3 (9·4)	0·53
Arrhythmia	0	7 (4·8)	1 (1·5)	2 (6·2)	0·36
COPD	6 (16·7)	26 (17·8)	11 (16·2)	4 (12·5)	0·95
Cirrhosis	0	5 (3·4)	5 (7·4)	3 (9·4)	0·18
Chronic kidney disease	2 (5·6)	17 (11·6)	8 (11·8)	6 (18·8)	0·49
Diabetes	1 (2·8)	29 (19·9)	14 (20·6)	9 (28·1)	0·03
Estimated blood loss, ml (median, IQR) [§]	100 [100–325]	300 [200–500]	300 [200–625]	500 [300–1750]	<0·001
Primary outcome (*n*, %)	1 (2·8)	28 (19·2)	27 (39·7)	14 (43·8)	<0·001
Hospital mortality	0	3 (2·1)	8 (11·6)	3 (9·4)	0·01
Postoperative complications	1 (2·8)	28 (19·2)	24 (35·3)	14 (43·8)	<0·001
Acute respiratory failure	0	7 (4·8)	5 (7·4)	0	0·26
AKI	0	6 (4·1)	12 (17·6)	8 (25)	<0·001
AKI needed RRT	0	2 (1·4)	6 (8·8)	5 (15·6)	0·002
Cardiac complications	0	2 (1·4)	0	0	n.a.
nSTEMI	0	1 (0·7)	0	0	n.a.
Pulmonary oedema	0	1 (0·7)	0	0	n.a.
Thromboembolic events	0	6 (4·1)	3 (4·4)	1 (3·1)	0·81
VTE	0	2 (1·4)	2 (2·9)	1 (3·1)	0·56
PE	0	5 (3·4)	1 (1·5)	0	0·64
Ischaemic stroke	0	2 (1·4)	0	0	n.a.
Infectious	1 (2·8)	22 (15·1)	20 (29·4)	10 (31·2)	0·001
Sepsis	0	8 (5·5)	7 (10·3)	4 (12·5)	0·14
Surgical site infection (SSI)	1 (2·8)	13 (8·9)	14 (20·6)	8 (25)	0·006
Pneumonia	0	9 (6·2)	7 (10·3)	2 (6·2)	0·27
Revision surgery for SSI	1 (2·8)	4 (2·7)	9 (13·2)	2 (6·2)	0·04
Revision surgery for haemorrhage	0	2 (1·4)	2 (2·9)	2 (6·2)	0·23
Length of stay (median, IQR)					
In ICU	1 [1–1]	1 [1–3]	2 [1–5]	3 [2–8]	<0·001
In hospital	7 [5–10]	10 [7–17]	14 [9–26]	16 [11–23]	<0·001

AKI, Acute Kidney Injury; F, Female; Hb, Haemoglobin; ICU, Intensive Care Unit; IQR, Interquartile Range; M, Male; nSTEMI, non-ST Elevation Myocardial Infarction; PE, Pulmonary Embolism; RRT, Renal Replacement Therapy; VTE, Venous Thrombo Embolism; SSI, Surgical Site Infection.

[§] Indicated variables with more than 20% of missing data.

(OR 1·5 [0·85–2·9], $P = 0·13$). In another model considering RBC transfusion, RBC transfusion was also independently associated with the primary outcome (OR 4·19 [2·12–8·39]; $P < 0·001$) (Fig. 4). Obesity (defined by a BMI > 30 kg/m^2) and abdominal surgery were the other variables independently associated with postoperative complications and/or hospital mortality in both models (Figs 3 and 4). When considering only patients with moderate-to-severe anaemia ($n = 100$), RBC transfusion was not associated with severe postoperative complications and/or hospital mortality anymore (OR 1·66 [0·68–4·07]; $P = 0·26$) (Fig. S4).

Table 3 Comparison between transfused and non-transfused patients over their hospital stay

	Non-transfused patients *n* = 228	Transfused patients *n* = 55	*P*
Age, years (mean, SD)	62·1 (12·5)	65·5 (10·5)	0·07
Surgery (*n*, %)			
Abdominal	104 (45·6)	41 (74·5)	<0·001
Neurosurgery	93 (40·8)	4 (7·3)	<0·001
Thoracic	28 (12·3)	9 (16·4)	0·58
Estimated blood loss, ml (mean, SD)[§]	340 (279)	1,430 (2,605)	<0·001
POSPOM (mean, SD)	28·3 (3·7)	29·3 (5)	0·08
RBC used (mean, SD)	n.a.	4·8 (6·1)	n.a.
In operating room	n.a.	1·65 (3·6)	n.a.
Postoperative period	n.a.	3·1 (5)	n.a.
Haemoglobin level, g/dl (mean, SD)			
Preoperative	14·2 (7·9)	12·1 (1·8)	0·047
Postoperative (nadir)	11 (1·7)	8·5 (1·8)	<0·001
Primary outcome (*n*, %)	41 (18)	29 (52·7)	<0·001
Hospital Mortality	3 (1·3)	11 (19·6)	<0·001
Postoperative complications	40 (17·5)	27 (49)	<0·001
Acute respiratory failure	8 (3·5)	4 (7·3)	0·38
AKI	10 (4·4)	16 (29·1)	<0·001
AKI needed RRT	2 (0·9)	11 (20)	<0·001
Cardiac complications	0	2 (2·6)	0·047
nSTEMI	0	1 (1·8)	n.a.
Pulmonary oedema	0	1 (1·8)	n.a.
Thromboembolic events	5 (2·2)	5 (9·1)	0·038
VTE	2 (0·9)	3 (5·5)	0·08
PE	4 (1·8)	2 (3·6)	0·74
Ischaemic stroke	1 (0·4)	1 (1·8)	0·85
Infectious	31 (13·6)	22 (40)	<0·001
Sepsis	10 (4·4)	9 (16·4)	0·004
Surgical site infection (SSI)	20 (8·8)	16 (29·1)	<0·001
Pneumonia	11 (4·8)	7 (12·7)	0·065
Revision surgery for SSI	7 (3·1)	9 (16·4)	<0·001
Revision surgery for haemorrhage	2 (0·9)	4 (7·3)	0·015
Length of stay (mean, DS)			
In ICU	2·9 (4·6)	7·8 (13·9)	<0·001
In hospital	14·1 (14·4)	26·3 (24·8)	<0·001

AKI, Acute Kidney Injury; ICU, Intensive Care Unit; IQR, Interquartile Range; nSTEMI, non-ST Elevation Myocardial Infarction; PE, Pulmonary Embolism; RRT, Renal Replacement Therapy; VTE, Venous Thrombo Embolism; SSI, Surgical Site Infection.

[§] Indicated variables with more than 20% of missing data.

Discussion

In this observational study of surgical oncologic critically ill patients, 86·9% of the patients were anaemic and 19·4% of them required RBC transfusion during their hospital stay. Moderate-to-severe anaemia and RBC transfusion were associated with postoperative complications and/or in-hospital mortality, and this association remained after adjustment for confounders. We identify elderly (age > 65 years old), obese patients (BMI > 30 kg/m^2) and patients who had undergone abdominal surgery to be more vulnerable to anaemia. When considering only patients with moderate-to-severe anaemia (Hb level < 10 g/dl), RBC transfusion was not associated with worse outcomes anymore, suggesting that worse outcomes associated with RBC transfusion are confounded by anaemia.

In oncologic peri-operative setting, literature regarding the impact of anaemia on patient outcomes is sparse [6,9–12]. Considering preoperative anaemia (according to WHO definition), our results were in line with recent

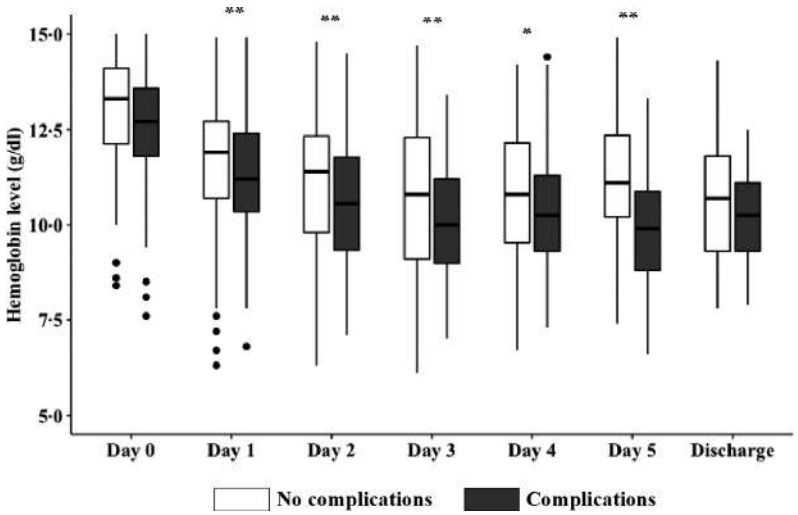

Fig. 1 Trends in haemoglobin from prior to surgery (Day 0) to Day 5 and at hospital discharge (g/dl) for patients with and without severe complications (postoperative complication and/or in-hospital mortality). (**$P < 0.05$; *$P < 0.1$). From Day 2 to Day 5 and at discharge, more than 20% of haemoglobin level were not available.

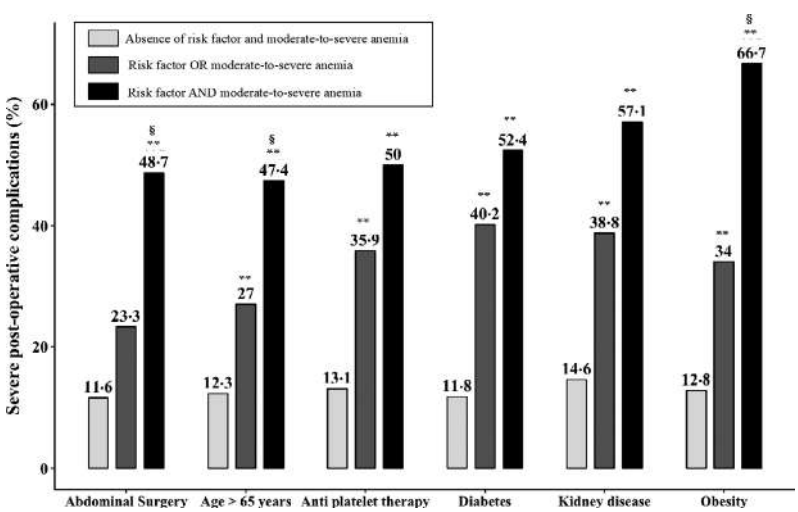

Fig. 2 Percentage of severe postoperative complication in patients with or without risk factor (antiplatelet agent, age > 65 years, abdominal surgery, diabetes, chronic kidney disease, obesity) and/or exposure to moderate-to-severe anaemia (defined as Hb level < 10 g/dl). In each of the 6 subgroups, percentages were compared between patients with neither risk factor nor moderate-to-severe anaemia (Hb < 10 g/dl) (White bar) with each of the 2 other group (grey bar: exposure to considered risk factor OR moderate-to-severe anaemia; and black bar: exposure to considered risk factor AND moderate-to-severe anaemia). [**$P < 0.05$ for comparison between reference group (White bar) vs. group 1 (grey bar) or group 2 (black bar); §$P < 0.05$ for comparison between group 1 (grey bar) and group 2 (black bar)].

studies [10–12,32]. However, in our study, the figures of anaemic patients are higher than those reported previously, possibly because of our inclusion criteria (major surgery and admission to ICU). The association between anaemia and postoperative complications has been reported elsewhere [6,33]. In non-cardiac surgery, Wu et al. found an inverse association between preoperative haematocrit level and cardiac complications or mortality [33]. In non-cardiac surgery, Musallam et al. reported that preoperative anaemia were associated with peri-operative complications (OR = 3·30 [3·20–3·40]) and 30-day mortality (OR = 6·12 [5·73–6·54]) [6]. In this study, cancer were an additional condition where anaemia was associated with postoperative complications [6]. Our study identified a 'dose effect' between anaemia and poor prognosis, with the most severely anaemic patients having the worse outcome. In addition, we identified three subgroups in whom the prognosis was significantly altered when patients were anaemic;

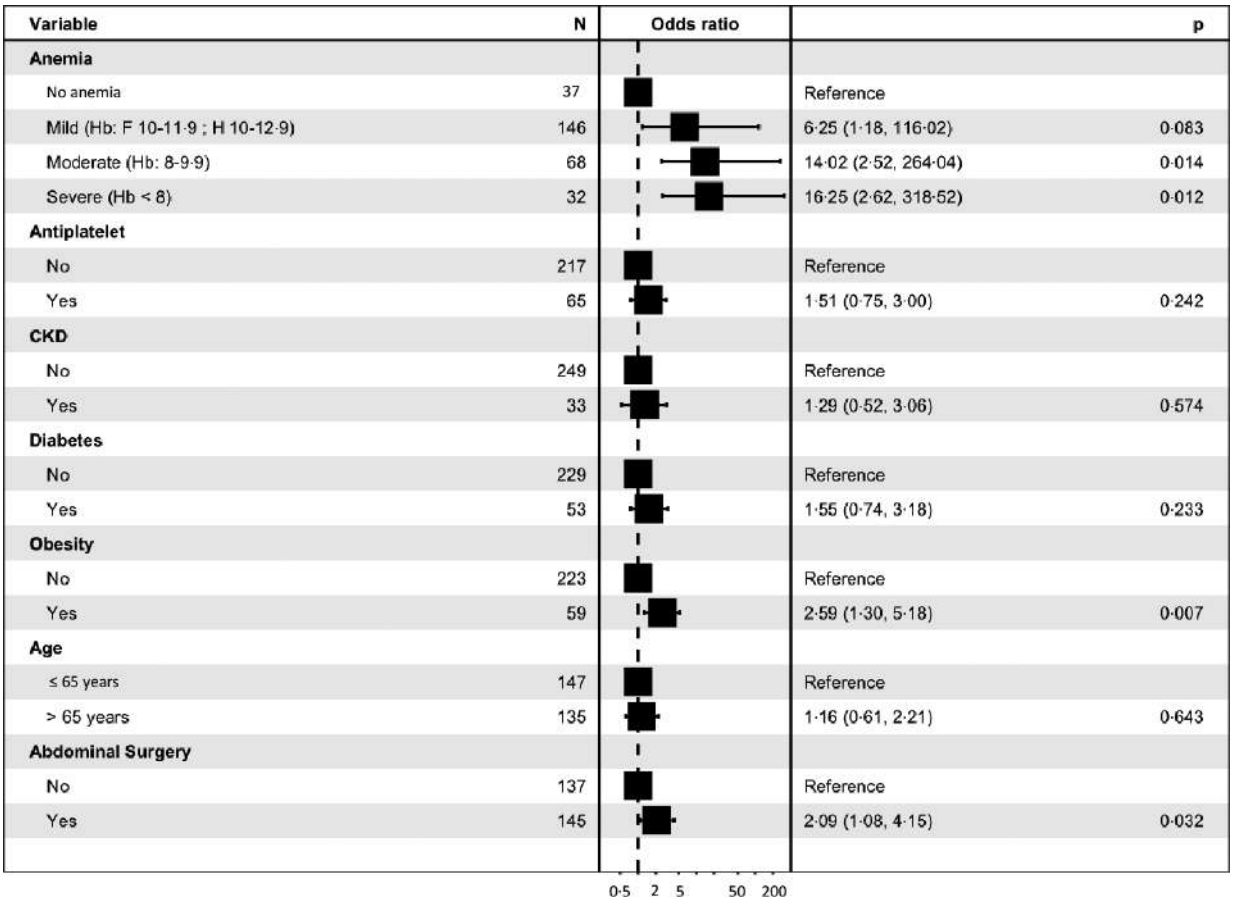

Fig. 3 Multivariate analysis determining the variables associated with severe postoperative complication and/or hospital death. CKD, Chronic Kidney Disease.

they were elderly, obese and patients with abdominal oncological surgery. Elderly patients are known to be less tolerant to anaemia [33,34]. Abdominal oncological surgery might be associated with higher blood loss and more severe anaemia. Obese patients might also be at higher risk of prolonged surgery, higher blood loss or cardiovascular events with higher peri-operative morbidity [35].

In peri-operative setting, RBC transfusion is the only available therapeutic that promptly corrects anaemia. Randomized controlled trials investigating the safety and the potential benefit of a restrictive transfusion strategy in cardiac and non-cardiac surgery have been published [18–21,36,37]. After hip surgery, a higher haemoglobin threshold (Hb < 10 g/dl) was not superior to lower haemoglobin threshold (Hb < 8 g/dl) in regards of survival and ability to walk at day 60 post-surgery (OR = 1·01 [0·84–1·22]) [18]. After cardiac surgery, three large randomized trials did not find any difference in the study primary outcomes supporting the safety of a 'restrictive' transfusion strategy (Hb threshold of 7·5–8 g/dl) compared to a liberal transfusion strategy (Hb threshold of 9–10 g/dl) in these patients [19,20,37]. At the opposite, a recent a pilot study reported better cerebral oxygenation parameters and less complications after vascular surgery in patients transfused at a high haemoglobin threshold (Hb < 9·7 g/dl) in comparison with those transfused at a low haemoglobin threshold (Hb < 8 g/dl) [38].

The optimal RBC transfusion threshold is unknown in this oncological setting [24]. In a large cohort of 20 000 patients undergoing surgery for abdominal malignancy, 30% of patients were exposed to RBC transfusion in the first 3 days after surgery [10]. Keeping with these findings, 20% of the patients included in our study received RBC transfusion, this figure rose to 27·4% when neurosurgical patients (with low risk of bleeding) were excluded. Several observational studies reported an association between RBC transfusion and postoperative morbidity and mortality after abdominal and thoracic surgery for malignant tumour [11,12,39–43]. These studies identified an association between RBC transfusion and tumour recurrence, cancer-related mortality or all-cause mortality [34,39,40,43]. Ecker et al. reported an association between

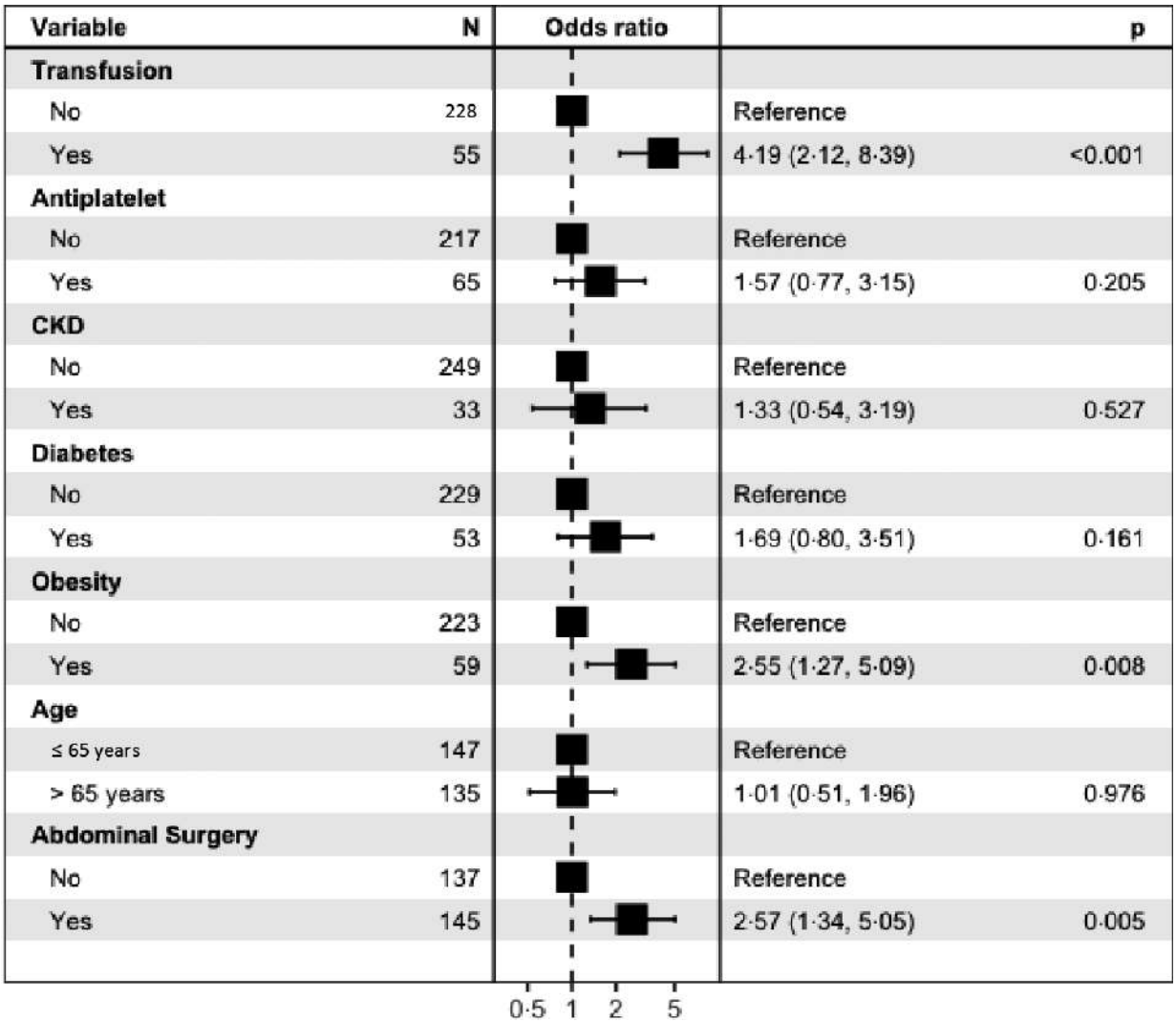

Fig. 4 Multivariate analysis to determine the variables associated to severe postoperative complication and/or hospital death. CKD, Chronic Kidney Disease.

RBC transfusion and surgical site infection (OR 1·51 [1·39–1·65]; $P < 0·001$), myocardial infarction (OR 2·03 [1·49–2·76]; $P < 0·001$) and acute kidney injury (OR 3·50 [2·85–4·31]; $P < 0·001$) after abdominal surgery for malignant tumour [10]. However, these studies did not consider different degrees of anaemia severity, as we did. In our study, the association between RBC transfusion and poor outcome was not found when only moderate-to-severe anaemia (Hb level < 10 g/dl) was considered. There is only one randomized controlled trial investigating the optimal transfusion strategy in oncologic surgery [21]. In this study, the patients randomized in the 'liberal' group (<9 g/dl) had a lower risk of death and less postoperative complications compared to the patients randomized in the 'restrictive transfusion strategy' group (<7 g/dl) [21]. However, this study suffers several biases [23,44] and further research is needed to provide high-quality evidence on the optimal transfusion threshold in this population [24].

Our study has some strengths. First, the dataset had only a few missing data and only a few patients were excluded from the analysis. Second, our statistical analysis considered different degrees of anaemia severity, allowing to distinguish subgroups of patients where RBC might be beneficial. Third, we performed subgroup analyses, based on patient comorbidities and type of surgery,

to identify patients who might be less tolerant to anaemia in this setting. Finally, the multivariate analysis was adjusted on the main confounders identified in the univariate analysis.

However, our study suffers some limitations. First, this is a retrospective single-centre study with the biases related to this design. Second, only critically ill patients were considered, limiting the external validity of the results. However, patients with major surgery or at risk of bleeding are usually admitted to ICU after surgery. Third, we could not be conclusive in regard of the causal association between anaemia or RBC transfusion and postoperative complications because of the observational design. Fourth, we collected the haemoglobin threshold only before the first transfusion episode in the operating room and before the 2 first transfusion episodes after surgery, leading to a potential bias. There was no calculation of an *a priori* sample size. However, an *a posteriori* power calculation considering the rates of postoperative complications and the study groups sample size found a power of 97%–99% for both analyses related to RBC transfusion and anaemia. Finally, we could not differentiate anaemia and transfusion effects, while transfusion benefit might outweigh anaemia adverse effects in the subgroup of patients with moderate-to-severe anaemia.

Conclusion

In this retrospective study, 86·9% of oncologic surgical patients admitted to ICU were anaemic. Anaemia was independently associated to poor outcome. We identified elderly (age > 65 years old), obese patients and patients with abdominal surgery to be less tolerant to anaemia. RBC transfusion also negatively impacts on patients' prognosis; however, this association was not found in patients with moderate-to-severe anaemia. Our study highlights the need for further research to determine the optimal transfusion strategies in surgical oncologic patients and to help clinicians to individualize their transfusion practices in this setting.

Conflict of interest

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to VOX SANGUINIS.

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Supporting Information

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

Fig. S1 Flow shart of the study.

Fig. S2 Trends in hemoglobin (Hb) from prior to surgery (Day 0) to Day 5 and at hospital discharge (g/dl) in: A) All patients (n = 283), B) Transfused and non-transfused patients, C) In-hospital survivors and non-survivors.
Fig. S3 Percentage of severe post-operative complications in patients with or without risk factor (Antiplatelet agent, age > 65 years, abdominal surgery, diabetes, chronic kidney disease, obesity) and/or exposition to RBC transfusion.
Fig. S4 Multivariate analysis evaluated impact of RBC transfusion on primary outcome (severe postoperative complication and/or hospital death) in sub-group of patients with moderate-to-severe anemia.
Table S1 Characteristics of surgical interventions and preoperative score
Table S2 Comparison between transfused patients considering the timing of the first RBC transfusion.
Table S3 Comparisons of patient's characteristics and outcomes considering existence of pre-operative anemia (based on the WHO definition of anemia).
Table S4 Comparisons of main patient's characteristics and outcomes between patients exposed to moderate anemia (defined as Hb level: 8–9.9 g/dl) and patients exposed to severe anemia (defined as Hb level <8 g/dl).
Table S5 Comparisons of main patient's characteristics and outcomes according to type of surgery.

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ORIGINAL PAPER

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Patterns of red-cell transfusion use in obstetric practice in Sweden 2003–2017: A nationwide study

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Abstract

Background There is a paucity of data on patterns of red-cell transfusions in obstetrical care, but some studies have suggested an increase in transfusion rates during the last decade. The purpose of this study was to investigate maternal characteristics, temporal trends and hospital variations in red-cell use in a large contemporary obstetric cohort in Sweden.

Study design and methods Nationwide observational cohort study of maternal red-cell transfusions for all deliveries in Sweden between 2003 and 2017.

Results The proportion of deliveries that received red-cell transfusions was stable during the study period, although the number of red-cell units administered per delivery declined. Among transfused women, most received a low-volume transfusion of 1 or 2 units. Red-cell transfusion was more common among the nulliparous, for instrumental and caesarean deliveries, and with increased maternal age. We saw large variations in transfusion rates between hospitals in Sweden, despite adjusting for age and parity.

Conclusions In comparison to other high-resource countries we see a high proportion of deliveries with maternal red-cell transfusions. However, we do not see an increase in red-cell use over time.

Background

While the overall incidence of red-cell transfusions has decreased in Sweden and other high-resource settings such as the US and England [1–3], there are studies that suggest increasing transfusion rates in obstetric patients [4–6]. The reasons for this are not fully understood.

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It has previously been observed that a significant fraction of maternal red-cell transfusions in obstetrics may be considered inappropriate [7]. In a 2017 study, it was observed that obstetricians were more likely to prescribe transfusion with two red-cell units, although estimated blood loss and haemoglobin level suggested that one unit would suffice [8]. A single centre study from the same year showed that almost half of red-cell transfusions were administered despite no ongoing blood loss and a haemoglobin level (Hb) of >7 g/dl [9].

A recent Swedish study showed that the majority of red-cell units administered to women between the ages 25–35 were used in an obstetric context [1]. Besides this, little is

known about the transfusion patterns in these patients. There is limited data on any long-term effects of allogeneic blood transfusion in the obstetric population, and given that blood products are a scarce and a costly resource, any upward trend in utilization warrant further investigation.

Therefore, we conducted a nationwide, long-standing study to describe maternal red-cell transfusion in Sweden. Specifically, we characterized trends over time and in relation to maternal age, as well as variation across hospitals.

Methods and materials

Data sources and setting

The study was based on the Swedish Medical Birth register and the Swedish portion of the third iteration of the Scandinavian donations and transfusion database (SCANDAT3-S). The registers were linked using the unique national registration number assigned to all Swedish residents.

The Swedish Medical Birth Register is a nationwide population-based health register established in 1973 and managed by the National Board of Health and Welfare, containing 96-99% of all births. The register contains demographic and gestational data, as well as information on diagnoses and medical procedures classified according to the Swedish version of the International Classification of Diseases (ICD) system and standardized procedural codes. Details on the register have previously been described [10]. Antenatal and in-hospital obstetric care is part of the government-funded public health-care system in Sweden, and virtually all women attend.

The SCANDAT databases are a series of bi-national databases that contains computerized information on blood donors, donations, blood products, transfusions and transfused patients since 1968 in Sweden and 1981 in Denmark. SCANDAT3-S is the third iteration of the Swedish portion of the database, containing data on blood transfusions and donations until 2017, extracted from Blood Bank laboratory information systems, with nationwide coverage in Sweden since 1996 [11].

Study design

All deliveries in women aged 15–50 years between January 1, 2003 and December 31, 2017 were identified in the Swedish Medical Birth Register. Information on all maternal red-cell transfusions was extracted from the SCANDAT3-S database.

Statistical analysis

A maternal peripartum red-cell transfusion was defined as a recorded administration of one or more red-cell units in the time period from 14 days prior to and until 14 days after delivery. If maternal transfusion occurred, the delivery was categorized as 'transfused'. The total number of transfused units administered during this period was also counted and categorized as '1 or 2 units', '3 to 9 units' or '10 units or more'.

Maternal demographic and gestational characteristics were described for transfused and non-transfused deliveries, and according to the number of administered units per delivery (in mentioned categories). Multi-foetal births were counted as one delivery.

Considered variables include maternal age at delivery, parity (nulliparous, one previous delivery and more than one previous delivery), body-mass index (BMI, kg/m^2), gestational length (preterm < 37 weeks, at term and post-term > 42 weeks), plurality (singleton or multi-foetal pregnancy), foetal presentation at delivery (cephalic, breach, flexed, other), delivery mode (spontaneous vaginal, instrumental vaginal or caesarean) and hospital category (county, regional or university hospital).

Results were presented as frequencies and percentages, and, where applicable, medians with interquartile ranges (IQR). Missing values were labelled as 'unknown'.

We calculated the percentage of transfused deliveries where a total count of one, two, three etc. maternal red-cell units were administered, and in relation we present the cumulative percentage of total red-cell unit use. We also analysed the distribution of administered red-cell units in relation to the day of delivery (i.e. a parturient that receives two red-cell units the day of delivery and on day two after delivery contributes with two units for each of those days).

To visualize overall transfusion trends, we applied logistic regression with year of delivery modelled as a restricted cubic spline with five equally placed knots. Time-trends were further stratified in categories according to the number of administered red-cell units, parity and delivery mode and presented as the number of transfused deliveries per 1000. In the analyses on maternal age and red-cell transfusion, we used as a restricted cubic spline, with knots manually placed (at ages 22, 30, 35, 40, 45, 48) for better fit. Results were stratified and presented as above. In supplemental material, we used a logistic regression to investigate red-cell unit usage over time and in relation to maternal age.

The proportion of deliveries with maternal transfusions was also stratified by hospital category (university, regional or county). In this analysis, only hospitals with more than 500 births per year were included (comprising 42 general hospitals with 97·8% of all recorded births). We calculated the number of transfusions per 1000 deliveries, using direct standardization to the year 2003 population, with regards to age and

parity. Estimates are presented with 95% confidence intervals. Of the excluded 2·2% of recorded births, most took place in 8 county hospitals with fewer than 500 births per year.

The creation of SCANDAT3-S database and the conduct of this study was approved by the regional ethics review board in Stockholm, Sweden (2018/167-31). All statistical analysis and data processing were conducted with SAS statistical software, version 9·4 (SAS Institute, Cary, NC).

Results

We identified 1 599 814 recorded deliveries in the period between January 1, 2003 and December 31, 2017. We excluded births to mothers aged below 15 (n = 77) or above 50 (n = 78). The remaining 1 599 659 deliveries to 959 868 women were included in further analyses. Among these, maternal transfusion occurred in relation to 48,088 (3·0%) deliveries. Of all the included women, 45 976 (4·8%) were transfused in conjunction with at

Table 1 Characteristics of study population presented overall and stratified by transfused/non-transfused

Number of subjects, N (%)	Overall 1 599 659 (100·0)	Non-transfused 1 551 571 (97·0)	Transfused 48 088 (3·0)
Age, N (%)			
≤24	221 641 (13·9)	215 097 (13·9)	6544 (13·6)
25–34	1 032 901 (64·6)	1 002 719 (64·6)	30 182 (62·8)
35–44	342 090 (21·4)	330 897 (21·3)	11 193 (23·3)
≥45	3027 (0·2)	2858 (0·2)	169 (0·4)
Median age (IQR)	30 (27–34)	30 (27–34)	31 (27–34)
BMI, N (%)			
<20	211 434 (13·2)	205 266 (13·2)	6168 (12·8)
20–24	637 191 (39·8)	619 172 (39·9)	18 019 (37·5)
25–29	418 088 (26·1)	405 177 (26·1)	12 911 (26·8)
30–34	139 428 (8·7)	134 895 (8·7)	4533 (9·4)
≥35	62 009 (3·9)	59 797 (3·9)	2212 (4·6)
Unknown	131 509 (8·2)	127 264 (8·2)	4245 (8·8)
Median BMI (IQR)	24 (22–27)	24 (21–27)	24 (22–27)
Gestational age, in weeks N (%)			
<37	166 628 (10·4)	158 056 (10·2)	8572 (17·8)
37–42	1 322 787 (82·7)	1 288 613 (83·1)	34 174 (71·1)
>42	109 743 (6·9)	104 436 (6·7)	5307 (11·0)
Unknown	501 (0·0)	466 (0·0)	35 (0·1)
Parity, N (%)			
0	705 889 (44·1)	677 731 (43·7)	28 158 (58·6)
1–2	590 940 (36·9)	577 836 (37·2)	13 104 (27·3)
≥3	302 830 (18·9)	296 004 (19·1)	6826 (14·2)
Pregnancy, N (%)			
Single	1 576 569 (98·6)	1 531 066 (98·7)	45 503 (94·6)
Multiple	23 090 (1·4)	20 505 (1·3)	2585 (5·4)
Presentation, (N%)			
Cephalic	1 406 074 (87·9)	1 367 596 (88·1)	38 478 (80·0)
Breech	52 801 (3·3)	50 937 (3·3)	1864 (3·9)
Flexed	67 661 (4·2)	64 738 (4·2)	2923 (6·1)
Other	33 619 (2·1)	31 249 (2·0)	2370 (4·9)
Unknown	39 504 (2·5)	37 051 (2·4)	2453 (5·1)
Delivery mode, N (%)			
Vaginal	1 237 717 (77·4)	1 211 099 (78·1)	26 618 (55·4)
Caesarean	262 746 (16·4)	248 346 (16·0)	14 400 (29·9)
Instrumental	99 196 (6·2)	92 126 (5·9)	7070 (14·7)
Hospital, N (%)			
County hospital	213 988 (13·4)	207 883 (13·4)	6105 (12·7)
Regional hospital	830 567 (51·9)	806 260 (52·0)	24 307 (50·5)
University hospital	555 104 (34·7)	537 428 (34·6)	17 676 (36·8)

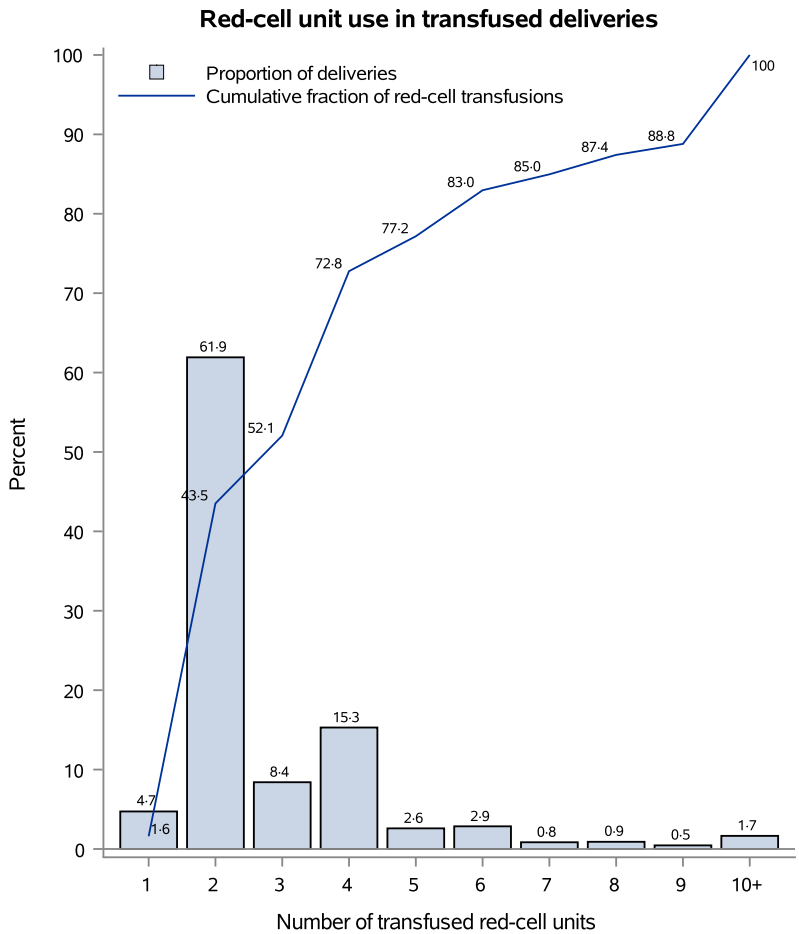

Fig. 1 The distribution of blood use. Bars illustrate the number of red-cell transfusion per delivery (e.g. 2 units were given in 62% of transfused deliveries). The solid line illustrates the cumulative proportion of all transfused red-cell units (e.g. 43·7% of units given to women who received 1–2 units). [Colour figure can be viewed at wileyonlinelibrary.com]

least one of their deliveries. Among transfused women, 2046 (4·5%) were transfused again in a subsequent delivery. The proportion of caesarean deliveries remained at approximately 17% (16·4–17·7%), whereas the proportion of instrumental deliveries decreased from 9·3% to 6·3% during the study period [12].

Demographic and gestational information on deliveries in which maternal red-cell transfusion did and did not occur is presented in Table 1. Compared to women with a delivery between week 37 until the end of week 42, maternal red-cell transfusions were more common in both preterm and post-term deliveries. Compared to the non-transfused parturient, transfused women more often had an instrumental vaginal or caesarean delivery. Among transfused, multi-foetal pregnancy was more common than among non-transfused patients. Maternal transfusions were also more common in nulliparous women compared to women with previous deliveries.

Details of the red-cell unit distribution in transfused deliveries is presented in Fig. 1. In total, 139 424 red-cell units were administered during the study period. In deliveries with maternal red-cell transfusions, a majority received 2 units (62%), and approximately 25% received more than 3 units. More than half (52%) red-cell units were used in low-volume transfusions with three units or less.

The distribution of red-cell units in relation to day of delivery is presented in Fig. 2. Approximately 42% of all units were administered during the day of delivery and 15% were administered on and onwards from the third day after delivery. The proportion of units administered prior to delivery was very small and previous to 3 days before delivery, the percentage was <0·1% per day.

Further demographic and gestational information stratified by the number of red-cell transfusions is presented in Table 2. In deliveries with a count of 10 or more units transfused, we noted that women of advanced age were

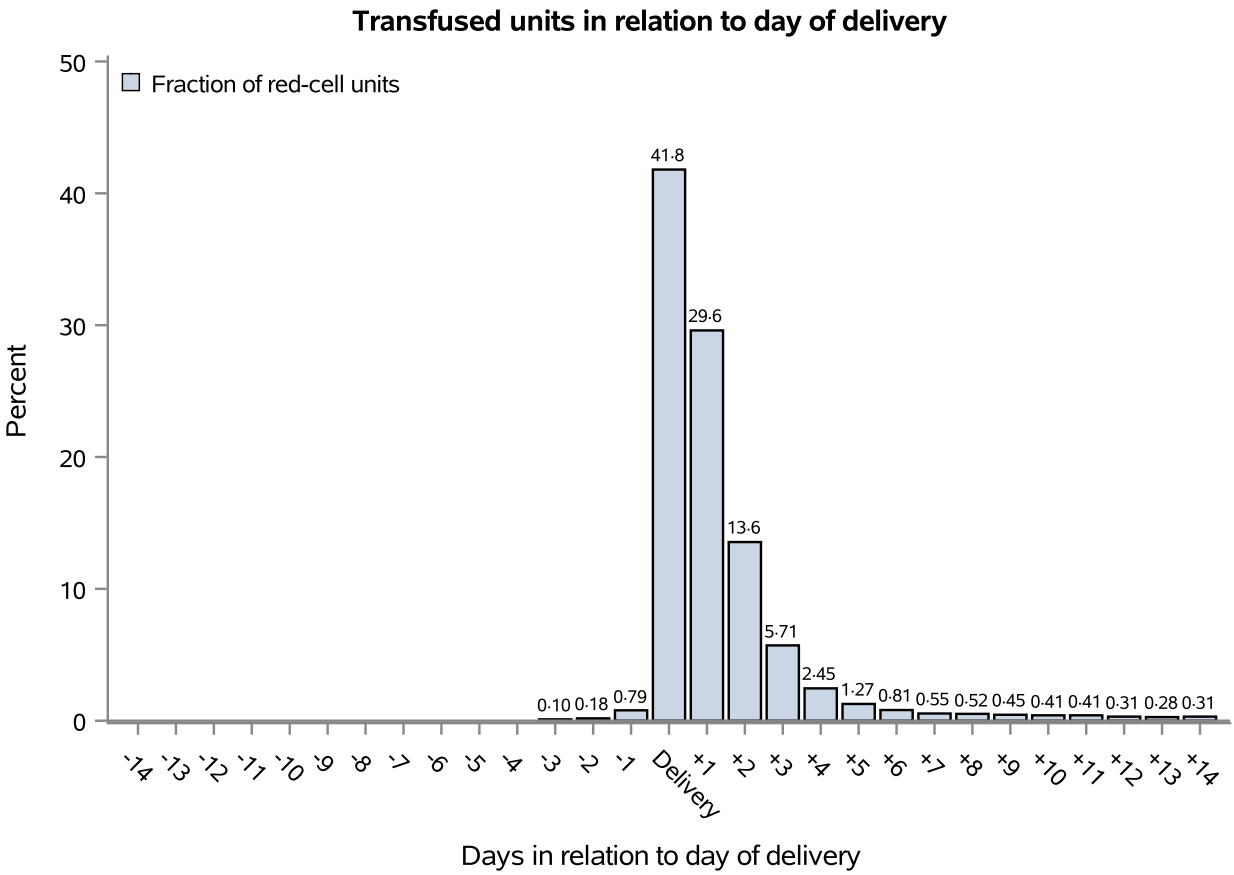

Fig. 2 The distribution of red-cell unit usage in per cent in relation to day of delivery. Previous to three days before delivery, the percentage is less than 0·1 per day. [Colour figure can be viewed at wileyonlinelibrary.com]

overrepresented, as were women with preterm deliveries, non-cephalic presentation at delivery, and caesarean delivery. Fewer deliveries were with nulliparous mothers among those that received 10 or more units, and BMI did not differ much between groups.

In Fig. 3, we present the number of transfused deliveries per 1000 over time. Overall, the number of transfused deliveries was approximately 30 per 1000 throughout the study period (A). The frequency of deliveries with maternal transfusion of 1 or 2 red-cell units increased from 17 to 22 per 1000 deliveries, whereas the proportion of deliveries with a count of 10 red-cell transfusions or more almost halved, from 0·67 to 0·32 per 1000 deliveries. Deliveries with 3–9 units also decreased from 10 to 7·6 per 1000 deliveries (B). Nulliparous women were transfused most frequently, and the trend was stable over time (C). While the proportion of transfused deliveries was stable among spontaneous vaginal and caesarean deliveries, it increased over time for instrumental deliveries from 67 to 81 per 1000 deliveries (D).

In Fig. 4, we present the overall number of transfused deliveries per 1000 in relation to maternal age. Overall, the count of deliveries with maternal transfusion was 30 per 1000 until age 35, after which we see a steady increase (A). Interestingly, the increase after age 40 was primarily seen in women receiving 1–2 units (B). Nulliparous women were more often transfused compared to parous women at all ages, with an increase in the transfused proportion with advancing maternal age (C). Spontaneous vaginal delivery was associated with the lowest risk of transfusion (D).

Figure 5 presents the overall number of transfused deliveries per 1000 across hospitals of different levels. We saw considerable variation, with a range from 20 to 54 transfused deliveries per 1000. The discrepancy was seen across all hospital categories and was not explained by adjusting for age and parity.

In supplemental Figure 1 (A), we present the total number of administered red-cell units per 1000 deliveries over time. Overall, red-cell unit usage decreased over the study period, from 90 to 80 units per 1000 deliveries. In (B), we present red-cell unit usage as a function of maternal age. At the maternal age of approximately 35, red-cell usage starts to increase. Across all strata,

Table 2 Characteristics of study population, stratified by number of transfusions

	Number of transfused units per delivery		
	1 or 2	**3–9**	**10 or more**
Number of subjects, N (%)	32 049 (66·6)	15 079 (31·4)	960 (2·0)
Age at delivery, N (%)			
≤24	4673 (14·6)	1800 (11·9)	71 (7·4)
25–34	20 260 (63·2)	9399 (62·3)	523 (54·5)
35–44	7013 (21·9)	3823 (25·4)	357 (37·2)
≥45	103 (0·3)	57 (0·4)	9 (0·9)
Median age (IQR)	30 (27–34)	31 (27–35)	33 (29–37)
BMI, N (%)			
<20	4259 (13·3)	1803 (12·0)	106 (11·0)
20–24	12 087 (37·7)	5615 (37·2)	317 (33·0)
25–29	8592 (26·8)	4050 (26·9)	269 (28·0)
30–34	2944 (9·2)	1481 (9·8)	108 (11·3)
≥35	1418 (4·4)	752 (5·0)	42 (4·4)
Unknown	2749 (8·6)	1378 (9·1)	118 (12·3)
Median BMI (IQR)	24 (22–27)	24 (22–27)	24 (22–28)
Gestational age, in weeks N (%)			
<37	5350 (16·7)	2888 (19·2)	334 (34·8)
37–42	23 226 (72·5)	10 399 (69·0)	549 (57·2)
>42	3453 (10·8)	1777 (11·8)	77 (8·0)
Unknown	20 (0·1)	15 (0·1)	0 (0)
Parity, N (%)			
0	18 872 (58·9)	8883 (58·9)	403 (42·0)
1–2	8774 (27·4)	4038 (26·8)	292 (30·4)
≥3	4403 (13·7)	2158 (14·3)	265 (27·6)
Pregnancy, N (%)			
Single	30 520 (95·2)	14 095 (93·5)	888 (92·5)
Multiple	1529 (4·8)	984 (6·5)	72 (7·5)
Presentation, N (%)			
Cephalic	25 752 (80·4)	12 074 (80·1)	652 (67·9)
Breech	1205 (3·8)	589 (3·9)	70 (7·3)
Flexed	1979 (6·2)	889 (5·9)	55 (5·7)
Other	1547 (4·8)	748 (5·0)	75 (7·8)
Unknown	1566 (4·9)	779 (5·2)	108 (11·3)
Delivery mode, N (%)			
Vaginal	18 149 (56·6)	8162 (54·1)	307 (32·0)
Caesarean	9411 (29·4)	4443 (29·5)	546 (56·9)
Instrumental	4489 (14·0)	2474 (16·4)	107 (11·1)
Hospital, N (%)			
County hospital	4121 (12·9)	1894 (12·6)	90 (9·4)
Regional hospital	16 336 (51·0)	7521 (49·9)	450 (46·9)
University hospital	11 592 (36·2)	5664 (37·6)	420 (43·8)

estimates were generally imprecise above age 43 due to scarce data.

Discussion

In this 15-year nationwide cohort study of obstetric transfusion practice in Sweden, we saw no overall change in the proportion of deliveries with maternal red-cell transfusions, but we saw a decrease in the number of administered units per delivery. This is consistent with the finding that the number of women receiving 3–9 red-cell units decreased over time with a simultaneous rise in the proportion receiving 1–2 units. Transfused women were more likely to be nulliparous, have a multi-foetal pregnancy, a preterm delivery and instrumental or caesarean delivery. These characteristics seem to be consistent with previous findings [5,6,13,14].

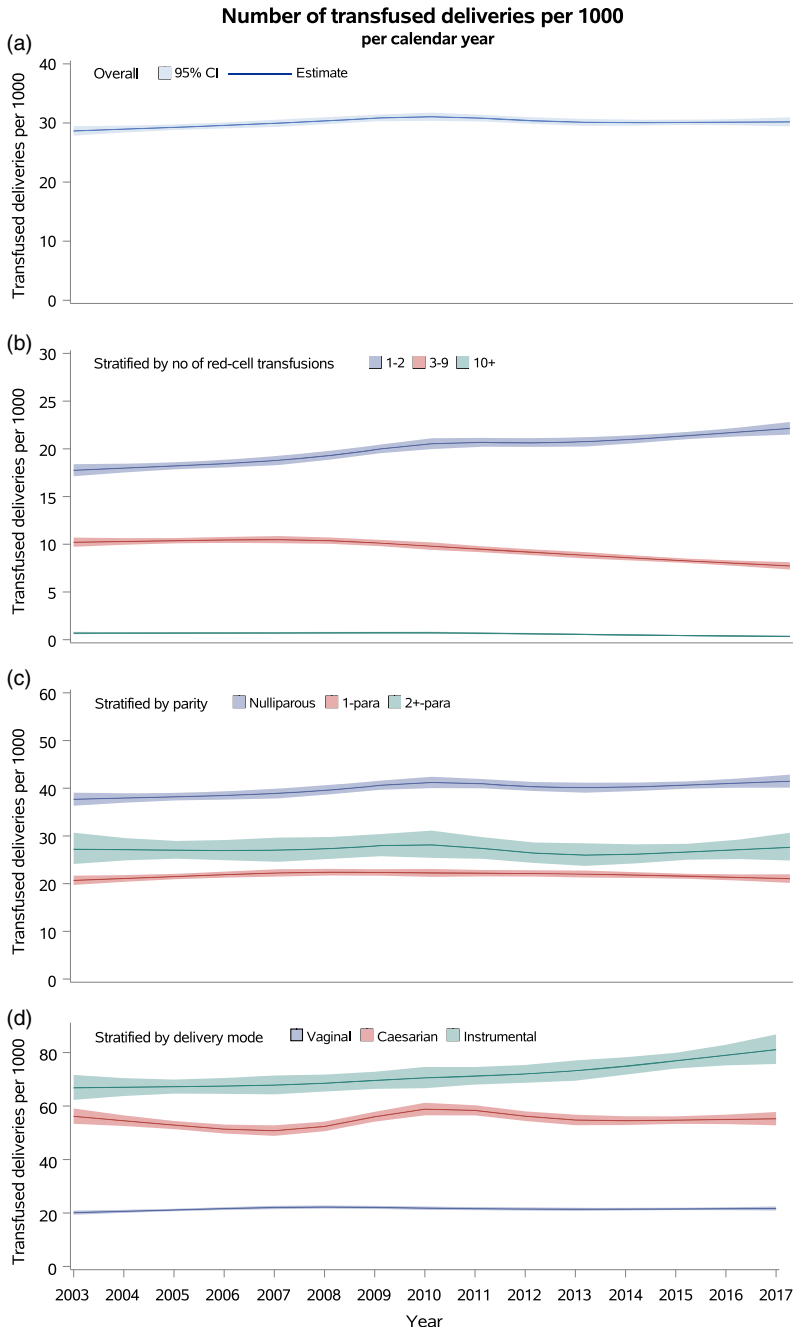

Fig. 3 The proportion of deliveries in which red-cell transfusion occurred as a function of time for (A) the entire cohort and subdivided (B) according to the total number of administered of red-cell units, (C) per parity and (D) per mode of delivery.

Overall, maternal red-cell transfusion occurred in 3% of deliveries, which is on the higher end compared with studies in other countries. A 2013 Finnish study on singleton births, the corresponding number was 2·3% in 2008 [6] and a 2012 Danish study found a transfusion rate of 1·9% [15]. We can only speculate on why there is a discrepancy, but there are methodological differences (e.g. we include virtually all deliveries of women between the ages of 17–50 and we include all transfusions within the 28 days encompassing delivery) that hinder direct comparison.

In case of uncontrolled massive haemorrhage, the red-cell unit obviously provides a life-saving bridge to recovery. However, there are reasons to be cautious when the patient is haemodynamically stable. In a study comparing

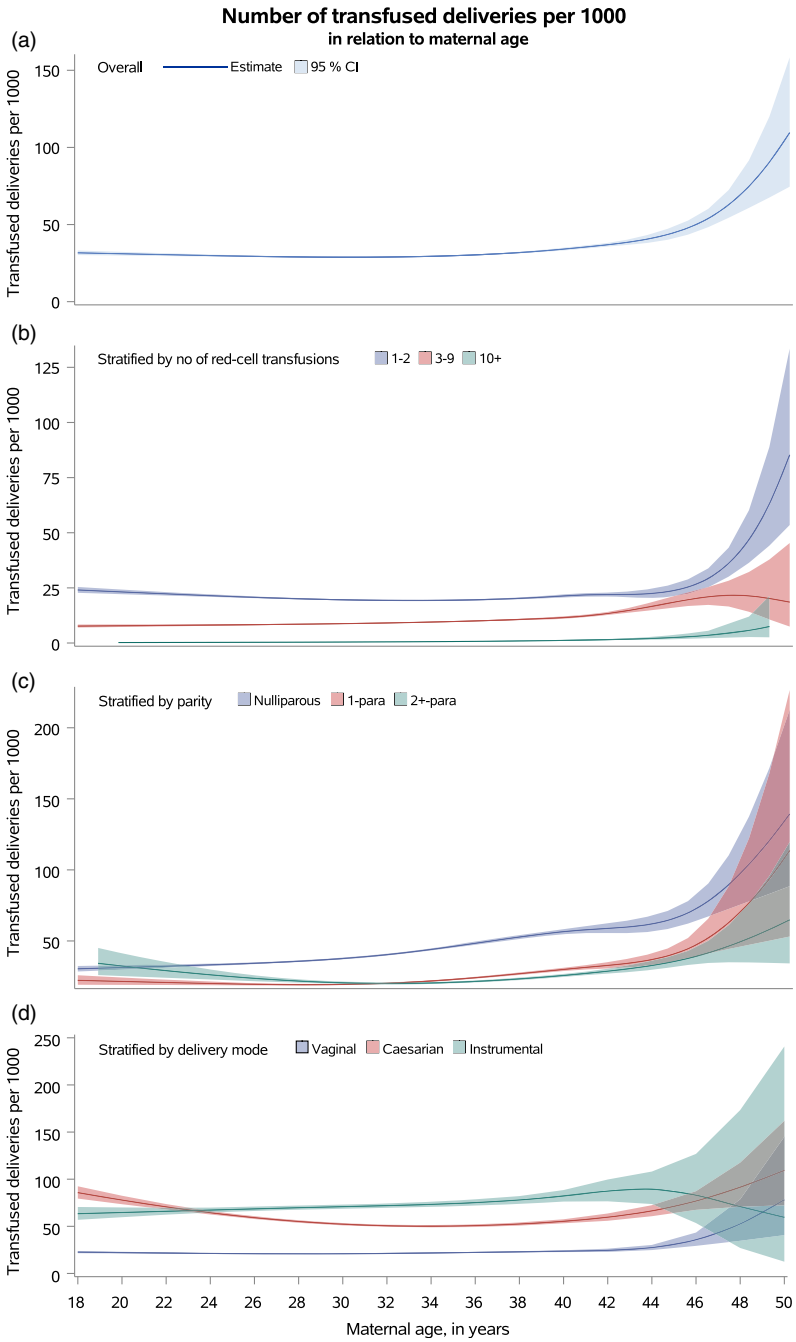

Fig. 4 The proportion of deliveries in which red-cell transfusion occurred as a function of maternal age for (A) the entire cohort and subdivided (B) according to the total number of administered of red-cell units, (C) per parity and (D) per mode of delivery.

health and health service utilization among low-risk women that suffered post-partum haemorrhage, it was noted that women that received a low-volume red-cell transfusion (1 or 2 units) had poorer maternal outcomes than otherwise comparable women receiving no transfusions [16]. In a non-inferiority trial looking at quality of life, little difference was found between maternal red-cell transfusions and alternatives like iron supplementation [17] and an observational study on births complicated by post-partum haemorrhage, maternal red-cell transfusion was associated with lower rates of breastfeeding at discharge [18].

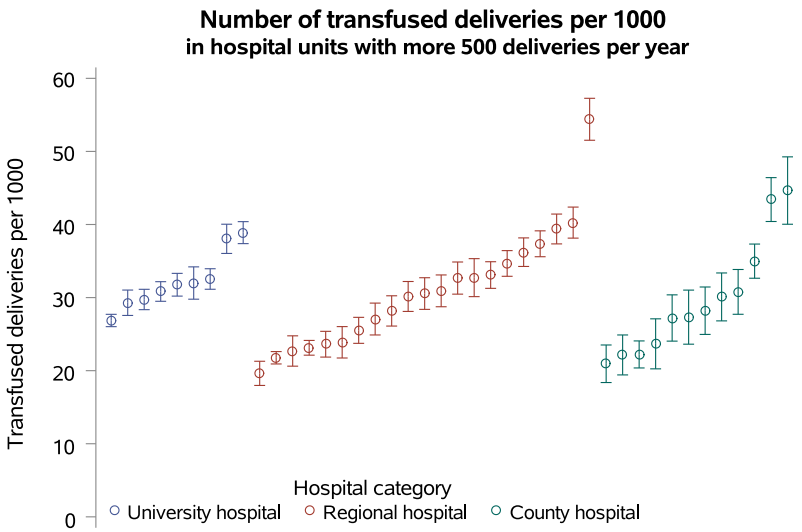

Fig. 5 The hospital-specific number of transfused deliveries per 1000, in all hospitals with more than 500 deliveries per year (comprising 97·8% of deliveries). Estimates were standardized to year 2003 accounting for age and parity, using direct standardization. Each circle represents an individual hospital and the error bars represent the 95% confidence limits.

From our data, we conclude that more than half of all units are administered after the day of delivery and that the 2-unit transfusion is most common. It is reasonable to think that a proportion of these units were administered to haemodynamically stable (albeit anaemic) patients. Current consensus guidelines, which were not in place during the study period, recommend transfusing 'one unit at the time' (a concept that was proposed decades ago [19]) and emphasize the use of iron supplementation in moderate to severe post-partum anaemia [20]. Over time, we did see a slight reduction in red-cell units use per delivery, and time will tell if there is continued reduction in overall red-cell exposure.

Previous studies have shown that advanced maternal age is associated with adverse pregnancy outcomes (e.g. stillbirth, preterm birth, pre-eclampsia) [21,22]. We found advancing maternal age to be accompanied with a larger proportion of transfused deliveries, a finding also seen in other studies [6,23]. In our study, women over 40 years were more often transfused, regardless of parity. We make no claim on a causal relationship between maternal age and red-cell transfusion on the basis of this study, but it is a topic for further study.

We saw a considerable variation in transfusion rates between hospitals, after adjusting for parity and maternal age. In an Australian study on 250 000 deliveries, difference in transfusion rates remained after careful adjustment for obstetrical case-mix, but there was no difference in patient outcomes in hospitals with lower transfusion rates compared to those with more liberal transfusion practice [24]. We cannot readily explain the differences in our study, but it is a finding that should spur further investigation.

There are important limitations to this study. In this broad overview of obstetric transfusion practice, we did not investigate the appropriateness of the individual red-cell transfusion (such as maternal Hb concentration, pre-partum anaemia etc.). Also, we did not consider other blood products than the red-cell unit.

The strength of the study is that it is, to our knowledge, the largest of its kind and has the advantage of being based on virtually complete, nationwide high-quality data over a long time period. The predictors (age, parity, delivery mode) are prospectively recorded and outcomes (red-cell transfusions) are robust and not prone to reporting or measurement error.

In our study of a Swedish obstetric cohort, we see a high proportion of deliveries with maternal red-cell transfusion in comparison to other high-resource countries. However, we do not see an increase over time, and the number of transfused units used per delivery has decreased over the study period.

Conflict of interests

The authors declare no conflict of interests.

Funding

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Fig. S1 The number of red-cell units administered per 1000 deliveries as a function of (A) time and (B) of maternal age.

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The risk to future pregnancies of transfusing Rh(D)-negative females of childbearing potential with Rh(D)-positive red blood cells during trauma resuscitation is dependent on their age at transfusion

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Background A risk assessment model for predicting the risk of haemolytic disease of the fetus and newborn (HDFN) in future pregnancies following the transfusion of Rh(D)-positive red blood cell (RBC)-containing products to females of childbearing potential (FCP) was developed, accounting for the age that the FCP is transfused in various countries.

Methods The HDFN risk prediction model included the following inputs: risk of FCP death in trauma, Rh(D) alloimmunization rate following Rh(D)-positive RBC transfusion, expected number of live births following resuscitation, probability of carrying an Rh(D)-positive fetus, the probability of HDFN in an Rh(D)-positive fetus carried by an alloimmunized mother. The model was implemented in Microsoft R Open, and one million FCPs of each age between 18 and 49 years old were simulated. Published data from eight countries, including the United States, were utilized to generate country-specific HDFN risk estimates.

Results The risk predictions showed similar characteristics for each country in that the overall risk of having a pregnancy affected by HDFN was higher if the FCP was younger when she received her Rh(D)-positive transfusion than if she was older. In the United States, the overall risk of HDFN if the FCP was transfused at age 18 was 3·4% (mild: 1·20%, moderate: 0·45%; severe: 1·15%; IUFD: 0·57%); the risk was approximately 0% if the FCP was 43 years or older at the time of transfusion.

Conclusion This model can be used to predict HDFN outcomes when establishing transfusion policies as it relates to the administration of Rh(D)-positive products for massively bleeding FCPs.

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Key words: haemolytic disease of the fetus and newborn, massive transfusion, pregnancy, red blood cell, trauma, whole blood, age, outcome, transfusion.

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Introduction

Evidence for the potential life-saving benefits of early intervention with blood products for massively bleeding trauma patients continues to accumulate [1–3]. In a

retrospective study of injured soldiers, an absolute mortality reduction of 14% was observed among the soldiers who were transfused with red blood cells (RBC), plasma or a combination of these two products within 20 min of their evacuation to hospital compared to those who were either not transfused or who received a transfusion more than 20 min after medical evacuation [3]. In a study of traumatically injured civilian patients who were evacuated to the hospital by helicopter, administering two units of plasma in addition to the standard of care produced a statistically significant reduction in 30-day mortality, [2] with patients who received RBCs in addition to plasma realizing the maximum survival benefit [4]. Thus, there is evidence that the earlier blood products can be administered to trauma patients, the lower their mortality will be; however, the use of blood products early in resuscitation would likely mean that the patient's ABO and Rh(D) type may be unknown at the time of transfusion. Practically speaking, this is not a problem as it relates to the ABO group since group O RBCs or low titre group O whole blood (LTOWB) would be transfused because these group O products will not lead to immediate, intra-vascular haemolysis caused by the recipient's pre-formed anti-A and/or anti-B antibodies. However, selecting the Rh(D) type of these products is not always straightforward. While Rh(D)-negative products are preferred, group O Rh(D)-negative blood products are a rare resource; in a recent study, only 10% of the RBCs distributed by blood centres around the world were group O Rh(D) negative, [5] and maintaining an adequate inventory of these products outside of the hospital at helicopter and/or ambulance bases can be infeasible due to the scarcity of these products. Group O Rh(D)-positive RBCs and LTOWB, on the other hand, are much more common; however, administering Rh(D)-positive RBC-containing products to females of childbearing potential (FCP; often defined as women between the ages of 15–49 years old) who are either Rh (D)-negative or whose Rh(D) type is not known could put their future pregnancies at risk for haemolytic disease of the fetus and newborn (HDFN). This risk makes the decision to utilize Rh(D)-positive blood products less straightforward, as it may offset some of the mortality benefit that could be gained by early transfusion protocols in the setting of unknown Rh(D) status when Rh (D)-positive blood products are the only available option.

In a recently published analysis, the risks to future pregnancies of transfusing Rh(D)-negative FCPs with Rh (D)-positive RBC-containing products during trauma resuscitation was estimated to be 0·3% and 2·0% for intrauterine fetal demise (IUFD) and moderate-to-severe HDFN, respectively [6]. While the authors considered many relevant factors, including the probability that the woman survives her trauma, becomes alloimmunized to Rh(D), has a pregnancy following the trauma and carries an Rh(D)-positive fetus, the model did not incorporate the effect of the FCP's age at the time of her exposure to Rh (D)-positive RBC-containing products on the risk of HDFN in subsequent pregnancies. Age at the time of trauma is an important variable, because younger FCPs are more likely to have pregnancies following their transfusion than older FCPs [7]. To provide a more specific HDFN risk assessment of transfusing Rh(D)-negative or Rh(D)-unknown FCPs based on her age at the time of the emergency transfusion, a stochastic model was developed that relates Rh(D) alloimmunization and adverse pregnancy outcomes to trauma mortality rates, fertility rates, age-dependent number of anticipated future pregnancies, and the population-dependent *RHD* genotype frequency, and this model was applied to data from different countries around the world.

Materials and methods

Model description and assumptions

The rate of HDFN was modelled using published data from the following countries: Australia, Brazil, China, India, Japan, Nigeria, Trinidad and Tobago and United States [7–41]. Critical assumptions used in the model, that is, the computation of HDFN risk based on the independent variables employed, and supporting literature are provided in Table 1.

Estimation of mortality rate for FCPs with traumatic injuries

The mortality rate for severe trauma, where severe trauma is defined as receipt of a blood product transfusion and/or an injury severity score >15, was modelled using a binomial random variate, and varied from 14·8% in Australia to 33·5% in Trinidad and Tobago (Table 1).

Derivation of anti-Rh(D) alloimmunization rate

Three retrospective studies from different countries have analysed the Rh(D) alloimmunization rate of hospitalized Rh(D)-negative patients who received at least one unit of Rh(D)-positive RBCs [23,24,40]. The rate of Rh(D) alloimmunization was estimated to be 22% in the United States, 30% in Germany, and 21% in Spain, with an overall alloimmunization rate of 72/351 or 21%. This 21% alloimmunization rate was modelled using a binomial random variate and was utilized for all of the countries simulated.

Table 1 Summary of assumptions and input parameters for trauma mortality rate, alloimmunization rate, fertility rate and childbearing habits, paternal factors including RHD genotype, and risk of HDFN for the eight countries involved in the simulation

Parameter		Australia	Brazil	China	India	Japan	Nigeria	Trinidad & Tobago	USA
Mortality, alloimmunization and fertility rates	In-hospital mortality from severe trauma (%)[a]	14·8 [17]	24·3 [33]	20·5 [41]	33·3 [21]	23.8 [30]	31.5 [38]	33.5 [14]	18.5 [35]
	Anti-D immunization rate (%) [23,24,40]	21							
	Average live births per FCP	1·70 [8]	1·72 [11]	1·70 [8]	2·20 [8]	1.42 [12]	5.40 [8]	1.70 [8]	1.77 [7]
Average maternal age (years)	1st birth	28·7 [9]	21·1 [19]	26·9 [25]	23·7 [36]	30.7 [12]	20.3 [9]	22.2 [20]	27.0 [7]
	2nd birth	31·2 [31]	23·3 (27-month IPI) [18]	30·2 [25]	25·8 [36]	32.6 [12]	21.6 (15-month IPI) [15]	24.2 (24-month IPI) [32]	29.4 [7]
	3rd birth	33·2 (24-month IPI) [32]	25·5 (27-month IPI) [18]	31·4 [25]	27·4 [36]	33.6 [12]	22.9 (15-month IPI) [15]	26.2 (24-month IPI) [32]	30.6 [7]
	4th birth	35·2 (24-month IPI) [32]	27·7 (27-month IPI) [18]	33·4 (24-month IPI) [32]	29·0 [36]	35.6 (24-month IPI) [32]	24.2 (15-month IPI) [15]	28.2 (24-month IPI) [32]	31.7 [7]
	5th birth	37·2 (24-month IPI) [32]	29·9 (27-month IPI) [18]	35·4 (24-month IPI) [32]	31·0 [36]	37.6 (24-month IPI) [32]	25.5 (15-month IPI) [15]	30.2 (24-month IPI) [32]	32.7 [7]
	6th birth	39·2 (24-month IPI) [32]	32·1 (27-month IPI) [18]	37·4 (24-month IPI) [32]	32·8 (22-month IPI) [39]	39.1 (24-month IPI) [32]	26.8 (15-month IPI) [15]	32.2 (24-month IPI) [32]	34.0 [7]
	7th birth	41·2 (24-month IPI) [32]	34·3 (27-month IPI) [18]	39·4 (24-month IPI) [32]	34·7 (22-month IPI) [39]	41.6 (24-month IPI) [32]	28.1 (15-month IPI) [15]	34.2 (24-month IPI) [32]	34.0 [7]
	8th birth	43·2 (24-month IPI) [32]	36·5 (27-month IPI) [18]	41·4 (2year interval) [32]	36·5 (22-month IPI) [39]	43.6 (24-month IPI) [32]	29.4 (15-month IPI) [15]	36.2 (24-month IPI) [32]	36.8 [7]
Standard deviation (SD) of maternal age (years)	1st birth [7]	6·2							
	2nd birth [7]	5·8							
	3rd birth [7]	5·6							
	4th birth [7]	5·4							
	5th birth [7]	5·5							
	6th birth [7]	5·2							
	7th birth [7]	5·2							
	8th birth [7]	4·9							
Paternal factors	*RHD*/*RHD* genotype %	33·1 [10]	38·1 [16]	93·6 [27]	53·6 [13]	89.4 [28]	69.0 [26]	58.6 [37]	35.0 [34]
	RHD/– genotype %	49·3 [10]	47·3 [16]	6·0 [27]	40·8 [13]	10.1 [28]	28.0 [26]	35.9 [37]	50.0 [34]
	–/– genotype %	17·5 [10]	14·6 [16]	0·4 [27]	5·6 [13]	0.5 [28]	3.0 [26]	5.5 [37]	15.0 [34]
	Probability of multiple fathers (%)	28 [22]							
Probability of HDFN (%)		31 [29]							

FCP, female of childbearing potential; HDFN, haemolytic disease of the fetus and newborn; IPI: interpregnancy interval.

[a]Represents mortality rate for patients with severe trauma who were received a blood product transfusion and/ or had an injury severity score greater than 15.

Derivation of total number of births per FCP

The total number of births each simulated FCP would have in her lifetime was modelled using a Poisson random variate and varied from an average of 1·7 live births per FCP for Trinidad and Tobago, China and Australia to 5·4 live births per FCP for Nigeria (Table 1). As only pregnancies that occur after the Rh(D)-positive RBCs and/or LTOWB units were administered to an Rh(D)-negative FCP during the trauma resuscitation would be at risk of HDFN, total births were probabilistically assigned as past or future events in an age-dependent manner using distributions of maternal age for the first through eighth births (means and SDs for each country shown in Table 1). The average maternal age at the first live birth was available for all eight countries and ranged from 20·3 years in Nigeria to 30·7 years in Japan; however, data specifying the average maternal age at subsequent births were only available for the United States. For the non-US countries, the World Health Organization's (WHO) recommended interpregnancy interval of 24 months was added to the average age for the highest birth order for which data were available in order to estimate the average ages for subsequent births up to eight [32]. The standard deviation (SD) of the maternal age for the first through eighth births was only available for the United States, and these SD values were utilized for the non-US countries (Table 1).

Derivation of the Rh(D) status of the fetus carried after Rh(D)-positive RBC-containing blood product transfusion during trauma resuscitation

If a simulated Rh(D)-negative FCP survived her injuries, became Rh(D)-alloimmunized and had one or more future pregnancies, the Rh(D) status of the father(s) of the future pregnancy(ies) was simulated based on population characteristics. For the United States, based on estimates in predominantly Caucasian populations, the model assumed that 35% of fathers would have an *RHD/RHD* genotype, and 50% would have an *RHD/–* genotype, resulting in 100% and 50% of fetuses, respectively, expressing the Rh (D) antigen; the remaining 15% of fathers would have been Rh(D)-negative, and thus, their fetuses would not have been at risk of HDFN [34]. The *RHD* genotype frequencies for the non-US countries that were used in the country-specific simulations are shown in the Table 1. Among the countries included in this study, the *RHD/RHD* genotype is most prevalent in China (93·6%) and Japan (89·4%), and least prevalent in Australia (33·1%), the United States (35·0%) and Brazil (38·1%). The model also accounted for multiple-partner fertility when the simulated FCP had multiple future births; among mothers with two or more children, it was estimated that 28% would have children with at least two different fathers based on data from the United States [22]. The 28% multiple-partner rate was also applied to the non-US countries as the data were unavailable outside of the United States.

Derivation of the risk stratification for HDFN severity

For every future Rh(D)-positive fetus carried by an Rh(D) alloimmunized FCP, the chance of developing any degree of HDFN was modelled using a binomial random variate and a total HDFN risk of 31% [29]. This means that 31% of all future pregnancies following the trauma resuscitation that featured an Rh(D)-positive fetus carried by an Rh(D) alloimmunized mother would be affected by some degree of HDFN. It was assumed that the 31% risk of experiencing HDFN would be the same in all countries. The distribution of HDFN severity among the 31% of affected future pregnancies in the United States has been reported as follows: mild (simple neonatal transfusion) 36%, moderate (neonatal exchange transfusion) 13%, severe (intrauterine transfusion or hydrops with live birth) 34%, or intrauterine fetal death (IUFD) 17% [29]. Data on the stratification of HDFN severity in countries outside the United States were not available, thus only the overall rate of HDFN stratified by FCP age in the non-US countries is presented.

Simulation implementation and web-based simulator

Simulations were implemented in Microsoft R Open version 3.5.3 (Microsoft Corporation, Redmond, WA, USA). Since the Rh(D) status of the FCP is frequently unknown during the initial trauma resuscitation, in the first set of simulations, one million FCPs of unknown Rh(D)-status and of each age from 18 to 49 years were simulated to receive at least one Rh(D)-positive RBC-containing product such as a conventional RBC unit or a unit of LTOWB. The Rh(D)-unknown HDFN rate was calculated as the number of cases of HDFN expected per 100 transfused FCPs of unknown Rh(D) status.

The second set of simulations addresses the risk of HDFN posed by transfusion of an Rh(D)-positive blood product to an FCP who is later confirmed to be Rh(D)-negative. One million Rh(D)-negative FCPs of each age from 18 to 49 years were simulated to receive at least one Rh(D)-positive RBC-containing product. The Rh(D)-negative HDFN rate was calculated as the number of cases of HDFN expected per 100 transfused Rh(D)-negative FCPs. Since data on the stratification of HDFN

severity was only available for the United States, a separate analysis was conducted for the United States simulation to determine the risk of mild, moderate or severe HDFN and IUFD.

A third simulation was used to estimate the total number of HDFN cases expected per year using real-world data from a large level 1 trauma centre in the United States with approximately 5000 annual trauma admissions. For this simulation, input data on the average age and number of Rh(D)-negative FCPs transfused per year were extracted from the University of Pittsburgh Trauma Registry for the period 2015–2019. The remaining assumptions for this simulation were based on the published data for the United States shown in the Table 1.

Since the same input data for Rh(D)-alloimmunization rate, multiple-partner fertility rate and HDFN risk were applied across all eight countries, a separate analysis was conducted to determine the sensitivity of the model output (HDFN rate) to variations in these three inputs. The upper and lower 95% exact binomial confidence limits were determined for the Rh(D)-alloimmunization rate (16% and 25%, respectively) and for the HDFN risk (26% and 35%, respectively) [23,24,29,40]. The upper and lower bounds selected for the multiple-partner fertility rate were 16% and 44% based on estimates for married couples and cohabiting unmarried couples, respectively [42,43]. Apart from these three inputs, the sensitivity analyses utilized input data representative of the United States (Table 1).

An R Shiny web-based simulator was created to allow transfusion services to input data for the model parameters specific to their to their institution and country in order to estimate the HDFN risk associated with transfusing Rh(D)-positive blood products to Rh(D)-negative FCPs (https://jnsanalytics.shinyapps.io/fcp_sim_app/).

Results and discussion

As shown in Figs. 1 and 2, the expected overall rate of HDFN (i.e., having a future pregnancy affected by HDFN of any severity) varies strongly with the FCP's age at the time of her Rh(D)-positive transfusion during her trauma resuscitation. In the United States, the Rh(D)-negative HDFN rate was approximately 3·4 per 100 Rh(D)-negative FCPs transfused at age 18 years (mild: 1·20 per 100 FCPs, moderate: 0·45 per 100 FCPs; severe: 1·15 per 100 FCPs; IUFD: 0·57 per 100 FCPs) and decreased to essentially 0 per 100 Rh(D)-negative FCPs by 43 years of age (Fig. 3 and Table S1).

The simulations shown in Fig. 1 address the population-level HDFN risk to FCPs of unknown Rh(D) status transfused with an Rh(D)-positive blood product. Included in these simulations are both Rh(D)-positive FCPs who would not have fetuses affected by anti-D HDFN and Rh (D)-negative FCPs who could have fetuses affected by HDFN. As expected, the Rh(D)-unknown HDFN rate was lowest for Japan and China, due to the <1% probability of the FCP being Rh(D)-negative (i.e. –/–) based on population *RHD* genotype data. In contrast, Australia, United States and Brazil had the highest estimated Rh(D)-unknown HDFN rates as a larger proportion of the FCP population (15%) would be Rh(D)-negative, and therefore, at risk of having a future pregnancy affected by HDFN.

The second set of simulations shown in Fig. 2 addressed the individual-level HDFN risk to an FCP who was transfused with an Rh(D)-positive blood product and later confirmed to be Rh(D)-negative. Rh(D)-positive FCPs were not included in these simulations. Nigeria had the highest estimated HDFN rate for a number of reasons including the high fertility rate, low average maternal age at birth, and a high *RHD/RHD* genotype frequency of 69% that puts Rh(D)-negative FCPs at higher risk of conceiving an Rh(D)-positive fetus (Table 1). While China and Japan had the lowest estimated overall HDFN rates when FCPs of unknown Rh(D) status were simulated in Fig. 1, they had the second and third highest overall HDFN risk, respectively, for specifically Rh(D)-negative FCPs transfused with an Rh(D)-positive blood product. The high Rh(D)-negative HDFN rates for China and Japan are explained by the high *RHD/RHD* genotype frequency in excess of 90%, which means that a large majority of fetuses will be Rh(D)-positive, as well as the older average maternal age at birth, which suggests that a young FCP may be more likely to have a future pregnancy following her trauma resuscitation compared with Australia or United States, per se (Table 1).

There are a few interesting observations for the other countries whose HDFN risks were simulated. First, although Brazil has a similar *RHD* genotype distribution to Australia and United States, the estimated overall HDFN rate for Rh(D)-negative FCPs in Brazil is consistently lower than that of Australia and United States for all FCP ages simulated (Fig. 2), likely because the average age of the first live birth in Brazil is more than 5 years earlier than in Australia and the United States, and the mortality rate from severe trauma is higher in Brazil (Table 1). The estimated overall HDFN rate was higher for India compared with Trinidad & Tobago even though both countries have similar *RHD* genotype frequencies; this higher rate is potentially explained by the higher fertility rate in India and the average maternal age at birth in India being about 1·5 years older than that in Trinidad & Tobago meaning that a young FCP would be more likely to have future pregnancies potentially affected by HDFN in India than in Trinidad & Tobago (Table 1).

Following institutional review board (IRB) approval, data were extracted from the University of Pittsburgh

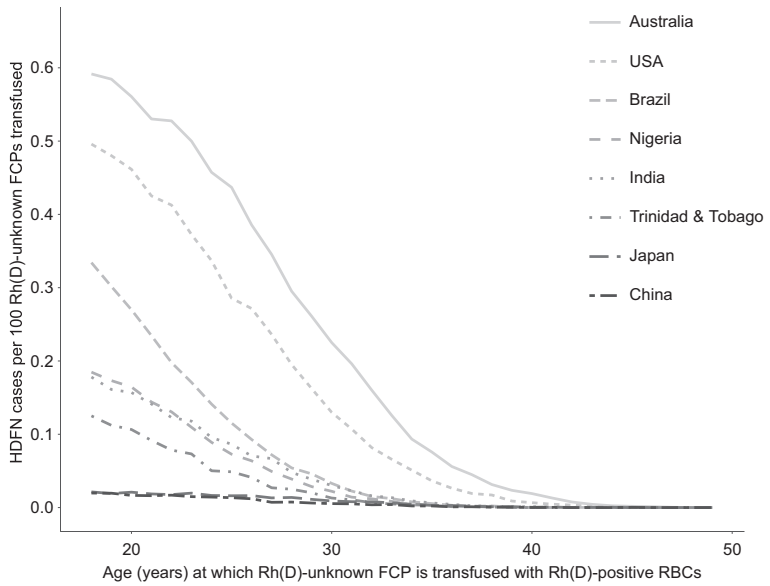

Fig. 1 Estimated overall rate of haemolytic disease of the fetus and newborn (HDFN) versus the age at which the female of childbearing potential (FCP) of unknown Rh(D)-status is transfused with an Rh(D)-positive RBC-containing product during trauma resuscitation for the eight countries simulated.

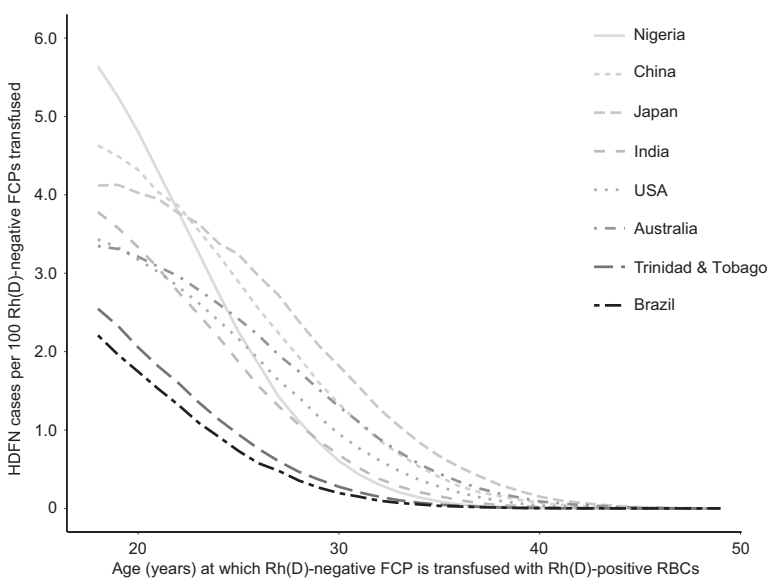

Fig. 2 Estimated overall rate of haemolytic disease of the fetus and newborn (HDFN) versus the age at which the Rh(D)-negative female of childbearing potential (FCP) is transfused with an Rh(D)-positive RBC-containing product during trauma resuscitation for the eight countries simulated.

Trauma Registry on the average age and number of Rh (D)-negative FCPs transfused per year. If the transfusion policy at this level 1 trauma centre allowed for the transfusion of Rh(D)-positive RBCs or LTOWB to Rh(D)-negative FCPs, approximately five such patients would be transfused with Rh(D)-positive RBCs annually, with an average (SD) FCP age of 33 ± 10 years. In one iteration of the simulation, over a 100-year period, this trauma centre would expect to transfuse 504 Rh(D)-negative FCPs with Rh(D)-positive RBCs of whom 416/504 (82·5%) would survive their injuries. It would be expected that 92/416 (22·1%) of these women would become Rh(D)-alloimmunized and go on to have a total of 67 pregnancies after their trauma resuscitation. Then, 34/67 (50·7%) of these fetuses/babies would be Rh(D)-positive; only 6/67 (9·0%) of these fetuses/babies would be expected to be affected by HDFN of any severity. Thus, the overall HDFN rate would be approximately 1·2 HDFN cases per 100 Rh

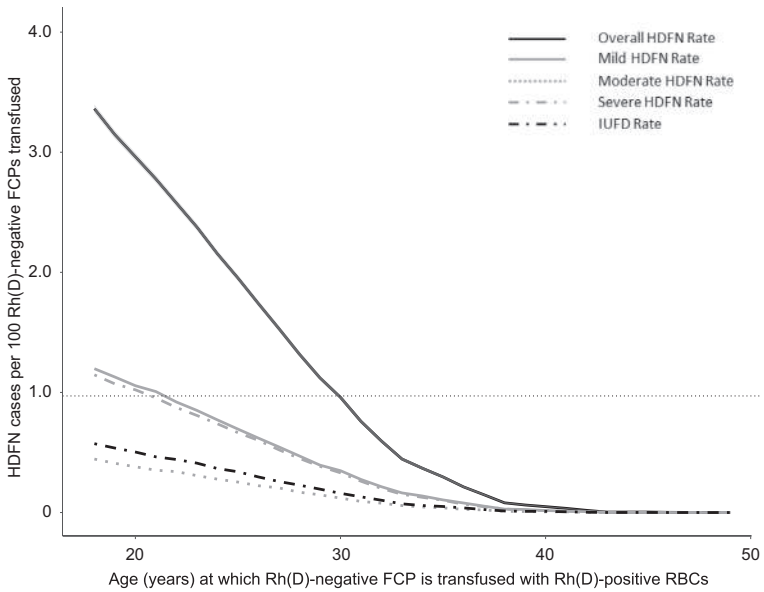

Fig. 3 Estimated rate of haemolytic disease of the fetus and newborn (HDFN), stratified by severity [mild, moderate, severe or intrauterine fetal demise (IUFD)], versus the age at which the Rh(D)-negative female of childbearing potential (FCP) is transfused with an Rh(D)-positive RBC-containing product during trauma resuscitation using assumptions for the United States. The dotted horizontal line represents the average overall HDFN rate of 0·97 HDFN cases per 100 Rh(D)-negative FCPs transfused across all ages from 18 to 49 years, assuming a uniform age distribution.

(D)-negative FCPs transfused over the simulated 100-year period. While experiencing HDFN during a pregnancy is surely physically and emotionally exhausting, the calculated low number of affected FCPs is small relative to the number of women whose lives would be saved by the pre-hospital or early in-hospital use of Rh(D)-positive RBC products. This low rate was used to support the ongoing practice of administering Rh(D)-positive LTOWB to FCPs who are bleeding from traumatic injuries at this hospital.

The lack of detailed data available from most countries regarding reproductive demographics and trauma outcomes leads to important limitations to the current study. The HDFN risk estimates described in this study are most applicable to the US medical system, as non-US country-specific data were not available for critical assumptions such as average maternal age for higher birth orders and multiple-partner fertility rates. Nonetheless, sensitivity analyses conducted for the United States data showed that the model was relatively insensitive to changes in multiple-partner fertility rates ranging from 16% to 44% (Fig. S1). Although the model was more sensitive to small changes in Rh(D) alloimmunization rate and HDFN risk (Figs S2 and S3), it is unlikely that estimates for these two model parameters depart substantially from 21% and 31%, respectively. In the absence of published literature for the average maternal age for higher birth orders, the interpregnancy interval specific to that country, when available, was used as a surrogate to calculate the expected average maternal age at higher birth orders. Otherwise, the WHO recommendation for a 24-month interpregnancy interval was used, which may not reflect the actual interpregnancy interval in these countries. The distribution of the maternal age was likewise unknown for non-US countries and, therefore, the generalization of the SD data derived from US data to non-US countries may have introduced inaccuracies in the model outputs. The trauma mortality rate estimates obtained in the literature also may not reflect equivalent trauma patient populations, since some studies reported mortality rates for injury severity score (ISS) >15 while others reported mortality rates for transfusion recipients and others reported mortality rates for 'severe trauma'. In addition, data underlying the critical assumptions may not be representative of the same historical period for all countries, for example, the only available data on the *RHD* genotypes for Trinidad & Tobago was published in 1969, and perhaps changes in the constitution of the population might have also changed the *RHD* gene frequencies. The probability distribution used for HDFN severity strata was based on a study that encompassed a 49-year period at a single centre in the United States and so they do not necessarily reflect the modern rates of severe HDFN and IUFD, which would be expected to be lower following the development of high-resolution ultrasound for diagnosis and management of HDFN [6,29,44]. Finally, while not all

hospitals may have access to a database of trauma patients as was available at the Level 1 trauma hospital described above, hospitals could use billing information or ICD codes to derive a list of trauma patients and then determine the demographics, such as age, sex, and transfusion rate, for those patients as needed for use in the model. Other data that are required for the model could be obtained from census data or, if local data is not available, approximated from international databases such as those that were used in building the models for the various countries studied herein.

The simulations described in this report demonstrate that the estimated overall risk of HDFN in future pregnancies for Rh(D)-negative FCPs transfused with Rh(D)-positive RBC-containing products varies from country to country but is expected to range from 2·2 to 5·7 cases per 100 transfused Rh(D)-negative FCPs if the transfusion occurs under the age of 20 years to negligibly low for patients over 40 years (Fig. 1). These data support changing the upper limit of childbearing age from the current 50 years of age in use at many institutions to 40 years of age. Transfusion services and hospitals that provide obstetric care can use these data, along with the web-based simulator (https://jnsanalytics.shinyapps.io/fcp_sim_app/) using inputs specific to their institution and country, to weigh the age-dependent risk of HDFN with the possible morbidity and mortality benefits of early transfusion in massively bleeding patients when designing their policies for providing blood products to Rh(D)-negative or Rh(D) status unknown FCPs with massive bleeding. The outputs from the model can also be used by the operators of emergency medical services (EMS), such as ambulances and rescue helicopters, when deciding if they wish to carry blood products that the evidence suggests is beneficial for trauma patients, [4] and if they are comfortable transfusing Rh(D)-positive RBC-containing blood products to FCPs whose Rh(D) type would likely be unknown at the time the transfusion is initiated. Hospitals that receive trauma patients and EMS should co-ordinate their transfusion policies as much as possible to assure the patient's smooth transition of care. As evidence continues to accumulate regarding the mortality benefits of early transfusion, including with potentially Rh(D)-mismatched blood products, these findings should help inform rational assessments of the risk-benefit ratio to transfusion policies in emergency resuscitation.

Sources of support

None.

Conflict of interests

The authors have disclosed no conflicts of interest.

Author contributions

JNS and MHY conceived the study, developed the model and wrote the manuscript; MNS and TP contributed to the model and revised the work for important intellectual content; CBB, SPE, JK, NOW, JLS, MT and DJT provided critical model assumptions and revised the work for important intellectual content.

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Supporting Information

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

Appendix S1. Table S1. Estimated overall rate of hemolytic disease of the fetus and newborn (HDFN) versus the age at which the Rh(D)-negative female of childbearing potential (FCP) is transfused with at least one Rh(D)-positive red blood cell (RBC)-containing product using assumptions for the USA. Exact binomial 95% confidence interval for the total HDFN rate are also shown.

Fig. S1. Sensitivity analysis for the effect of variations in multiple-partner fertility rate on the overall HDFN rate, defined as the number of HDFN cases per 100 Rh(D)-negative FCPs transfused. Only the multiple-partner fertility rate was varied; the other model inputs reflected data for the USA, as shown in Table 1. The relative difference in the HDFN rate was approximately ±15-20% at age 18.

Fig. S2. Sensitivity analysis for the effect of variations in Rh(D)-alloimmunization rate on the overall HDFN rate, defined as the number of HDFN cases per 100 Rh(D)-negative FCPs transfused. Only the allo-immunization rate was varied; the other model inputs reflected data for the USA, as shown in Table 1. The relative difference in the HDFN rate was approximately ±20-25% at age 18.

Fig. S3. Sensitivity analysis for the effect of variations in HDFN risk on the overall HDFN rate, defined as the number of HDFN cases per 100 Rh(D)-negative FCPs transfused. Only the HDFN risk was varied; the other model inputs reflected data for the USA, as shown in Table 1. The relative difference in the HDFN rate was approximately ±15% at age 18.

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ORIGINAL PAPER

Association of ABO blood group with postoperative total bleeding volume in patients undergoing total hip arthroplasty

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Abstract

Background and objectives As individuals with blood types A, B and AB have approximately 25% higher levels of vWF than those with type O, the risk of developing venous thrombotic events has been investigated in a number of clinical studies, but whether individuals with type O blood experience increased bleeding remains to be clarified. The purpose of this study was to evaluate an association of ABO blood group with intraoperative bleeding and postoperative total bleeding in patients undergoing total hip arthroplasty.

Materials and methods We prospectively recruited 84 women who were undergoing total hip arthroplasty. The differences between blood groups in mean age, body weight, preoperative and postoperative Hct levels, and postoperative/preoperative Hct ratio, intraoperative bleeding volume (IBV), and total bleeding volume (TBV) were evaluated.

Results Twenty-six patients had type A blood, 17 had type B, 9 had type AB, and 30 had type O. There were no significant differences in mean age, body weight or operating time between the different ABO blood groups. While there was no significant difference in these Hct levels or IBV among the different blood groups, there was a significant difference in TBV between type O and type AB, and between type O and non-type O.

Conclusion Our study in patients undergoing total hip arthroplasty suggests that patients in blood group O tend to have large amounts of bleeding.

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Introduction

Previous studies suggested that patients with non-type O blood, especially in patient with type AB, have twice the risk of developing venous thromboembolism (VTE) as those with type O blood [1–3]. One possible explanation for these findings is that patients with type O blood have a different coagulation profile than those with other blood types. Several studies have reported that patients with type A, B and AB blood had 25%–30% higher plasma levels of vWF than those with O blood [4]. vWF plays a decisive role in primary haemostasis by mediating the adhesion of blood platelets to the subendothelium of the damaged vessel walls and promoting the aggregation of activated platelets.

Therefore, the tendency that VTE is unlikely to occur in patients with type O blood may mean, conversely, that those patients have a predisposition to bleeding and a higher incidence of bleeding problems than patients with non-O blood types. While some investigators in gynaecology reported that women with type O blood suffer greater postpartum blood loss than women with other blood types [5–8], whether individuals with type O blood experience increased bleeding remains to be clarified. Here, we report an association of ABO blood group with

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intraoperative bleeding and postoperative total bleeding volume in patients undergoing total hip arthroplasty.

Patients and methods

This study was approved by our institutional review board, and informed consent was obtained from all patients. Eighty-two women underwent primary total hip arthroplasty at our hospital from June 2017 to September 2019. Their mean age and weight were 65·5 years (range: 43–80 years) and 55·8 kg (range: 40–76 kg), respectively, and the hip disease was osteoarthritis in all 82 patients. All operations were performed by one surgeon via the posterior approach with the patient in the lateral decubitus position. Prostheses were similar in all operations. Patients were mobilized on the first postoperative day, with full weight bearing being allowed, and all of them followed a standard postoperative rehabilitation programme. From the first postoperative day until 3 weeks after the operation, the nonsteroidal anti-inflammatory drug celecoxib was administered orally at a dose of 100 mg twice a day for postoperative pain relief, and from the fourth day after surgery, the anticoagulant drug factor Xa inhibitor (edoxaban tosilate hydrate, 15 mg) was administered once a day for 10 days to prevent deep venous thrombosis. Exclusion criteria included haematologic disorders, predisposition to bleeding, daily anticoagulant therapy, previous hip operation, intolerance of general anaesthesia, and arthroplasty for hip fracture.

Blood (800 ml) was taken preoperatively from all patients for autologous blood storage, of which 400 ml was transfused immediately after surgery and 400 ml was transfused the following day. All subjects also underwent intraoperative autologous blood salvage using an Xtra autotransfusion system (LivaNova, Tokyo, Japan). All blood was collected from the operating field using a heparinized double-lumen suction catheter, then washed and concentrated automatically using Xtra. After repeating the washing and concentration steps, half of the collected blood can ultimately be used as intraoperative autologous salvage blood. Therefore, the total intraoperative bleeding volume (IBV) was calculated as twice the amount of salvaged blood.

As used in past studies [9], perioperative estimated bleeding volume (EBV) was calculated using Gross' formula [10] from changes in haematocrit (Hct) levels before and after surgery and patient's blood volume (PBV) was calculated using Nadler's formula [11].

Nadler's formula for women:

$$\begin{aligned}\mathrm{PBV\ (L)} = {} & [\mathrm{height\ (m)}^3 \times 0.3561[+]\mathrm{weight\ (kg)} \times 0.03308] \\ & +0.1833\end{aligned}$$

Gross' formula:

$$\begin{aligned}\mathrm{EBV} = {} & \mathrm{PBV\ (ml)} \times [\mathrm{preoperative\ Hct\ (\%)} - \\ & \mathrm{postoperative\ Hct\ (\%)}] \\ & \div \mathrm{mean\ perioperative\ Hct\ (\%)}\end{aligned}$$

where preoperative Hct = Hct level 1 day before surgery, postoperative Hct = Hct level 4 days after surgery, and mean perioperative Hct (%) = [preoperative Hct (%) + postoperative Hct (%)] ÷ 2.

As previously described [9], if either reinfusion or allogenic transfusion was performed, the EBV calculated from the change in Hct levels was smaller than expected because intraoperative and postoperative blood transfusions increase postoperative Hct levels. Because both predeposit autologous blood transfusion and intraoperative autologous blood salvage were performed, we needed to add the volume of transfused blood to the volume calculated with the above formula for EBV. Therefore, we calculated the total bleeding volume with the following formula:

$$\begin{aligned}\mathrm{Total\ bleeding\ volume\ (TBV)} = {} & \mathrm{EBV} \\ & + \mathrm{predeposit\ autologous\ blood\ transfusion\ volume} \\ & + \mathrm{intraoperative\ autologous\ blood\ salvage\ volume}\end{aligned}$$

We also calculated each patient's ratio of postoperative Hct (day 4) to preoperative Hct (day −1).

Statistical analysis

To compare differences in mean age, body weight, pre- and postoperative Hct levels, postoperative Hct to preoperative Hct ratio, IBV, and TBV between the three groups (type O blood group, type AB blood group, and type non-O non-AB [type A + type B] blood group), we used multiple comparison analysis with a Bonferroni/Dunn test, and to compare differences in mean age, body weight, preoperative and postoperative Hct levels, postoperative Hct to preoperative Hct ratios, IBV, and TBV between individuals with type O blood and those with non-type O blood, we used unpaired *t*-tests. Statistical analysis was performed in StatView 5.0 for Mac (SAS Institute Inc., North Carolina). $P < 0{\cdot}0167$ was considered significant in the Bonferroni/Dunn test and $P < 0{\cdot}05$ in the unpaired *t*-tests.

Results

The number of patients with each blood type was as follows: type non-O non-AB, 43 (type A, 26; type B, 17);

type AB, 9; and type O, 30. Thus, 52 patients had non-O type blood. We found no significant differences in mean age, body weight or operating time between the three blood groups (Table 1). The mean preoperative and postoperative Hct levels and postoperative Hct to preoperative Hct ratios for each blood group are shown in Table 2. The mean difference in the postoperative Hct to preoperative Hct ratio between the type O and type AB groups was 4·5%, but this difference did not reach statistical significance. Although we found no significant difference in IBV between the various blood groups, we did find a significant difference in TBV between type O and both type AB ($p = 0{\cdot}0115$) and non-type O ($p = 0{\cdot}0276$) (Table 3).

Discussion

Some studies reported a higher bleeding risk in patients with type O blood who experienced upper gastrointestinal haemorrhage [12,13]. Other studies reported higher postpartum blood loss: Kahr et al. [5] found that women with type O blood had greater postpartum blood loss than women with non-O type blood; Drukker et al. [6] found that women with type O blood were at 1·14-fold greater risk of postpartum haemorrhage; and Bade et al. [7] found that women undergoing caesarean delivery had significantly more blood loss if they had type O blood rather than type non-O blood and that type O blood was associated with a 1·09-fold greater risk for transfusion than type non-O blood. In contrast, other studies in patients with peptic ulcer bleeding [14] or cerebral haemorrhage [15] or after cardiac surgery [16] did not confirm such increases in bleeding tendency associated with type O blood. Thus, the relationship between bleeding risk and blood type has not yet been fully elucidated. Our study in patients undergoing total hip replacement supports the findings of differences between blood group types in that it found significant differences in total bleeding volume between type O and both type non-O and type AB.

Most previous studies on the incidence of VTE combined non-O blood groups into a single group and compared them with type O; however, some reports indicate that patients with type AB blood are at the highest risk of VTE. A significantly increased risk of VTE in people with type AB blood was found by Newman et al. [17] in total joint arthroplasty and by Larsen et al. [18] in pregnancy and the puerperium. The steady-state levels of circulating vWF are lowest in type O blood, followed by type A, type B and finally type AB [4]; these differences may explain why patients with type AB blood have the highest risk of developing VTE.

This study has some limitations. First, it was not randomized. Second, it included only female patients; we enrolled only women because differences in physique between men and women result in differences in blood volume, which in turn are expected to lead to differences in bleeding loss. Furthermore, more women than men receive total hip arthroplasty for developmental dysplasia of the hip in Japan. However, future studies should include also male patients. Third, we examined four samples of different sizes. The difference in sample sizes for each blood type was related to the difference in blood type frequency, which varies depending on ethnicity. In Japan, the ratio of type A to type B to type AB to type O is 4:2:1:3; in our study, the ratio was 3:2:1:3. Future studies need to compare the four blood types in samples of the same size. Last, the accuracy of our method for measuring intraoperative bleeding may be questionable. In total hip arthroplasty, in addition to the blood observed during surgery a large amount of hidden blood loss occurs in the form of bleeding into and between the muscles; this blood loss cannot be measured and can amount to about 60% of the total blood loss [19]. Because the amount of intraoperative bleeding cannot be accurately measured by any method, we believe that estimating the amount of intraoperative bleeding from the amount of intraoperative autologous blood salvage was a valid approach.

We hope that more research will be conducted to examine the relationship between bleeding and ABO blood type during and after surgery. If relationships between ABO blood type and bleeding tendency are confirmed in a future large-scale study, surgeons may be able

Table 1 Patients' demographics

Blood type	Number	Mean age (years)	Mean body weight (Kg)	Mean operating time (min)
A	26	65·8	54·8	108·2
B	17	64·7	54·5	102·9
Non-O and Non-AB (A + B)	43	65·4	54·7	106·1
AB	9	69·3	52·9	111·3
O	30	64·6	58·2	109·3
Non-O (A + B + AB)	52	66·1	54·4	107·0

Table 2 The mean preoperative and postoperative Hct levels

Blood type	Preoperative Hct (%)	Postoperative Hct (%)	Postoperative Hct/Preoperative Hct (%)
A	38·3	35·4	92·4
B	38·4	35·6	92·9
Non-O and Non-AB (A + B)	38·3	35·5	92·6
AB	38·4	36·7	95·5
O	39·8	36·1	90·9
Non-O (A + B + AB)	38·3	35·7	93·1

Table 3 The mean intraoperative bleeding volume (IBV) and total blood volume (TBV)

Blood type	IBV (ml)	TBV (ml)
A	401·5	1256·2
B	372·4	1234·3
Non-O and Non-AB (A + B)	390·0	1247·6
AB	317·8	1106·8*
O	433·0	1353·3*
Non-O (A + B + AB)	377·5	1223·2*

*There was a significant difference in TBV between type O and both type AB ($P = 0·0115$) and type non-O ($P = 0·0276$).

to estimate the risk of intraoperative bleeding on the basis of the patient's ABO blood type. Moreover, such knowledge could be useful for planning blood management in patients who need surgical treatment.

In conclusion, we found significant differences in total bleeding volume after total hip arthroplasty between patients with type O blood and both those with type non-O and those with type AB. Our results support those of previous studies suggesting that patients with type O blood tend to bleed more, and they may provide useful information for patient blood management.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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See also http://www.isbtweb.org/congresses/

4.5.2021	IPFA/PEI – The International Workshop on Surveillance and Screening of Blood-borne Pathogens
13–15.5.2021	The Canadian Society for Transfusion Medicine (CSTM) are holding their annual scientific conference virtually in 2021.
26–27.05.21	21st Congress of the European Society for Hemapheresis
5–9.6.2021	ISBT In Focus, the 31st regional congress of the ISBT, will be a virtual event in 2021
17.9.2021	11th BIC International Conference – Advances in Haemostasis and Bleeding Disorders
22–24.9.2021	Deutsche Gesellschaft für Transfusionsmedizin und Immunhämatologie e.V.
23–26.9.2021	16th International Congress on Myelodysplastic Syndromes (MDS 2021)
13–16.11.2021	32nd Regional congress of ISBT, Brisbane, Australia