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# Vox Sanguinis

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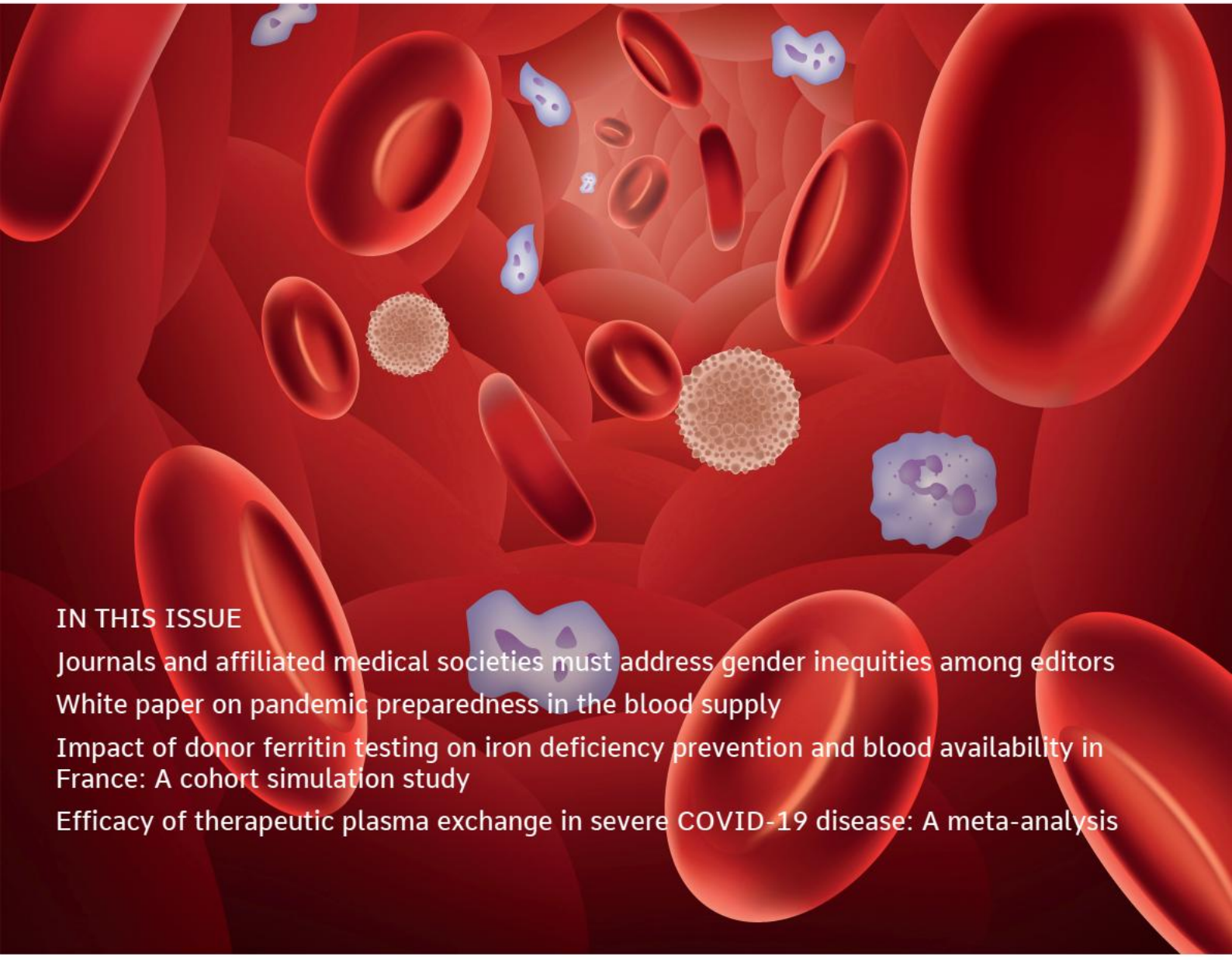

IN THIS ISSUE



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# Vox Sanguinis

International Journal of Blood Transfusion

**Official Journal of the International Society of Blood Transfusion**

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# Vox Sanguinis

## International Journal of Blood Transfusion

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*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 10 main sections:

1. 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;
2. 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;
3. Donors and Donations: Donor recruitment and retention; donor selection; donor health
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6. International Forum: Section in which topics related to any aspects of the transfusion chain (from technical to scientific, including socioeconomic and ethical facets) are discussed by invited participants and summarized by guest editors
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8. Review: Comprehensive reviews or short comments of any aspect related to transfusion medicine;
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10. 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;

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

# Knowledge assessment of a donor adverse reaction severity grading tool by the National Blood Donor Vigilance Programme of India

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## Abstract

**Background and Objectives:** The severity grading tool (SGT) was recently designed by the Association for Advancement of Blood and Biotherapies (AABB) to have more objectivity in severity assignment for an adverse donor reaction after blood donation. A study was performed in India to assess the knowledge (post-training) and determine the degree of agreement of the SGT between participating centres and the subject expert group.

**Materials and Methods:** This prospective cross-sectional survey-based study was conducted by the National Coordinating Centre (NCC) of the National Blood Donor Vigilance Programme (NBDVP) of India. Thirty-five real-world case scenarios, validated by seven national and two international experts, were sent to the participating centres, and their responses received (diagnosis and severity grade) were compared and analysed.

**Results:** A total of 50 blood centres participated in the study. The overall agreement between the participating centres and the expert group was 66.4%, with a fair Kendall's coefficient of concordance ($W$) of 0.271 ($p$-value < 0.05). The degree of agreement was observed to be more than 80% for 12 centres, 60%–80% for 27 centres and <60% for 11 centres. The overall degree/percentage of agreement for cases with single and multiple types of donor adverse reaction was 71.3% and 42.6%, respectively.

**Conclusion:** The SGT will be an efficient mode to have uniform objective reporting of the adverse donor reactions and may be implemented in the NBDVP of India. This study also highlights the need for training of the blood centres on the basic definitions and categorization of the donor's adverse reaction.

## Keywords

donor adverse reaction, donor vigilance, haemovigilance, severity grading tool

## Highlights

- The severity grading tool (SGT) knowledge assessment by the Indian donor vigilance programme showed 'fair' concordance between the participating centres and the expert group.
- The study showed an overall 66.4% agreement.

  

- The study also highlights that there is a need to train the reporting centres in basic definitions and categorization of SGT as it becomes implemented in the national programme in India.

## INTRODUCTION

Blood donation by a healthy blood donor is the cornerstone of providing safe blood and blood components to patients requiring transfusion. The process of blood donation is safe, and a very small proportion of donors may experience local or generalized donor adverse reactions (DARs) or donor adverse events (DAEs). For regulatory and ethical requirements, all blood centres in the country capture and record any DAR reported with whole blood and apheresis donations. However, presently, reporting them to the National Blood Donor Vigilance Programme (NBDVP) of India is voluntary. The NBDVP recently reported an incidence of DAR at 0.24% as compared with 0.36%–3.25% for various institutions in India [1].

The NBDVP of India was launched in 2015 and is still in its initial phases of implementation. The aim of the national programme is to collect data on DAR and work on providing evidence-based recommendations for donor safety in the country. The National Coordinating Centre (NCC), which is the National Institute of Biologicals (NIB), Ministry of Health, Government of India, is closely working with blood centres and regulatory authorities to have more regular and standardized reporting in accordance with international donor vigilance practices.

From its inception, the NBDVP of India adopted harmonized international standard definitions to have harmonized reporting in all the centres [2]. These Standards for Surveillance of Complications Related to Blood Donation (SSCRBD) have played a very important role in providing appropriate definitions and categorization of the donor's adverse reactions/events. It was observed that SSCRBD established the definitions for the complications but uniformity in severity grading and imputability reporting was still not standardized. Recently, International Haemovigilance Network (IHN) reported 11 years of data from 24 haemovigilance systems (HVS) from across the world and showed that there is a variability of reporting practices and severity data between the HVS impairing the feasibility of comparison [3]. The severity was reported as mild/moderate/severe based on independent definitions and criteria adopted by different centres or countries and lacked uniformity due to a lack of internationally accepted guidelines. This lack of guidance on severity assessment was also highlighted during the validation of the SSCRBD definitions was done [4].

In January 2018, to work on the same issue, the Donor Haemovigilance Working Group of the Association for Advancement of Blood and Biotherapies (AABB), the Haemovigilance Working Party of the International Society of Blood Transfusion (ISBT) and the chair of the Plasma Protein Therapeutics Association (PPTA) medical policy formed the severity grading subgroup to undertake the task of developing the severity grading tool (SGT) for blood DAE [4]. Recently, this SGT, validated by AABB, was also endorsed by ISBT, IHN and European Blood Alliance [5]. The SGT aims to standardize the severity reporting of DAR or DAE and remove subjectivity and interpersonal variations (Table 1).

Keeping this notion in mind that implementing SGT in the national programme can help in uniform reporting for the severity of DAR as well as help in comparing the national data with international haemovigilance data and guide to prioritize recommendations for donor safety. This knowledge assessment study was planned by NCC among the regularly reported blood centres.

The aim of the study was to validate the SGT by determining the degree of agreement between the participating centres and the subject expert group. Further, the objectives were to determine the variation in the degree of agreement between single or multiple DARs as well as to study the causes of disagreement.

## MATERIALS AND METHODS

This was a prospective cross-sectional survey-based study conducted by the NCC of the NBDVP, which is the National Institute of Biologicals, Noida, India, from 9 August 2021 to 6 September 2021 through an activated link in the Donor-Vigil software. Thirty-five questions based on real-world case scenarios of DAR representing different scenarios (with different severity) were prepared by two subject experts (Supporting Information S1). The distribution of the type of real-world case scenarios were both from whole blood and apheresis donations causing either a single or double DARs in the survey questions (Table 2).

This study was conducted in three stages. The first stage involved studying 35 real-world cases by the subject experts, the second stage was an online webinar for all the blood centres enrolled in the study and in the third stage, cases were sent to all the enrolled centres for them to respond.

In the first stage, the case scenarios were sent to both national ($n = 7$) and international ($n = 2$) subject experts, who independently reviewed and validated them. In the second stage, nodal officers of all the study enrolled centres participated in an online webinar (to explain the SGT in detail) on 27 July 2021 (2 h) before the cases were sent to them. The categories of DAR and the process to use the SGT were explained in this webinar.

For the third stage, the cases were distributed by NCC to the enrolled participating centres, through the reporting software, that is, Donor-Vigil for reporting of DAR. A period of 1 month was given for submitting responses. These numbers of minimum centres to be

**TABLE 1** Definitions of severity grades.

| Severity grade | General factors to consider in assigning severity donor adverse event (DAE) severity tool | DAE examples |
|---|---|---|
| Grade 1 | No outside medical care (OMC)<br>AND<br>Short duration ≤2 weeks<br>AND<br>No limitation on activities of daily living (ADL)<br>AND<br>Resolved with no or minimal intervention | • Arterial puncture, pressure bandage applied, resolved without intervention or sequelae<br>• Vasovagal event that resolves with comfort care and/or oral hydration<br>• Citrate reaction resolved with oral calcium or reduction in infusion rate |
| Grade 2 | OMC, no hospitalization<br>OR<br>Duration >2 weeks to ≤6 months<br>OR<br>Limitations on ADL for ≤2 weeks | • Superficial thrombophlebitis resolved with oral antibiotics, no sequelae<br>• Vasovagal event that requires transport to ER for IV hydration<br>• Lacerations requiring sutures |
| Grade 3 | Not life-threatening<br>AND any of the following:<br>Hospitalization<br>OR<br>Duration >6 months<br>OR<br>Limitations on ADL >2 weeks<br>OR<br>Require surgery<br>OR<br>Other serious complications (Category E) | • Arteriovenous fistula requiring surgical repair<br>• Fracture, dental injury or concussion<br>TIA and other cardiovascular events, which are not life-threatening |
| Grade 4[a] | Immediate medical intervention required to prevent death | • LOC with fall and intracranial bleed anaphylaxis requiring intubation or tracheostomy |
| Grade 5[a] | Death related to DAE | Death |

[a]Grade 4 and Grade 5 are not shown in the grading severity of blood donor adverse events tool (Supporting Information S1).
Abbreviations: ER, emergency room; IV, intravenous; LOC, loss of consciousness; TIA, transient ischemic attack.

**TABLE 2** Details of the distribution of categories of DAR in the 35 real-world cases.

| Type of donation | Single DAR cases | Double DAR cases | Total DAR cases |
|---|---|---|---|
| Whole blood | 21 | 2 | 23 |
| Apheresis | 8 | 4 | 12 |
| Total | 29 | 6 | 35 |

Abbreviation: DAR, donor adverse reactions.

included in the study were selected using stratified sampling with proportional allocation. A minimum of 25 centres were required to be enrolled in the study. These numbers were selected using stratified sampling with proportional allocation. The weights were generated for these three categories in such a manner so that these categories would generate the representative of these categories.

The inclusion criteria for the blood centre to be enrolled in the knowledge assessment study were that the blood centre should be a centre enrolled in the national programme (NBDVP), it should have regular reporting for the last 2 years, it should collect blood through both in-house and outdoor camps facility as well as should provide consent to participate in the study.

Responses to the survey questions were received in the form of diagnosis and severity grading. The responses from the centres were compared with the responses by the subject experts, and the degree of agreement was calculated.

## Analysis of the responses and scoring

The responses were calculated in two parts: diagnosis and severity grade (using the SGT). For a response to be correct, it should match the correct diagnosis and severity grade assigned by the expert group.

### Analysis of disagreement

There were two categories of disagreement, that is, 'incorrect diagnosis' and 'incorrect severity grading'. The incorrect diagnosis was further categorized into (i) single DAR reported as double DAR; (ii) signs and symptoms not matched with the diagnosis; (iii) DAR category is correct, but diagnosis is incorrect and (iv) total incorrect diagnosis (i.e., both diagnosis and severity score did not match with the expert).

### Scoring of the responses

Each correct response was awarded a score of '1' while incorrect responses either for diagnosis or severity grading, or both, were awarded the score of '0'. The maximum score that each centre can score was '35', which means that the centre has correctly diagnosed and provided the correct severity grading to all 35 cases. Based on the total number of centres enrolled and the number of cases received by each centre, the total possible correct responses were analysed, that is, 50 centres received 35 cases (each) and the total possible correct responses were 1750 (50 × 35).

## Outcome analysis

The responses (SGT scoring) from the participating centres were analysed and the degree of the agreement was evaluated as the percentage agreement with expert grading. Inter-rater reliability was calculated using Kendall's coefficient of concordance (*W*) for ordinal data. *W* was interpreted using the following guidelines: 0 as poor; 0–0.2 as slight; 0.2–0.4 as fair; 0.4–0.6 as moderate; 0.6–0.8 as substantial and 0.8–1 as almost perfect agreement.

$$\text{Percentage of agreement}\,(\%)\ \text{was calculated by}: \frac{\text{Each centre's score (out of 35)} \times 100}{\text{Maximum score (35)}}$$

# RESULTS

A total of 53 centres responded to the survey questionnaires out of a total of 65 blood centres that received the cases. Of the total responders, three centres submitted incomplete responses, so only 50 centres (with complete responses) were included in the analysis of the study (Table 3).

## Distribution of cases prepared for the study

The distribution of severity grading of DARs among the cases prepared was in the majority of Grade 2 and most single DAR-type cases were provided (Tables 4 and 5).

**TABLE 3** Details of the participant centres in the study.

| Type of participating centres | Minimum required | Enrolled in the study |
|---|---|---|
| Hospital-based (government) blood centres | 6 | 16 (32%) |
| Hospital-based (private/charitable/trust) blood centres | 16 | 27 (54%) |
| Stand-alone blood centres | 3 | 7 (14%) |
| Total | 25 | 50 |

**TABLE 4** Details of the distribution of severity grading in cases with a single type of DARs ($n = 29$).

| Categories of DAR | Total | Severity grading | | |
|---|---|---|---|---|
| | | 1 | 2 | 3 |
| A | 11 | 2 | 6 | 3 |
| B | 7 | 1 | 4 | 2 |
| C | 4 | 3 | 1 | 0 |
| D | 4 | 2 | 1 | 1 |
| E | 3 | 0 | 0 | 3 |
| Total | 29 | 8 (27.5%) | 12 (41.4%) | 9 (31%) |

Abbreviation: DAR, donor adverse reactions.

**TABLE 5** Details of the distribution of severity grading in cases with multiple types of DARs ($n = 6$).

| Categories of DAR | B + C ($n = 1$) | A1 + C ($n = 3$) | A1 + B ($n = 2$) |
|---|---|---|---|
| Severity grading | 2 and 2 | 1 and 1, 1 and 3, 2 and 2 | 1 and 1 |

Abbreviation: DAR, donor adverse reactions.

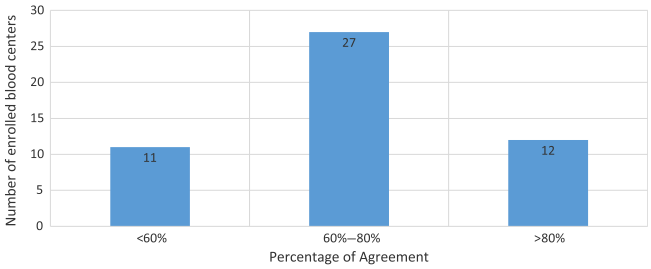


**FIGURE 1** The distribution of the overall degree of agreement (%) between the experts and the participant centres in the study.

## Degree of agreement and coefficient of concordance (*W*)

### Overall cases

The degree of agreement among the expert group was more than 90%. Among the participants, there were a total of 1164 correct responses out of the total of 1750 responses. The overall degree of agreement between the participant centres and the expert group was 66.4% (Figure 1).

The overall agreement, Kendall's coefficient of concordance (*W*) was *fair* with 0.271, with a statistically significant association (*p*-value < 0.05). Twelve participating centres reported as high as >80% degree of agreement with the expert group, whereas 27 centres reported between 60%–80% and <60% by 11 centres (Figure 1). Of the 35 DAR cases, more than 80% of the agreement was achieved in 13 cases followed by 60%–80% and <60% in 11 DAR cases each.

**TABLE 6** Details of the distribution of correct responses (scores) by the participant centres for cases with single DAR ($n = 29$).

| Categories of DARs | A ($n = 11$) | B ($n = 7$) | C ($n = 4$) | D ($n = 4$) | E ($n = 3$) | Total ($n = 29$) |
|---|---|---|---|---|---|---|
| Whole blood | 311 | 281 | 0 | 94 | 106 | 792 |
| Apheresis | 54 | 0 | 144 | 44 | 0 | 242 |
| Total correct | 365 | 281 | 144 | 138 | 106 | 1034 |
| Max correct score | 550 | 350 | 200 | 200 | 150 | 1450 |
| Percentage | 66.3% | 80.2% | 72% | 69% | 70.6% | 71.3% |

Abbreviation: DAR, donor adverse reactions.

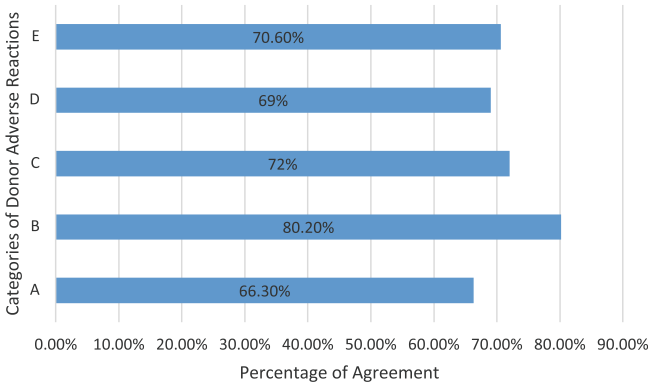


**FIGURE 2** The distribution of degree of agreement between the expert group and the participant centres for various categories of DARs. DAR, donor adverse reactions.

### Cases with single DAR

The overall degree/percentage of agreement between the participant centres and the expert group for cases ($n = 29$) with a single type of DARs was 71.3% (Table 6 and Figure 2).

### Cases with multiple DAR

The overall degree/percentage of agreement between the participant centres and the expert group for cases ($n = 6$) with multiple types of DARs was 42.6% (Table 7).

### Cases with local complications type of DAR

Local DARs have many subcategories, and the distribution of these cases and their responses showed variation with the maximum agreement in category 'A3' and minimum in 'A1' (Table 8).

## Details of disagreement

Most of the disagreement in whole blood donation cases were in categories A (local complications) and B (generalized complications), whereas in apheresis donation DAR cases, it was in category C of DARs (Table 9). The major cause of disagreement was an *incorrect diagnosis* of the DAR cases by 53.8% of centres and the rest were due to *incorrect severity grading* by 46.1% (Figure 3).

When analysing the *causes of disagreement* due to *incorrect diagnosis*, it was found that the reasons were when subcategory diagnosis was incorrect even after initial appropriate category diagnoses ($n = 78$; most common with category A; local complications type of DARs), such as 'hematoma' misdiagnosed as 'local inflammation' or 'other painful arm'.

Other reasons for 'disagreement' were due to the wrong diagnosis of the DARs ($n = 74$) and the identification of single DARs as multiple ($n = 47$). Many centres also reported errors when the diagnosis was correctly identified, but the incorrect signs and symptoms were assigned to the diagnosis ($n = 25$). These types of errors were most common with the generalized type of DARs where symptoms such as the history of injury and loss of consciousness (LOC) were incorrectly identified.

The most common disagreement due to the 'incorrect severity grading' assignment was for category A followed by category B in cases with whole blood donation and category D followed by category A in apheresis-related donation DARs.

On further analysing the category A DARs cases ($n = 9$), it was found that the disagreement was most common in category A1 followed by A2 due to both 'incorrect diagnosis' and 'incorrect severity grading' assignments (Table 10). The most common cause of 'incorrect diagnosis' in category A was due to inappropriate identification of subcategories, most commonly in subcategories A1 followed by A2 and A4 by the reporting centres.

## DISCUSSION

The NBDVP of India is actively collecting data on DARs from the year 2016 onwards. From the beginning of the national programme, the definitions and categories of DARs were adopted from SSCRBD [2]. These definitions provided a basic framework for the reporting format of the national programme. Since these SSCRBD recommendations lack objective criteria to identify the severity of the adverse donor reactions, a severity tool was recently designed and validated [4–6]. This study was one of the first attempts to conduct a knowledge assessment of the new SGT

**TABLE 7** Details of the distribution of correct responses (scores) by the participant centres for cases with multiple DARs ($n = 6$).

| Categories of cases | A1 + C ($n = 3$) | B + C ($n = 1$) | A1 + B ($n = 2$) | Total ($n = 6$) |
|---|---|---|---|---|
| Whole blood | 0 | 0 | 59 | 59 |
| Apheresis | 60 | 9 | 0 | 69 |
| Total correct score | 60 | 9 | 59 | 128 |
| Max correct score | 150 | 50 | 100 | 300 |
| Percentage | 40% | 18% | 59% | 42.6% |

Abbreviation: DAR, donor adverse reactions.

**TABLE 8** Details of the distribution of correct responses (scores) by the participant centres for cases with local complications type of DAR ($n = 11$).

| Categories of cases | A1 ($n = 5$) | A2 ($n = 3$) | A3 ($n = 2$) | A4 ($n = 1$) | Total ($n = 11$) |
|---|---|---|---|---|---|
| Whole blood | 139 | 100 | 39 | 33 | 311 |
| Apheresis | 14 | 0 | 40 | 0 | 54 |
| Total correct | 153 | 100 | 79 | 33 | 365 |
| Max correct score | 250 | 150 | 100 | 50 | 550 |
| Percentage | 61.2% | 66.6% | 79% | 66% | 66.3% |

Abbreviation: DAR, donor adverse reactions.

**TABLE 9** Details of the distribution of causes for 'incorrect responses' or 'disagreement' by the participant centres for cases with single DAR ($n = 29$).

| | | Diagnosis incorrect, grading not assessed | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Categories of DAR | | Single but double reported | Diagnosis is correct but incorrect S/S | Categories are correct but incorrect diagnosis | Incorrect diagnosis | Total incorrect diagnosis | Diagnosis (correct), grading (incorrect) | Total |
| Whole blood ($n = 21$) | A ($n = 9$) | 3 | 0 | 61 | 12 | 76 | 63 | 139 |
| | B ($n = 7$) | 1 | 25 | 0 | 2 | 28 | 41 | 69 |
| | C ($n = 0$) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | D ($n = 2$) | 0 | 0 | 0 | 3 | 3 | 3 | 6 |
| | E ($n = 3$) | 2 | 0 | 0 | 18 | 20 | 24 | 44 |
| Apheresis ($n = 8$) | A1 ($n = 1$) | 3 | 0 | 8 | 10 | 21 | 15 | 36 |
| | A3 ($n = 1$) | 0 | 0 | 6 | 3 | 9 | 1 | 10 |
| | C ($n = 4$) | 35 | 0 | 0 | 11 | 46 | 10 | 56 |
| | D ($n = 2$) | 3 | 0 | 3 | 15 | 21 | 35 | 56 |
| Total ($n = 29$) | $N = 29$ | 47 | 25 | 78 | 74 | 224 | 192 | 416 |
| | | | | | | 53.8% | 46.1% | |

Abbreviation: DAR, donor adverse reactions.

in an Indian setting before considering it for implementation in the national programme.

One of the first attempts to validate this SGT tool was done by AABB in 2020, with 93 centres from 40 different countries and graded 34 total DARs cases [6]. The AABB study showed the overall agreement was *almost perfect*, with Kendall's coefficient of concordance (*W*) of 0.84 (CI 0.78–0.90) compared with our study where the overall agreement was *fair* with Kendall's coefficient of concordance (*W*) of 0.271. In our study, the degree of agreement was higher with the generalized reactions (category B) and lowest in local reactions (category A, Table 6), whereas the degree of agreement in the AABB study was higher in the local complication category [6].

One of the main reasons for an overall lower degree of agreement and coefficient of concordance (*W*) in our study was incorrect diagnosis (53.8%), and the most common cause of incorrect diagnosis was

wrong identification of subcategories of DARs, wrong identification of DARs as well as reporting a single DAR as a double. There may be the following reasons for lesser agreement in our study when compared with the AABB study:

1. This was the first of its kind of knowledge assessment done by the NBDVP of India, so it was a new way to read and respond to the cases by the participant centres.
2. There were many new terms and definitions in SGT, which may not have been understood appropriately by participating centres although a training webinar was conducted before the initiation of the study.
3. The AABB validation study was responded by the 'in charge/head' of the blood centre or the expert group representing the international organizations, whereas in our study, these cases were responded to by the nodal officers, who can be medical/technical/nursing officers of blood centres reporting to the national programme from a cross-sectional representation of blood centres across India. The level of training and knowledge will be very different at all these levels and may have caused a higher degree of disagreement in our study.
4. Another reason may be subletting of work or response to these cases by juniors or untrained staff, as one of the enrolled centres in the study answered incorrectly to all the 35 cases provided. This highlights that doctors or trained staff may not have responded to the cases.

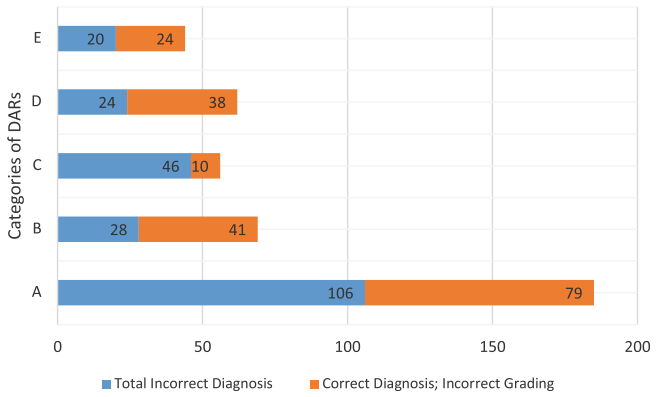


**FIGURE 3** Details of the distribution of reasons for 'incorrect responses' or 'disagreement' by the participant centres for cases with single DAR ($n = 29$). DAR, donor adverse reactions.

Despite the lower degree of agreement compared with the AABB study, this study provides many valuable inputs to the NBDVP of India, such as it provides clinical and practical insights into the level of understanding of DARs by the participating centres. It also provides a framework for the rephrasing of SGT in India in alignment with easily understood and locally used medical terms. It provides an opportunity to improve the understanding/reporting as well as provide uniformity in DARs reporting by the participating centres. It helped to educate/update the participating centres with the recent advancements in the field of donor vigilance.

There were a few limitations to this study such as the terms, and definitions were directly adopted from AABB; these were not modified into locally understandable language. We also did not define the minimum qualification criteria and responsibilities of the nodal officers. Cases for Grades 4 and 5 (as per AABB) were not included in this study.

We can conclude that this study demonstrated a *fair* agreement with the overall degree of agreement of 66.4% between the enrolled blood centres with subject experts on the assignment of severity grading to DAR cases using the new SGT. The standardization will not only help us capture more reliable data but also provide an opportunity to define best practices in blood donor safety and identify priority areas for research. As per the findings and results of the knowledge assessment study, the expert group made the following recommendations:

1. The NBDVP of India should make efforts to train the reporting centres on the definitions and categorization of DARs.
2. The NCC, that is, NIB, should conduct webinars on a regular basis to discuss the finding of the study with the enrolled centres as well as explain the cases that had an agreement rate of <60%.
3. The SGT may be implemented in the NBDVP of India on an optional basis for the enrolled blood centres.

**TABLE 10** Details of the distribution of reasons for 'incorrect responses' or 'disagreement' by the participant centres for cases with single DAR for category A ($n = 9$).

| | | Diagnosis incorrect, grading not assessed | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Categories of DAR | | Single but double reported | Diagnosis is correct but incorrect S/S | Categories are correct but incorrect diagnosis | Incorrect diagnosis | Total incorrect diagnosis | Diagnosis (correct), grading (incorrect) | Total |
| Whole blood ($A = 9$) | A1 ($n = 4$) | 1 | 0 | 29 | 4 | 34 | 27 | 61 |
| | A2 ($n = 3$) | 2 | 0 | 16 | 5 | 23 | 27 | 50 |
| | A3 ($n = 1$) | 0 | 0 | 0 | 2 | 2 | 9 | 11 |
| | A4 ($n = 1$) | 0 | 0 | 16 | 1 | 17 | 0 | 17 |

Abbreviation: DAR, donor adverse reactions.

In conclusion, the SGT is a very useful method to report the severity of a donor's adverse reaction as well as to remove subjectivity in reporting. This tool will not only help in collecting uniform data on the severity of an adverse donor reaction but also help in providing meaningful data for inter-organizational data comparison. Our cross-sectional study is one of the first attempts to validate this tool apart from the original knowledge assessment work. Our study shows a 'fair' correlation between the use of severity by the enrolled centres when compared with experts. As per our study, SGT may be implemented in the NBDVP of India in the near future to have more objective reporting of the severity in the national programme. This study also brought out the need for training of the blood centres on basic definitions and categorization of the donor's adverse reaction.

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## CONFLICT OF INTEREST STATEMENT

The authors have declared no conflicts of interest for this article.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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## SUPPORTING INFORMATION

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

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REVIEW

Vox Sanguinis ISBT International Society of Blood Transfusion

# Men who have sex with men and risk for transfusion-transmissible infections in blood donors in Western countries: A systematic review update

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## Abstract

**Background and Objectives:** This systematic review update summarizes evidence concerning transfusion-transmissible infections (TTIs) in male blood donors reporting sex with another man (MSM) or after easing the MSM deferral period.

**Materials and Methods:** We searched five databases, including studies comparing MSM versus non-MSM donors (Type I), MSM deferral periods (Type II) or infected versus non-infected donors (Type III) in Western countries, and used GRADE to determine evidence certainty.

**Results:** Twenty-five observational studies were included. Four Type I studies suggest that there may be an increased risk for overall TTIs, human immunodeficiency virus (HIV), hepatitis B virus (HBV) and syphilis in MSM donors, but the evidence is very uncertain. There was insufficient evidence of MSM with low-risk sexual behaviour. A Type II study indicates that easing the MSM deferral period to 1 year may have little to no effect on TTI risk. TTI prevalence in blood donors under 5-year, 1-year, 3-month or risk-based deferral in eight other Type II studies was too low to provide clear conclusions on the effect of easing the deferral. Three Type III studies reported that MSM may be a risk factor for HIV. Increased risk of HBV, hepatitis C virus and HTLV-I/II could not be shown. The evidence from Type III studies is very uncertain.

**Conclusion:** There may be an increased risk of HIV in MSM blood donors. Shortening the deferral from permanent to 1 year may have little to no effect on TTI risk. However, there is limited, unclear evidence from observational studies concerning the impact of introducing 3-month or risk-based deferrals.

## Keywords

donor selection/deferral, men who have sex with men, transfusion-transmissible infections

## Highlights

- Based on 25 observational studies, we conclude that (1) there may be an increased risk of transfusion-transmissible infections (TTIs), and human immunodeficiency virus (HIV) specifically, in blood donors reporting male-to-male sex, but the evidence is very uncertain and (2) it is currently difficult to draw solid conclusions on the impact of easing the men who

  

have had sex with another man (MSM) deferral given that TTI prevalence is very low in blood donors.

- Most studies (with two exceptions) were performed under a permanent or 1-year MSM deferral period. There is limited research on the impact of shorter or risk-based deferral policies or differential risk behaviour among MSM (eligible) donors.
- When informing policy, the current results should be supplemented with other available data (e.g., on compliance with the donor questionnaire, donor comfort, modelling studies and surveillance reports) and other criteria (e.g., feasibility and cost-effectiveness of alternative deferral periods). In addition, studies focusing on potential novel risks, including monkeypox transmission and the impact of use of pre-exposure prophylaxis on blood safety, would be relevant.

## INTRODUCTION

Deferral of donors who are at elevated risk for transfusion-transmissible infections (TTIs) is one of the strategies that has been adopted to safeguard the blood supply. Among other risk factors, the donor history questionnaire contains a range of items related to sexual behaviour, one of which regards men who have had sex with another man (MSM). Current MSM deferral periods vary from indefinite to 3 months, and several countries have implemented or are planning to adopt individual risk-based criteria instead of a blanket policy for MSM [1–3].

Epidemiological evidence has linked MSM to an increased risk of TTIs. For example, sex between men accounts for 53% of new human immunodeficiency virus (HIV) diagnoses in 2020 in the EU/EEA (only including cases for which the route of transmission was known) [4], while MSM has been estimated to represent only 0.03%–6.55% of men in European countries [5]. Similarly, elevated prevalence in MSM has been found for syphilis [6], hepatitis B or C virus (HBV or HCV) [7] and monkeypox [8].

Whether such elevated risk justifies excluding MSM from donating blood has been a matter of debate. Arguments against deferral periods for MSM include discrimination and stigmatization, a lack of acknowledging differential risk among MSM, and the residual risk for transmission via transfusion being low given the current testing methods [9, 10]. The other side argues that the right of receiving safe blood transcends the wish of being allowed to donate, and that the existing empirical data (cf. above) justify the exclusion of a population group that overall elicits high-risk behaviour. In addition, removal of the deferral period for MSM would require accompanying measures to retain the infection risk for recipients as low as possible, including a more in-depth screening of all donors for sexual risk behaviours, possibly leading to donor loss due to discomfort [11]; and transition from pooled towards individual genomic screening to increase sensitivity, especially in case of potential pre-exposure prophylaxis (PrEP) use [12].

The current systematic review summarizes published studies on the relation between MSM or an MSM deferral policy and the risk of TTI markers in donated blood. Of note, this is only one aspect that may inform decisions, and an analysis of all available resources to inform policy is outside the scope of this review. In line with the first and previous versions of this systematic review [13], the focus is exclusively on blood donors or people eligible to give blood, contrary to existing systematic reviews investigating TTIs in the general population [7, 14, 15]. Given that several countries have recently changed their deferral policies, and new studies have become available, the previous version of this review [13] is outdated, and an update is justifiable and timely.

Three types of relevant studies include (1) 'Type I studies', comparing the prevalence of TTI markers in blood products of MSM versus non-MSM donors or people eligible to give blood; (2) 'Type II studies', comparing prevalence, incidence and/or residual risk in blood donors before versus after a change in the MSM deferral period; and (3) 'Type III studies', comparing the number of MSM in infected blood donors versus non-infected blood donors (case-control studies).

## MATERIALS AND METHODS

The current systematic review, reported according to the PRISMA statement [16] (see Data S1, pp. 2–4), is an update of De Buck et al. [13]. No protocol was published beforehand, but we followed the methodology by De Buck et al. [13], with two exceptions: (1) a range of European countries (i.e., Belarus, Bulgaria, Cyprus, Czech Republic, Hungary, Ireland, Moldova, Poland, Romania, Russia, Slovakia, Turkey, Ukraine, United Kingdom) was added to the inclusion criteria to include all European countries; and (2) the presence of HTLV-I/II markers in blood donors was included as a relevant outcome. Titles and abstracts of the references obtained during the previous search up until 26 March 2014 [13] were screened for the additional countries and HTLV-I/II markers, but none of those studies were deemed relevant for inclusion in this review.

### Search strategy and study selection

MEDLINE (PubMed interface), Embase, Cochrane Central Register of Controlled Trials, Cinahl and Web of Science were searched from 1 January 2014 to 3 February 2022 (the previous search by De Buck

et al. [13] dates from 26 March 2014). The search strategies are provided in Data S1 (pp. 5–7). Study selection was performed in parallel by two independent reviewers (V.B. and N.S.) using EndNote X9 (see PRISMA flow chart, Figure 1). Reference lists of included studies and the first 20 related items in PubMed were scanned for potentially relevant studies (no additional studies were included via these routes).

## Selection criteria

### Population

Inclusion: blood donors or people eligible to give blood, living in areas most relevant for the blood service of the Belgian Red Cross: Australia, Canada, Europe, New Zealand, USA. Exclusion: a population containing, but not exclusively consisting of, either blood donors or people eligible to give blood.

### Intervention/risk factors

Inclusion: MSM after 1977 (Type I and III studies) or a change in MSM deferral policy (Type II studies).

### Comparison

Inclusion: men *not* reporting sex with other men or the general donor population (Type I studies), occurrence of TTIs before a change in

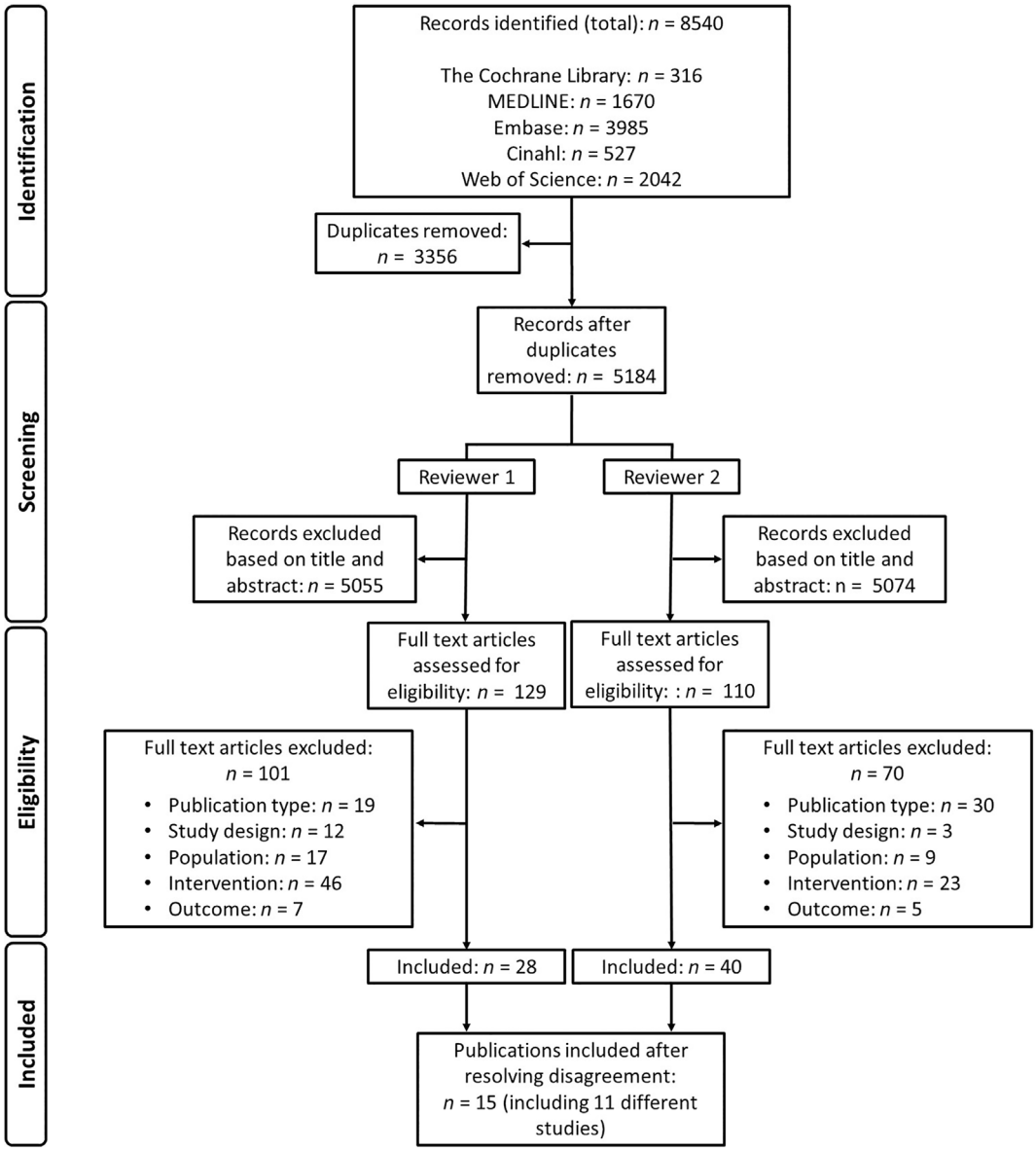


**FIGURE 1** PRISMA flowchart. Fifteen publications were retained (9 research articles and 6 conference abstracts), reporting about 11 different observational studies.

**TABLE 1** Overview of four observational Type I studies.

| Study | MSM | Control | HIV | HBV (anti-HBC) | HBV (HBsAg) | HCV | Syphilis | HTLV-I/II |
|---|---|---|---|---|---|---|---|---|
| Miller, 2018, USA [19] | 520 MSM donors who were previously deferred under permanent MSM deferral, but allowed to donate under 1-year MSM deferral | 4,829,679 donors | **OR: 3.00 [1.44; 6.38]** ('all infectious diseases' combined) | | | | | |
| | 72 MSM donors who returned to donate after their 1-year MSM deferral was terminated | | **OR: 6.30 [1.55; 25.84]** ('all infectious diseases' combined) | | | | | |
| Sanchez, 2005, USA [20] | 92 MSM donors with last contact ≤1 year ago | 24,563 non-MSM donors | **OR: 2.90 [1.50; 5.80]** (HIV, HBV, HCV, syphilis, HTLV-I/II, ALAT combined) | | | | | |
| | 54 MSM donors with last contact 1–5 years ago | | OR: 4.30 [0.90; 21.70] (HIV, HBV, HCV, syphilis, HTLV-I/II, ALAT combined) | | | | | |
| | 3 of 134 MSM donors with last contact >5 years ago | | OR: 1.00 [0.50; 2.20] (HIV, HBV, HCV, syphilis, HTLV-I/II, ALAT combined) | | | | | |
| Alpaugh, 1985, Israel [21] | 10 MSM eligible to give blood with >1 sexual partner during last 3 years (**'high risk'**) | 209 donors | **RR: 36.62 [4.63; 289.33]** | **RR: 37.51 [4.75; 296.45]** | NA | – | – | – |
| | 5 MSM eligible to give blood with 0–1 sexual partner during last 3 years (**'low risk'**) | | RR: 4.88 [0.21; 144.41] | RR: 5.00 [0.21; 147.21] | NA | – | – | – |
| van Bilsen, 2019, The Netherlands [22] | 183 MSM eligible to give blood with anal intercourse with casual partner(s) without condom use during preceding year (**'medium-high risk'**) | 583 age-matched repeat male donors | NA | **RR: 9.03 [3.61; 22.55]** | RR: 9.52 [0.39; 232.73] | NA | **RR: 34.91 [1.94; 628.40]** | NA |
| | | 361 new male donors | NA | **RR: 4.19 [1.84; 9.53]** | RR: 9.52 [0.39; 232.73] | RR: 0.66 [0.03; 16.02] | RR: 4.93 [0.97; 25.17] | NA |
| | 197 MSM eligible to give blood with no anal intercourse/monogamous relationship with a steady male partner (including condomless sex with that partner)/ consistent condom use during anal intercourse with casual partner(s) during preceding year (**'low risk'**) | 583 age-matched repeat male donors | NA | **RR: 7.40 [2.91; 18.81]** | RR: 8.85 [0.36; 216.33] | NA | NA | NA |
| | | 361 new male donors | NA | **RR: 3.44 [1.48; 7.96]** | RR: 8.85 [0.36; 216.33] | RR: 0.61 [0.02; 14.89] | RR: 0.37 [0.02; 7.58] | NA |

*Note*: Risk ratio (RR) or odds ratio (OR) with 95% confidence intervals between brackets. In some cases, RR was not estimable due to zero cases (NA). Data on the prevalence of markers for human immunodeficiency virus (HIV), hepatitis C virus (HCV), syphilis, human T-lymphotropic virus I/II (HTLV-I/II), HBV core antibody (anti-HBC), HBV surface antigen (HBsAg) and/or alanine aminotransferase (ALAT) were collected under permanent [20, 21] or 1-year [19, 22] male blood donors reporting sex with another man (MSM) deferral. Statistically significant results are shown in bold font ($p < 0.05$). A detailed description of population characteristics and applied testing methods is available under 'Characteristics of included studies (Data S1, pp. 8–20). Separate data for first-time and repeat donors are provided in [20]; the prevalence of recent infections (0%–1%) is provided in [22].

MSM deferral policy (Type II studies) or (male) donors with a negative or false-positive test result (Type III studies).

## Outcome

Inclusion: prevalence, incidence or estimated residual risk of markers of HIV, HBV, HCV, Chlamydia, Treponema pallidum (causing syphilis) and human T-lymphotropic virus I/II (HTLV-I/II).

## Study design and publication type

Inclusion: (randomized) controlled trials, before-and-after, cohort, case-control, cross-sectional. Exclusion: non-controlled studies, case reports, case series, letters, comments, opinion pieces, narrative reviews, modelling studies (i.e., estimations/predictions of infection risk under hypothetical scenarios, such as a potential MSM deferral period).

## Data collection and synthesis

Study design, population, comparisons or risk factors, outcome measures (relative occurrences of TTI markers expressed as risk ratio, odds ratio (OR), incidence rate ratio; or changes in incidence or residual risk, Tables 1–4) and study quality were extracted by two reviewers (V.B. or E.D.B. and N.S.). If the size of infected and total samples was provided for each comparison group, risk ratio, 95% confidence interval (CI) and *p*-value (from the test for overall effect or 'Z-test') were calculated using Review Manager [17]. In addition, we extracted information on how 'MSM' was defined in each study questionnaire (e.g., 'ever' or 'during past year'), timing of data collection and under which MSM deferral policy (if the deferral policy was not mentioned, this information was added by the reviewers based on timing of data collection). All extracted information is available in Data S1 (pp. 8–33). Data were not pooled in meta-analyses due to heterogeneity of the studies. Instead, data were grouped according to the type of comparison (Type I, II and III) and narratively synthesized.

**TABLE 2** Prevalence per million donations from eight observational Type II studies.

| Study | Donor population (*N*) | Deferral period for MSM | | | | | Relative risk (long/short deferral) [95% CI] |
|---|---|---|---|---|---|---|---|
| | | Perm. | 5 y. | 1 y. | 3 m. | IRA | |
| **TTIs combined (HBV, HCV, HIV and syphilis)** | | | | | | | |
| Davison, 2021, UK [23] | Male (11,979,739) | 141 | – | 131 | – | – | 1.07 [0.97; 1.18] |
| | Male (recent infections) | 17 | – | 17 | – | – | 1.00 [0.76; 1.32] |
| **HIV** | | | | | | | |
| Seed, 2010, Australia [31] | Male (4,698,823) | 7.6 | | 5.0 | – | – | 1.51 [0.73; 3.15] |
| O'Brien, 2016, Canada [29] | Male (3,012,607) | 4.1 | 3.8 | – | – | – | 1.06 [0.32; 3.53] |
| O'Brien, 2019, Canada [30] | All (NA) | 0.05–5.1 | 2.0–5.3 | 0.0–3.5 | – | – | – |
| Caffrey, 2022, Canada [28] | All (10,212,709) | 3.4 | 3.6 | 2.5 | 2.5 | – | 1.00 [0.28; 3.55][a] |
| Grebe, 2020, USA [25] | Male first-time (2,282,961) | 121 | – | 150 | – | – | 0.81 [0.64; 1.02] |
| Steele, 2020, USA [32] | Male first-time\|*repeat* (2,655,619\|*11,917,140*) | 120\|*209* | – | Y1: 133\|*209*<br>Y2: 134\|*146*<br>Y3: **169**[b]\|*166* | – | – | – |
| Suligoi, 2013, Italy [1] | First-time (860,793) *repeat (3,642,498)* | 161<br>*30* | – | – | – | 138<br>*39* | 1.17 [0.68; 2.01]<br>*0.96 [0.61; 1.51]* |
| **HBV** | | | | | | | |
| Steele, 2020, USA [32] | Male first-time\|*repeat* (2,655,619\|*11,917,140*) | 408\|*10* | – | Y1: 447\|*11*<br>Y2: 439\|*8*<br>Y3: 406\|*7* | – | – | – |
| **HCV** | | | | | | | |
| Steele, 2020, USA [32] | Male first-time\|*repeat* (2,655,619\|*11,917,140*) | 1136\|*37* | – | Y1: 1211\|*39*<br>Y2: 1099\|*42*<br>Y3: 1048\|***27***[b] | – | – | – |

*Note*: Outcomes include markers for human immunodeficiency virus (HIV), hepatitis B or C virus (HBV or HCV) under different male blood donors reporting sex with another man (MSM) deferral policies (i.e., permanent [perm.], 5 years [5 y.], 1 year [1 y.], 3 months [3 m.] or individual risk assessment [IRA]). Statistically significant results are shown in bold font ($p < 0.05$). The last column shows relative risk (RR) with a 95% confidence interval (CI) (RR < 1 reflects a relative increase in prevalence after implementing the new deferral). In some studies, data were collected per year (e.g., the first year after the change in deferral [Y1], etc.). Davison et al. [23] provided data for HIV, HBV, HCV and syphilis combined (shown here) and separately (Data S1, pp. 27–30).

[a]RR for change from 1-year to 3-month MSM deferral (see Data S1, pp. 26 and 30 for other deferrals' RRs).

[b]Steele et al. [32] found a statistically significant increase in HIV prevalence (and decrease in HCV prevalence) before versus the third year after implementation of the 1-year MSM deferral period, but it should be noted such a statistically significant increase/decrease was also found in first-time female donors.

**TABLE 3** Residual risk per million donations from three observational Type II studies.

| Study | Donor population | Deferral period for MSM | | | | |
|---|---|---|---|---|---|---|
| | | Perm. | 5 y. | 1 y. | 3 m. | IRA |
| **HIV** | | | | | | |
| Caffrey, 2022, Canada [28] | All | 0.05 | 0.07 | 0.08 | 0.05 | – |
| Grebe, 2020, USA [25] | Male first-time (RBC)<br>Male first-time (plasma) | 0.52<br>0.84 | – | 0.63<br>1.03 | – | – |
| Steele, 2021, USA [27] | All repeat | 0.55 | – | 0.39–0.47 | – | – |
| **HBV** | | | | | | |
| Steele, 2021, USA [27] | All repeat | 0.49 | – | 0.56–0.58 | – | – |
| **HCV** | | | | | | |
| Steele, 2021, USA [27] | All repeat | 0.30 | – | 0.30–0.43 | – | – |

*Note*: Residual risk is the risk of undetected infection after donor selection and testing, calculated by multiplying the daily incidence rate in blood donors with the length of the infectious window period. Outcomes are markers for human immunodeficiency virus (HIV), hepatitis B or C virus (HBV or HCV) under different male blood donors reporting sex with another man (MSM) deferral policies (i.e., permanent [perm.], 5 years [5 y.], 1 year [1 y.], 3 months [3 m.] or individual risk assessment [IRA]).
Abbreviation: RBC, Red Blood Cells.

## Certainty of evidence

Two reviewers (V.B. and N.S.) independently assessed the certainty of evidence in duplicate using the GRADE approach (see Data S1, pp. 42) [18].

# RESULTS

## Study selection and study characteristics

Figure 1 contains a flowchart of the study identification and selection process. Fifteen publications were retained (9 research articles and 6 conference abstracts), reporting about 11 different observational studies (2 Type I, 7 Type II and 2 Type III studies). The original 2015 systematic review [13] identified 14 studies (2 Type I, 2 Type II and 10 Type III studies), yielding a total amount of 25 studies (4 Type I, 9 Type II and 12 Type III studies), summarized in Tables 1–4. Studies were performed in Australia, Canada, Denmark, France, Israel, Italy, Switzerland, The Netherlands, UK or USA. The methods used to screen for TTIs varied between studies.

## Synthesis of findings

### Type I studies: Comparing the prevalence of TTIs in MSM versus non-MSM donors

Four studies compared the prevalence of different TTIs in MSM versus non-MSM donors or people eligible to give blood (Table 1). (Eligible) donors reporting MSM had a statistically significantly increased risk of overall TTIs [19, 20], HIV [21], HBV (based on HBV core antibody, but not on HBV surface antigen) [21, 22] and syphilis [22]. An increased risk of HCV could not be demonstrated, and the relative risk for HTLV-I/II was not estimable due to zero observed cases during the study period [22].

The study by Miller et al. [19] compared 'reinstated' MSM donors who gave blood after changing the MSM deferral period from permanent to 1 year or after termination of their 12-month MSM deferral versus the general blood donor population (without adjustment for confounders). Sanchez et al. [20] subdivided MSM into three groups based on the time since last male-to-male sexual contact (i.e., ≤12 months, 12 months–5 years or >5 years), and only found a statistically significantly increased risk of TTIs in the group with last MSM contact within 12 months before donation, compared to male non-MSM blood donors (adjusted for first-time or repeat donor status and test seeking in weighted logistic regression models).

The studies by Alpaugh et al. [21] and van Bilsen et al. [22] subdivided MSM who qualified as potential donors into subgroups based on sexual risk behaviour. For 'high-risk' sexual behaviour (see Table 1 for definitions), there is a statistically significantly increased risk of HIV [21], syphilis [22] and HBV (core antibody but not surface antigen) [21, 22]. In eligible donors with 'low-risk' male-to-male sexual behaviour (see Table 1 for definitions), statistically significantly increased risks of HIV, syphilis and HBV surface antigen could not be demonstrated [21, 22]. The study by van Bilsen et al. [22] did show a statistically significantly increased risk of HBV core antibody in the low-risk group, but this effect could not be demonstrated in the smaller study by Alpaugh et al. [21]. In addition, the observed increased risk in the low-risk group may reflect past behaviour (i.e., prior to the year in which sexual risk behaviour was assessed) given that (1) there was no evidence for increased risk of *recent* HBV infections in the low-risk group (recent was defined as antibody seroconversion within the preceding year) and (2) the fact that the presence of HBV core antibody may point to either current or past infection, whereas HBV surface antigen (which was not statistically significantly increased in low-risk MSM) indicates current HBV infection. Based on these two

**TABLE 4** Twelve Type III (case-control) studies.

| Study | Donor population | Risk factor | Outcome | | | | |
|---|---|---|---|---|---|---|---|
| | | | HIV | HBV (anti-HBC) | HBV (HBsAg) | HCV | HTLV-I/II |
| Allison, 2012, USA [47] | 469 cases versus 217 false-positive controls | MSM | – | – | – | **RR: 8.79 [1.18; 65.25]** | – |
| Bruhn, 2021, USA [34, 38] | 224 HIV cases, 11 male recent HBV cases, 18 male recent HCV cases, 553 false-positive controls (for HBsAg or anti-HIV) | MSM or sex with MSM during the past 12 months | **aOR: 16.7 [3.8; 74.4]** | – | – | OR: 1.50 [0.40; 5.60] | – |
| Busch, 1994, USA [35] | 129 cases versus 131 age-matched controls (all males) | MSM | **OR: 45.0 [10.66; 189.84]** | – | – | – | – |
| Christensen, 2001, Denmark [39] | 37 HBV cases versus 553 false-positive controls | Sex with homo/bisexual male | – | aOR: 5.44 [0.52; 50.20] | – | – | – |
| Custer, 2014/5, USA [36, 37] | 149 HIV cases, 190 HBV cases, 45 HTLV-I/II cases versus 761 false-positive controls (all males) | MSM or sex with MSM | **aOR: 62.3 [27.6; 140.4]** | – | / (HBV NAT) | – | RR: 2.60 [0.61; 11.18] |
| Kaldor, 1992, Australia [40] | 220 cases versus 210 false-positive controls | MSM | – | – | – | RR: 6.90 [0.83; 150] | – |
| Moore, 1997, UK [41] | 35 cases versus 11 controls matched on age, sex, race/ethnicity, blood centre, first-time/repeat donor status | Homosexuality | – | RR: 1.67 [0.09; 32.33] | – | – | – |
| Murphy, 2000, USA [42] | 758 cases versus 1039 controls | Men with 1-lifetime male sexual partners | – | – | – | aOR: 0.9 [0.2; 4.4] | – |
| | | Men with ≥2-lifetime male sexual partners | – | – | – | aOR: 1.1 [0.2; 5.4] | – |
| Neal, 1994, UK [43] | 35 cases versus 150 controls matched on age, sex, venue of donation | Male with a homosexual sex partner | – | – | – | aRR: 7.0 [0.9; 5.5] | – |
| Serfaty, 1993, France [44] | 35 cases versus 35 controls matched on age and sex (all without prior transfusion or intravenous drug abuse) | Homosexuality | – | – | – | RR: 7.0 [0.37; 130.69] | – |
| Tullen, 1993, Switzerland [45] | 68 cases versus 103 controls with high ALAT levels | Homosexuality | – | – | – | NA | – |
| Van der Poel, 1991, The Netherlands [46] | 45 cases versus 761 false-positive controls | MSM | – | – | – | OR: 5.05 [0.42; 60.20] | – |

*Note*: Risk ratio (RR), odds ratio (OR) or adjusted RR/OR (aRR/aOR), with 95% confidence intervals between brackets. Adjustments were made for donor sex and sexual orientation [34, 38]; age, gender, tattoos, imprisonment, stay in endemic areas, operative procedures, number of sexual partners, commercial sex and sexual partners from endemic regions [39]; donor status, age group, race/ethnicity, income, sex, injecting drug use, detention, tattoo, piercing, other sexual risk behaviours, donation period and missing categories for 'low income' and 'sex with HIV-positive partner' [36, 37]; or intravenous drug use [42, 43]. Data on the prevalence of markers for human immunodeficiency virus (HIV), hepatitis C virus (HCV), human T-lymphotropic virus I/II (HTLV-I/II) or hepatitis B virus (HBV) core antibody (anti-HBC) or HBV surface antigen (HBsAg) were collected under permanent (all studies) and/or 1-year [34, 38] male blood donors reporting sex with another man (MSM) deferral period. Statistically significant results are shown in bold font ($p < 0.05$). In some cases, OR was not estimable due to zero cases (NA), the particular outcome was not assessed (–) or the particular outcome was assessed without providing data (/).

studies, there is a limited amount of research that differentiates low- versus high-risk male-to-male sexual history.

## Type II studies: Comparing prevalence, incidence or residual risk of TTI markers in donors before versus after changing MSM deferral strategy

Nine studies assessed changes in prevalence (number of cases divided by total number of donations during a given period; Table 2), incidence (rate of *new* cases during a given period; Data S1 pp. 27–39) and/or residual risk (Table 3) for markers of TTIs in blood donors after a change in the MSM deferral period. One of those studies suggested that shortening the MSM deferral period from permanent to 1 year did not affect the TTI rate (HIV, HBV, HCV and syphilis combined) in male donors [23]. In eight other studies, focusing on the implementation of a 5-year (three studies), 1-year (five studies), 3-month (one study) or individual risk assessment (IRA) (one study) [1, 23–31], significant effects of changing the MSM deferral period could not be demonstrated. However, the low population risk and use of stringent blood donor eligibility criteria result in very low baseline TTI prevalence in blood donors, making it difficult to detect possible effects of easing the deferral. Indeed, despite the large sample sizes (1–3.5 million donors), studies had imprecise effect estimates (i.e., wide confidence intervals) [1, 25, 27–29, 31] due to low numbers of cases (i.e., 4–212 detected infections during the study period before or after the change). The results of those eight studies should thus not be seen as evidence for the absence of a change in TTIs after shortening the MSM deferral period. The 2020 study by Steele et al. [32] did report a statistically significant increase in HIV prevalence (and decrease in HCV prevalence) for first-time male donors during the period before versus the third year after the implementation of the 1-year MSM deferral period, but such an increase/decrease was also found in first-time female donors.

Four studies assessed changes in the proportion of infected (male) donors reporting MSM before versus after shortening the deferral. For HIV, three out of four studies found the percentage of infected donors reporting MSM to numerically increase after implementation of the 1-year MSM deferral (not statistically significant; 39%–51% [23]; 13%–38% [31]) or IRA (11%–22% [1]), whereas another study reported that none of the three HIV cases that were detected after the implementation of a 1-year deferral reported MSM [30]. For syphilis, the data by Davison et al. [23] suggested a statistically significant increase in the percentage of cases reporting MSM after the introduction of the 1-year deferral (from 7% to 13%; based on own calculations). For HCV or HBV, statistically significant effects of implementing the 1-year deferral on the proportion of positive donors reporting MSM could not be demonstrated (i.e., from 0.4% to 0.7% or 0.9% to 1.7%, respectively) [23].

The MSM deferral periods that are currently most relevant for informing policy in Western countries are the relatively short deferrals (e.g., 3–4 months) and IRA. However, only two studies assessed TTI risk under these policies. One (Italian) study [1] reported HIV prevalence under IRA as 60 cases per million donations, which was numerically albeit not statistically significantly, higher than under permanent MSM deferral (44 cases per million donations). Relative risk (i.e., risk under permanent deferral/risk under IRA) was 0.73, 95% CI [0.52–1.03] in all donors, indicating an estimated increase in HIV prevalence by factor 1.4 under IRA (1.3-fold decrease to 1.9-fold increase). Another included study [28] compared HIV risk before versus after the implementation of a 3-month MSM deferral period in Canada. Prevalence of an HIV-positive test in blood donors was found to be low (2.52 cases/million donations), with a relative risk of 1.00, 95% CI [0.28–3.55], implying that the true effect may range from a threefold decrease to a threefold increase in prevalence (with 95% certainty). Residual risk of transmission via transfusion was estimated at 0.05 cases per million donations (95% CI [0.001; 0.37]), compared to 0.08 cases per million donations (95% CI [0.02–0.22]) under 1-year deferral. Residual risk refers to the risk of undetected infection after donor selection and testing and was simulated by multiplying the daily incidence rate of HIV in blood donors with the length of the infectious window period (i.e., the time during which an infectious blood donation may not be detected by testing with nucleic acid multiplex in 6-unit minipools [33]).

## Type III studies: Comparing infected versus non-infected donors for risk factors

Three out of 12 studies included HIV, all of which identified MSM as a risk factor for HIV [34–37] (Table 4). Only one of these studies adequately controlled for confounders (i.e., donor status [first time or repeat], age group, race/ethnicity, income, sex, multiple partners, sex for money or drugs, sex with HIV-positive partner, injecting drug use, detention, jail or group home, tattoo or piercing) [36], whereas the others age-matched controls to cases without control for other confounders [35] or only adjusted odds ratio for donor sex and sexual orientation [34]. Nine studies assessed the risk of HBV, HCV or HTLV-I/II. A statistically significantly increased risk of HBV, HCV and HTLV-I/II could not be demonstrated [36–46]; except for one study, showing that MSM blood donors have a statistically significantly increased risk of HCV compared to non-MSM blood donors, but there was no control for confounding variables [47], as was the case in other studies.

## Certainty of the evidence

In two out of four Type I studies, blood donors [19] or eligible donors [21] who reported MSM were presumably compared to the general donor population (males and females) without controlling for donor sex, age and status (first-time or repeat). Sanchez et al. [20] adjusted ORs for donor status. In van Bilsen et al. [22], MSM who participated in the Amsterdam cohort studies and qualified as potential blood donors were age-matched to repeat male blood donors (but not to new male donors, in which there was no control for existing between-group differences in age). Sanchez et al. [20] used an

anonymous self-report questionnaire on sexual history 30–60 days after donation, possibly leading to the occurrence of recall bias. The study populations in three studies [20–22] were not necessarily representative of MSM blood donors in 2022, due to the year of data collection [20, 21] and/or the fact that MSM were eligible to give blood based on questionnaire responses, but not actual blood donors (donors are expected to self-assess their blood as safe and suitable for a recipient, which may not be the case for non-donors) [21, 22].

In all Type II studies, the risk for TTIs in (male) blood donors was compared in the months-years preceding versus the months-years following a change in MSM deferral policy. Such study design does not allow to control for fluctuations in factors that may affect risk but are unrelated to the MSM deferral change.

For several Type III studies, there was no matching of cases and controls [34, 38–41, 45–47] and no control for confounding variables such as other risk behaviours, donor sex, age and status [38, 40, 41, 46, 47]. Four studies did control for intravenous drug use [42–45], and one for donor sex and sexual orientation [34], but it was unclear whether this was sufficient. Two studies [36, 37, 39] calculated ORs adjusted for age, sex, race, income and/or behavioural risk factors as part of logistic regression analyses, and one study [35] performed subgroup analyses in males reporting MSM with no other behavioural risk factors. Three Type III studies used self-report questionnaires to identify behavioural risk factors [45], two of which were anonymous [39, 42], as well as one Type I study [20]. Other Type III studies used face-to-face or telephone interviews [34–36, 38, 40, 41, 43, 44, 46, 47]. If questionnaires/interviews were completed after donation and blood tests, the occurrence of recall bias cannot be excluded. In addition, interviews come along with a risk of favourable answers (e.g., not revealing risk factors after failure to comply with deferral).

All included studies were observational, resulting in an initial low level of evidence according to the GRADE approach. The level of evidence was downgraded because of the risk of bias (see paragraphs above for limitations in study designs) and imprecision (low number of events compromising statistical power, wide confidence intervals and/or lack of data) (Data S1 pp. 40–48). There were no reasons to downgrade for inconsistency or indirectness or to upgrade the level of evidence. Overall, the certainty of evidence is rated as 'very low' for all outcomes, implying that the estimates of effect are uncertain.

## DISCUSSION

This systematic review summarizes the evidence on the association between MSM (eligible) blood donors or MSM deferral policies and risk for TTIs. The scope of the current systematic review is geographically restricted to Australia, Canada, Europe, New Zealand and USA. The results should therefore not be generalized to areas with different epidemiology of TTIs. Twenty-five different published observational studies are included: 14 of which were retained in 2015 [13] and 11 in 2022.

First, four studies compared blood donors or people eligible to give blood reporting MSM to blood donors not reporting MSM (ever or during the one or three preceding year(s)), suggesting that there may be an elevated risk of TTIs combined, HIV, syphilis and HBV (core antibody but not surface antigen) in MSM (Table 1). Note that the evidence is very uncertain due to study limitations (see 'Certainty of the evidence' above). Studies considering differential risk behaviour among MSM are rare, and two existing studies, one of which is a conference abstract, consist of small samples with few infections that are identified during relatively short follow-up periods. In addition, the subgroups of MSM defined as those with 'high-risk' behaviour in those studies are broader than what is currently being used in blood centres that adopt IRA.

Second, nine studies assessed fluctuations in TTI risk in blood donors after easing the MSM deferral period [1, 23–31]. One study suggested that easing the MSM deferral period to 1 year may have little to no effect on TTI risk. Only one study focused on the effect of changing from a 1-year to 3-month deferral [28] and suggested that this may have little to no effect on HIV prevalence. However, the 95% CI was so wide that a threefold decrease/increase in HIV prevalence could not be ruled out. Another study estimated that adopting IRA may slightly increase HIV prevalence with a factor of 1.4, but not ruling out a twofold increase, compared to permanent deferral [1]. Despite the large sample sizes used in these two latter studies, the imprecision in effect estimates (i.e., wide confidence intervals; see Table 2) can (at least partly) be attributed to the low HIV prevalence in blood donors (which is, in turn, due to the low population risk and the use of stringent blood donor eligibility criteria).

Third, 12 studies compared the proportion of MSM in infected versus non-infected blood donors (Table 4). It was suggested that MSM may be a risk factor for HIV [34–37], but the certainty of evidence was very low. A statistically significantly increased risk of HBV, HCV or HTLV-I/II could not be demonstrated overall [36–46]. None of the Type 3 studies provided information on the time since last MSM contact, number of sexual partners (except [42]) or details on sexual risk behaviour (e.g., anal sex, condom use).

Overall, several research gaps can be identified based on the currently available observational studies, especially when it comes to making practical recommendations.

First, there is a limited amount of data concerning 3-month or risk-based deferral policies. Rather, the vast majority of studies have been performed under a permanent or 1-year MSM deferral period and often included MSM who were non-compliant with the deferral rules. Given that several countries have eased their MSM deferral to 3 months or individual risk-based criteria, data on the safety and effectiveness of these alternative strategies are expected to be underway [3]. Existing modelling studies do estimate HIV prevalence, incidence and/or residual risk in blood or plasma donations under the scenario of a 3-month, 4-month or no MSM deferral period [48–50]. Those modelling studies are not included in this systematic review given that they rely on hypothetical scenarios rather than observed data. Nevertheless, they do provide a valuable contribution to decision-making, especially if potential novel risks (such as PrEP use and monkeypox) are considered.

Second, it would be relevant to gain more insight into variable risk profiles among MSM in eligible blood donors to inform risk-based deferral policies. In this regard, recently acquired infections could be assessed to disentangle the risks associated with recent versus long-ago behaviour, given that the presence of antibodies in blood represents lifetime sexual risk behaviour [22].

Third, studies regarding the risk of HIV transmission by donors using PrEP or antiretroviral therapy (ART) have not been identified (except for one *modelling* study [51]). Given that PrEP and ART are being more frequently used and that this interferes with HIV testing, research concerning the impact of PrEP and ART use on sexual risk behaviour, blood safety and compliance with the donor questionnaire could provide useful for informing future policy-making [52]. In addition, future studies could include the risk of monkeypox transmission.

The current systematic review focuses on observational studies concerning TTI risk in MSM blood donors and after easing the MSM deferral. When informing policy-making, other sources of currently available information should be used as well, including findings on compliance and donor comfort when using alternative questions [11, 53, 54], modelling studies [48–50] and surveillance reports [55, 56]. In addition, other criteria, including the feasibility and cost-effectiveness of alternative deferral periods, should be further examined and considered when making decisions.

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## CONFLICT OF INTEREST STATEMENT

All authors are employees of Belgian Red Cross-Flanders, responsible for supplying adequate quantities of safe blood products to hospitals in Flanders and Brussels.

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## SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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# The impact of donor biological variation on the quality and function of cold-stored platelets

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## Abstract

**Background and Objectives:** Room temperature-stored platelets (RTPs) maximize platelet viability but limit shelf life. The aims of this study were to investigate the impact of donor variability on cold-stored platelets (CSPs) and RTP, to determine whether RTP quality markers are appropriate for CSP.

**Materials and Methods:** Double platelet donations ($n = 10$) were collected from consented regular male donors stored in 100% plasma. A full blood count, donor age, weight, height and body mass index (BMI) were collected at the time of donation. Platelet donations were split equally into two bags, and assigned to non-agitated CSP or agitated RTP. The quality and function of platelets were assessed throughout the standard 7 days of storage and at expiry (day 8). Non-parametric statistical analyses were used to analyse results given the small sample size.

**Results:** As expected, there were significant differences between CSP and RTP throughout storage including a reduction in CSP concentration as well as a loss of swirling. Furthermore, a significant increase in CSP exhibiting activation and apoptotic markers was observed. Platelet concentrations were further impacted by donor BMI, and donors with the highest BMI (>29) had the lowest platelet concentration and activation response at the end of CSP storage.

**Conclusion:** Platelet quality and functionality play a vital role in transfusion outcomes; however, blood components are inherently variable. This study demonstrated, for the first time, the specific impact of donor BMI on CSP quality and function and highlights the requirement for novel quality markers for assessing CSPs.

## Keywords

apheresis platelets, BMI, cold storage, donor biological variation, haemostatic function

## Highlights

- Donor body mass index (BMI) may impact platelet quality and function.
- Current platelet quality markers are not appropriate for cold-stored platelets (CSPs).
- Donor BMI impacts CSP activation.

## INTRODUCTION

Platelet transfusions are an essential tool to restore normal haemostasis in haemorrhagic patients with thrombocytopenia or functionally abnormal platelets. However, despite significant advances in the last decades, questions persist about the appropriate transfusion dose, the quality of platelet components, storage conditions and the risk of bacterial growth. The recent blood supply and donation challenges during and since the global COVID-19 pandemic, coupled with the ever-present challenge of balancing donation with blood supply, have led to the exploration of new contingency measures which may expand the repertoire of blood components available for transfusion, such as cold-stored platelets (CSPs) [1, 2]. Refrigerated platelets were routinely used in the 1970s to treat actively bleeding patients [3]. However, CSPs' short survival time in circulation limited their utility and they were replaced with room temperature-stored platelets (RTP) [4, 5]. RTP storage conditions maximize platelet viability but limit shelf life to between 5 and 7 days, as room temperature (RT) storage increases the risk of bacterial transfusion-transmitted infection [6, 7]. In addition, RTP storage can irreversibly compromise platelet's functionality, resulting in platelet aggregation in vivo and platelet storage lesion (PSL) in vitro [8]. The efficacy of RTP has been extensively tested on thrombocytopenic patients, but the evidence of their effectiveness in other clinical settings, such as critical bleedings, is still less well documented [9].

The clinical applications of CSP are now being re-explored, as they may overcome some of the logistical limitations of RTP, especially in the military and trauma settings. The cold temperature reduces the platelet's metabolism thereby negating the need for constant agitation during storage, enabling easier transportation and longer storage [10, 11]. However, cold storage induces a unique series of physiological changes in platelets, referred to as 'cold storage lesion' including shape and structural changes, such as membrane rafting and clustering of the GP1b receptor, followed by an increased externalization of phosphatidylserine (PS) on the outer layer of the platelet membrane [11–16]. PS exposure induced by platelet activation leads to increased apoptosis, resulting in accelerated platelet clearance from the circulation in vivo. In contrast, these changes lead to procoagulant platelets with important haemostatic effect [15, 17–19]. However, at present, there is a lack of controlled clinical trials investigating CSP applications in vivo [20]. The United States (U.S.) Food and Drug Administration (FDA) has issued regulatory requirements allowing for the use of CSP for up to 14 days. The Mayo Clinic has implemented a programme for transitioning from RTP, at expiry, to CSP, which may be used for transfusion in trauma patients showing excellent clinical outcomes and a reduction in wastage [21].

Standard production and clinical practice guidelines for the efficient and safe use of CSP in blood transfusion in Europe are in development [2]. However, further information is required to appropriately evaluate their quality, as well as the impact that donor biological variation may have on the quality and function of CSP. Donors' biological variation has been previously reported as the greatest source of variability in stored blood components [22]. For example, body mass index (BMI) from overweight and obese male donors has a significant impact on the haemolysis level in stored red cells [23]. Furthermore, factors such as age, sex and BMI have been shown to impact the development of PSL [24, 25]. The high degree of variation in platelet responsiveness between donors has been shown to impact platelets' apheresis function, as well as their levels of activation [26–28]. Platelets from older males have a reportedly different metabolome compared with those obtained from females and younger males, implying increased energy metabolism [29]. Lastly, collection time may also be a factor impacting platelet activation state among apheresis donors [30], and it is widely accepted that donor characteristics influence room temperature storage [25, 31, 32].

Despite these reported differences, the current clinical trial data suggest that variation in donor platelet function does not affect the outcome of prophylactic transfusion [33]. It is clear that the factors impacting donor-related platelet responsiveness are not fully understood. There is some evidence that higher BMI leads to changes in platelet characteristics; however, the underlying mechanisms impacting this are not fully understood [34]. The aims of this study were to assess the impact of donor variation on apheresis-derived CSP and RTP, based on these assessments, determine whether RTP quality markers are appropriate for CSP.

## MATERIALS AND METHODS

### Study population and ethical approval

Double platelet donations ($n = 10$) were collected from regular male apheresis donors attending the Irish Blood Transfusion Service (IBTS) donation clinic. Ethical approval was obtained from Trinity College Dublin Research Ethics Committee. All donors were provided with a Donor Information Leaflet and informed consent was given prior to donation. Donors were randomly selected based on donation eligibility and their blood type, and none of them withdrew. All donors were male, blood group O and RhD positive. In addition to the apheresis donation, a full blood count (FBC), the mean platelet volume (MPV), donor age (years), weight (kg) and height (m) were collected for the study. BMI was calculated in Excel.

### Platelet collection and storage

Apheresis donations were collected in standard polyvinyl chloride bags using the Trima Accel Automated Blood Collection System (Terumo) and gamma irradiated by the Gammacell 3000 Elan blood irradiator, as per manufacturers' instructions. Each platelet donation was split equally into two bags, stored in 100% plasma, and randomly assigned to non-agitated CSP ($n = 10$) or agitated RTP ($n = 10$). The CSPs were stored at 4°C within 1 h of donation, and the RTPs were stored at 22°C under continuous gentle agitation in a flat-bed temperature-controlled agitator (Helmer). Both CSP and RTP were stored for 7 days, as per standard of care, and deemed expired on day 8. CSP and RTP were stored overnight before testing on day 1.

## Platelet assessment

On day 1, day 4 and day 8 of storage, an aliquot (20 mL) was aseptically retrieved from each donor bag. Platelets stored at 4°C were left at RT for 5–10 min under agitation before sampling. All assessments were carried out as per manufacturers' guidelines, unless otherwise stated. The quality and function of the platelets on days 1, 4 and 8 (expiry) were assessed using the following techniques.

### Quality

FBC, which enabled calculation of the MPV and platelet count, was carried out using a Sysmex XN haematology analyser. Visual swirling assessment scored from 0 (no swirling) to 3 (optimal swirling) as per Bertolini and Murphy [35]. Platelet aggregates were assessed with the score system for size (score A: 0 <1 mm to 4 >3 mm) and number (score B: 0 no aggregates to 4 >20) as recommended by van der Meer et al., where the acceptable global score for aggregates is <5 [36]. The BacT/Alert 3D system was used as a quality control measure to exclude bacterial contamination in either CSP or RTP at expiry only (day 8).

### Metabolic activity

The oxygen ($PO_2$) and carbon dioxide ($PCO_2$) partial pressures, and pH levels, were measured using a blood gas analyser (Roche Omni S.). The platelet glucose concentration was measured using the Roche Cobas c311 automated analyser.

### Platelet apoptosis—PS exposure analysis

RTP and CSP were acquired with a FACSCanto II benchtop flow cytometer system (BD Biosciences) and required a minimum of 10,000 platelet events. Apoptotic platelets with membrane expression of PS were detected using fluorescein isothiocyanate (FITC)-conjugated Annexin V (BD Bioscience). The maximum platelet expression of PS, and thus the maximum binding potential to Annexin V, was calculated following the addition of a calcium ionophore (Sigma-Aldrich) to the positive control. A calcium-free phosphate-buffered saline (PBS) solution was used as the assay's negative control. Platelet concentrate samples were washed in PBS and centrifuged at 500*g* for 5 min (Biorad® Diacent 12). 1× working solution of Annexin V binding buffer (BD Bioscience) was used to suspend the positive control/sample under test, and flow cytometric analysis was carried out within an hour of staining with the FITC-labelled antibody at medium flow.

### Platelet activation—CD62P expression

CSP and RTP were diluted to a final platelet concentration of $200 \times 10^6$/mL and incubated with 5 mM of the agonist thrombin receptor activating peptide (TRAP) (Sigma-Aldrich) to measure platelets in vitro responsiveness to the agonist and maximum activation response. Briefly, 4.4 µL of 5 mM TRAP was diluted to a final concentration of 40 µM in N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES)-buffered saline. Prior to staining, the platelets were fixed with 1% CellFIX™ (BD Bioscience) to stop further activation and incubated a second time with CD62P FITC-CD62P antibody (BD Bioscience) to detect activated platelets expressing CD62P. A negative control containing FITC-IgG monoclonal antibody (BD Bioscience) was included for each analysis to control for auto-fluorescence and non-specific binding. Samples were analysed immediately at low flow rate.

## Statistical analysis

Statistical analyses were carried out using IBM SPSS statistical package version 25.0. Microsoft Excel was used to manage data, tabulate results and build graphs. The Wilcoxon signed rank test performed paired analysis between RTP and CSP quantitative parameters. Friedman's test assessed the significance of differences between repeated measures of each platelet's parameter. Pearson correlations were performed to test the significance of the relationship between donor and platelet parameters. Chi-squared analysis was used to compare quality assessment markers.

**TABLE 1** Donor population.

| Donor demographic | *N* | Mean | Median | Maximum | Minimum |
|---|---|---|---|---|---|
| Gender—male | 10 | | | | |
| Blood group—O Rh+ | 10 | | | | |
| Age (years) | | 49.0 ± 9 | 49.0 | 63.0 | 37.0 |
| Body mass index (kg/m$^2$) | | 29.5 ± 3.8 | 29.0 | 37.1 | 24.5 |
| Underweight <18.5 | 0 | | | | |
| Normal weight <18.5–24.9 | 1 | | | | |
| Overweight 25–29 | 5 | | | | |
| Obese ≥30 | 4 | | | | |
| Platelet count (>200 × 10$^9$ cells) | | 253.0 ± 39 | 253.0 | 324.0 | 197.0 |
| Mean platelet volume (fL) | | 10.0 ± 0.6 | 10.0 | 10.8 | 9.0 |

# RESULTS

## Study participant demographics

The donor study population comprised 10 paired apheresis platelet donations collected from 10 regular male donors with blood group O Rh(D) positive, which, given the limited sample size, allowed for the exclusion of both gender and blood group as confounding variables. Donor demographics and platelet assessments on the day of donation are listed in Table 1. All donor platelet counts at day 0 were within the standard acceptance criteria of >200 × $10^9$ cells/L [7]. Platelet donations were all collected before noon in an attempt to mitigate collection time impacting our results.

## Comparison of room temperature and CSPs

As expected, there were significant differences between CSP and RTP throughout storage ($p < 0.05$). A significant drop in platelet concentration was observed in CSP following the first day of storage, where

**TABLE 2** Room temperature and CSP quality and functional parameters throughout 8 days of storage.

| Platelet parameter | Day of storage | RTP | | CSP | | Paired analysis ($p$ value) |
|---|---|---|---|---|---|---|
| | | Median | $p$ values | Median | $p$ values | |
| Swirling | 1 | 3 | – | 0 | – | <0.05* |
| | 4 | 3 | – | 0 | – | <0.05* |
| | 8 | 2 | – | 0 | – | <0.05* |
| Aggregates score | 1 | 0 | – | 0 | – | N/S |
| | 4 | 0 | – | 5 | – | <0.05* |
| | 8 | 0 | – | 5 | – | <0.05* |
| PLT concentration ($10^9$ cells/L) | 1 | 884 | | 983 | | 0.022* |
| | 4 | 917 | | 750 | | 0.017* |
| | 8 | 953 | 0.001 | 686 | 0.003* | 0.005* |
| MPV (fL) | 1 | 9.4 | | 10.4 | | 0.005* |
| | 4 | 9.4 | | 10.1 | | 0.005* |
| | 8 | 10.0 | <0.0001* | 9.6 | <0.0001* | 0.005* |
| Glucose (mmol/L) | 1 | 18.7 | | 19.1 | | 0.029* |
| | 4 | 16.2 | | 18.6 | | 0.005* |
| | 8 | 11.0 | <0.0001* | 16.9 | <0.0001* | 0.005* |
| pH (37°C) (kPa) | 1 | 7.3 | | 7.1 | | 0.005* |
| | 4 | 7.3 | | 7.2 | | 0.005* |
| | 8 | 7.1 | 0.001* | 7.3 | 0.001* | 0.005* |
| $PCO_2$ (37°C) (kPa) | 1 | 5.0 | | 7.7 | | 0.008* |
| | 4 | 3.0 | | 4.8 | | 0.005* |
| | 8 | 2.4 | <0.0001* | 3.8 | <0.0001* | 0.005* |
| $PO_2$ (37°C) (kPa) | 1 | 17.8 | | 20.9 | | 0.021* |
| | 4 | 20.2 | | 20.6 | | 0.047* |
| | 8 | 21.0 | 0.002* | 21.6 | 0.067 | 0.878* |
| CD62P expression (%) | 1 | 25.0 | | 51.0 | | 0.005* |
| | 4 | 32.7 | | 65.2 | | 0.005* |
| | 8 | 53.1 | <0.0001* | 70.7 | <0.0001* | 0.005* |
| Responsiveness to TRAP (%) | 1 | 95.8 | | 95.3 | | 0.959 |
| | 4 | 94.9 | | 92.0 | | 0.074 |
| | 8 | 91.1 | 0.025* | 89.3 | <0.0001* | 0.047 |
| PS exposure (%) | 1 | 4.9 | | 16.6 | | 0.007* |
| | 4 | 5.6 | | 17.4 | | 0.005* |
| | 8 | 12.3 | <0.0001* | 28.0 | 0.025* | 0.005* |

Abbreviations: CSP, cold-stored platelets; MPV, mean platelet volume; PS, phosphatidylserine; RTP, room temperature platelet; TRAP, thrombin receptor activating peptide.

*Statistical significance following Friedman test statistical analysis or paired Wilcoxon signed rank test.

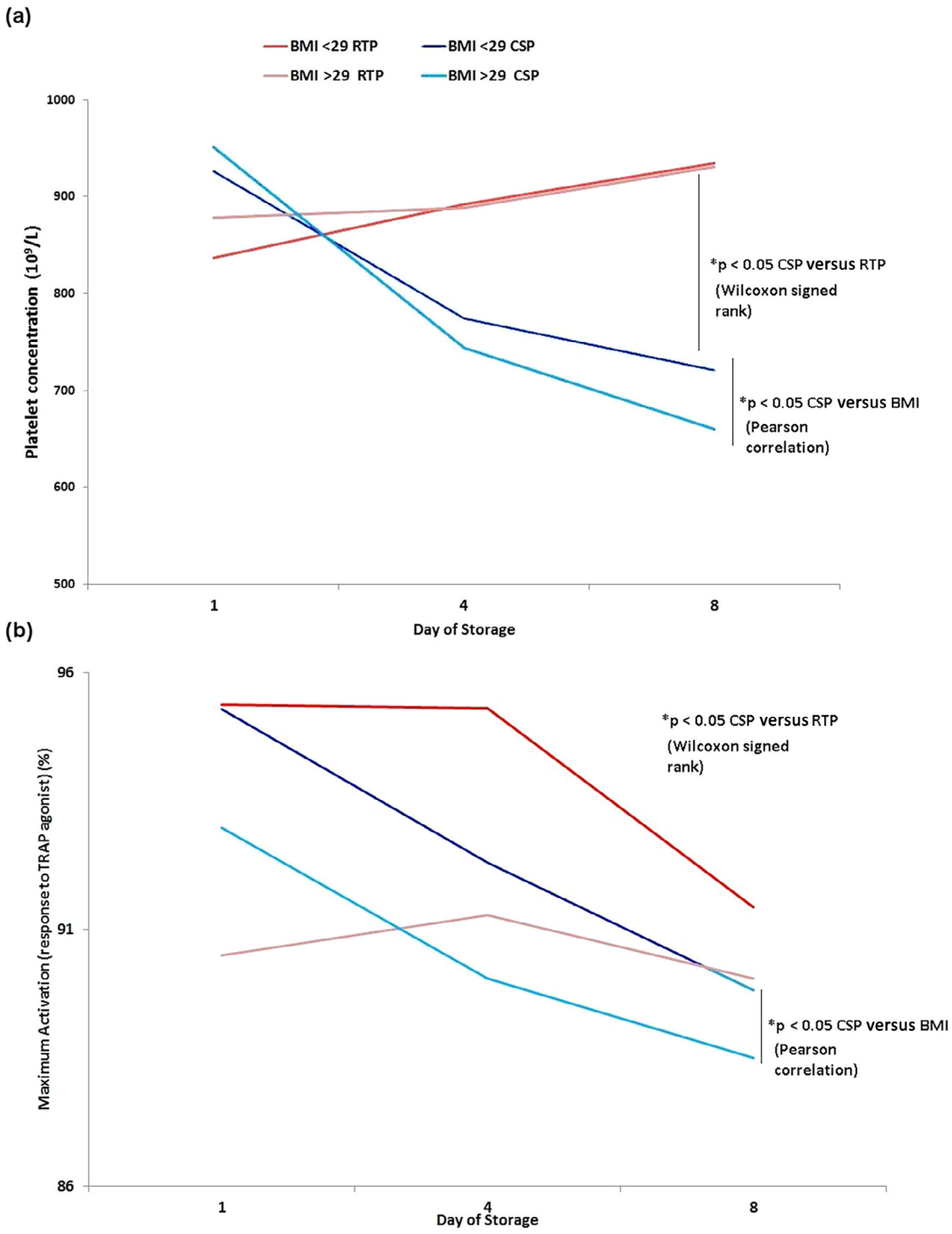


**FIGURE 1** Impact of donor body mass index (BMI) on (a) platelet concentration and (b) activation response throughout storage. Donors were categorized into two groups: BMI ≤ 29 kg/m$^2$ and BMI ≥ 29 kg/m$^2$. (a) The graph shows a significant difference between room temperature platelet (RTP) and cold-stored platelet (CSP) concentrations, which is further impacted by donor BMI. The correlation between BMI and platelet concentration in CSP was significant on days 4 and 8 ($p$ values <0.05). No significant correlation was observed between RTP platelet concentration values and BMI. (b) A significant differential impact on the donor's BMI was detected following thrombin receptor activating peptide (TRAP)-induced CD62P expression between RTPs and CSPs. The correlation of BMI versus CSP was significant on day 8 (Pearson $p$ values <0.05). No significant correlation was observed between RTP maximum activation response and BMI ($p$ values >0.05).

RTP platelet concentrations improved steadily until expiry. Consistent MPV was observed in both groups during storage, indicating uniformity of size, with a small decrease in CSP on day 8, which was coupled with the drop in platelet concentration (Table 2). A significant increase in the percentage of platelets exhibiting activation and apoptotic markers was observed in CSP, with higher levels of expression of both

CD62P and Annexin V-PS ($p < 0.05$, Table 2). The maximum activation response induced by the agonist TRAP was not statistically different between RTP and CSP on days 1 and 4, with values >90% in both groups, although a significant difference was detected by day 8 of storage, with a decreased activation response to agonist in CSP (<90%; Table 2).

## Correlation of donor variation with platelet quality and function

The impact of donor variables (Age, BMI, Baseline Donor Platelet Count/MPV) on platelet and glucose concentration, $PO_2/PCO_2$ levels, pH, PS exposure, CD62P expression and activation response was investigated for both the CSP and RTP. Individual donor data points are presented in Table S1. The mean donor age was 49 ± 9 years (range 37–63 years); age only had a marginal correlation $r = -0.641$ ($p = 0.046$) with $PCO_2$ in CSP on day 4; however, age was not significantly correlated with CSP and RTP variables overall (Tables S2 and S3).

The donors had an average BMI of 29.5 ± 3.8 and were categorized in two groups, BMI ≤ 29 ($n = 6$) and BMI > 29 ($n = 4$). The expected differences in CSP and RTP quality and functional parameters were further impacted by donor BMI. A strong correlation in CSP was observed between BMI and platelets concentration on day 4 ($r = -0.674$; $p = 0.033$) and day 8 ($r = -0.695$; $p = 0.026$) of storage, respectively. Overall, the higher the donor BMI, the lower the platelet concentration in CSP, and donors with BMI >29 had the lowest platelet concentration at the end of storage ($p < 0.05$, Figure 1). Furthermore, CSP from donors with a BMI >29 had the lowest activation response to the thrombin agonist at the end of storage (Figure 1, $p < 0.05$). There was no corresponding significant relationship in RTP. BMI was also associated with MPV on day 8 of storage ($r = -0.689$; $p = 0.027$). Statistical comparisons between donors age or BMI, baseline donor platelet count and MPV, and quality and functional platelets outputs are shown in Tables S2 and S3.

## Standard quality assessment

Visual assessment scores were also significantly different between the two platelet groups. The swirling phenomenon, which is a routine quality assessment for RTP, was non-existent in CSP. In addition, aggregates with grainy appearance were observed on days 4 and 8 in CSP with a total score of 5 (size <2 mm scored 2 and numbers 11–20 scored 3). No aggregates were observed in RTP (Table 2). RTP consumed more carbon dioxide than CSP throughout the 8 days of storage; however, the oxygen levels were comparable for both groups (Table 1). There was increased oxygen concentration in RTP, whereas levels remained unchanged over storage at 4°C. This correlates with the significantly higher glucose concentration in CSP compared with RTP ($p < 0.05$), indicating decreased metabolic activity (Table 2). All platelets, regardless of storage temperature, had pH levels within the EDQM acceptance criteria of >6.4, and all bacteria growth investigations were negative.

# DISCUSSION

Blood services must balance maintaining an adequate platelet supply with minimizing wastage. The standard of care requires storing platelets up to 7 days at RT with constant agitation to ensure gas exchange and aerobic metabolism [37]. As agitation of CSP is not required, the logistical challenges related to platelet storage, shelf life and transportation are reduced, as well as the limiting bacterial contamination and metabolic activity [22, 24, 33]. Blood products are inherently variable [22, 24, 33] and there is limited data on the specific donor-related factors influencing the optimal storage of platelets following donation. This study focused on the differences between RTP and CSP, and the donor variation impact on this, and demonstrated, for the first time, the potential impact of donor BMI on the function of CSP. As per current practice, day 8 quality and bacterial testing is carried out on all stored platelets prior to disposal. These data add to the limited data available on donor contribution to component quality and show a correlation between BMI and CSP platelet concentration and levels of activation response.

Many factors are included in the evaluation of donor eligibility, such as social behaviours, iron levels and minimum blood volume. Consideration of donor variables, known to impact component quality, may benefit platelets during storage. Our findings indicate that high BMI ranges in western male donors might reduce platelet concentration and activation response in CSP. Extrapolation of this relationship suggests that CSP transfusion efficacy may also vary depending on the donor's BMI, pointing the possibility that donor characteristics for room temperature may not be appropriate indicators for cold storage and vice versa. There are already reported known links between donor's sex, age and body weight, and post-transfusion patient outcome [22]. Storage performance of platelet has previously been associated with donor health and lifestyle, and higher glucose consuming platelets are impacted by donor BMI [31]. Specifically, increased BMI may lead to changes in platelet characteristics [38, 39]. The impact on inventory management and donor recruitment would need to be assessed to mitigate any changes to platelet provision.

There is a clear requirement for novel quality markers for assessing the quality and efficacy of CSP, although cold storage does not result in loss of platelets' quality and function, it clearly impacts platelet morphology such as the loss of discoid shape and swirling [40]. Cold storage can result in greater expression of apoptosis marker PS on the platelet membrane and the release of intracellular calcium [12, 14, 16]. A greater CSP PS-externalization was detected in this study, possibly reflecting the development of cold-induced PSL and apoptosis, but also the potential pro-inflammatory and pro-thrombotic properties of activated CSP [41]. These differences have been observed in multiple publications [14, 42, 43, 10, 44–46] and in this study; however, alternatives to the standard visual makers of quality have not

yet been identified. The grainy aggregates observed did not impact functionality, and therefore, this was not an indicator of platelet unsuitability [36]. Guidelines on managing platelets with aggregates vary from centre to centre, and the evidence on the possible negative effects of platelet aggregates and their impact on the haemostatic function and clinical outcomes, it is still very limited [47]. It is possible that aggregation in CSP is related to the storage medium, as CSP in platelet additive solution have fewer aggregates than those stored in plasma [42]. Moreover, pH, which is one of most significant indicators of PSL, was maintained at an acceptable level through refrigeration [7]. This study did not attempt to determine the length of time that CSPs are viable in storage, although the potential of CSP prolonged storage time is acknowledged [22, 24, 33].

The findings presented are based on a small pilot donor study which is a notable limitation. However, although the sample size may have been insufficient to clearly understand the relationship between BMI and CSP quality, our results clearly point to a greater association between BMI and CSP compared with RTP. The high average BMI of study participants reflected the current demographic profile of the Irish platelet donor panel, particularly the prevalence of overweight and obese male donors, and represents an average donor's BMI of most of the western countries [48, 49]. Unfortunately, given the small sample size, possible associations with gender, blood group and aggregate formation could not be assessed. Aggregates only occurred in CSPs at the end of storage as a result of the cold temperature. A larger and diverse donor cohort study is required to corroborate these findings.

It is evident that the safe and effective use of CSP still requires appropriate quality control indicators and clinical trial evaluation [1, 2]. A deeper understanding of the impact of donor variability is, therefore, necessary to achieve greater consistency of platelet quality. It is proposed that including the donor characteristics in studies investigating the underlying PSL mechanisms will help to standardize the quality of platelets for transfusion. These findings support BMI as a possible factor impacting the quality of CSP, and its potential inclusion as a novel quality assessment measure may improve and standardize the quality of CSP. However, further extensive studies replicating the current findings are needed to confirm the study's outcome.

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A.L. performed methodology, data curation, statistical analysis, and prepared the scientific paper. H.C. conceptualized and designed the study and performed methodology, specifically microscopy, platelet production and storage flow cytometric analysis. S.F.-O'D. performed methodology, specifically, flow cytometry and flow cytometric analysis. S.F. conceptualized and designed the study and received ethical approval. Á.F. done reagent purchasing and assessed health and safety risk. A.F. administered laboratory and project resources. T.H. reviewed and edited the article. A.W. carried out project oversight and data curation, supervised the principal project and reviewed and edited the article. Open access funding provided by IReL.

## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

Data are available following a specific request to research@ibts.ie.

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## SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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# Risk of variant Creutzfeldt–Jakob disease in a simulated cohort of Canadian blood donors

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## Abstract

**Background and Objectives:** No transfusion-associated cases of variant Creutzfeldt–Jakob disease (vCJD) have occurred in more than 20 years. Yet, many countries have maintained blood donor deferral criteria for vCJD. We developed a risk simulation model to reassess the need for vCJD-related deferral criteria in Canada.

**Materials and Methods:** The model provides results separately for Héma-Québec (HQ) and Canadian Blood Services (CBS). The model used a Monte Carlo simulation approach to estimate the risk of having a vCJD-contaminated blood donation ('risk of vCJD') in a simulated cohort of 10 million donors followed for up to 85 years. The model assumed current deferral criteria for vCJD were lifted, which would allow new 'at-risk' donors to give blood. The model accounted for disease prevalence, donors' travel/immigration history, *PRNP* genotype at codon 129, demographics and the type of labile blood product.

**Results:** In the base case, the risk of vCJD was estimated at zero at both blood services. In the most pessimistic scenario, the risk of vCJD was $6.4 \times 10^{-9}$ (i.e., 1 in 157 million donations) at HQ, or ≤1 in 77 million based on the upper bound of the 95% confidence interval (CI). At CBS, this risk was $4.8 \times 10^{-8}$ (i.e., 1 in 21 million donations), or ≤1 in 16 million based on the upper bound of the 95% CI.

**Conclusion:** vCJD poses minimal risks to the Canadian blood supply. Current vCJD deferral criteria may, therefore, be lifted with virtually no impact on safety, while significantly expanding the donor base.

## Keywords

blood donors, donor deferral, Monte Carlo simulations, risk model, vCJD

## Highlights

- We developed a risk model to reassess the need for deferral criteria related to variant Creutzfeldt–Jakob disease (vCJD) in Canada.
- In the base case, no donations would be infectious among the 10 million simulated donors followed over 85 years; in the most pessimistic scenario, it would take ≥335 years to collect one contaminated donation at Héma-Québec and ≥20 years to collect one at Canadian Blood Services (based on the upper bound of the 95% confidence interval).
- vCJD poses minimal risks to the Canadian blood supply, so that the current vCJD deferral criteria may be lifted with virtually no impact on safety.

  

## INTRODUCTION

Variant Creutzfeldt–Jakob disease (vCJD) is a rare, incurable neurodegenerative prion disease characterized by early psychiatric symptoms, persistent painful sensory symptoms, ataxia, cerebellar ataxia, and dementia [1]. The disease emerged in the United Kingdom (UK) in 1995 [2, 3], approximately 9 years after the beginning of a large outbreak of bovine spongiform encephalopathy (BSE) in UK cattle [4]. The vast majority of hitherto reported cases have thus probably been acquired through meat consumption.

However, vCJD can also be acquired through blood transfusion or receipt of a plasma-derived product. To date, five individuals are strongly suspected to have acquired a vCJD infection from a vCJD-implicated blood product, including three cases of clinical vCJD and two of asymptomatic vCJD [5]. All of these individuals received a vCJD-implicated, non-leukodepleted product between 1996 and 1999 [5].

Despite the >20-year absence of new transfusion-associated cases, transfusion-transmission of vCJD remains theoretically possible. vCJD has a protracted incubation period that varies widely depending on the carrier's genotype at codon 129 of *PRNP*: For dietary exposure, the average incubation period may last 15 years (median: 12 years) in methionine homozygotes (MM) and may span several decades in valine-methionine heterozygotes (MV) and valine homozygotes (VV) [6]. Given such a long incubation period, there may remain silent carriers capable of transmitting the disease through blood transfusion—especially among MV and VV individuals.

Blood services have, thus, implemented measures to mitigate the risk of transfusion transmission of vCJD. In the late 1990s and early 2000s, many resource-rich countries adopted universal leukodepletion [7, 8] to reduce the risk of vCJD [9–11] and other immune and infectious complications resulting from blood transfusion [12, 13]. Given the unclear performance of available blood screening tests and potential logistical problems, several jurisdictions have also implemented donor deferral criteria based on time spent in high-risk countries, such as the UK, France and Ireland. While theoretically effective, donor deferral limits the donor base and is thus suboptimal for blood services.

Given this limitation and the waning vCJD epidemic, an increasing number of countries (e.g., the UK, the United States [US] and Australia) are reassessing the need for donor deferral [8, 14, 15], often using risk simulation models. However, no such evaluation has been conducted in Canada, where blood donors who have resided in high-risk countries are still indefinitely deferred from donating blood. Therefore, this study estimated the risk of transfusion transmission of vCJD in Canada, assuming current vCJD deferral criteria were lifted.

## MATERIALS AND METHODS

### Model overview

The model used a Monte Carlo simulation approach to estimate the number of vCJD-contaminated blood donations in a simulated cohort of blood donors. The model assumed current deferral criteria for vCJD were lifted (Table 1), which would allow new 'at-risk' donors to give blood. The model accounted for several variables that may influence the risk of having a contaminated donation, including donors' travel/immigration history, *PRNP* genotype at codon 129, demographics and the type of blood product donated. Labile blood products (i.e., red blood cells [RBCs], apheresis platelets and fresh-frozen plasma) were considered in the model.

Canada has two independent blood services: Héma-Québec (HQ) operates in Québec and Canadian Blood Services (CBS) operates throughout the other provinces of the country. CBS was anticipated to have more at-risk donors than HQ as the former operates in predominantly English-speaking provinces where migration flows to the UK are more significant. The model, therefore, provides results separately for both blood services.

### Simulated cohort and model structure

A simulated cohort of 10 million first-time donors was observed for each blood service between 2023 and 2108 ('observation period'), beyond which no new clinical cases are predicted to arise [16]. The cohort comprised donors currently eligible for blood donation and at-risk donors who would become eligible should existing deferral criteria be lifted.

The subset of currently eligible donors comprised donors aged at least 18 years (for HQ) or 17 years (for CBS) simulated using internal data. The subset of at-risk donors comprised individuals who would be deferred under current criteria based on the length of stay in an at-risk country.

A Monte Carlo simulation with a Markov chain procedure was employed to estimate the probability of collecting a vCJD-contaminated blood donation. The Monte Carlo algorithm performed simulations of each donation individually and independently from the joint distribution based on conditional relationships among selected variables.

**TABLE 1** Current vCJD-related criteria at HQ and CBS.

| HQ | CBS |
|---|---|
| ≥3 months spent in France or the UK between 1980 and 1996 | ≥5 years spent in France or Ireland between 1980 and 2001 |
| ≥6 months in Saudi Arabia between 1980 and 2007 | ≥3 months spent in the UK between 1980 and 1996 |
| ≥5 years spent in the rest of Western Europe[a] between 1980 and 2007 | Dura mater transplant received |
| Blood transfusion received in Western Europe[a] since 1980 | |

Abbreviations: CBS, Canadian Blood Services; HQ, Héma-Québec; UK, United Kingdom; vCJD, variant Creutzfeldt–Jakob disease.
[a]Includes Austria, Belgium, France, Denmark, Germany, Luxembourg, Netherlands, Switzerland, Ireland, UK, Italy, Portugal and Spain.

**TABLE 2** Model inputs.

| Parameter | Value | Simulation distribution | References |
|---|---|---|---|
| Donation-related parameters | | | |
| Number of annual blood donations | Blood-bank-specific parameter | Point estimates | Internal data (Table S1 and Supporting Information File S1)[a] |
| Deferral rate for a stay in the UK, France or other WE countries—high estimate | Blood-bank-specific parameter | Point estimates | Internal data (Table S1)[a] |
| Units derived from a single WBD | Blood-bank-specific parameter | Point estimates | Internal data (Table S1)[a] |
| RBC | | | |
| Plasma | | | |
| Platelets | | | |
| Donor-related parameters | | | |
| Age, gender and country of infection—at-risk donors | See Supporting Information File S2 | Point estimates | Internal data and [17] |
| Age and gender—other donors | Blood-bank-specific parameter | Point estimates | Internal data (Table S1)[a] |
| Year of death | See Supporting Information File S3 | Point estimates | [18] |
| vCJD-related parameters | | | |
| UK | | | |
| High estimate | Mean = 1 in 2028 (min = 1 in 1250, max = 1 in 3500) | Triangular | [19] |
| Low estimate | Mean = 1 in 588,235 (min = 1 in 267,380, max = 1 in 5,300,000) | Triangular | [20] |
| France | | | |
| High estimate[b] | Mean = 1 in 12,892 (min = 1 in 7946, max = 1 in 22,250) | Point estimates | [2, 19] |
| Low estimate[b] | Mean = 1 in 3,746,720 (min = 1 in 1,703,057, max = 1 in 33,757,962) | Point estimates | [2, 20] |
| Other WE countries[c] | | | |
| High estimate[d] | Mean = 1 in 21,234 (min = 1 in 13,088, max = 1 in 36,647) | Point estimates | [2] |
| Low estimate[d] | Mean = 1 in 6,159,166 (min = 1 in 2,799,626, max = 1 in 55,494,118) | Point estimates | [2] |
| Year of infection | Year of report − 15 | Point estimates | [2] |
| Infectivity window | • Mean = 75%<br>• Min = 50%<br>• Max = 90% | Triangular | [21, 22] |
| Distribution of genotypes at codon 129 of *PRNP* | | | |
| MM | $\beta$(156, 242) | Beta | [21, 26] |
| MV | $\beta$(195.5, 220.5) | Beta | [21, 26] |
| VV | 100-MM%-MV% | Beta | [21, 26] |
| Incubation period (by dietary exposure) | | | |
| MM donors | Lognorm (2.48,0.67) | Log-normal | [6, 20, 21] |
| VV and MV donors | Lognorm (3.47,0.42) | Log-normal | [6, 20, 21] |
| Efficacy of leukodepletion | $\beta$(15,1.67) | Beta | [16, 23] |

Abbreviations: MM, methionine homozygote; MV, methionine-valine heterozygote; RBC, red blood cell; UK, United Kingdom; vCJD, variant Creutzfeldt–Jakob disease; VV, valine homozygote; WBD, whole blood donation; WE, Western European.

[a]Blood-bank-specific parameters are described in Table S1.

[b]Estimate calculated by multiplying the UK prevalence by the relative prevalence of vCJD in France compared with the UK. France reported 84.3% fewer vCJD cases than the UK. The point estimate for prevalence was therefore (1–0.843) times the point estimate in the UK.

[c]Includes Austria, Belgium, Denmark, Germany, Luxembourg, Netherlands, Switzerland, Ireland, Italy, Portugal and Spain.

[d]Estimate calculated by multiplying the UK prevalence by the relative prevalence of vCJD in WE countries compared with the UK. WE countries reported 89.9% fewer vCJD cases than the UK. The point estimate for prevalence was therefore (1–0.899) times the point estimate in the UK.

## Assumptions

As mentioned above, the model assumed that current vCJD deferral criteria were lifted, thereby making eligible at-risk donors who were previously deferred. The demographics and blood donation frequency of these donors were presumed to be similar to those of currently eligible donors. Their life expectancy was also presumed to be the same as that of the general population (up until the end of the incubation) and to remain constant throughout the observation period.

Due to the lack of published data, MV and VV individuals were assumed to have the same incubation period (assessed since the time of dietary exposure). After the incubation period (i.e., once they become symptomatic), donors were either dead or no longer considered at-risk as they should be identified and deferred by pre-donation screening. In other words, pre-donation screening was presumed to capture all donors with clinical vCJD. Furthermore, donors were only considered infectious (i.e., capable of transmitting the disease) later during the incubation period (the 'infectivity window'). For example, a donor with an incubation period of 20 years may have an infectivity window of 75%, in which case the donor would be infectious during the last 15 years (75% × 20 years) of the incubation period. This infectivity window did not depend on the donor's genotype at codon 129 of *PRNP*.

Universal leukodepletion is the only risk mitigation measure currently used in Canada for vCJD. Its effectiveness was presumed to be the same for all types of labile blood products.

## Model inputs

The following blood-bank-specific parameters were derived from internal data: the number of annual blood donations, the number of product units obtained with a single whole blood donation and the deferral rates for a travel history in the UK, France or Western Europe (Table S1). Of note, the CBS deferral rate was based on the predicted donor loss resulting from the introduction of this measure in 2000–2001 and thus accounts for self-deferral. By contrast, the HQ deferral rate was based on the observed deferral rate after the implementation of the vCJD deferral (i.e., donors presenting at a centre/blood drive and deferred after pre-donation screening) and hence does not account for self-deferral (see Discussion section for more information).

The demographics of current non-at-risk donors were derived from internal data, and those of at-risk donors were derived from 2021 Canadian census data [17] (Table 2). Data on donors' year of death were also obtained from 2021 Canadian census data [18].

Two estimates of vCJD prevalence in the UK were considered: a high estimate of 1 in 2028 individuals derived from the Appendix-II study, which reflects the prevalence of $PrP^{sc}$-positive appendices excised between 2000 and 2012 from UK individuals born in 1941–1985 [19]; and a low estimate of 1 in 588,235 individuals derived from Garske and Ghani's stochastic model [20], which reflects the prevalence of $PrP^{sc}$-positive cases after 2010 in the UK (Table 2). This estimate was derived from Yang et al. [6] by dividing the 100 predicted future cases by the UK population (i.e., 60 million), which yielded a prevalence of 1.7 per million individuals (i.e., $1/1.7 \times 10^{-6} = 1$ in 588,235 individuals). Unlike the Garske and Ghani study, the Appendix-II study stratified prevalence estimates by genotype at codon 129 of *PRNP* [19, 20], which was considered in the model. For France and other Western European countries, the prevalence of vCJD was estimated based on how the number of vCJD cases reported in these regions compared with that in the UK. For example, as of April 2021, France had reported 84.3% fewer vCJD cases than the UK [2]. The high-prevalence estimate in France was therefore (100%–84.3%) × 1 in 2028, which gives 1 in 12,889.

The estimated incubation periods since dietary exposure for MM (median = 12 years, mean = 15 years), MV and VV individuals (median = 32 years, mean = 35 years) were derived from other risk simulation models (Table 2) [6, 20, 21]. The year of infection was derived from the year of reporting of the 233 probable and confirmed vCJD cases worldwide (virtually all in MM individuals) [2], considering a 15-year incubation period for MM individuals; donors for whom the resulting year of infection was after 1996 were not considered at risk. The infectivity window was based on prior experiments of sheep infected with scrapie, in which an 80% transmission rate was observed with blood samples collected at >50% of the estimated incubation period [22]. The effectiveness of leukodepletion was based on that used in other risk simulation models [16, 23].

**TABLE 3** Scenarios considered in the model.

| Scenario | vCJD prevalence in the UK | Second wave | Proportion of at-risk donors |
|---|---|---|---|
| Optimistic | Low (1 in 588,235) | Yes | HQ: 1.52%[a]<br>CBS: 2.40%[a] |
| Base case | Low (1 in 588,235) | Yes | HQ: 1.90%<br>CBS: 3.10% |
| Pessimistic (i) | High (1 in 2028) | No | HQ: 1.90%<br>CBS: 3.10% |
| Pessimistic (ii) | High (1 in 2028) | Yes | HQ: 1.90%<br>CBS: 3.10% |

Abbreviations: CBS, Canadian Blood Services; HQ, Héma-Québec; UK, United Kingdom; vCJD, variant Creutzfeldt–Jakob disease.
[a]20% fewer at-risk donors than the base case.

## Scenarios

Scenario analyses were conducted to test the sensitivity of model outputs. The base case, which was considered more likely, considered a low-prevalence estimate and assumed a second wave of vCJD will occur. The high-prevalence estimate appeared less plausible: In a model validation exercise, Yang et al. found that this estimate largely overestimated the number of observed clinical vCJD cases in the US and the number of transfusion-associated cases in the UK [6].

Three alternative scenarios were evaluated: one optimistic scenario and two pessimistic scenarios (Table 3). These scenarios differed

**TABLE 4** Model results.

| | Héma-Québec | | | | Canadian Blood Services | | | |
|---|---|---|---|---|---|---|---|---|
| | Optimistic | Base case | Pessimistic (i) | Pessimistic (ii) | Optimistic | Base case | Pessimistic (i) | Pessimistic (ii) |
| At-risk donors, *n* | 151,579 | 188,825 | 189,308 | 188,306 | 266,261 | 324,313 | 324,345 | 303,083 |
| Infectious donors, *n* | 0 | 0 | 0 | 3 | 0 | 0 | 8 | 26 |
| Probability of collecting an infected donor, *p* (95% CI) | 0.0 | 0.0 | 0.0 | $3.0 \times 10^{-7}$ ($6.2 \times 10^{-8}$–$8.8 \times 10^{-7}$) | 0.0 | 0.0 | $8.0 \times 10^{-7}$ ($3.5 \times 10^{-7}$–$1.6 \times 10^{-6}$) | $2.6 \times 10^{-6}$ ($1.7 \times 10^{-6}$–$3.8 \times 10^{-6}$) |
| At-risk donor-years | 0 | 0 | 0 | 32 | 0 | 0 | 55 | 206 |
| Contaminated donations in inventory (after leukodepletion), *n* | 0 | 0 | 0 | 7.0 | 0 | 0 | 13.3 | 54.1 |
| Contaminated RBC donations | 0 | 0 | 0 | 6.7 | 0 | 0 | 12.8 | 52.0 |
| Contaminated plasma donations | 0 | 0 | 0 | 2.0 | 0 | 0 | 3.8 | 15.4 |
| Contaminated platelets donations | 0 | 0 | 0 | 0.1 | 0 | 0 | 0.2 | 0.8 |
| Risk of collecting a vCJD-contaminated donation, *p* (95% CI)[a] | 0.0 | 0.0 | 0.0 | $6.4 \times 10^{-9}$ ($2.6 \times 10^{-9}$–$1.3 \times 10^{-8}$) | 0.0 | 0.0 | $1.2 \times 10^{-8}$ ($6.7 \times 10^{-9}$–$2.0 \times 10^{-8}$) | $4.8 \times 10^{-8}$ ($3.6 \times 10^{-8}$–$6.3 \times 10^{-8}$) |

Abbreviations: CI, confidence interval; p, probability; RBC, red blood cell; vCJD, variant Creutzfeldt–Jakob disease.

[a]CIs are not reported when the estimated risk is 0, since the upper bound of the 95% CI would exclusively depend on the sample size.

based on their prevalence assumptions (low or high), the presence or absence of a second wave, and the proportion of at-risk donors currently being deferred, which was (arbitrarily) reduced by 20% relative to the base case in the optimistic scenario. For scenarios that assumed no second wave, MV or VV donors were assumed to have the same incubation period as MM donors.

## Statistical analysis

The probability of having a vCJD-contaminated donation in inventory was calculated based on the below formula:

$$r_{cd} = \frac{n_{cd}}{\sum_{d=1}^{N} \sum_{y=Y_{min}}^{Y_{max}} \boldsymbol{A} \times \boldsymbol{B}}$$

where $r_{cd}$ corresponds to the risk of collecting a contaminated donation; $n_{cd}$ is the number of contaminated donations collected from infectious donors between $Y_{min} = 2023$ and $Y_{max}$ = year of the last donor death; $N$ is the total number of donors sampled; $Y_{min}$ is the start of the at-risk period; $Y_{max}$ is the end of the at-risk period; $\boldsymbol{A}$ is a Bernoulli random variable indicating whether donor $d$ of sex $s$ from blood bank $bb$ is still alive at year $y$ and age $a$, with parameter $p = P$(donor still alive|$y$, $s$, $a$, $bb$) where $y$ = year of donation; and $\boldsymbol{B}$ is a random variable representing the number of donations during year $y$ of donor $d$ at age $a$, sex $s$, and from blood bank $bb$.

Confidence intervals (CIs) were calculated using binomial distribution around the probability. Model outputs were generated using R 4.2.1, RStudio 2022.07.2 and Compute Canada Narval server.

## RESULTS

In the base case, the simulated HQ cohort of 10 million donors comprised 188,825 (1.9%) at-risk donors, and the CBS cohort comprised 324,313 (3.2%) at-risk donors. Over 85 years, no donors were predicted to be infectious at either blood service, and no donations would be contaminated (Table 4). The risk of having a vCJD-contaminated donation was thus 0.0 for both blood services.

Similar results were obtained in the optimistic scenario but not in pessimistic scenarios (i) and (ii). In pessimistic scenario (i), there were zero infectious donors at HQ and eight at CBS (Table 4). The resulting number of contaminated donations in inventory was 0.0 at HQ and 13.3 at CBS. At HQ, the risk of having a contaminated donation was, therefore, estimated at 0.0. At CBS, this risk was $1.2 \times 10^{-8}$ (i.e., 1 in 85 million donations), or ≤1 in 49 million donations based on the upper bound of the 95% CI.

In pessimistic scenario (ii), there were three infectious donors at HQ and 26 at CBS. The resulting number of contaminated donations in inventory was seven at HQ and 54 at CBS. At HQ, the risk of having a contaminated donation was estimated at $6.4 \times 10^{-9}$ (i.e., 1 in 157 million donations), or ≤1 in 77 million based on the upper bound

of the 95% CI. At CBS, this risk was $4.8 \times 10^{-8}$ (i.e., 1 in 21 million donations), or ≤1 in 16 million based on the upper bound of the 95% CI.

## DISCUSSION

The risk of having a vCJD-contaminated donation was minimal at both blood services. In the base case, the model predicted that no contaminated blood donations would be collected at either blood service among the 10 million donors followed over 85 years; the estimated risk was, therefore, zero. In the most pessimistic scenario assessed (i.e., pessimistic scenario [ii]) and conservatively assuming that the true risk is the upper bound of the 95% CI, the estimated risk was ≤1 in 77 million donations at HQ and ≤1 in 16 million at CBS. In this scenario and with the current number of annual donations (HQ: ~230,000, CBS: ~800,000), it would take ≥335 years for one contamination to occur at HQ and ≥20 years for one to occur at CBS. vCJD, thus, appears to pose minimal risks to the safety of the Canadian blood supply.

The two pessimistic scenarios yielded higher figures, but their prevalence assumption is likely an overestimate. Both scenarios considered the high estimate from the Appendix-II study, which found that 1 in 2028 UK appendices excised between 2000 and 2012 tested positive for $PrP^{sc}$ [19]. Intriguingly, the Appendix-III study found that the prevalence of $PrP^{sc}$-positive appendices did not significantly differ between individuals potentially exposed to BSE (i.e., those in Appendix-II) and presumably unexposed individuals who had their appendix removed in 1962–1979 or those born in 1996–2000 [24]. These puzzling results might indicate that the BSE agent entered the UK food chain earlier than previously thought or that the specificity of immunohistochemistry is too low given the rarity of $PrP^{sc}$ accumulation. Regardless, some questioned the clinical relevance of $PrP^{sc}$ accumulation in the appendix, suggesting it may simply reflect dietary exposure to BSE rather than an ongoing infection [25]. Furthermore, in another model of transfusion-transmission of vCJD, the Appendix-II prevalence largely overestimated the cumulative number of primary cases of clinical vCJD in the US and the number of transfusion-associated cases in the UK, while the low estimate better reproduced the official numbers [6].

What is more, should one donation be contaminated, only a fraction of recipients will develop clinical vCJD, although this was not explored in our study. In a previous risk simulation model developed by the UK Department of Health & Social Care, 7.0%–45.5% of infections acquired from RBC transfusions, 5.6%–41.3% of those acquired from plasma, and 3.4%–23.3% of those acquired from platelets will progress to clinical vCJD (depending on the scenario assumed) [16]. According to another risk simulation model in the US, only 4.3% of the cumulative number of transfusion-acquired infections from 1980 to 2011 would result in clinical vCJD (using a high-prevalence estimate) [6].

The base case risk estimates (i.e., 0 contaminated donations) are also smaller than those reported in other countries where vCJD deferral criteria have recently been lifted. In Australia, the risk of having a vCJD-contaminated donation was estimated at 1 in 29.9 million [21], and these results prompted the Australian regulator (i.e., the Therapeutic Goods Administration) to lift the vCJD deferral in 2022 [15]. In the US, the risk of receiving a contaminated RBC unit was estimated at 1 in 134 million donations (based on the UK prevalence of vCJD estimated by Garske and Ghani) [6]. The US Food and Drug Administration has withdrawn the vCJD deferral, initially for all countries except those considered at higher risk (i.e., France, Ireland and the UK), and subsequently for all countries [14]. The proportion of at-risk donors may be a key driver of differences among different regions. In the current scenario analyses, the risk of contamination at HQ and CBS differed largely because of the proportion of at-risk donors, which was higher at CBS as it operates in predominantly English-speaking provinces where migration flows to the UK are more significant.

The present risk simulation model thus supports lifting vCJD deferral criteria in Canada. The estimated risk to recipients appears minimal, and epidemiological data on vCJD are reassuring. Indeed, there has been no suspected transfusion-transmission case of vCJD since 1999 [5], possibly (in part) because of the implementation of universal leukodepletion in the UK (also in 1999) [8]. Furthermore, the vCJD epidemic has considerably waned since the early 2000s, with fewer than five cases reported worldwide between 2015 and 2021 [2]. Nonetheless, should these criteria be lifted, surveillance efforts will need to be maintained owing to concerns over a potential second wave driven by MV and VV individuals.

Lifting the vCJD deferral may entail significant benefits for HQ and CBS. Notably, by our calculation, doing so would increase the HQ donor base by ≥2890 donors and the CBS donor base by ≥13,950 donors. Other potential benefits are more difficult to quantify. For example, more people may view blood donation as a more inclusive activity once the vCJD deferral is lifted, thus possibly encouraging more to donate. Moreover, fewer first-time donors would be indefinitely deferred, and these donors may be more easily retained as active donors.

Our model is nonetheless subject to some limitations. To begin, the risk of infection and clinical vCJD were not assessed. However, these risks must be lower than that of collecting a contaminated donation, which already appeared minimal. Moreover, the model did not account for donors currently excluded due to a blood transfusion received in the UK after 1980, as well as the risk of tertiary transmission via organ donation; such risk was deemed negligible since no case of tertiary transmission has ever been reported. Another limitation is that the deferral rate at HQ only includes deferrals observed at donation sites and hence does not account for self-deferrals. However, this (constant) rate was applied throughout the nearly 100 years of follow-up, which is highly precautionary since vCJD deferrals will inevitably dwindle as the donor base is replenished with younger, unexposed donors and at-risk donors die or are deferred for other health reasons. Lastly, the model assumed no donor may have been infected after 1996, but the Appendix-III study found some $PrP^{sc}$-positive appendices in UK individuals born between 1996 and

2000 [24]. Albeit unlikely, some donors might, therefore, have been infected after 1996.

In conclusion, the results of this risk simulation model demonstrate that the risk of having a vCJD-contaminated donation is minimal in Canada. Current vCJD deferral criteria may, therefore, be lifted with virtually no impact on safety, while significantly expanding the donor base. Although no transfusion-associated cases of vCJD have occurred in more than 20 years, HQ and CBS plan to maintain vCJD surveillance efforts as the prospect of a second wave of vCJD cannot be ruled out.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

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

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## SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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# Paediatric clinical decision support: Evaluation of a best practice alert for red blood cell transfusion

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## Abstract

**Background and Objectives:** Providing red blood cell (RBC) transfusion to paediatric patients with a haemoglobin (Hb) level of <7 g/dL is the current best practice, but it is often difficult to ensure appropriateness of RBC transfusion on a health system level. Electronic health record (EHR) clinical decision support systems have been shown to be effective in encouraging providers to transfuse at appropriate Hb thresholds. We present our experience with an interruptive best practice alert (BPA) at a paediatric healthcare system.

**Materials and Methods:** An interruptive BPA requiring physician response was implemented in our EHR (Epic Systems Corp., Verona, WI, USA) in 2018 based on Hb thresholds for inpatients. The threshold was initially <8 g/dL and later changed to <7 g/dL in 2019. We assessed total activations, number of RBC transfusions and hospital metrics through 2022 compared to the 2 years prior to implementation.

**Results:** The BPA activated 6956 times over 4 years, slightly less than 5/day, and the success rate, with no RBC transfusions within 24 h of order attempt, was 14.5% (1012/6956). There was a downward trend in the number of total RBC transfusions and RBC transfusions per admission after implementation, non-significant ($p = 0.41$ and $p =$ >0.99). The annual case mix index was similar over the years evaluated. The estimated cost savings based on acquisition costs for RBC units were 213,822 USD or about $51,891 per year.

**Conclusion:** BPA implementation led to sustained change in RBC transfusion towards best practice, and there were long-term savings in RBC expenditure.

## Keywords

best practice alert, clinical decision support, erythrocytes, haemoglobin threshold, paediatric transfusion, patient blood management

## Highlights

- Clinical decision support can be used to reduce red blood cell (RBC) transfusions that do not fit evidence-based criteria in the paediatric setting.
- This study showed an overall savings of ~50,000 USD a year based on RBC acquisition costs.
- Best practice alerts offer a reliable intervention for sharing transfusion guidance in settings where prospective triaging may not be feasible.

## INTRODUCTION

Over the past decade, red blood cell (RBC) transfusions have decreased in the United States, likely reflecting moves towards restrictive transfusion strategies as supported by the paediatric and adult literature [1–3]. A concurrent reduction in blood donation has been observed, a phenomenon that has been exacerbated by the Coronavirus Disease 2019 (COVID-19) pandemic [4]. As such, many hospitals have undertaken efforts to prevent unnecessary RBC transfusions that do not fit evidence-based criteria. These interventions include the use of electronic health record (EHR) pop-up alerts known as best practice alerts (BPAs), though contemporary data amongst paediatric hospitals are sparse [5, 6].

BPAs are not unique to transfusion medicine but represent one part of clinical decision support (CDS) systems; CDS is used throughout the EHR to provide assistance to ordering providers [7]. Recent meta-analysis data demonstrate that CDS systems lead to an overall 5.8% increase in the desired care being provided to the patient [7]. Though this difference may seem incremental, it is meaningful at a health system level. This is especially true in certain situations or populations, as the same meta-analysis illustrated higher decision support agreement in the paediatric setting (14.7%) [7]. However, it should be noted that recent data from a paediatric hospital show the number of alerts is increasing, with a baseline amount of pop-ups of 7250 a week at one tertiary care children's hospital, which can lead to alert fatigue in providers, possibly reducing compliance [8].

Though adult data showing improved outcomes with a restrictive haemoglobin (Hb) threshold of <7 g/dL are appropriate, data are somewhat less robust in paediatrics and certain populations require further assessment [1]. Two large randomized controlled trials of low-birth-weight infants demonstrated that liberal transfusion strategies led to no significant difference in outcomes, including the prevalence of neurodevelopmental impairment at 2 years of age, a critical endpoint when considering neonatal transfusion [9, 10]. Similarly, data in paediatric intensive care unit (PICU) patients showed decreased transfusion with no increase in adverse events when using an Hb threshold of <7 g/dL [11]. Meta-analysis data and randomized trial data in patients with sepsis support these findings in paediatric patients, but patients with myocardial infarction or traumatic brain injury have not been extensively investigated and may require transfusion at higher Hb levels to improve oxygenation [1, 12].

We sought to determine the impact of an RBC transfusion BPA in a contemporary tertiary care paediatric hospital. We compared RBC transfusion rates before and after implementation. We analysed reasons for not following the BPA. We also followed BPA usage temporally to assess long-term effects of this intervention on ordering practice.

## MATERIALS AND METHODS

Children's Health is an academically affiliated paediatric healthcare system in North Texas comprised of two acute care hospitals that are

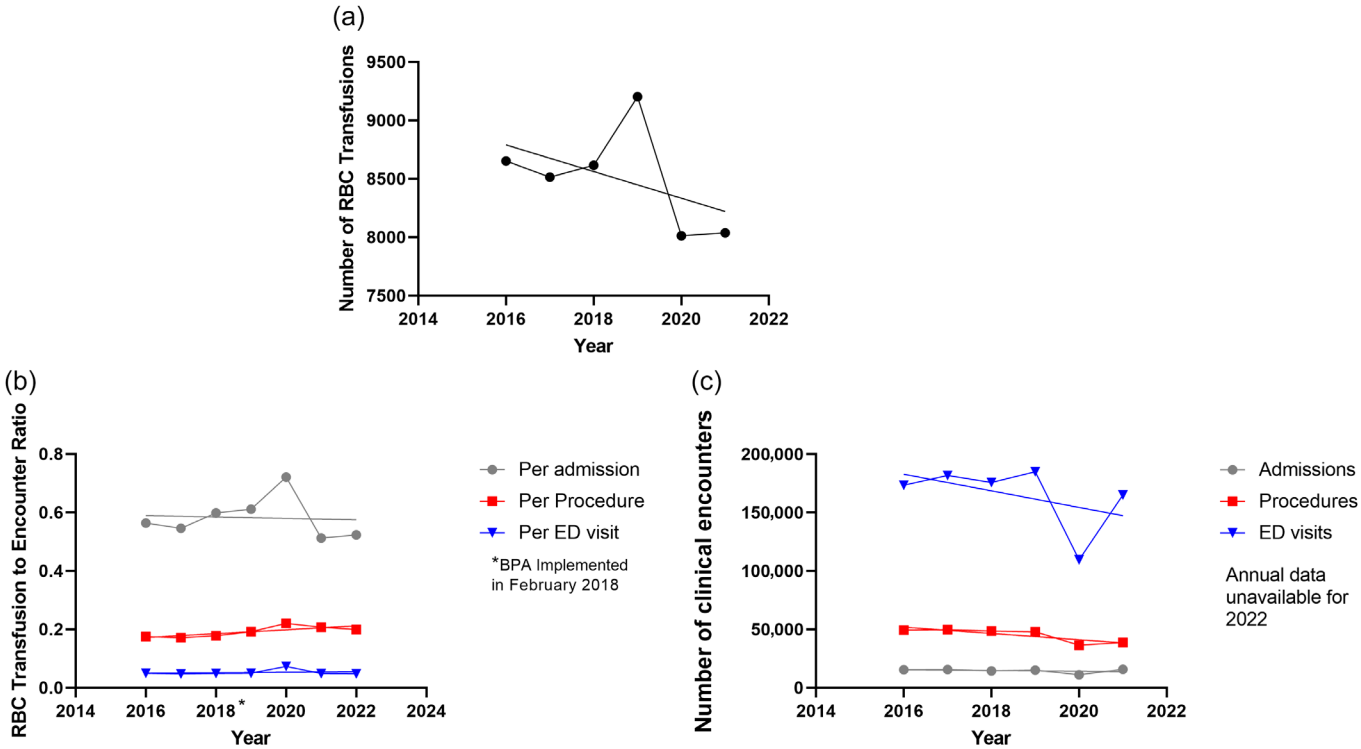


**FIGURE 1** (a) Number of red blood cell (RBC) transfusions per year at a tertiary children's care hospital network from 2016 to 2022. (b) Ratio of RBC transfusions versus type of clinical encounter at a tertiary children's care hospital network from 2016 to 2022. (c) Number of clinical encounters at a tertiary children's care hospital network from 2016 to 2022. BPA, best practice alert; ED, emergency department.

licensed for a total of 601 beds and 21 ambulatory specialty care centres. These facilities care for more than 200,000 unique patients annually, including approximately 20,000 surgeries and 100,000 emergency department (ED) visits. Volume and intensity of care were followed using a case mix index, a measure of hospital output based on hospital charges and reimbursement [13]. This study was submitted to the University of Texas Southwestern Institutional Review Board (IRB) and the Children's Health research department, and it was determined that IRB approval was not necessary. No patient guardian signatures were required.

An EHR (Epic Systems Corp., Verona, WI, USA)-based CDS intervention (Figure 1) was developed consisting of an interruptive BPA with a suggested initial transfusion threshold Hb of <8 g/dL for stable inpatients, this was later lowered to <7 g/dL. The alert was designed to trigger based on inpatient location and the last recorded Hb level. This included all floor patients within the hospital outside of a specific care setting, described below. The BPA displays immediately when the rule is met, and a recommendation for avoiding transfusion above a prescribed level in stable patients is provided as well as a link to a reference and a list of the patient's most recent Hb values [11]. The BPA must be acknowledged and answered prior to placing the RBC order.

The interruptive BPA was automated to display when an RBC transfusion was ordered for a patient with an Hb over the predetermined threshold. This excluded specific patients, described below, who were critically ill or had cardiac disease. Trauma blood, including the massive transfusion protocol, as well as ordering within the operating room would also not activate the BPA.

In detail, patients from the BPA included the cardiology and cardiovascular intensive care unit. The haematology/oncology floors were also excluded because (1) haematology patients are often transfused based on criteria other than Hb and (2) chemotherapy protocols often require thresholds of <8 g/dL or greater. Finally, surgery and the post-anaesthesia care unit were excluded due to their need to respond to acute bleeding rather than previous results. Trauma ordering is performed via a verbal order and was, therefore, excluded from the BPA. The system's limited ability to track the location of outpatients (and the accepted clinical practice of transfusing to a higher threshold to prevent unneeded hospital admission) meant that outpatients were excluded from the BPA.

If the provider chose to proceed with transfusion, they would encounter an alert, which they could ultimately override. The alert provided an 'acknowledgement reason' drop-down query that required the ordering physician to provide an answer as to why the blood transfusion was ordered: patient unstable, patient actively bleeding, (congenital) heart disease, standard treatment protocol (Neuroblastoma Protocol), symptomatic anaemia, procedure needed or see comment free text option.

Prior to implementation, a year's worth of patients who would have activated the BPA were individually reviewed (D.K.N.) for appropriateness. There were multiple meetings with relevant stakeholders to determine suitability of the computerized intervention. Relevant service lines were made aware of the change through meetings, and it was determined that a large-scale educational effort would be too far-reaching.

**TABLE 1** Quarterly best practice alert data from March 2018 to April 2022.

| Quarter | Number of activations | RBC transfusions avoided | Percentage of RBC transfusions avoided | USD saved on RBC |
|---|---|---|---|---|
| 2nd, 2018 | 316 | 75 | 23.7% | $15,375.00 |
| 3rd, 2018 | 302 | 67 | 22.2% | $13,735.00 |
| 4th, 2018 | 270 | 68 | 25.2% | $13,940.00 |
| 1st, 2019 | 321 | 94 | 29.3% | $19,270.00 |
| 2nd, 2019 | 345 | 63 | 18.3% | $12,915.00 |
| **3rd, 2019** | 526 | 86 | 16.4% | $17,630.00 |
| **4th, 2019** | 495 | 90 | 18.2% | $18,450.00 |
| **1st, 2020** | 473 | 42 | 8.9% | $8,610.00 |
| **2nd, 2020** | 438 | 40 | 9.1% | $8,200.00 |
| **3rd, 2020** | 496 | 62 | 12.5% | $12,710.00 |
| **4th, 2020** | 436 | 57 | 13.1% | $12,198.00 |
| **1st, 2021** | 390 | 42 | 10.8% | $8,988.00 |
| **2nd, 2021** | 425 | 55 | 12.9% | $11,275.00 |
| **3rd, 2021** | 500 | 56 | 11.2% | $11,480.00 |
| **4th, 2021** | 492 | 59 | 12.0% | $12,508.00 |
| **1st, 2022** | 494 | 49 | 9.9% | $10,388.00 |
| Mean ± SD | 419.9 ± 84.3 | 62.81 ± 16.7 | 15.9 ± 6.3 | 12,980 ± 3357 |

*Note*: There were two periods in the study: (1) from February 2018 to June 2019; (2) from July 2019 to April 2022. Quarters in bold correspond with a transfusion threshold haemoglobin of <7 g/dL. Data shown from the first full quarter onward.
Abbreviation: RBC, red blood cell.

The BPA was implemented on 16 February 2018 with an initial Hb threshold of <8 g/dL after presentation to various hospital committees and clinical services. The threshold Hb level was eventually decreased to <7 g/dL in July 2019. The number of RBC transfusions averted (i.e., the numerator) was compared with the number of initial activations of the BPA (i.e., the denominator); this was used to determine the effectiveness of the CDS. RBC transfusions were considered averted if the BPA was activated, the order was cancelled followed and there was no subsequent RBC transfusion within 24 h time from BPA activation. BPA activity was assessed by quarter to adjust for days of the month, and quarters were assessed to determine seasonal variation in compliance.

Statistical analysis was performed using GraphPad Prism (release 9.2.0, GraphPad Software, San Diego, CA, USA). Simple linear regression was performed to assess change over time. Normality was assessed using the Shapiro–Wilk test. Descriptive statistics were performed, and means are shown with standard deviation for parametric groups, while the median and interquartile range are shown for non-parametric groups. Groups were compared using an unpaired *t*-test or Mann–Whitney test where appropriate and differences were considered significant if $p < 0.05$. Quarterly data were assessed using analysis of variance repeated-measures testing, and by convention, matching was considered effective if $p < 0.05$ [14].

## RESULTS

The BPA has been activated a total of 6956 times from 16 February 2018 to 31 March 2022, or approximately 4.6 times/day. No safety issues were reported to the Blood Bank, the hospital quality and safety committees, or through the patient safety incident reporting system. Quarterly data are displayed in Table 1. The number of total transfusions has trended down since implementation (2016: 8652, 2017: 8514, 2018: 8615, 2019: 9202, 2020: 8013 and 2021: 8037) though there is no significant difference in the total number of RBC transfusions (Mann–Whitney, $p = 0.41$) (Figure 1a) or RBC transfusions per admission (Mann–Whitney, $p = >0.99$) (Figure 1b) in a context of similar care volumes (Figure 1c). The annual case mix index for all inpatients for the years 2016–2021 increased slightly with individual values as follows: 2016-2.47, 2017-2.55, 2018-2.77, 2019-2.76, 2020-2.78 and 2021-2.76. The success rate, with no RBC transfusions within 24 h was 14.5% (1012/6956). The estimated cost savings based on the acquisition cost of RBC units at the time of firing are $213,822 or about $51,891 a year.

The Hb threshold for BPA triggering was changed from <8 to <7 g/dL in July 2019. The number of BPA activations per quarter increased significantly from 310.8 (SD = 27.6) to 469.5 (SD = 41.24) (*t*-test, $p = <0.001$), though the percentage of successful activations decreased significantly from 23.7% (SD = 4.1) to 12.3% (SD = 2.9) (*t*-test, $p = <0.001$). The number of transfusions per quarter decreased from 15,047 (SD = 2522) to 12,040 (SD = 3354) though this change was non-significant (*t*-test, $p = 0.10$). The total savings for the BPA at 8/dL was $81,385 and the total savings for the BPA at <7 g/dL was $132,437.00. The average cost savings for the BPA at <8 and <7 g/dL were $45.44/activation and $25.64 per activation, respectively.

A large proportion of BPAs were immediately overridden, 70.5% (4902/6956), while the remaining patients would go on to be transfused in the 24 h after activation. The most common reason for overriding the BPA was 'procedure needed' (Figure 2). A screen capture of the BPA is provided (Figure 3).

The number of BPA activations was similar from quarter to quarter as matching was considered effective ($p = 0.0015$) (Table 2). It should be noted that the highest mean activation rate was in quarter 3 (456, SD = 103.5). The raw number of RBC transfusions avoided did not effectively match ($p = 0.10$), though the percentage of transfusions avoided per BPA did ($p = 0.0005$), suggesting similar rates of compliance year round.

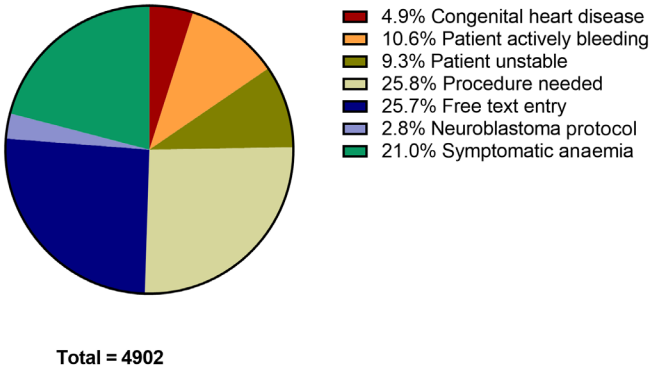


**FIGURE 2** Percentage of override reasons for a restrictive red blood cell transfusion best practice alert from 16 February 2018 to 1 April 2022.

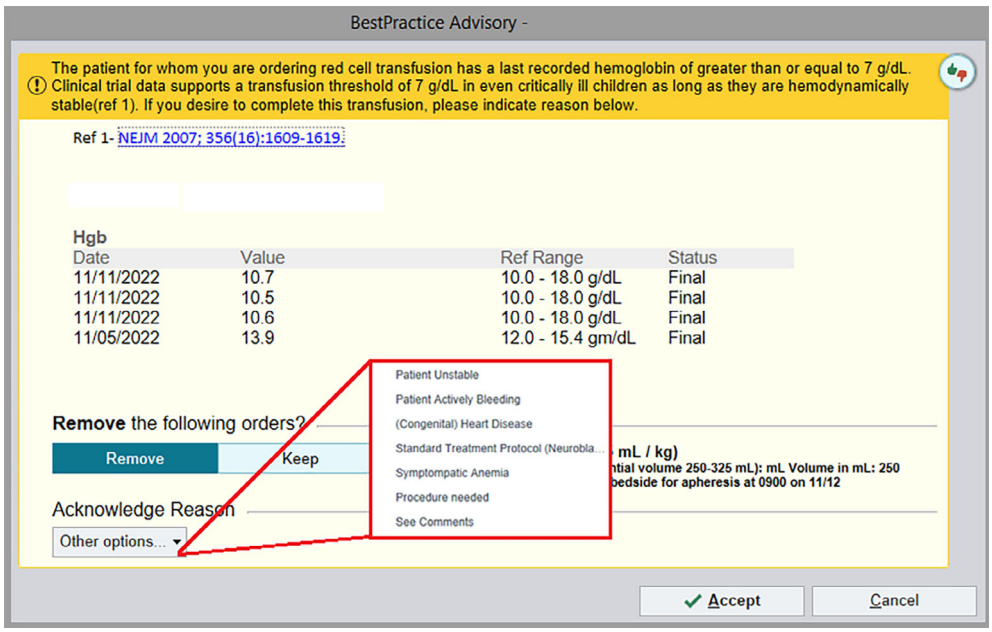


**FIGURE 3** Screen capture of best practice alert.

**TABLE 2** Mean quarterly best practice alert data from March 2018 to April 2022.

| | First quarter mean ± SD | Second quarter mean ± SD | Third quarter mean ± SD | Fourth quarter mean ± SD | Matching effective (*p* value) |
|---|---|---|---|---|---|
| Number of activations | 419.5 ± 79.6 | 381.0 ± 59.7 | 456.0 ± 103.5 | 423.3 ± 105.7 | 0.0015 |
| RBC transfusions avoided | 56.75 ± 25.1 | 58.25 ± 14.7 | 67.75 ± 13.0 | 68.50 ± 15.1 | 0.10 |
| Percentage of RBC transfusions avoided | 14.73 ± 9.7% | 16.00 ± 6.4% | 15.58 ± 5.0% | 17.13 ± 6.0% | 0.0005 |

*Note*: Data presented to assess seasonal variation. Data determined from the first full quarter onward.
Abbreviation: RBC, red blood cell.

## DISCUSSION

We successfully implemented a BPA for RBC transfusion appropriateness at a large, tertiary care children's hospital system. We demonstrated sustained use and cost savings in keeping with a prior systematic review and meta-analysis of data assessing transfusion behavioural interventions [6]. Likewise, our achievement of the desired outcome, avoidance of RBC transfusion, rate of 14.5% is similar to recent meta-analysis data showing a rate of 14.7% success of BPAs in the paediatric setting [7].

There was a slight decrease in compliance during the first two quarters of 2020, likely related to the COVID-19 pandemic, though this was not associated with an overall increase in transfusions for the year. We speculate this may have been associated with physician stress and burnout, though we had also lowered our BPA's Hb threshold roughly 9 months prior. Physician burnout has been associated with extended EHR use and increased frequency of pop-up alerts may lead to more burnout and decreased compliance with BPAs [8, 15, 16]. As such, these tools should be used in a targeted fashion, to not only ensure evidence-based practice but similar to support clinical staff's well-being. As we have fewer than five activations a day, this may represent a right-size approach, where the appropriate caregiver is seeing the alert in the appropriate context; likewise, care must be given to avoid activation in critically ill or haemorrhaging patients to prevent delay [17].

Blood shortages have been sustained, even nearly 3 years after the beginning of the COVID-19 pandemic. With that in mind, many institutions routinely utilize prospective auditing for other types of blood orders, while other groups may reserve this tactic during times of shortage [18, 19]. Alternate interventions (including educational outreach, feedback to clinicians and reminders) represent behavioural modifications, and care should be given when choosing an appropriate intervention to reduce transfusion [5, 6]. As much of this work is labour intensive, a BPA may represent an optimal intervention at sites with fewer transfusion medicine physicians. They can also be considered in private labs where coverage is provided by generalists for both anatomic and clinical pathology issues, especially in a paediatric setting.

One of the strengths of this investigation is the length of time studied, which demonstrates annual variation, but an overall decrease in RBC transfusion. The number of transfusions increased in 2019 for unclear reasons, though there was an incremental increase in ED visits, invasive procedures and admissions for that year. As RBC transfusion rates were only slightly higher, but not significantly different, prior to BPA implementation, this may suggest that education about transfusion practice was happening in other ways to avoid unnecessary RBC exposure. It should be noted, however, that the case mix index was higher in the years following the implementation of the BPA, suggesting a higher intensity of care compared to the years prior to the BPA implementation. Nonetheless, as transfusion education has historically represented a gap in undergraduate medical education as well as paediatrics residency, a BPA may be a vital way to share important transfusion knowledge, especially at training facilities [20–23]. It should be noted that the highest number of mean activations was seen in quarter 3, which covers the months of July, August and September. Interestingly, these months had a high level of compliance, suggesting that newer residents may be more open to BPA intervention, an area that could potentially be explored with further investigation.

Most prior paediatric studies were performed in a small number of centres around the early 2010s and focused primarily on intensive care unit patients or in one instance patients with sickle cell disease [24–27]. Adams et al. performed a similar intervention in both PICU and acute care wards, and when compared with an historical cohort, showed a significant reduction in transfusion, lower mean Hb levels and no significant difference in mortality after implementation of an interruptive BPA [27]. Given the small amount of recent research examining paediatric patients, our findings represent a contemporary, large-scale paediatric study demonstrating the efficacy of an RBC transfusion BPA in this population. Given the high rate of prior success, it is important to investigate if these interventions are generalizable to current practice in additional hospitals [27].

The field of transfusion medicine has maintained for years that restrictive RBC transfusion practice is most appropriate. As recently highlighted in an editorial by Mo and Higgins, it will be important to trend data relating to long-term outcomes in cardiac surgery patients, as our indications for overriding demonstrate that physicians will transfuse as they deem appropriate for the patient's clinical situation, and a 'hard-stop' with this BPA would require additional levels of clinical buy-in and safeguards [28]. Tailoring a BPA with your EHR support to match your institution's populations may be appropriate to reduce unnecessary firing for certain groups or patients on certain floors and alleviate pop-up fatigue. Thus, future research should focus on patient outcomes relating to these EHR interventions (as previous systematic review data shows limited information on long-term clinical outcomes), as well as the impact of potential under transfusion [5, 29].

Study limitations include the nature of single-centre retrospective analysis. Likewise, we used a conservative definition of compliance with the BPA as a cancelled order and no RBC transfusion within 24 h. This would, unfortunately, categorize patients who did not qualify for transfusion at the time of firing because of adequate Hb as a failure, even if this changed (i.e., Hb became <7 g/dL) and they were subsequently transfused. However, this conservative method served to not inflate the success rate or mischaracterize any associated cost saving.

Our utilization of a BPA for RBC transfusion has proven to be successful for many years at a large paediatric tertiary care centre. Given our success, along with prior work showing a reduction in transfusion, these methods may be generalizable to other paediatric care settings [27]. If groups consider the implementation of a similar BPA, they should keep in mind the current EHR setup including the number of pop-ups, as well as populations who may or may not need exclusion. The use of a restrictive RBC BPA showed a similar success rate compared to other paediatric alerts and may represent a viable intervention to reduce transfusion in some care settings.

## ACKNOWLEDGEMENTS

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D.K.N., H.S.L. and R.M. conceptualized the study. D.K.N. and R.M. performed data collection. B.D.A. performed data analysis and wrote the initial manuscript. All authors contributed to critical edits and preparation of the final manuscript.

## CONFLICT OF INTEREST STATEMENT

The authors have no relevant conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

# A retrospective analysis of haemolytic reactions to intravenous immunoglobulin using data from the Transfusion-Transmitted Injuries Surveillance System (Ontario)

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## Abstract

**Background and Objectives:** Haemolysis can occur following intravenous immunoglobulin (IVIG) infusion. Haemovigilance data were analysed using a novel approach for including two control groups with no haemolysis to IVIG. Objectives included a summary of all reactions to IVIG, rate estimates and analysis of haemolytic reactions including risk factors.

**Materials and Methods:** Canadian haemovigilance data from Ontario (2013–2021), IVIG distribution and transfusion data from the blood supplier, and data from a large local transfusion registry were used. An 'other-reactions' control group included patients with IVIG reactions that were not haemolytic, and registry patients with no-reaction were the 'no-reaction controls'. Descriptive analysis and two logistic regression models for the different control groups were performed.

**Results:** One thousand one hundred and seventy reactions were included. Most common were febrile non haemolytic (26.1%), minor allergic (24.5%) and IVIG headache (15.3%) followed by haemolytic 10.9% (128/1170). Haemolytic reaction rates decreased over time: rates since 2020 estimated between 1.5 and 2.9/1000 kg IVIG used. The regression model for other-reaction controls identified two risk factors for haemolysis: non-O blood group recipients compared with group O recipients ($p$ value = 0.0106) and IVIG dose per 10 g increase (OR 1.359; 95% CI 1.225–1.506). The model using no-reaction controls gave similar results and also showed no pre-medication was associated with a higher risk of haemolysis (OR 29.084; 95% CI 1.989–425.312).

**Conclusion:** The frequency of haemolytic reactions has decreased over time. We confirmed non-O blood group recipients and IVIG dose as risk factors for haemolysis and raise the hypothesis that no pre-medication may increase the risk of haemolysis.

## Keywords

haemolysis, haemovigilance, intravenous immunoglobulin, risk factors

## Highlights

- Haemolytic reactions are the fourth most common reaction to intravenous immunoglobulin (IVIG) preceded by febrile non-haemolytic, allergic and IVIG headache; however, their frequency has decreased since 2018.
- Analyses of haemovigilance data are limited by the lack of a control group; however, this study used a novel methodological approach to identify two different control cohorts for use in our analysis.
- The analysis confirmed that non-group-O patients and IVIG dose were risk factors for IVIG haemolysis and raised the hypothesis that lack of pre-medication could increase this risk.

## INTRODUCTION

Immunoglobulin (IG) products are produced from plasma donations (apheresis and/or whole blood) which undergo routine blood product screening and are then pooled (≥1000 plasma units/pool) and fractionated to make the intravenous (IVIG) and subcutaneous immunoglobulin (SCIG) products. Although IG products have an exceptional safety record for infectious disease transmissions, infusion of the product can be accompanied by signs and symptoms of acute reactions in some patients [1].

Haemolysis (acute or delayed) can be mild to severe reactions that can occur during or following an IVIG infusion [2]. The primary cause of these reactions appears to be ABO antibodies in the IVIG product that can bind to red blood cells of group A, B and/or AB individuals resulting in intravascular and/or extravascular haemolysis [3]. To decrease the risk of haemolysis some manufacturers have modified the manufacturing process, to reduce ABO antibody concentration in the product [4]. Others have suggested that screening and excluding donors with high anti-A and anti-B titres may be effective [2, 5]. Risk factors for IVIG haemolysis appear to be receiving a high dose of IVIG (≥2 g/kg), non-group-O blood type recipients and median anti-A and anti-B titres of 16 and 8, respectively [2, 6–9].

Few studies have used haemovigilance data to explore haemolysis associated with IVIG [6, 10, 11] and the lack of a control group and denominator data has limited these studies to descriptive analyses. In Canada, the Transfusion Transmitted Injuries Surveillance System (TTISS) programme is funded by the Public Health Agency of Canada to capture adverse transfusion events [12]. The Michael DeGroote Centre for Transfusion Research (MCTR) coordinates the TTISS programme for the province of Ontario (TTISS-ON). The objectives of this study using TTISS-ON data included: identifying the frequency and risk rate of reactions to IVIG, performing a comprehensive analysis of haemolytic reactions including risk factors and identifying current limitations of surveillance data to understand transfusion reactions.

## MATERIALS AND METHODS

This retrospective study analysed reactions to IVIG reported to TTISS-ON between 2013 and 2021. Research ethics approval for the study was obtained from the Hamilton Integrated Research Ethics Board #13375.

Three data sources were used (Figure 1). Reaction data from all reporting hospitals were extracted from the TTISS-ON database (2013–2021). Hospitals submit reaction data using the national TTISS case report form [13], with data being entered and managed using REDCap electronic data capture tools hosted at McMaster University in Hamilton, Canada. Common reaction definitions were defined by the national TTISS programme with reactions being categorized by severity and imputability at the hospital level. Currently, all Ontario hospitals report severe and life-threatening reactions to TTISS-ON. Twenty-eight of the hospitals (representing 40% of the blood products transfused in the province) are sentinel sites and report all reactions including non-severe (see Data S1 for specific details on TTISS). Haemolytic reactions were categorized as acute haemolytic transfusion reactions (AHTR), delayed haemolytic transfusion reactions (DHTR) and delayed serological transfusion reactions (DSTR); Data S1. Data elements submitted to TTISS-ON were cleaned to ensure validity and capture missing data.

Denominator data for total grams of IVIG used per year were provided for the years 2014–2021 by the Ontario Regional Blood Coordinating Network [14] using hospital distribution data stored in a web portal provided by the blood supplier. Clinical, demographic and laboratory data for patients receiving IVIG who did not have reactions were extracted from the Transfusion Research Utilization Surveillance and Tracking database (TRUST). TRUST contains demographic and clinical data, laboratory test results and transfusion data on every patient admitted to four academic Hamilton hospitals over a 20-year period [15]. For this project, TRUST data were extracted for the years 2013–2021 with the exception of pre-medication data which were available only for the years 2013–2018. All data were encrypted on a secure SQL server housed and maintained by the Computer Services Unit, McMaster University.

Data from all Ontario hospital sites were analysed to describe the number of reactions by type and trends over time. All other analyses were performed with data reported by the sentinel sites. This included: rates by reaction type and exploratory analyses of haemolytic reactions to IVIG. Two control groups were included for the exploratory analysis: (1) patients in the TTISS-ON database who had reactions to IVIG not categorized as haemolytic (referred to as 'other-reaction controls') and (2) patients identified from the TRUST

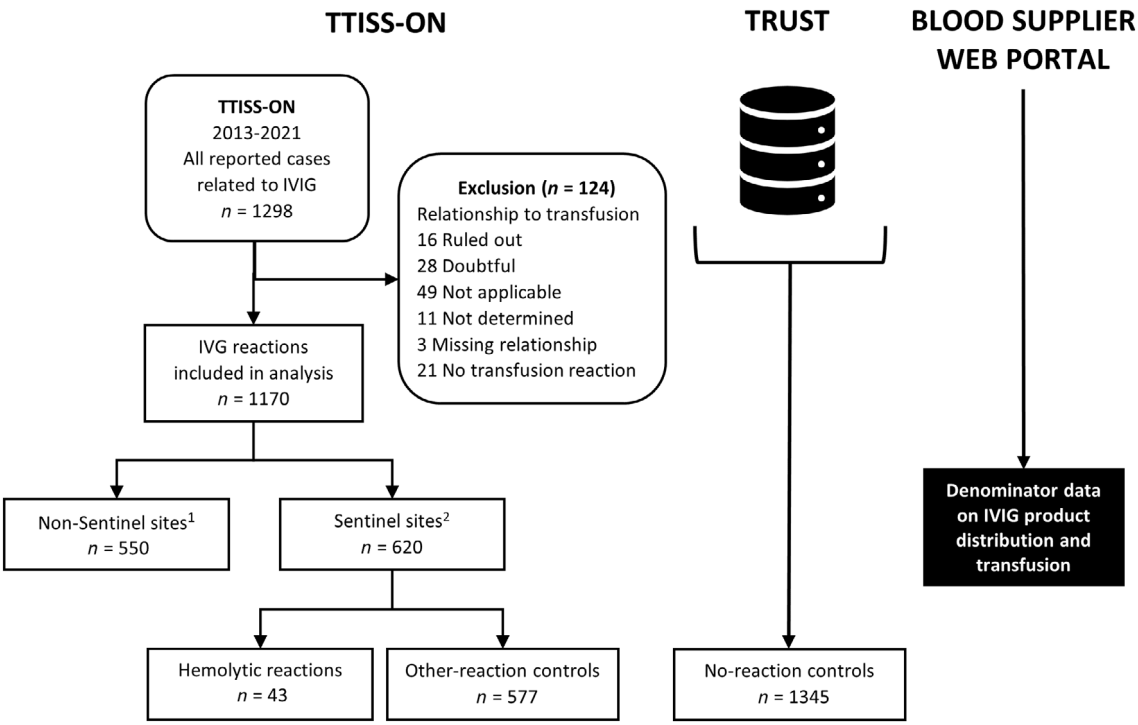


**FIGURE 1** Flow diagram of data sources. [1]Sites that are required to report moderate to severe reactions only. [2]Sites that report all reactions (mild, moderate and severe); the analysis of reaction frequency and rate included only reactions reported between 2014 and 2021 ($n = 568/620$ reactions). TRUST, Transfusion Research Utilization Surveillance and Tracking database; TTISS-ON, Transfusion Transmitted Injuries Surveillance System—Ontario.

database who received IVIG and did not have reactions (referred to as 'no-reaction controls').

## Statistical analysis and sample size

Descriptive analyses of continuous data were described using measures of central tendency (mean and standard deviation, or median, interquartile range and min–max values). Categorical data were analysed as proportions.

Reaction rates by reaction type were calculated using sentinel site data from 2014 to 2021 as this was the time period when denominator data were available. Reaction rates were calculated using the number of reactions in the time period divided by the total grams of IVIG administered during that time period.

The time between infusion and reaction presentation was explored. Multivariate logistic regression analyses were performed to identify risk factors for IVIG haemolytic reactions using two models: each model separated by all patients and just adults. The first model included cases and other-reaction controls from TTISS-ON and the second model included cases and no-reaction controls from TRUST. Independent risk factors controlled for in all models were recipient sex, age, ABO group, service category (haematology/oncology, obstetrics/gynaecology, surgical and medical), pre-medication before transfusion (yes/no) and dose of IVIG administered. The joint hypothesis was tested using chi-bar-square statistic with $p$ values obtained by simulation [16]. A formal sample size was not calculated as this was a sample of convenience based on the availability of data from TTISS-ON. Analyses were performed with SAS 9.4 (Gary, NC, USA).

# RESULTS

During the study period, 1298 IVIG-associated adverse events were reported. There were 124 cases excluded (Figure 1) leaving 1170 reactions for analysis. The sentinel sites reported 620 reactions: 43 (6.9%) were haemolytic reactions; the remaining 577 reactions were included in the 'other-reactions control group'. From TRUST, there were 1345 patients who received IVIG with no reaction on their first infusion and were included in the 'no-reaction control group' (Figure 1).

Reactions to IVIG reported by all hospitals by year are summarized in Table 1. The most common reactions were febrile non-haemolytic transfusion reactions (FNHTR)—26.1%, minor allergic (24.5%) and headache (15.3%) and the least common (<1%) were transfusion-related alloimmune neutropenia (TRAIN), bacterial and hyperhaemolysis. The frequency of DHTR appeared to decrease around 2018, but trends were not present for all other reaction types. The demographic information for this cohort is presented in Table S1.

Analysis of sentinel sites data 2014–2021 showed a similar pattern of the most and least common reactions. The most frequent types of reactions to IVIG included: FNHTR (30.8%), minor allergic

**TABLE 1** Number and frequency of reactions to intravenous immunoglobulin (IVIG) by type for all Ontario hospitals reporting to TTISS-ON from 2013 to 2021.

| | Type of reactions reported by year—number (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Reaction type** | **2013 ($n = 64$)** | **2014 ($n = 117$)** | **2015 ($n = 113$)** | **2016 ($n = 130$)** | **2017 ($n = 124$)** | **2018 ($n = 135$)** | **2019 ($n = 214$)** | **2020 ($n = 143$)** | **2021 ($n = 130$)** | **Total ($n = 1170$)** |
| Febrile non-haemolytic | 18 (28.1) | 21 (17.9) | 16 (14.2) | 22 (16.9) | 32 (25.8) | 42 (31.1) | 75 (35.0) | 53 (37.1) | 28 (21.5) | 307 (26.1) |
| Minor allergic | 11 (17.2) | 39 (33.3) | 39 (34.5) | 37 (28.5) | 30 (24.2) | 35 (25.9) | 29 (13.6) | 30 (21.0) | 38 (29.2) | 288 (24.5) |
| IVIG headache | 10 (15.6) | 16 (13.7) | 17 (15.0) | 19 (14.6) | 28 (22.6) | 17 (12.6) | 37 (17.3) | 16 (11.2) | 20 (15.4) | 180 (15.3) |
| Delayed haemolytic | 10 (15.6) | 14 (12.0) | 14 (12.4) | 13 (10.0) | 8 (6.5) | 6 (4.4) | 5 (2.3) | 2 (1.4) | 4 (3.1) | 76 (6.5) |
| Non-specific pain | 1 (1.6) | | 1 (0.9) | 2 (1.5) | 1 (0.8) | 5 (3.7) | 22 (10.3) | 14 (9.8) | 11 (8.5) | 57 (4.9) |
| Severe allergic/anaphylactic | 3 (4.7) | 8 (6.8) | 4 (3.5) | 4 (3.0) | 6 (4.8) | 9 (6.7) | 10 (4.7) | 6 (4.2) | 2 (1.5) | 52 (4.4) |
| Acute haemolytic | 4 (6.3) | 4 (3.4) | 4 (3.5) | 10 (7.5) | 4 (3.2) | 1 (0.7) | 6 (2.8) | | 6 (4.6) | 39 (3.3) |
| TACO | | 2 (1.7) | | 5 (3.7) | 5 (4.0) | 5 (3.7) | 8 (3.7) | 2 (1.4) | 6 (4.6) | 33 (2.8) |
| Aseptic meningitis | 2 (3.1) | 2 (1.7) | 2 (1.8) | 5 (3.7) | 1 (0.8) | 3 (2.2) | 6 (2.8) | 3 (2.1) | 4 (3.1) | 28 (2.4) |
| TAD | | | 1 (0.9) | 1 (0.7) | 2 (1.6) | | 9 (4.2) | 2 (1.4) | 2 (1.5) | 17 (1.4) |
| Hypotensive | | | 2 (1.8) | 2 (1.5) | 3 (2.4) | 2 (1.5) | | 4 (2.8) | 2 (1.5) | 15 (1.3) |
| Delayed serological | 1 (1.6) | 1 (0.9) | 2 (1.8) | 3 (2.3) | | 1 (0.7) | | 1 (0.7) | 4 (3.1) | 13 (1.1) |
| Hypertension | 1 (1.6) | | | | 1 (0.8) | 2 (1.5) | 1 (0.5) | 5 (3.5) | 2 (1.5) | 12 (1.0) |
| TRAIN | | | | | | 3 (2.2) | | | | 3 (0.3) |
| Possible TRALI | | | | | 1 (0.8) | | 1 (0.5) | | | 2 (0.2) |
| Bacterial infection[a] | | | 1 (0.9) | | | | | | | 1 (0.1) |
| Hyperhaemolysis | | | | | | | 1 (0.5) | | | 1 (0.1) |
| Other | | 5 (4.3) | 8 (7.1) | | | 1 (0.7) | | | | 14 (1.2) |
| Unknown | 2 (3.1) | 3 (2.6) | 1 (0.9) | 1 (0.8) | | 1 (0.7) | 2 (0.9) | 1 (0.7) | 1 (0.8) | 12 (1.0) |

*Note*: Non-sentinel sites are required to report only moderate and severe reactions.

Abbreviations: TACO, transfusion-associated circulatory overload; TAD, transfusion-associated dyspnoea; TRAIN, transfusion-related alloimmune neutropenia; TRALI, transfusion-related acute lung injury.

[a]This reaction was categorized as possible bacterial contamination; however, failure to change the cap on the central line prior to administration could have contributed to an increased risk of contamination. Unlikely due to product contamination; however, cultures on the IVIG product were not performed. The patient culture grew *Enterobacter cloacae*.

(29.0%) and IVIG headache (15.3%) accounting for 75.2% of all adverse events. Haemolysis occurred in 6.9% (39/568) of reactions: DHTR (5.1%), AHTR (1.4%) and DSTR (0.4%). Reactions with a frequency of less than 1% included: hypotension, hypertension, transfusion-associated dyspnoea (TAD), TRAIN, DSTR, bacterial infection and hyperhaemolysis (Figure 2). Sentinel sites did not report any confirmed cases of TRALI although two cases of possible TRALI were reported by non-sentinel hospitals (Table 1). The event rate for each reaction type per 1000 kg of IVIG used ranged from 31.2/1000 kg of IVIG used for FNHTR to 0.2/1000 kg of IVIG used for hyperhaemolysis and bacterial infection (Figure 2 and Table S2).

The sentinel sites reported 43 cases of haemolysis associated with IVIG over the 9-year period (2013–2021): AHTR in ten cases (23.2%), DHTR in 31 cases (72.1%), and two case (4.7%) classified as a DSTR. Demographics, infusion data and reaction severity by type of haemolytic reaction are summarized in Table 2. Patient demographics were similar between the three categories of haemolytic reactions; however, the mean dose of IVIG infused was highest in the patients with DHTR (mean 113.8 g; SD 62.1) compared with AHTR (mean 80.9; SD44.4) and DSTR (mean 42.5; SD24.7). Most haemolytic reactions were classified as non-severe (53.4%) with 41.9% classified as severe and 4.7% as life threatening. Severe reactions presented most frequently as DHTR (16/18; 88.9%).

The median number of days (interquartile range) from infusion until reaction presentation was 0 (0–1) for AHTR, 2 (1–5) for DHTR and 5 (0–10) for DSTR. The trends (2014 and 2021) for haemolytic reactions by product manufacturer are shown in Figure 3. Between 2014 and 2015, the highest rates of reported reactions (adjusted for product use) occurred with products from Company D. After 2015, reaction rates for all products decrease. The current estimate of reaction rates from 2020 and 2021 are 1.5 and 2.9/1000 kg IVIG use, respectively.

Demographic data on patients with haemolysis and the two control groups are summarized in Table 3. The results for each variable were similar between the three groups with the exceptions of the ABO group and dose. Patients with haemolysis had a higher frequency of blood groups A, B and AB compared with O, whereas blood group frequencies in the control groups were similar to the population frequencies. The dose of IVIG was higher in the cases compared with controls. The results of the multivariate logistic regression analyses are summarized in Figure 4. The first model included cases of haemolysis and other-reaction controls performed with all patients and just adults. The second model included cases of haemolysis and no-reaction controls derived from the TRUST (all patients and just adults). In all models, an increased risk of haemolysis was associated with individuals of blood groups A, B or AB (largest odds ratios with group AB vs. group O), and the $p$ value of the joint contrast comparison (non-O blood group recipients to group O recipients) was significant ($p = 0.0106$). A higher dose of IVIG was also significant in all models. In the second model (cases and no-reaction controls), the risk of haemolysis was significantly higher when pre-medications

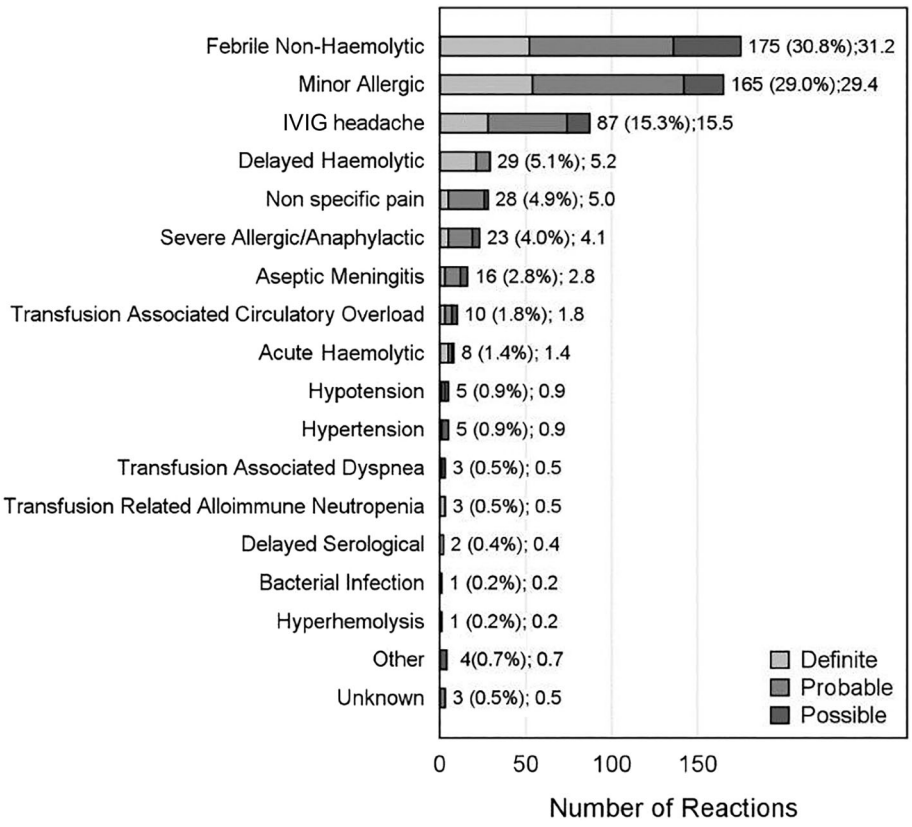


**FIGURE 2** Frequency and rate of reactions by type reported by sentinel sites (2014–2021; 570 cases). Beside each bar is the number of reactions (percentage), and reaction rate (number of reactions per 1000 kg of intravenous immunoglobulin [IVIG] used).

**TABLE 2** Demographics, intravenous immunoglobulin (IVIG) infusion data and reaction severity by type of haemolytic reaction (acute haemolytic transfusion reactions, delayed haemolytic transfusion reactions and delayed serological transfusion reactions).

| | Acute haemolytic ($n = 10$) | Delayed haemolytic ($n = 31$) | Delayed serological ($n = 2$) |
|---|---|---|---|
| Sex *n* (%) | | | |
| Male | 5 (50.0) | 13 (41.9) | |
| Female | 5 (50.0) | 18 (58.1) | 2 (100.0) |
| Age (years) mean/standard deviation; median (interquartile range) | 47.5/27.6; 53 (19–63) | 45.2/26.5; 53 (21–65) | 50.5/30.4; 51 (29–72) |
| Blood group, *n* (%) | | | |
| A | 6 (60.0) | 19 (61.3) | 2 (100.0) |
| B | 1 (10.0) | 4 (12.9) | |
| O | | 1 (3.2) | |
| AB | 3 (30.0) | 7 (22.6) | |
| Service category, *n* (%) | | | |
| Haematology/bone marrow transplant | 1 (10.0) | 6 (19.4) | |
| Oncology | | 2 (6.5) | |
| Medical | 8 (80.0) | 23 (74.2) | 2 (100.0) |
| Obstetrics/gynaecological/perinatal | 1 (10.0) | | |
| Department, *n* (%) | | | |
| Intensive care unit | | 6 (19.4) | |
| Medical ward/surgical ward | 5 (50.0) | 20 (64.5) | 1 (50.0) |
| Obstetrics | 1 (10.0) | | |
| Outpatient clinics | 4 (40.0) | 5 (16.1) | 1 (50.0) |
| Pre-medication, *n* (%) | | | |
| No (includes missing) | 10 (100.0) | 31 (100.0) | 1 (50.0) |
| Yes | | | 1 (50.0) |
| Dose available, *n* (%) | 8 (80.0) | 29 (93.5) | 2 (100.0) |
| Dose (g) mean/standard deviation; median (interquartile range) | 80.9/44.485; (44–123) | 113.8/62.1; 110 (65–150) | 42.5/24.7; 43 (25–60) |
| Severity grade | | | |
| Grade 1 (non-severe) | 7 (70.0) | 14 (45.2) | 2 (100.0) |
| Grade 2 (severe) | 2 (20.0) | 16 (51.6) | |
| Grade 3 (life threatening) | 1 (10.0) | 1 (3.2) | |
| Outcome | | | |
| Minor or no sequelae | 9 (90.0) | 30 (96.8) | 2 (100.0) |
| Death | 1 (10.0) | | |
| Not determined | | 1 (3.2) | |

(antipyretics, antihistamines, corticosteroids, diuretics, sedatives and epinephrine) were not administered before transfusion. Patient age and sex were not significant factors. The complete summaries from the regression models are provided in Tables S3 and S4.

The research team identified limitations using surveillance data which included: the structure of the reporting form which resulted in some missing data; failure to capture key test results that may be applicable to specific types of reaction; a lack of diagnosis, other clinical data and reason for transfusion and inability to link multiple reactions on the same patient. These are issues that can be resolved by altering the structure of the report form and database.

## DISCUSSION

In this study, we identified the most common reactions associated with IVIG and their rates calculated per 1000 kg of IVIG usage. Febrile non-haemolytic, minor allergic reactions and IVIG headache were the most common reactions with haemolytic being the fourth most frequent adverse event representing approximately 6.9% of all IVIG-associated adverse events reported. We confirmed the previously identified risk factors for IVIG haemolysis as non-O blood group recipients and a higher dose of IVIG was administered. Overall reaction rates for IVIG haemolysis have decreased over time with current estimates for 2020 and 2021 of 1.5–2.9 reactions per 1000 kg used, respectively.

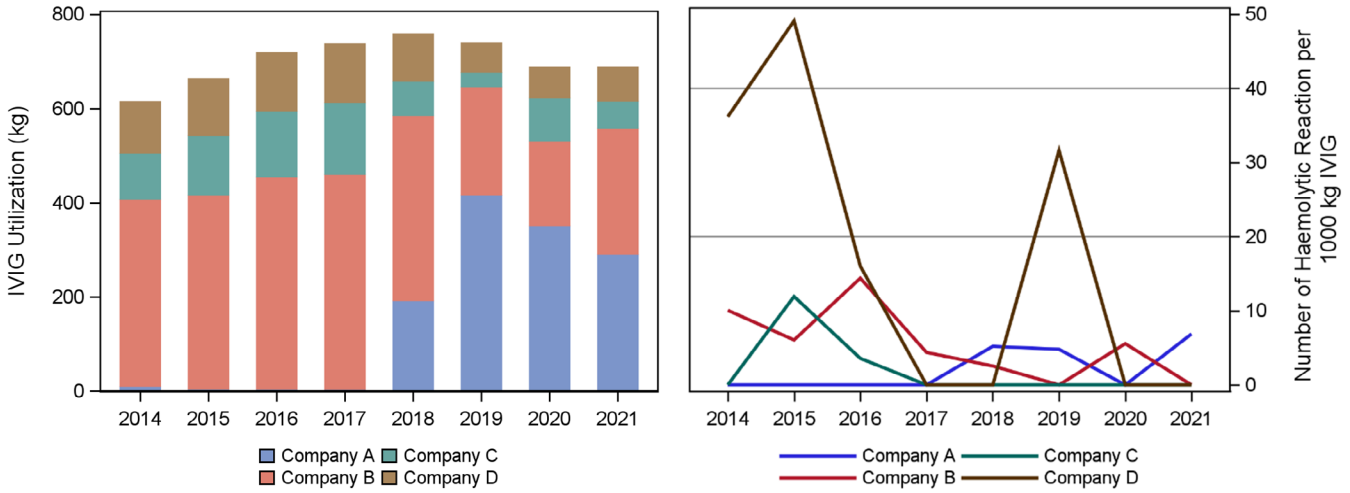


**FIGURE 3** Summary of trends in haemolytic reactions by the manufacturer between 2014 and 2021. Bar chart on the left represents the proportion of company products used per year during the 8-year period. Reaction rates are adjusted for product use and expressed as reactions per 1000 kg of intravenous immunoglobulin (IVIG) used.

We acknowledge that a haemolysis rate of reaction calculated by 1000 kg of IVIG used is not helpful when communicating risk to patients and health care providers; however, this limitation is consistent throughout the literature. For example, a systematic review recently published by Cuesta et al. [2] reports the rate as an incidence frequency of haemolysis and a report by Walderhorst et al. [10] reports the rate as haemolysis/1000 kg sold. The inability to calculate a meaningful rate estimate results from a lack of information on a total number of patients receiving IVIG and the number of repeated infusions of IVIG at the patient level. Such information is required to specify a risk per infusion at the patient level or risk per patient. We were able to calculate the rate per 1000 kg of IVIG used because the TTISS-ON programme has access to the total grams of IVIG that are used in Ontario each year, but patient-level data and repeated reactions per patient are not available.

A description of the serological work-up associated with haemolytic reactions was not possible as the current TTISS case report form structure resulted in missing data. We were able to assess the timing associated with acute, delayed and serological reactions which were consistent with post-infusion reaction times reported by Pendergrast et al. [17]. However, we question whether the definitions of acute, delayed and serological haemolytic reactions are appropriate in the context of IVIG haemolysis. These terms evolved from haemolytic reactions primarily to red blood cells where antibody formation usually represented an amnestic response or in some cases a primary response to foreign antigen exposure. When passive ABO antibodies in IVIG are infused, some patients have immediate haemolysis but more frequently there is delayed onset of haemolysis. In 2009, Health Canada's IVIG Haemolysis Pharmacovigilance Group proposed a definition of IVIG haemolysis comprised of at least a 10 g/L drop in haemoglobin, evidence of abnormal laboratory test results and clinical signs of haemolysis [18] rather than the traditional categories used in haemovigilance systems. Changes will be made to the TTISS-ON database to accommodate this definition and ensure that all relevant information is collected to make a standardized conclusion about IVIG haemolysis.

Patient factors can have a contributing role in the risk and onset of IVIG-associated haemolysis as described in the literature. Patient risk factors identified include increased macrophage phagocytic activity, absence of immunosuppressive drugs, patient co-morbidities, underlying health issues, inflammatory status and routine use of medications (non-steroidal anti-inflammatory drugs, broad-spectrum antibiotics and gastrointestinal medications) [2, 6, 7, 17, 19].

Risk factors identified in this study (non-O blood group recipients and dose) have been previously reported in numerous prospective and retrospective studies [2]. Patients of blood groups A, B and AB were at greater risk of haemolysis compared with group O patients likely because of the passive transfer of ABO antibodies in the IVIG product. In our regression analysis, group AB individuals had the largest odds ratio compared with individuals who were in groups A and B suggesting a higher risk of haemolysis which is consistent with some published reports [17]; however, reported risk by blood group is variable with some studies suggesting that group A individuals may be at greater risk than group AB [2]. Haemolysis in group O patients is rare but has been observed and is typically attributed to other non-ABO blood group antibodies that are present in some IVIG products. In our cohort, there was one haemolytic reaction to IVIG reported in a group O patient; however, on further investigation resulting from our study, this patient had received an ABO incompatible transplant which was probably the cause of the haemolysis observed. The regression model including the cases and the no-reaction controls group (TRUST data) indicated that not receiving pre-medication was associated with a higher risk of haemolytic reactions. This finding has not been reported in other studies. Our results could be due to chance or be related to under-reporting of pre-medication in the TTISS database. Pre-medication is typically used to prevent

**TABLE 3** Demographics and intravenous immunoglobulin (IVIG) infusion data for the cases and the two control groups.

| | Haemolytic reactions ($n = 43$) | Other-reaction controls ($n = 577$) | TRUST controls[a] ($n = 1345$) |
|---|---|---|---|
| Sex, *n* (%) | | | |
| Male | 18 (41.9) | 247 (42.8) | 589 (43.8) |
| Female | 25 (58.1) | 330 (57.2) | 756 (56.2) |
| Age available, *n* (%) | 42 (97.7) | 575 (99.7) | 1345 (100.0) |
| Age (years) mean/standard deviation/median (interquartile range) | 46.0/26.2; 53 (21–65) | 49.6/22.9; 55 (32–68) | 36.7/30.3; 39 (4–65) |
| Blood group available, *n* (%) | 43 (100.0) | 531 (92.0) | 1331 (99.0) |
| A | 27 (62.8) | 192 (36.2) | 535 (40.2) |
| B | 5 (11.6) | 75 (14.1) | 185 (13.9) |
| O | 1 (2.3) | 244 (46.0) | 556 (41.8) |
| AB | 10 (23.3) | 20 (3.8) | 5 (4.1) |
| Service category[b], *n* (%) | | | |
| Haematology/oncology | 9 (20.9) | 149 (25.8) | 244 (18.1) |
| Medical | 33 (76.7) | 401 (70.5) | 985 (73.2) |
| Surgical | | 12 (2.1) | 97 (7.2) |
| Obstetrics/gynaecology/perinatal | 1 (2.3) | 7 (1.2) | 19 (1.4) |
| Department[c], *n* (%) | | | |
| Intensive care unit | 6 (14.0) | 11 (1.9) | 200 (14.9) |
| Emergency department | | 11 (1.9) | 85 (6.3) |
| Medical ward/surgical ward | 26 (60.5) | 144 (25.0) | 1044 (77.6) |
| Obstetrics | 1 (2.3) | 4 (0.7) | 16 (1.2) |
| CHR | | 1 (0.2) | |
| Outpatient clinics | 10 (23.3) | 405 (70.3) | |
| Pre-medication available, *n* (%) | 43 (100.0) | 577 (100.0) | 802 (59.6) |
| No | 42 (97.7)[d] | 524 (90.8) | 622 (77.6) |
| Yes | 1 (2.3) | 53 (9.2) | 180 (22.4)[e] |
| Missing pre-med type | 1 (100) | 5 (9.4) | |
| Antipyretic | | 21 (39.6) | 81 (45.0) |
| Antihistamine | | 9 (17.0) | 47 (26.1) |
| Corticosteroid | | 1 (1.9) | 75 (41.7) |
| Diuretic | | | 23 (12.8) |
| Sedative | | | 11 (6.1) |
| Other | | 8 (15.1) | 11 (6.1) |
| ≥2 types of pre-meds | | 24 (45.3) | 53 (29.4) |
| Dose available, *n* (%) | 39 (90.7) | 362 (62.7) | 1345 (100.0) |
| Dose (g) mean/standard deviation; median (interquartile range) | 103.4/60.1; 110 (55–130) | 35.6/28.6; 25 (15–55) | 39.8/28.0; 33 (20–60) |
| Dose by service category (g) mean/standard deviation; median (interquartile range) | | | |
| Haematology/bone marrow transplant | 95.0/49.6; 98 (65–130) | 29.6/24.3; 20 (15–40) | 45.1/24.1; 35 (25–65) |
| Oncology | 65.0/7.1; 65 (60–70) | 23.7/17.8; 20 (10–30) | 65.0/27.2; 68 (55–90) |
| Medical | 109.3/63.8; 113 (50–140) | 38.2/30.5; 30 (17–60) | 49.5/26.2; 40 (30–65) |
| Surgical | | 38.5/24.6; 30 (20–65) | 62.8/25.6; 65 (40–75) |
| Obstetrics/gynaecological/perinatal | 55[f] | 54.5/26.6; 59 (34–75) | 54.9/22.8; 60 (35–70) |

Abbreviation: TRUST, Transfusion Research Utilization Surveillance and Tracking.

[a]Only the patient's first inpatient admission receiving IVIG and no documented evidence of a transfusion reaction.

[b]TRUST controls summarize the attending physician service.

[c]TRUST controls: location defined based on the first dose of IVIG received.

[d]TTISS cases: missing response counted as no pre-medication.

[e]TRUST controls: pre-medications given within 4 h of the first IVIG dose (antipyretic, antihistamine, corticosteroid, diuretic, sedative and other, e.g., epinephrine).

[f]Only one patient in this category.

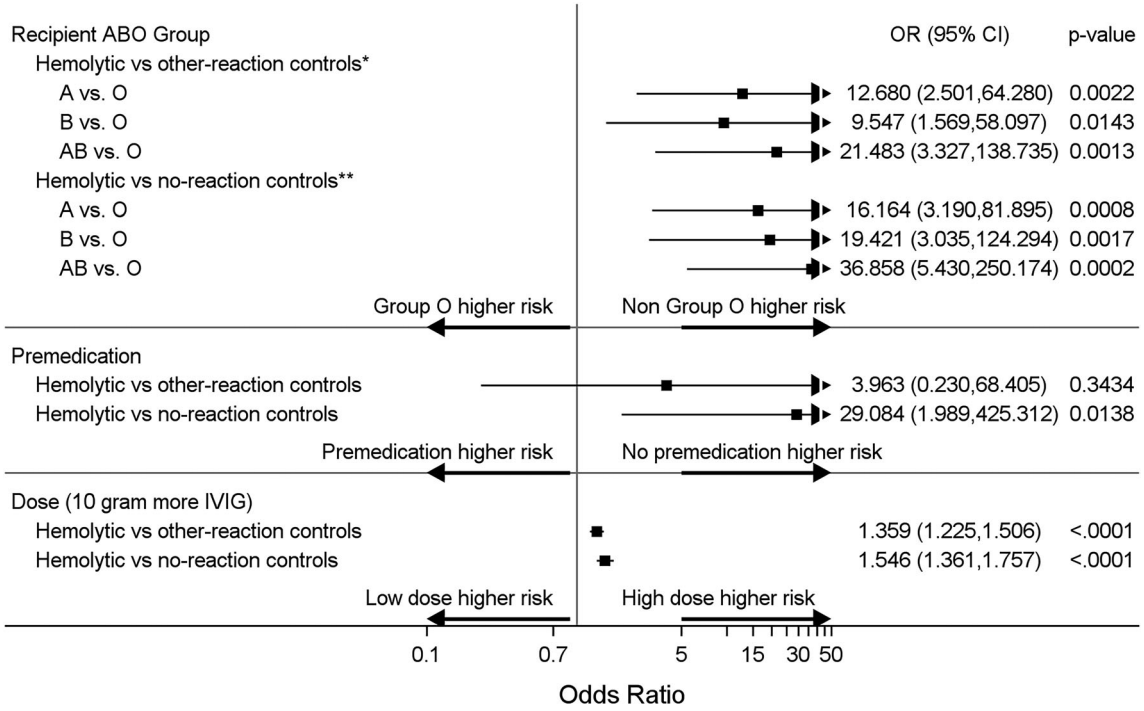


**FIGURE 4** Forest plots of the regression models for all patients using the other-reaction controls (patients with reactions to intravenous immunoglobulin (IVIG) not classified as haemolytic) and no-reaction controls (patients from Transfusion Research Utilization Surveillance and Tracking who received IVIG and did not react).

complications such as febrile non-haemolytic transfusion reactions, allergic reactions and IVIG headache; hence, it is unclear why such medications would lower the risk of a haemolytic event; however, one could hypothesize that pre-medications like antipyretics, analgesics, antihistamines and/or steroids could decrease the response of different biological response modifiers also making haemolysis less likely. We were not able to determine which type(s) of pre-medication could be providing this benefit as only one of the 43 cases reported pre-medication use and did not specify the type. This finding requires further investigation. Although we did not evaluate the manufacturing method or titre of ABO antibodies in the IVIG products used, our finding of a significant decrease in the frequency of haemolytic reactions between 2013 and 2021 is consistent with the manufacturing strategies implemented to decrease the concentration of ABO antibodies in the IVIG products [2].

We were able to identify risk factors because of the novel approach to selecting two different control groups for this study. Rationale for selecting the other-reaction control group from our surveillance data was based on the assumption that all other reactions were not caused by antibody directed against the red cell membrane. Availability of TRUST data allowed us to include a second control group (no-reaction control group) by selecting all patients with no documented reaction following IVIG administration. Generalizability of the TRUST control cohort to provincially reported TTISS-ON cases is unknown, but TRUST includes data from four different hospitals which cover a wide variety of patient populations and speciality services. There could be limitations with these two control groups; however, our findings suggest that they were appropriate given that our results are consistent with current literature. We hypothesize that these strategies to assemble a control group in combination with surveillance data could provide a more informative analysis to understand other types of reactions.

There are other limitations to our study. The current way of collecting data prevented the analysis of serological findings and resulted in some missing data for certain variables. Also, some reactions may be missed or not captured within TTISS-ON because the system involves voluntary reporting from the clinical services to Transfusion Medicine (TM) and from TM to TTISS-ON.

This study has provided information on the risk rate of different types of reactions to IVIG and has categorized reactions by frequency (highest to lowest) which is a useful tool for healthcare professionals when thinking about a differential diagnosis. The use of control groups in the analyses validated previously published risk factors for haemolysis to IVIG and suggested that lack of pre-medication may be another risk factor. This novel methodological approach may be applicable to the investigation of other types of reactions that are seen following transfusions and provide new opportunities for analysing haemovigilance data.

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K.B. and N.H. designed the research study with methodological input from K.W., J.N., M.S. and N.A. The operations of the research were guided by K.B., N.H., Y.L., K.W., J.N., M.S. and M.K.; Y.L. acquired and organized the data for analysis; J.P. assisted with records review; Y.L. performed the analysis; K.B. and N.H. wrote the first draft of the manuscript; K.B., N.H., K.W., Y.L., J.N., M.S., N.A., M.K. and J.P. reviewed drafts and provided feedback.

## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from Public Health Association of Canada. Restrictions apply to the availability of these data, which were used under license for this study. Data are available from the author(s) with the permission of Public Health Association of Canada.

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## SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

# World human neutrophil antigens investigation survey

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[18]Platelet/Leucocyte Serology Laboratory, Sanquin Diagnostic Services, Amsterdam, The Netherlands

[19]Department of Transfusion Medicine and Cell Therapy, Medical University of Vienna, Vienna, Austria

[20]National Blood Centre, Thai Red Cross Society, Bangkok, Thailand

[21]Japanese Red Cross Society Central Blood Institute, Tokyo, Japan

[22]Institute of Hematology and Transfusion Medicine, Warszawa, Poland

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## Abstract

**Background and Objectives:** Human neutrophil antigens (HNAs) are categorized into five systems: HNA-1 to HNA-5. Given the importance of neutrophils in immunity, we sought to create awareness of the role of HNA diagnostic services in managing immune neutropenia and transfusion-related acute lung injury. To provide health communities all around the world with access to these services, we conducted a survey to create a directory of these HNA diagnostic services.

**Materials and Methods:** An Excel table-based survey was created to capture information on the laboratory's location and was emailed to 55 individuals with known or possible HNA investigation activity. The collected data were then summarized and analysed.

**Results:** Of contacted laboratories, the surveys were returned from 23 (38.2%) laboratories; 17 have already established HNA diagnostic (of them 12 were regular participants of the International Granulocyte Immunobiology Workshop [ISBT-IGIW]), 4 laboratories were in the process of establishing their HNA investigation and the remaining 2 responder laboratories, did not conduct HNA investigations. In established laboratories, investigation for autoimmune neutropenia (infancies and adults) was the most frequently requested, and antibodies against HNA-1a and HNA-1b were the most commonly detected.

**Conclusion:** The directory of survey respondents provides a resource for health professionals wanting to access HNA diagnostic services. The present study offers a comprehensive picture of HNA diagnostics (typing and serology), identifying weak points and areas for improvement for the first time. Identifying more laboratories involved in HNA diagnostics with limited access to international societies in the field will globally improve HNA diagnostics.

## Keywords

alloantibody, autoantibody, autoimmune, HNA, neutropenia, neutrophil, TRALI

## Highlights

- Investigation for autoimmune neutropenia (infancies and adults) is the most frequently requested analysis for neutrophil serology worldwide.
- Antibodies against human neutrophil antigen (HNA)-1a and HNA-1b are the most commonly detected antibodies involved in autoimmune neutropenia.
- Although neutrophil serology services are distributed across the globe, the number of services available to African, Middle Eastern and Western Pacific populations is limited.

## INTRODUCTION

Neutrophils are the major subtype of granulocytes and have a pivotal role in innate and adaptive immunity. Human neutrophil antigens (HNAs) are distributed on five different glycoproteins on the surface of neutrophils and have been designated as HNA-1 to HNA-5 [1]. Eleven HNA alleles have been described so far [2]. HNA incompatibilities during pregnancy or transfusion may induce the production of HNA antibodies [3]. HNA antibodies have been implicated in the mechanism of neonatal alloimmune neutropenia (NAIN), autoimmune neutropenia (AIN), transfusion-related acute lung injury (TRALI), immune neutropenia after bone marrow transplant and febrile non-haemolytic transfusion reactions [2, 4].

Neutropenia may arise from a defect in neutrophil production in bone marrow or increased neutrophil destruction for various reasons [5]. Immune neutropenia involves the attachment of neutrophil reactive antibodies to neutrophil antigens, leading to their clearance from circulation [6]. Depending on the cause, treatment for neutropenia varies. It is therefore important to distinguish immune neutropenia from other causes. Therefore, the availability of services to detect

**TABLE 1A** Directory of participating established laboratories.

| | Region | HNA investigating laboratories and contact details | QA (quality assessment) |
|---|---|---|---|
| 1 | Americas | Dr Elyse Moritz, email: elysemoritz@yahoo.com.br<br>Clinical and Experimental Oncology, Escola Paulista de Medicinia, Universidade Federal de Sao Paulo, Brazil. | ISBT-IGIW |
| 2 | | Rebecca Dangerfield, email: rebecca.dangerfield@redcross.org; Margaret Keller, email margaret.keller@redcross.org<br>American Red Cross Neutrophil Immunology Laboratory, St Paul, Minnesota, USA. | ISBT-IGIW |
| 3 | | Dr Brian Curtis, email: brian.curtis@bcw.edu<br>Platelet & Neutrophil Immunology Lab, Versiti, Milwaukee, WI, USA. | ISBT-IGIW |
| 4 | Europe | Dr Marlies Schoenbacher, email: marlies.schoenbacher@meduniwien.ac.at<br>Dr Günther Körmöczi, email: guenther.koermoeczi@meduniwien.ac.at<br>Department of Transfusion Medicine and Cell Therapy, Medical University of Vienna, Austria | INSTAND EQA |
| 5 | | Dr Kaspar René Nielsen, email: k.nielsen@rn.dk<br>Klinisk immunologi, Aalborg universitetshospital Nord, Denmark. | ISBT-IGIW |
| 6 | | Dr Laure Croisille, email: laure.croisille@efs.sante.fr<br>Laboratoire HLA-ILP E.F.S Ile-de-France, Créteil, France. | ISBT-IGIW |
| 7 | | Dr Marie Audrain, email: marie.audrain@chu-nantes.fr<br>Service d·Immunologie, Laboratoire de Biologie, Nantes, France. | ISBT-IGIW and INSTAND EQA |
| 8 | | Dr Harmut Kroll, email: hartmut.kroll@bsd-nstob.de<br>Dr Claudia Grabowski, email: claudia.grabowski@bsd-nstob.de<br>Institut für Transfusionsmedizin Dessau, DRK-Blutspendedienst NSTOB, Dessau, Germany. | INSTAND EQA |
| 9 | | Dr Ulrich Sachs, email: ulrich.sachs@med.uni-giessen.de<br>Dr Behnaz Bayat, email: behaz.bayat@immunologie.med.uni-giessen.de<br>Institute for Clinical Immunology and Transfusion Medicine, Giessen, Germany. | ISBT-IGIW and INSTAND EQA |
| 10 | | Dr Leendert Porcelijn, email: l.porcelijn@sanquin.nl<br>Platelet/Leucocyte Serology Laboratory, Sanquin Diagnostic Services, Amsterdam, The Netherlands. | ISBT-IGIW |
| 11 | | Dr Malgorzata Uhrynowska, email: muhrynowska@ihit.waw.pl<br>Institute of Haematology and Transfusion Medicine: Warszawa, Poland. | ISBT-IGIW |
| 12 | | Dr Anthony Poles, email: anthony.poles@nhsbt.nhs.uk<br>Department of Histocompatibility & Immunogenetics, NHS Blood & Transplant, Bristol, United Kingdom. | ISBT-IGIW |
| 13 | | Dr Marjeta Macek Kvanka, email: marjeta.macek@ztm.si<br>Blood transfusion centre of Slovenia, Ljubljana, Slovenia | ISBT-IGIW |
| 14 | South East Asia | Dr Oytip Nathalang, email: oytipntl@hotmail.com<br>Faculty of Allied Health Sciences, Thammasat University, Pathumtani 12120, Thailand. | |
| 15 | Western Pacific | Gail Pahn, email: gpahn@redcrossblood.org.au<br>Platelet & Granulocyte Reference Laboratory, Australian Red Cross Lifeblood, Brisbane, Australia. | ISBT-IGIW |
| 16 | | Dr Janette Kwok, email: kwoksy@ha.org.hk<br>Queen Mary Hospital, Division of Transplantation and Immunogenetics, Department of Pathology, Hong Kong SAR. | INSTAND EQA |
| 17 | | Dr Daisuke Takahashi, email: d-takahashi@jrc.or.jp<br>Japanese Red Cross Society Central Blood Institute, Tokyo, Japan. | |

anti-neutrophil antibodies enables the physician to confirm immune neutropenia thus avoiding the invasive bone marrow biopsy [7]. This is particularly useful in cases of infant and childhood neutropenia. The implication of HNA antibodies, particularly anti-HNA-3a in clinically severe TRALI has also significantly emphasized the importance of HNA antibody detection.

The International Society of Blood Transfusion (ISBT)-Granulocyte Immunology Working Party (GIWP) organizes an annual quality assessment workshop to assess a laboratory's ability to carry out neutrophil serology and genotyping investigations. There are currently only 18 reference laboratories participating in this quality assessment workshop, but we expect that there are other laboratories performing similar investigations around the world as well. Given the significant role of neutrophils in immunity, we conducted a survey to identify and catalogue laboratories around the globe performing neutrophil serology and genotyping investigations. The goal is to create a worldwide directory of granulocyte investigating laboratories conducting neutrophil serology and genotyping investigations to increase awareness of these diagnostic services and to make these specialized services more accessible to health communities all around the world.

## MATERIALS AND METHODS

The survey consisted of an Excel table that captured information on the laboratory's location, the range of neutrophil investigations (e.g., AIN, TRALI, neutrophil reactive antibodies in transplants and convalescent plasma of COVID-19 patients) conducted, list of techniques used for serology and molecular investigations, and whether they participated in a quality assessment programme(s) in the 12-month period from January 2019 to January 2020. The survey was emailed to 55 individuals with known or possible HNA investigation activity, and recipients were encouraged to forward the survey to any other laboratories that may conduct HNA investigations. A literature search (keywords: HNA frequency, human neutrophil antigens, neutrophils, neutrophil antigen 'country') was conducted to gather reports on HNA frequencies from different populations.

## RESULTS

A total of 23 surveys were returned equating to a return rate of 41.8%. From the responses received, 17 established laboratories regularly conducted HNA investigations, 3 (17.6%) are in the Americas, 10 (58.8%) in Europe, 1 in South-East Asia (5.9%) and 3 (17.6%) in the Western Pacific area (Table 1A). Of these 17 laboratories, 15 (88.2%) participated in a granulocyte quality assessment program, the most common ($n = 11$) being the International Society of Blood Transfusion-International Granulocyte Immunobiology Workshop (ISBT-IGIW) and the other being the INSTAND External Quality Assessment (EQA) programme (Table 1A). Four institutes (National Blood Centre Thailand, South Africa, Iran and South Korea) are in the process of establishing their HNA investigation services and have been clustered as 'Laboratories in development' (Table 1B). The remaining two responses did not conduct HNA investigations: the New Zealand Blood Transfusion Service referred their investigations to Australia, and Dr Olnaiyi Olanrewaju from the Irrua Specialist Teaching Hospital, Irrua/Ambrose Alli University, Ekpoma, Edo State Nigeria reported that they had cases of immune neutropenia but did not have a laboratory to refer the samples to.

Serological testing was conducted by 16 of 17 established laboratories and all 4 laboratories in development (Table 2) but the range of techniques varied. All 16 laboratories from first group and 1 from second group conducted granulocyte immunofluorescence tests (GIFT) and used a typed panel of granulocytes. All these laboratories complimented the GIFT with the granulocyte agglutination test (GAT) or another technique, except in Aalborg and Créteil. LabScreen Multi was used by 6 established laboratories and 3 of 4 laboratories in development (Table 2) [8]. Monoclonal antibody immobilization of granulocyte antigen (MAIGA) [9] was conducted by 12 established laboratories, with Sao Paulo and Tehran in the process of optimizing the assay. All laboratories investigating CD16 used two monoclonal antibodies (mAb) except for USA, Versiti. MEM-166 was the most common CD177 mAb and Bear 1 for CD11b. Sixteen established laboratories and two laboratories in development conducted genotyping for HNA-1, HNA-3, HNA-4 and HNA-5, but only five from first group also genotyped for HNA-2 (Table 3).

NAIN investigations were conducted by 15 established laboratories (Table 4). Of the samples tested in the survey period, 88 samples were positive, and the most common antibodies detected were anti-HNA-1a and anti-HNA-1b. Samples were referred by physicians from

**TABLE 1B** Directory of participating laboratories in development.

| | | |
|---|---|---|
| 1 | Africa | Dr Derrick Nelson, email: derrick.nelson@sanbs.org.za<br>Specialized Laboratory Services, South African National Blood Service, Johannesburg, South Africa. |
| 2 | Eastern Mediterranean | Dr Esmaeili Behnaz, email: esmaeili.behnaz@yahoo.com<br>Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran. |
| 3 | W.Pac | Dr Hyungsuk Kim, email: hyungsuk.kim79@gmail.com<br>Seoul National University Hospital, Seoul, Korea. |
| 4 | South East Asia | Dr Pawinee Kupatawintu, email: pawinee.k@redcross.or.th<br>Dr Atthapol Srisuddee, email: atthapol.s@redcross.or.th<br>National Blood Centre, Thai Red Cross Society, Bangkok, Thailand. |

Abbreviations: EQA, External Quality Assessment; HNA, human neutrophil antigens; ISBT-IGIW, International Society of Blood Transfusion-International Granulocyte Immunobiology Workshop.

**TABLE 2** Human neutrophil antigen (HNA)-antibodies screening assay and monoclonal antibodies (mAb) used in monoclonal antibody immobilization of granulocyte antigen (MAIGA).

| | Serology | GIFT | GAT | Typed panel | No. typed cells | Laboratory screen | Other techniques | MAIGA | Monoclonal antibodies used in MAIGA | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | CD16 | | | | | CD177 | | CD11a (LFA-1α) | CD11b | CD18 | HLA class I |
| 1 | Brazil, Sao Paulo | Y | Y | Y | 3 | Y | | inP | 3G8 | LNK16 | | | | MEM166 | | | Bear1 | | |
| 2 | USA, ARC | Y | Y | Y | 5 | | | Y | 3G8 | LNK16 | DJ130c | BCW238.7 | | | 7D8 | | | IB4 | |
| 3 | USA, Versiti | Y | ? | Y | 2–10 | | | Y | | | MBC238.7 | | | | 7D8 | | | | |
| 4 | Austria, Vienna | Y | Y | Y | 3 | | WIFT | Y | 3G8 | LNK16 | DJ130c | | | MEM-166 | | HI111 | Bear1 | 7E4 | W6/32 |
| 5 | Denmark, Aalborg | Y | | Y | 4 | | D-GIFT | | | | | | | | | | | | |
| 6 | France, Créteil | Y | | Y | 3–6 | | | Y | 3G8 | LNK16 | | | | MEM166 | | | | | |
| 7 | France, Nantes | Y | Y | Y | 4 | | | Y | 3G8 | LNK16 | | | | MEM-166 | | HI111 | Bear1 | | |
| 8 | Germany, Dessau | Y | Y | Y | 4 | | | Y | 3G8 | LNK16 | DJ130c | | | MEM166 | 7D8 | 25.3.1 | Bear1 | | B1G6 |
| 9 | Germany, Giessen | Y | Y | Y | 4 | | | Y | 3G8 | LNK16 | | | | MEM166 | 7D8 | | Bear1 | | W6/32 |
| 10 | The Netherlands, Sanquin | Y | Y | Y | 2 | | | Y | 3G8 | LNK16 | DJ130c | 238,7 | | MEM166 | TAG4 | | | IB4 | |
| 11 | Poland, Warsaw | Y | Y | Y | 2 | Y | | Y | 3G8 | LNK16 | DJ130c | | | MEM166 | | | Bear1 | 7E4 | |
| 12 | United Kingdom, NHS | Y | | Y | 2+ | Y | rHNA-3a/3b cell lines, GCLT, LIFT | Y | 3G8 | LNK16 | MBC238.7 | | | MEM166 | 7D8 | | Bear1 | 7E4 | |
| 13 | Slovenia, Ljubljana | Y | Y | Y | 3+ | Y | | Y | | | | | | | | | | | |
| 14 | Thailand, Thammasat University | N | N | | | N | | N | | | | | | | | | | | |
| 15 | Australia, Lifeblood | Y | Y | Y | 3 | | | Y | 3G8 | LKN16 | DJ130c | | | MEM166 | | 25.3.1 | Bear1 | | W6/32 |
| 16 | Hong Kong, Queen Mary | Y | Y | Y | 3 | Y | | N | 3G8 | LNK16 | DJ130 | MEM154 | 3H1029 | | | | | | |
| 17 | Japan, JRC | Y | | Y | 3–5 | Y | ICFA | N | | | | | | | | | | | |
| *Laboratories in development* | | | | | | | | | | | | | | | | | | | |
| 1 | South Africa, SANBS | | | | | Y | | N | | | | | | | | | | | |

(Continues)

**TABLE 2** (Continued)

| Serology | GIFT | GAT | Typed panel | No. typed cells | Laboratory screen | Other techniques | MAIGA | Monoclonal antibodies used in MAIGA: CD16 | CD177 | CD11a (LFA-1α) | CD11b | CD18 | HLA class I |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 Iran, Tehran | Y | Y | Y | 2 | | LIFT | inP | LNK16 | MEM166 | | | | |
| 3 Korea, Seoul | | | | | Y | | N | | | | | | |
| 4 Thailand, Red Cross | | | | | Y | | N | | | | | | |

Abbreviations: GAT, granulocyte agglutination test; GCLT, granulocyte chemiluminescence test; GIFT, granulocyte immunofluorescence test; HNA, human neutrophil antigen; ICFA, immunocomplex capture fluorescence analysis; LIFT, lymphocyte immunofluorescence test; MAIGA, monoclonal antibody immobilization of granulocyte antigen; WIFT, white cell immunofluorescence test.

patients with neutropenia (absolute neutrophil count [ANC] < 1500 cells/μL for adults and ANC < 1000 per microliter). The number of investigations for AIN was higher and the positive rates ranged from 9% to 52.2% (Table 5). Requests for AIN investigations were mainly for patients aged 2–36 months and in adults (Table 5). Only 16 established laboratories conducted TRALI investigations, and the number of samples tested in each laboratory varied considerably (Table 6). The majority of TRALI-associated antibodies were in donor samples and only three established laboratories detected antibodies in the patient.

## DISCUSSION

The survey results show that the distribution of HNA investigation capability among laboratories is varied. There is a large cluster of laboratories in Europe (58.8%), three in America (17.6%), three in Western Pacific (17.6%) and one in South East Asia (5.8%; Tables 1A and 1B). There are presently no established laboratories in Africa or Eastern Mediterranean, but there is a laboratory in development in Tehran, Iran.

NAIN is a disorder of the foetus that results from maternal alloimmunization against paternal HNA expressed on fetal neutrophils [4, 10]. NAIN is analogous to haemolytic disease of the foetus/newborn (HDFN) which affects red blood cells and foetal/neonatal alloimmune thrombocytopenia (FNAIT) which affects platelets [11]. In NAIN, the mother develops alloantibodies against HNAs expressed on foetal neutrophils that may cause elimination of neutrophils leading to neutropenia in foetus [11]. Of the 17 established laboratories that regularly investigate NAIN, the majority (58.8%) are located in Europe, and there are none in Africa or Easter Mediterranean (Tables 1A and 1B). The glycoproteins carrying epitopes for HNA-1 and HNA-2 are the most frequently involved in the NAIN cases in this study. Seven laboratories identified alloantibodies against HNA-2 as the second most frequent alloantibodies involved in NAIN cases. In the reports from four laboratories (Creteil and Nantes in France, Sanquin in the Netherlands and NHS in UK), alloantibodies against HNA-1c were described in the cases of NAIN. Interestingly, only the UK laboratory reported alloantibodies against HNA-3a in cases of NAIN. There have been rare reports on NAIN associated with high-frequency antigens HNA-4a [12] and HNA-5a [13]. As HNA-4b frequency is very low in many populations, this may explain the rare reports of NAIN cases mediated by alloantibodies against this antigen. In addition, the low frequency of antibodies against HNA-4 and HNA-5 can also be correlated to the non-immunogenic structure of CD11b and CD11a where HNA-4 and HNA-5 epitopes are located.

The survey results show that AIN is the most requested HNA investigation, conducted in 15 established laboratories and the laboratories in development in Iran (Table 5). Specifically, primary AIN occurs mainly in infants and children. The AIN autoantibody is elusive because the antibody concentration can change. Hence, AIN investigation often requires testing of multiple patient sera at different times to detect the antibody [14]. The antibody specificity has

**TABLE 3** Human neutrophil antigen (HNA)-genotyping conducted by participants.

| Genotyping | Y/N | HNA-1 | HNA-2 | HNA-3 | HNA-4 | HNA-5 |
|---|---|---|---|---|---|---|
| 1. Brazil, Sao Paulo | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 2. USA, ARC | Y | HNA-1 | HNA-2 | HNA-3 | HNA-4 | HNA-5 |
| 3. USA, Versiti | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 4. Austria, Vienna | Y | HNA-1 | HNA-2 | HNA-3 | HNA-4 | HNA-5 |
| 5. Denmark, Aalborg | Y | HNA-1 | HNA-2 | HNA-3 | HNA-4 | HNA-5 |
| 6. France, Créteil | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 7. France, Nantes | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 8. Germany, Dessau | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 9. Germany, Giessen | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 10. The Netherlands, Sanquin | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 11. Poland, Warsaw | Y | HNA-1 | HNA-2 | HNA-3 | HNA-4 | HNA-5 |
| 12. United Kingdom, NHS | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 13. Slovenia, Ljubljana | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 14. Thailand, Thammasat University | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 15. Australia, Lifeblood | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 16. Hong Kong, Queen Mary | Y | HNA-1 | HNA-2 | HNA-3 | HNA-4 | HNA-5 |
| 17. Japan, JRC | Y | HNA-1 | | HNA-3 | | |
| *Laboratories in development* | | | | | | |
| 1. South Africa, SANBS | N | | | | | |
| 2. Iran, Tehran | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 3. Korea, Seoul | Y | HNA-1 | | HNA-3 | HNA-4 | HNA-5 |
| 4. Thailand, Red Cross | N | | | | | |

**TABLE 4** Human neutrophil antigen (HNA) antibody specificities detected in neonatal alloimmune neutropenia investigations.

| | | | | Antibody specificity (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Neonatal alloimmune neutropenia investigations | | Samples tested (*n*) | Samples positive (*n*) | HNA-1a | HNA-1b | HNA-1c | HNA-2 | HNA-3a | HNA-4a | HLA-class II | HLA-class I | CD16 Iso Ab |
| 1 | Brazil, Sao Paulo | 2 | 1 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | USA, ARC | 6 | 2 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | USA, Versiti | 40 | 3 | 50 | 25 | 0 | 25 | 0 | 0 | 0 | 0 | 0 |
| 4 | Austria, Vienna | 5 | 3+ | 0 | 33.3 | 0 | 33.3 | 0 | 0 | 0 | 0 | 33.3 |
| 5 | Denmark, Aalborg | 7 | 1 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | France, Créteil | 37 | 8 | 62 | 25 | 13 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | France, Nantes | 24 | 6 | 0 | 33 | 17 | 17 | 0 | 0 | | 0 | 33 |
| 8 | Germany, Dessau | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | Germany, Giessen | 38 | 8 | 25 | 25 | 0 | 25 | 0 | 0 | 0 | 12.5 | 12.5 |
| 10 | The Netherlands, Sanquin | 12 | 5 | 20 | 0 | 20 | 20 | 0 | 0 | 0 | 0 | 40 |
| 11 | Warsaw, Poland | | 380[a] | 42 | 13 | 0 | 0 | 0 | 2 | 6 | | 25 |
| 12 | United Kingdom, NHS | 55 | 7 | 43 | 0 | 14 | 29 | 14 | 0 | 0 | 0 | 0 |
| 13 | Slovenia, Ljubljana | 6 | 1 | 50 | 30 | 0 | 20 | 0 | 0 | 0 | 0 | 0 |
| 14 | Australia, Lifeblood | 4 | 1 | 0 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | Hong Kong, Queen Mary | 36 | 10 | 100 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

[a]Total number of diagnosed neutropenia (auto- or alloimmune).

also been reported to change with time [15]. Diagnosis of primary AIN is especially useful as it supports exclusion of other causes of neutropenia [16].

Although in many cases of adult AIN no antigen specificity is described, antibodies against HNA-1a and HNA-1b have been detected as the most common target antigens for autoantibodies involved in cases of AIN in adults [17]. In contrast to primary AIN in infancy, AIN in adults is not a self-limiting condition and manifests a higher frequency in females (up to 70% of cases) [6].

Transfusion of blood products containing HNA alloantibodies to recipients with the cognate antigen has been known to induce reactions such as TRALI [18]. The role of neutrophils in TRALI is well established [19]. However, some alloantibodies such as alloantibodies against HNA-3a have been implicated in severe and some fatal TRALI

**TABLE 5** Patient age distribution and positivity rate in neonatal autoimmune neutropenia investigations.

| Autoimmune neutropenia investigations | | Sample tested (*n*) | Positive results | | Patient age distribution (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *n* | % | Newborn | 2–36 months | Primary | Adult |
| 1 | Brazil, Sao Paulo | 33 | 11 | 33.3 | 0.0 | 36.4 | 18.2 | 45.5 |
| 2 | USA, ARC | 95 | 32 | 33.7 | 0.0 | 43.2 | 0.0 | 56.8 |
| 3 | USA, Versiti | 1981 | 526 | 26.6 | 0.1 | 47.0 | 18.2 | 34.8 |
| 4 | Austria, Vienna | 75 | 15 | 20.0 | 21.4 | 64.3 | 0.0 | 14.3 |
| 5 | Denmark, Aalborg | 90 | 20 | 22.2 | 0.0 | 54.1 | 18.9 | 27.0 |
| 6 | France, Créteil | 1169 | 105 | 9.0 | 0.0 | 30.1 | 0.0 | 69.9 |
| 7 | France, Nantes | 774 | 87 | 11.2 | 1.2 | 26.2 | 0.0 | 72.6 |
| 8 | Germany, Dessau | 63 | 9 | 14.3 | 0.0 | 36.4 | 36.4 | 27.3 |
| 9 | Germany, Giessen | 990 | 255 | 25.8 | 0.0 | 17.9 | 13.5 | 68.6 |
| 10 | The Netherlands, Sanquin | 270 | 79 | 29.3 | 57.0 | | | 43.0 |
| 11 | Warsaw, Poland | 380 | | | 0.0 | 91.5 | 0.0 | 8.5 |
| 12 | United Kingdom, NHS | 2004 | 488 | 24.4 | 0.6 | 58.5 | 30.4 | 10.5 |
| 13 | Slovenia, Ljubljana | 30 | 8 | 26.7 | 0.0 | 43.2 | 18.9 | 37.8 |
| 14 | Australia, Lifeblood | 141 | 58 | 41.1 | 0.0 | 2.1 | 1.4 | 96.5 |
| 15 | Hong Kong, Queen Mary | 11 | 0 | 0.0 | 0.0 | 18.2 | 36.4 | 45.5 |
| LiD | Iran, Tehran | 23 | 12 | 52.2 | 0.0 | 40.0 | 40.0 | 20.0 |

**TABLE 6** Transfusion-related acute lung injury (TRALI) investigation findings.

| TRALI investigations | | Samples tested (*n*) | Patient antibody | | Donor antibody | |
|---|---|---|---|---|---|---|
| | | | *n* | % | *n* | % |
| 1 | USA, ARC | 60 | 0 | 0 | 6 | 10.0 |
| 2 | USA, Versiti | 73 | | | 1 | 1.4 |
| 3 | Austria, Vienna | 3 | 0 | 0 | 2 | 66.7 |
| 4 | Denmark, Aalborg | 9 | | | 1 | 11.1 |
| 5 | Germany (Dessau) | 276 | 1 | 0.3 | 1 | 0.3 |
| 6 | Germany (Giessen) | 37 | 0 | 0 | 3 | 8.1 |
| 7 | France, Nantes | 29 | 0 | 0 | 0 | 0.0 |
| 8 | The Netherlands, Sanquin | 2 | 0 | 0 | 0 | 0.0 |
| 9 | Poland, Warsaw | 22 | | | 1 | 4.5 |
| 10 | United Kingdom, NHS | 78 | Not tested | | 4 | 20.0 |
| 11 | Slovenia, Ljubljana | 3 | | | 1 | 33.3 |
| 12 | Thailand Red Cross | 7 | 1 | 14.3 | 1 | 14.3 |
| 13 | Thailand, Thammasat University | 2 | 0 | 0 | 2 | 100.0 |
| 14 | Australia, Lifeblood | 2 | 0 | 0 | 1 | 50.0 |
| 15 | Hong Kong | 0 | 0 | 0 | 0 | 0 |
| 16 | Japan, JRC | 72 | 9 | 12.5 | 28 | 38.9 |

**TABLE 7** The human neutrophil antigens (HNAs)-allele frequencies in different populations.

| | HNA-1 | | | HNA-2 | | HNA-3 | | HNA-4 | | HNA-5 | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Population** | **1a** | **1b** | **1c** | **Positive** | **Negative** | **3a** | **3b** | **4a** | **4b** | **5a** | **5b** | **Author, year** |
| Asian | | | | | | | | | | | | |
| Southern Thai | 0.619 | 0.365 | 0.012 | | | 0.808 | 0.192 | 0.973 | 0.027 | 0.656 | 0.344 | Intharanut et al., 2019 [42] |
| Central Thai | 0.548 | 0.452 | 0.004 | | | 0.718 | 0.282 | 0.975 | 0.025 | 0.771 | 0.229 | Intharanut et al., 2019 [42] |
| Northern Thai | 0.677 | 0.323 | 0 | | | 0.775 | 0.225 | 0.965 | 0.035 | 0.748 | 0.252 | Intharanut et al., 2019 [42] |
| Northeastern Thai | 0.696 | 0.301 | 0 | | | 0.785 | 0.215 | 0.972 | 0.028 | 0.676 | 0.324 | Intharanut et al., 2019 [42] |
| Thai | | | | 0.995 | 0.005 | | | | | | | Nathalang et al., 2018 [41] |
| Chinese (Han, Guangzhou) | 0.667 | 0.333 | 0 | 1 | 0 | 0.738 | 0.262 | 0.996 | 0.004 | 0.854 | 0.146 | Xia et al., 2011 [33] |
| Chinese (Hong Kong) | 0.678 | 0.315 | 0 | 0.983 | 0.017 | 0.71 | 0.29 | 0.995 | 0.005 | 0.852 | 0.148 | Tam et al., 2018 [35] |
| Chinese (Han, Zhejiang) | 0.613 | 0.387 | 0 | | | 0.654 | 0.346 | 1 | 0 | 0.896 | 0.104 | He and Zhang, 2014 [34] |
| Korean | | | | | | 0.695 | 0.305 | 0.986 | 0.014 | 0.959 | 0.041 | Han and Han, 2015 [38]; Han and Han, 2006 [39] |
| Indian | 0.433 | 0.444 | 0.086 | 0.9927 | 0.012 | 0.812 | 0.188 | 0.955 | 0.045 | 0.237 | 0.763 | Gogri et al., 2022 [36] |
| Japanese | 0.623 | 0.377 | 0 | 0.987 | 0.013 | 0.654 | 0.346 | 1 | 0 | 0.84 | 0.16 | Matsuhashi et al., 2012 [37] |
| Burmese | 0.605 | 0.395 | 0.031 | | | 0.747 | 0.253 | 0.971 | 0.029 | 0.559 | 0.441 | Simtonget al., 2018 [32] |
| Karen | 0.725 | 0.275 | 0 | | | 0.845 | 0.155 | 0.956 | 0.044 | 0.693 | 0.307 | Simtong et al., 2018 [32] |
| Malays (Total) | 0.706 | 0.294 | 0.037 | | | 0.758 | 0.242 | 0.977 | 0.023 | 0.708 | 0.292 | Manaf et al., 2015 [40] |
| European | | | | | | | | | | | | |
| German | 0.36 | 0.631 | 0.019 | | | 0.801 | 0.199 | 0.889 | 0.111 | 0.665 | 0.335 | Grabowski et al., 2019 [44] |
| Danish | 0.348 | 0.623 | 0.029 | | | 0.814 | 0.186 | 0.881 | 0.119 | 0.724 | 0.276 | Nielsen et al., 2012 [26] |
| English (Caucasoid) | 0.318 | 0.668 | 0.014 | | | 0.768 | 0.232 | 0.882 | 0.118 | 0.736 | 0.264 | Cardoso et al., 2013 [43] |
| Russian (St. Petersburg) | 0.384 | 0.584 | 0.032 | | | 0.804 | 0.196 | 0.898 | 0.102 | 0.708 | 0.292 | Krobinets et al., 2020 [45] |
| Turkish | 0.42 | 0.564 | 0.03 | | | 0.737 | 0.263 | 0.881 | 0.119 | 0.754 | 0.246 | Hauck et al., 2011 [46] |
| African | | | | | | | | | | | | |
| Zambian | 0.395 | 0.345 | 0.25 | | | 0.975 | 0.025 | 0.895 | 0.105 | 0.5 | 0.5 | Nielsen et al., 2012 [26] |
| American | | | | | | | | | | | | |
| United States (Black population) | 0.59 | 0.77 | 0.23 | | | | | | | | | Kissel et al., 2000 [31] |
| United States (African American) | | | | | | 0.929 | 0.071 | | | | | Bowens et al., 2012 [30] |
| United States (Hispanic/Latino) | | | | | | 0.826 | 0.174 | | | | | Bowens et al., 2012 [30] |
| United States (Native) | | | | | | 0.946 | 0.054 | | | | | Bowens et al., 2012 [30] |
| Brazilian | 0.315 | 0.637 | 0.048 | | | 0.81 | 0.19 | 0.822 | 0.178 | 0.711 | 0.289 | Santos et al., 2016 [27]; Lopes et al., 2014 [28]; Cardone et al., 2006 [29] |

(Continues)

**TABLE 7** (Continued)

| Population | HNA-1 | | | HNA-2 | | HNA-3 | | HNA-4 | | HNA-5 | | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1a | 1b | 1c | Positive | Negative | 3a | 3b | 4a | 4b | 5a | 5b | Author, year |
| Middle Eastern | | | | | | | | | | | | |
| Iranian | 0.34 | 0.63 | 0.03 | | | 0.63 | 0.37 | 0.85 | 0.15 | 0.72 | 0.28 | Esmaeili, 2022 [3] |
| Syrian | 0.375 | 0.58 | 0.04 | | | 0.742 | 0.258 | 0.86 | 0.14 | 0.66 | 0.34 | Hauck-Dlimi et al., 2018 [47] |

cases. This may be due to the fact that HNA-3a is expressed not only on neutrophils but also on other cells such as endothelial cells, monocytes and platelets [18, 20]. Conscious of the role of HNA antibodies in TRALI, the ISBT Working Party on Granulocyte Immunobiology published recommendations for leukocyte antibody investigations in TRALI [21]. Data from this survey confirm that in the majority of cases the antibodies are in the transfused blood product (Table 6). As two-thirds of TRALI cases are associated with alloantibodies; both HLA Class I and II, and HNA [22], these investigations provide a vital way to identify and manage donors with the culprit antibodies.

During COVID-19 pandemic, transfusion of plasma obtained from COVID-19-immunized healthy donors containing neutralizing antibodies against COVID-19 antigen to COVID-19 patients (known as COVID-19 convalescent plasma [CCP]) was considered as promising therapy. It is worth noting that previous reports indicate the development of TRALI in COVID-19-infected patients after transfusion with CCPs [23, 24]. Therefore, analysis of convalescent plasma for detection of anti-HNAs and HLAs antibodies was conducted. Among participants of this current survey, four laboratories have tested CCPs. However, the frequency of anti-HNAs positive samples among investigated CCPs in these laboratories is not yet reported.

Investigation for the presence of HNA antibodies usually involves screening test, most commonly GIFT and GAT [25, 26]. Use of HNA-typed panel cells at the screening stage may facilitate identification of antibody specificity. The MAIGA provides another way to confirm antibody specificity but is liable to false negatives. Among this survey's participants, 16 established laboratories and 1 laboratory in development conducted GIFT, 11 of 17 established laboratories and one laboratory in development conduct GAT and 12 established laboratories conduct MAIGA with two laboratories optimizing the MAIGA (Table 2). Six established laboratories and three laboratories in development use the LabScreen Multi test kit [21]. The combination of GIFT, GAT and MAIGA assays is considered as the 'gold standard' for HNA testing.

It is interesting to note that although there are only five established laboratories in the Asia Pacific, since 2012, there have been at least 12 publications of HNA frequencies from that region (Table 7). Genotyping has made this feasible. There is a gap in our knowledge on HNA frequencies in African, the Middle Eastern and Western Pacific populations (Table 7). HNAs allele frequency analysis among different populations indicated a different pattern for HNA-1 alleles between Asian and Western populations; in many Asian populations, no HNA-1c allele has been detected. Currently, we do not know if the deviation in allele frequency is due to genotyping techniques applied in the region or if there is a real absence of HNA-1c in some Asian populations.

To evaluate the accuracy and effectiveness of a laboratory's ability to detect HNA antibodies, the ISBT-GIWP conducts the International Granulocyte Immunobiology Working Party (IGIWP) every year. In 2022, each of the 18 participating laboratories received four blinded samples (four DNA and four serum samples) to analyse. Of the 18 participating laboratories, only 13 responded to this survey. It is hoped that this survey will encourage other laboratories to

participate in IGIWP workshop. In addition to IGIWP, there is the INSTAND EQA for Granulocyte Immunobiology. This is an intradisciplinary non-profit, scientific-medical association designated by the German Medical Association to promote quality assurance. For each evaluation, INSTAND distributes blinded samples for HNAs genotyping and detection of anti-HNA-alloantibodies.

This is the first study to provide a comprehensive synopsis of HNA typing and serology conducted in diagnostic laboratories across the whole world. The number of samples received and analysed provides a picture of HNA-related diseases such as adults and infants AIN, immunization frequencies against HNA and is indication of the population-related HNA involved in alloimmune neutropenia. The data provide a summary of investigation techniques employed that may help interested health services develop HNA investigation capability and improve the activities of established laboratories. Importantly, the list of participants (Tables 1A and 1B) provides a useful contact list for physicians and also regional hospitals wanting to contact HNA-diagnostic services.

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B.B. and L.C. designed the research study, prepared the questionnaire and mailing list, collected data and wrote the manuscript, J.L. prepared the synopsis on HNA allele frequencies in different populations, M.A., L.C., B.C., R.D., B.E., C.G., M.K., H.Ki., H.Kr., M.M.K., J.K., E.M., O.N., D.N., K.R.N., G.P., A.P., L.P., U.J.S., M.S., G.F.K., P.K., D.T. and M. U. provided the data. B.F. provided valuable input into the drafting and finalization of the manuscript. Open Access funding enabled and organized by Projekt DEAL.

## CONFLICT OF INTEREST STATEMENT

There are no conflicts identified.

## DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

# Extracorporeal photopheresis and the cellular mechanisms: Effects of 8-methoxypsoralen and UVA treatment on red blood cells, platelets and reactive oxygen species

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## Abstract

**Background and Objectives:** Extracorporeal photopheresis (ECP) is a widespread cellular therapy for graft-versus-host disease, autoimmune diseases and Sézary disease. One of the main effects of ECP is the apoptosis of leukocytes, but the therapeutic mechanisms are not completely known. The aim of this study was to investigate the effects on red blood cells, platelets and the induction of reactive oxygen species.

**Materials and Methods:** We used human cells from healthy blood donors to simulate in vitro the composition in an apheresis bag. Cells were treated with 8-methoxypsoralen (8-MOP) and UVA. Red blood cell stability, platelet activity and induction of reactive oxygen species were analysed.

**Results:** After 8-MOP and UVA treatment, the red blood cells showed high cell integrity with low levels of eryptosis and no increase of free haemoglobin or red blood cell distribution width (RDW). Red blood cell immune-associated antigens CD59 and CD147 were hardly affected by the treatment. Platelet glycoproteins CD41, CD62P and CD63 indicated strong platelet activation after 8-MOP and UVA treatment. Reactive oxygen species were slightly but not significantly induced by the treatment.

**Conclusion:** The effect of the ECP therapy is probably not exclusively mediated by leukocytes. Platelet activation is another striking effect caused by the treatment of the apheresis product with 8-MOP/UVA. However, since we could hardly identify any evidence for eryptosis or haemolysis, it is unlikely that red blood cell eryptosis is part of the therapeutic mechanism. Further research on this topic seems to be promising.

## Keywords

apoptosis, ECP, eryptosis, photopheresis, platelet activation, reactive oxygen species

## Highlights

- The effect of extracorporeal photopheresis on leukocytes is well known, but its effects on red blood cells and platelets in the apheresis bag have not yet been clarified.
- In this study, we found that 8-methoxypsoralen (8-MOP) and UVA treatment had a strong impact on platelet activation.
- In contrast, red blood cells remain very stable after 8-MOP and UVA treatment, but potential interactions of treated red blood cells with the immune system need to be evaluated further.

## INTRODUCTION

Extracorporeal photopheresis (ECP) is a well-known second-line therapy for graft-versus-host disease (GvHD) [1, 2]. Furthermore, ECP can be used as a therapy for different autoimmune diseases [3, 4]. The indications for ECP also include the therapy of solid organ rejection after lung and heart transplantation [5, 6], too. Apostolova et al. could demonstrate that ECP can be used even for the therapy of adverse events after anti-PD1 immunotherapy [7].

A great advantage of ECP over other immune-suppressive therapies such as corticosteroid administration is the induction of immune tolerance in the host [8]. In contrast to generalized immune suppression, ECP stands out with low rates of opportunistic infections, which is very important in the treatment of GvHD patients [9]. So far, the mechanisms by which the ECP induces immune tolerance are not completely understood, and research on this topic is still ongoing. A major mechanism of ECP is the induction of leucocytic apoptosis by extracorporeal treatment with the DNA-intercalator 8-methoxypsoralen (8-MOP) and the subsequent activation of 8-MOP with electromagnetic irradiation with ultraviolet-A (UVA, 315–380 nm) frequencies [10–12]. The following cellular clearance of the apoptotic bodies is an important feature in the restoration of a disbalanced immune homeostasis [13]. Other findings after ECP therapy are the induction of regulatory T cells (Tregs) [14, 15] and the induction of tolerogenic dendritic cells [16, 17]. The ECP-induced monocyte-to-dendritic cell maturation is probably mediated by platelets that are activated by the photopheresis procedure [18]. Maybe additional mechanistic effects and epiphenomena, which are triggered by the ECP, contribute to these changes in the immune cells and platelets.

When investigating further ECP effects on the immune system, the exact ingredients of the ECP irradiation bag should be evaluated. As discussed above, most studies focus on leukocytes because these cells are the most dominant cell fraction in the ECP irradiation bag. However, the platelets seem to be playing an important role as well. In addition, it should be noted that red blood cells (RBCs) outnumber white blood cells (WBCs) around 10-fold in the ECP irradiation bag. Little to nothing is known about the effects of the photopheresis procedure on the RBCs and their interactions with the immune system. In our in vitro study with human blood cells, we examined the influence of the 8-MOP and UVA treatment on the RBCs. We analysed the eryptosis, the free haemoglobin (fHb) levels, RBC distribution width (RDW) and immune-associated RBC antigens. Furthermore, we analysed the activation of platelets through the 8-MOP and UVA treatment, and finally, we investigated the potential of the treatment on reactive oxygen species (ROS) production, an important feature of the innate immune response.

## MATERIALS AND METHODS

### Blood collection and preparation

For our study, we used blood samples from healthy blood donors. Blood donation took place in the Department of Transfusion Medicine of the University Medical Center in Göttingen. All blood donors, female and male, with an age of 18–65 years were included in the study. Blood was used for which donors gave written permission that residual parts of their blood donation may be used for scientific investigations. The study meets all criteria of the Declaration of Helsinki and permission was granted by the local ethics committee of the University Medical Center Göttingen (approval no. 6/10/20).

We used buffy coats from whole blood donations for the isolation of mononuclear cells. The buffy coats were produced by centrifugation of the whole blood at 4000*g* for 10–20 min followed by a plasma and RBC elimination step with a Macopress separation system (Macopharma, Tourcoing, France). RBCs were drawn from the buffy coat after haematocrit measurement. The isolation of mononuclear cells from the buffy coats was performed 24 h after blood donation with Ficoll-Paque Plus using sodium citrate as an anticoagulation substrate (GE Healthcare Biosciences AB, Uppsala, Sweden). For this isolation, we diluted 10 mL from the buffy coat with 10 mL phosphate-buffered saline (PBS) containing 3.8% (w/v) sodium citrate as anti-coagulation substrate (GE Healthcare Biosciences AB, Uppsala, Sweden). Afterwards, 20 mL of the diluted buffy-coat solution was carefully pipetted on top of 30 mL Ficoll-Paque Plus solution. A centrifugation protocol with 600g for 20 min followed by two platelet reduction steps with 100g for 10 min was used. The resulting cell suspension contained the WBCs and platelets for the WBC and platelet experiments. Afterwards, we reproduced the cell proportions of the apheresis bag in a 1 in 10 dilution. For this purpose, we measured the WBC concentration and prepared a cell solution with $3.5 \times 10^6$ WBCs/mL and added RBCs that were drawn from the buffy coat for a final haematocrit of 0.2%.

## Cellular treatment with 8-MOP and UVA-irradiation

Cells for control and treatment conditions were isolated together in one batch, suspended in PBS (described above) and control and treatment cohorts were pipetted in separate 75-$cm^2$ cell culture flasks before 8-MOP and UVA treatment. We used 8-MOP produced by the in-house pharmacy of the University Medical Center Göttingen. Cell suspensions in 10 mL PBS were incubated with 200 ng/mL 8-MOP and irradiated with 2 J/$cm^2$ UVA light (Vilber Lourmat, Marne la Vallee, France). In this system, the UVA light is applied all around the cell culture flasks. After 8-MOP and UVA treatment, all cells were centrifuged with 350g for 10 min to remove 8-MOP from the cell suspension. Treated and control cells were resuspended in RPMI 1640 cell culture medium (Gibco, Thermo Fischer, Waltham, MA, USA), with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (penicillin and streptomycin, Gibco) and cultured at 37°C and 5% $CO_2$ in the incubator.

## Cell counting, free haemoglobin measurement and flow cytometry

RBC and WBC counting and RBC distribution width (RDW) measurements were performed with a CELL-Dyn Ruby system (Abbott, IL, USA).

The amount of free fHb in the cell suspension was determined by a HemoCue Low Hemoglobulin analyser (HemoCue AB, Sweden).

As a marker for WBC apoptosis and RBC eryptosis, we used annexin V FITC (fluorescein isothiocyanate) for phosphatidylserine binding (BioLegend, San Diego, CA, USA). For RBC detection, we used CD235a Pacific Blue (Clone HI-264), for WBC detection, CD45 FITC (Clone HI-30) and for further immune assays, CD147 APC (Clone HIM-6) and CD59 PE (Clone p282) from BioLegend.

## Detection of PLT glycoprotein expression

PLTs (platelets) were activated with adenosine 5-diphosphate (ADP, 100 µM) or thrombin-receptor-activating-peptide 6 (TRAP, 30 µM) and PBS was used as a control.

For subsequent detection of PLT glycoprotein expression CD41 PE (Clone HIP-8), CD42b PE (Clone HIP-1), CD62P APC (Clone AK4) and CD63 APC (Clone H5C6) antibodies from BioLegend were used. Measurement of median fluorescence intensity was performed with a FACSCanto II analyser (BD Biosystems, San Jose, CA, USA).

## ROS detection

The release of hydrogen peroxide ($H_2O_2$) was quantified with an Amplex UltraRed assay. Superoxide dismutase (SOD, 100 U/mL) was used for the conversion of $O_2^{\cdot -}$ to $H_2O_2$ that served as a reactant for Amplex UltraRed fluorescence (50 µM) catalysed by horseradish peroxidase (HRP, 500 U/mL). Fluorescence was measured in 96-well black/clear bottom plates with a CLARIOstar microplate reader (BMG Labtech, Ortenberg, Germany). Every test condition was measured in triplicates. Phorbol 12-myristate 13-acetate (PMA, 1 µM) was used to induce maximal ROS production. Measurements were performed with the CLARIOstar (version 5.21 R2) software.

## Statistics

Calculations for statistical significance were performed with two-way repeated measures analysis of variance (two-way ANOVA). After the two-way ANOVA, subgroups were compared with Sidak multiple comparison tests. Statistical significance was defined for $p$ values <0.05 ($^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$). Calculations were performed with the Graph Pad Prism 6 analysis software (San Diego, CA, USA).

# RESULTS

## Robust RBC integrity after 8-MOP and UVA treatment

After treatment with 8-MOP and UVA, our in vitro data showed a significant apoptosis rate in the CD45 leukocytes of 60.5% after 24 h in culture (Figure 1a). After 48 h in culture, the apoptosis level of the leukocytes rose up to 83.9% and reached 90.5% after 72 h (Figure 1a).

The RBCs showed a minimal increase on day 1 in the corresponding annexin V eryptosis assay, which was not significant on days 2 and 3 (Figure 1b). The untreated control cultures and the 8-MOP and UVA-treated cultures revealed less than 0.4% of annexin V positive RBCs within the 72 h culture time period (Figure 1b).

The leucocyte cell number was already decreased 24 h after 8-MOP and UVA treatment (Figure 1c). After 72 h in culture, only a few 8-MOP and UVA-treated leukocytes were countable (Figure 1c). The corresponding RBC numbers of the in vitro cultures indicated no significant decrease in the 8-MOP and UVA-treated RBCs after 72 h (Figure 1d).

Measurement of free fHb, which is released by damaged RBC membranes, was performed over 48 h. We observed no increase in fHb after 48 h in the 8-MOP and UVA-treated RBCs (Figure 1e).

The RDW is a clinical parameter for the heterogeneity of the RBC volume. The RDW value is associated with several types of anaemia and some infectious diseases. We compared the RDW value of untreated and 8-MOP and UVA-treated in vitro cultures for 48 h. We found no abnormalities in the RDW values: All RDW values were in the reference range of 11.5–14.5 (Figure 1f).

## Impact of 8-MOP and UVA treatment on CD59 and CD147 surface antigens

Subsequently, we quantified the surface antigen CD59, which has been suggested to act as a complement inhibitor and is expressed on

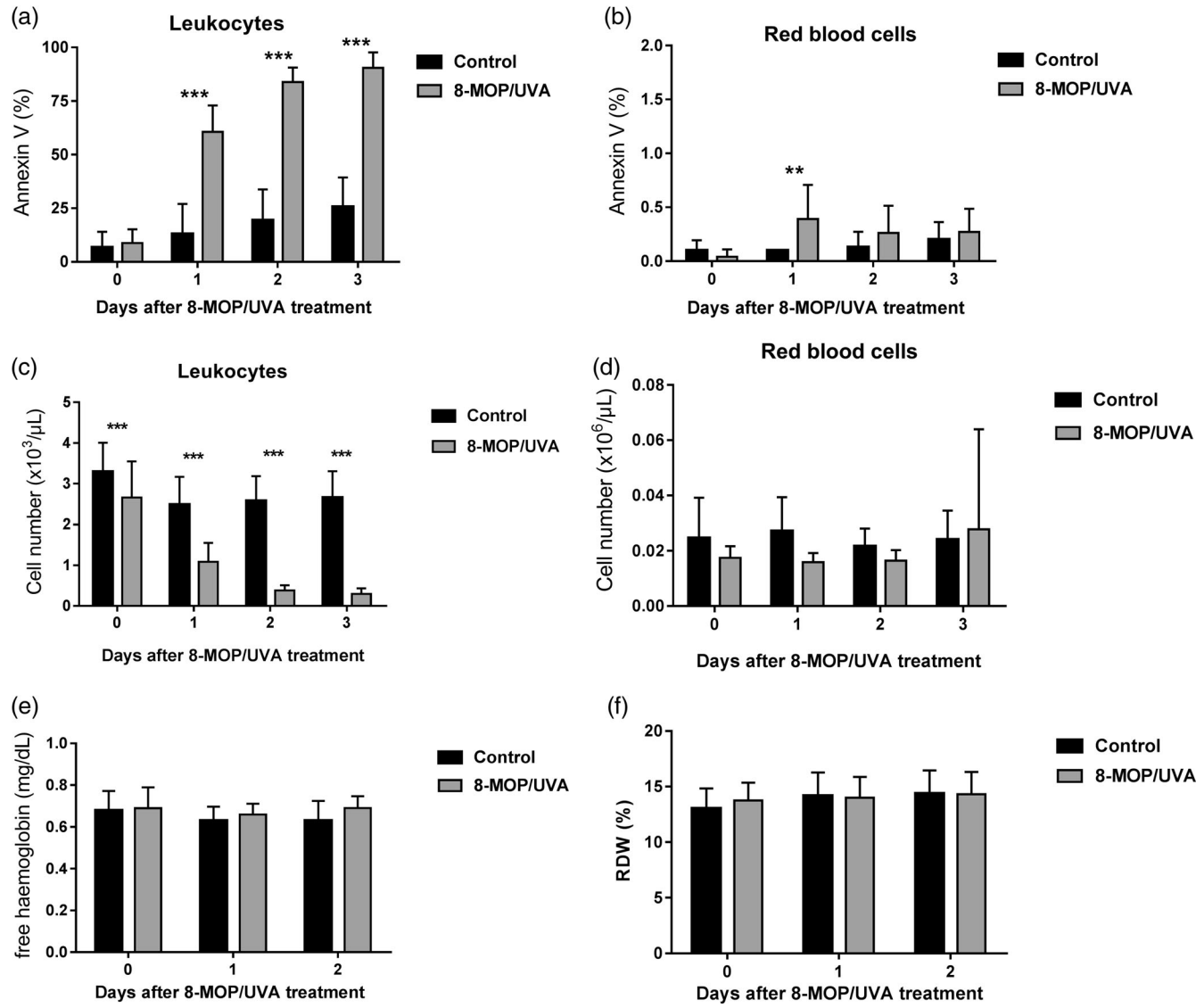


**FIGURE 1** Effects of 8-methoxypsoralen (8-MOP) and UVA treatment on red blood cell (RBC) and leucocyte eryptosis/apoptosis and cell counts. (a) Percentage of annexin V positive CD45 leukocytes and (b) percentage of annexin V positive CD235a RBCs. (c) Leucocyte and (d) RBC counts. (e) Concentration of free haemoglobin (mg/dL) and (f) RBC distribution width (RDW) values. Experiments were performed in 10 replicates and RDW measurements in nine replicates (bars represent the mean + SD; $^{**}p < 0.01$; $^{***}p < 0.01$).

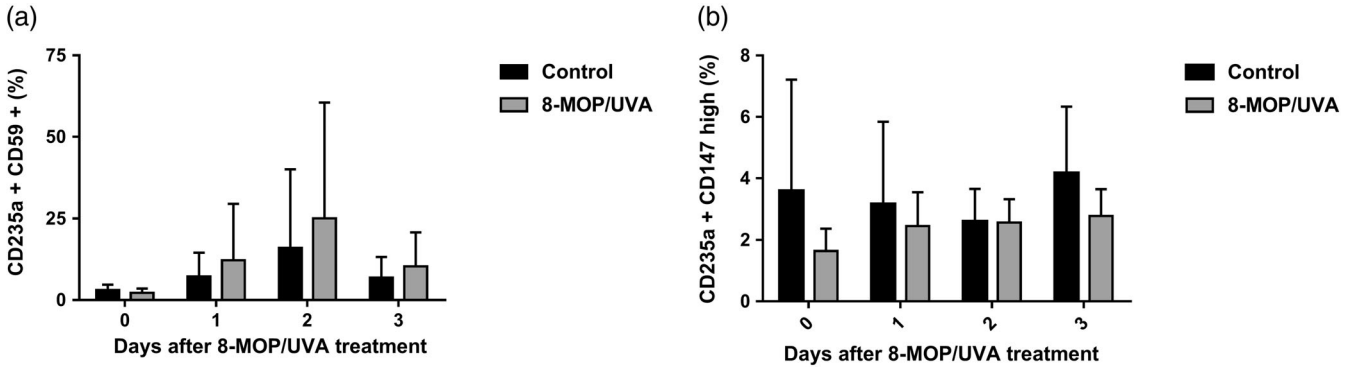


**FIGURE 2** Expression of red blood cell (RBC) and immune system-associated CD59 and CD147 antigens after 8-methoxypsoralen (8-MOP) and UVA treatment. (a) Percentage of CD59 and CD235a double positive cells from all CD235a RBCs ($N = 7$). (b) Percentage of $CD147^{high}$ and CD235a double positive cells from all CD235a RBCs ($N = 8$). Bars represent the mean + SD.

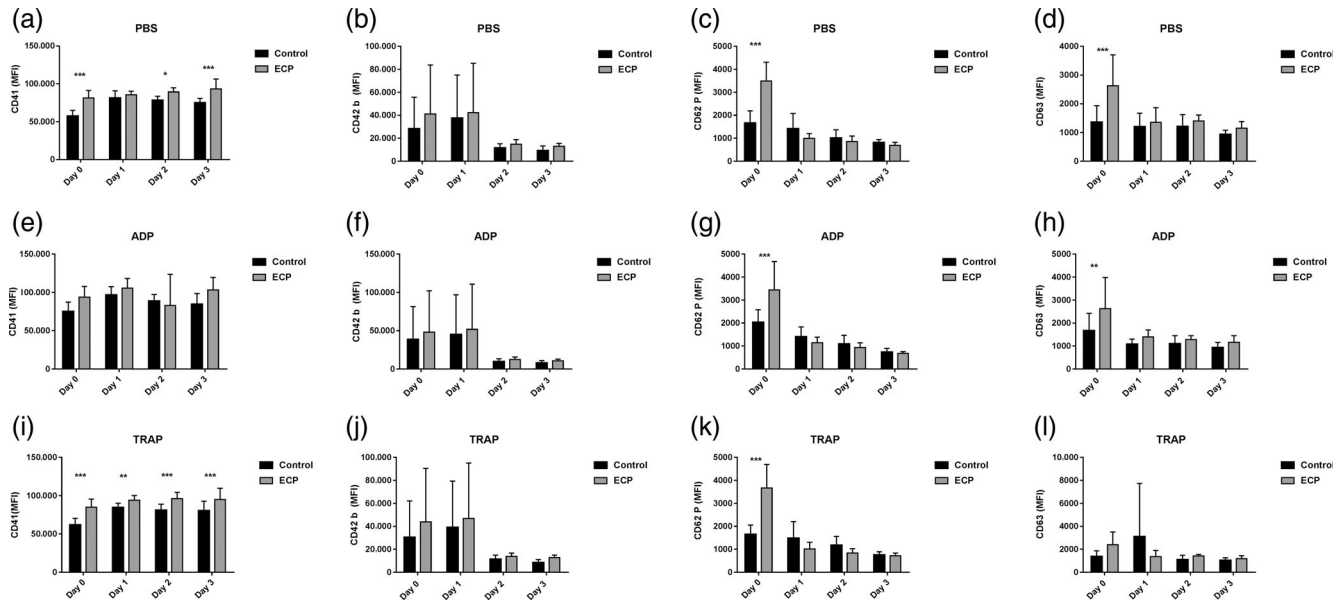


**FIGURE 3** Effects of 8-methoxypsoralen (8-MOP) and UVA treatment on the expression of platelet activation markers and platelet cell culture numbers. (a–d) Basal expression of CD41, CD42b, CD62P and CD63 platelet glycoproteins in phosphate-buffered saline buffer, 0–3 days after 8-MOP and UVA treatment of the cell cultures. (e–h) Expression of CD41, CD42b, CD62P and CD63 glycoproteins in adenosine 5-diphosphate (ADP)-activated cell solutions of the corresponding cell cultures 0–3 days after 8-MOP and UVA treatment. (i–l) Expression of CD41, CD42b, CD62P and CD63 glycoproteins in thrombin-receptor-activating-peptide 6 (TRAP)-activated cell solutions of the corresponding cell cultures 0–3 days after 8-MOP and UVA treatment. All experiments were performed in five replicates (bars represent the mean + SD; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.01$). ECP, extracorporeal photopheresis; MFI, median fluorescence intensity.

the RBC membrane. We performed flow cytometry analyses of untreated and 8-MOP and UVA-treated in vitro cultures over a time period of 72 h. There were no significant differences in the CD59 expression in the cultures (Figure 2a). Nevertheless, we could observe a tendency for higher CD59 expression in the 8-MOP and UVA-treated in vitro cultures (Figure 2a).

Another antigen, which is expressed on the RBC surface, is CD147. The CD147 protein belongs to the immunoglobulin superfamily and is expressed on the cell membrane of mature RBCs. The flow cytometry analyses of our 72 h in vitro cultures revealed no statistically significant differences in the CD147 expression between the untreated and the 8-MOP/UVA-treated cultures. However, a tendency for a lower CD147 expression in the 8-MOP/UVA group could be observed (Figure 2b).

## Increased platelet glycoprotein expression after 8-MOP/UVA treatment

In addition to leukocytes and RBCs, platelets, whose function depends on their activation state, also represent an important cell type in the cell suspension of the apheresis bag in the ECP system. Several glycoproteins on the surface of the platelet cell membrane serve as clinical indicators of platelet activation. In platelets that were already activated in vivo or by the in vitro treatment, the subsequent stimulation with ADP or TRAP will not result in an increased expression of the corresponding platelet glycoproteins. ADP or TRAP was replaced by PBS to control for the glycoprotein expression of the test samples without artificial activation.

In the non-stimulated PBS assays, we observed a significantly higher expression of CD41, CD62P and CD63 on the platelet membrane directly after treatment with 8-MOP and UVA (Figure 3a,c,d). The effect on platelet activation by the 8-MOP and UVA treatment was significant directly after treatment but disappeared already after 24 h in culture (Figure 3a,c,d). CD42b glycoprotein expression showed no significant differences in the PBS test condition of untreated and 8-MOP and UVA-treated cultures (Figure 3b).

After our observation that the platelets were significantly activated by the 8-MOP und UVA treatment, we used the platelet activation agents ADP and TRAP. This subsequent platelet test activation with ADP or TRAP showed similar results in the glycoprotein expression of CD41, CD42b, CD62P and CD63 as in the unstimulated PBS test condition (Figure 3e–l). The platelets had no more activation potential (Figure 3e–l) after their activation by the 8-MOP and UVA treatment (Figure 3a–d).

## Influence of 8-MOP and UVA treatment on cellular ROS generation

We used an $H_2O_2$ detection assay for the analysis of ROS production. The assay was based on the addition of SOD for the conversion of $O_2^{\cdot -}$ to $H_2O_2$. Without an external stimulus, the baseline fluorescence of the $H_2O_2$ assay was relatively low and increased slightly with time (Figure 4a).

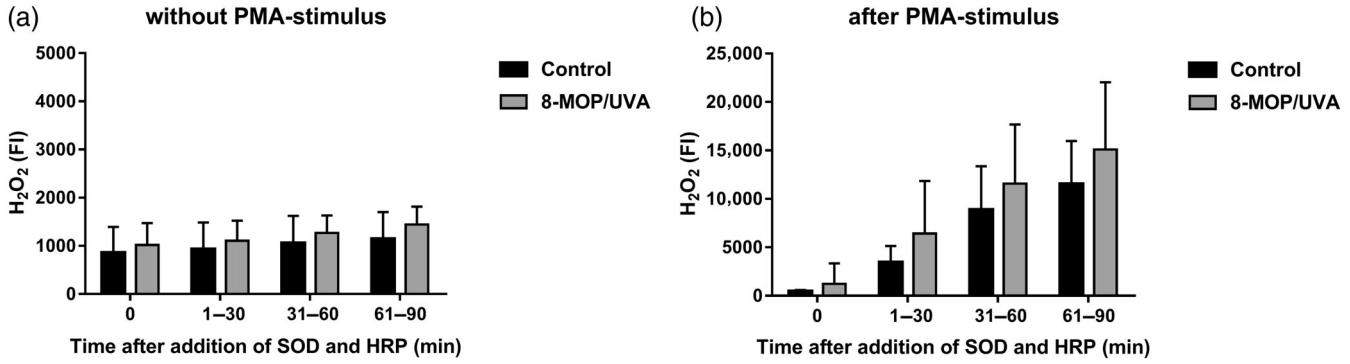


**FIGURE 4** Hydrogen peroxide production in 8-methoxypsoralen (8-MOP) and UVA-treated cell cultures. (a) Fluorescence intensity (FI) of the $H_2O_2$ signal in the cultures without prior phorbol 12-myristate 13-acetate (PMA) stimulation. (b) FI of the $H_2O_2$ signal in the cultures directly after PMA stimulation ($N = 10$). Bars represent the mean + SD. HRP, horseradish peroxidase; SOD, superoxide dismutase.

Average values after 61–90 min showed increased levels of $H_2O_2$ of around 50% (untreated control) and 40% (8-MOP and UVA-treated samples). There were no significant differences in $H_2O_2$ production between the untreated control and 8-MOP and UVA-treated samples. After stimulation with PMA, the average $H_2O_2$ levels were strongly increased with the highest levels after 61–90 min after stimulation (Figure 4b). Notably, the 8-MOP and UVA-treated cells generated more $H_2O_2$ than the untreated (but PMA stimulated) cells after 90 min. As shown in Figure 4b, at each time point, the 8-MOP and UVA-treated cells generated more $H_2O_2$. However, these results reached no statistical significance due to the high donor-based variations.

## DISCUSSION

A well-known ECP mechanism is the induction of apoptosis in the leukocytes of the patient. With our annexin V apoptosis assay, we could demonstrate that the apoptosis of the leukocytes in the cell sample was strongly induced already 24 h after the 8-MOP and UVA treatment. In concordance with our previous studies, the leucocytic apoptosis level reached a level of more than 90% after 72 h in culture [11, 19]. This finding was confirmed by several other groups, too [10, 12].

On the other hand, little is known about the impact of 8-MOP and UVA treatment on the high number of RBCs in the ECP bag. In contrast to the high apoptosis susceptibility of the leukocytes after 8-MOP and UVA treatment, we observed no increase in the RBC eryptosis. Even after 72 h in culture, the phosphatidyl-based annexin V assay did not detect any disturbances in the RBC membrane. In contrast to the markedly reduced leucocyte cell number after 8-MOP and UVA treatment, the RBC count was stable even after 72 h in culture. These results were confirmed by our measurement of the free haemoglobin levels. We found no difference between the untreated and 8-MOP and UVA-treated cells, which is a clear sign of stable RBC membrane integrity. The RBC robustness against 8-MOP and UVA treatment was further underlined by the normal RDW values. The RDW value is a parameter for RBC size variance, especially in several kinds of anaemia. The 8-MOP and UVA treatment seem to have no significant influence on these metabolic and morphologic RBC parameters. However, our measurements of free haemoglobin and RDW values are not sensitive enough to fully determine any adverse effects on the RBCs. Free haemoglobin could be removed by the experimental centrifugation steps and RDW values are donor dependent. Our measurements still lack information about RBC deformability, morphology, potassium leak, osmotic/oxidative fragility and exosome release. Measurements of these parameters are necessary for future studies on this topic.

After our observations on the RBC integrity and size distribution, we examined the expression of CD59 and CD147, parameters that indicate the possible link between the RBCs and the immune system. We observed a slight tendency for higher CD59 expression on the RBC membranes in the 8-MOP and UVA samples, but due to the high standard deviation, these results were not statistically significant. Since CD59 functions as an inhibitor of the complement system, a slightly enhanced CD59 expression after 8-MOP and UVA treatment could maybe result in an inhibition of the complement system. This consideration fits the observation that CD59 is decreased in systemic lupus erythematosus [20]. ECP as a potential therapy for autoimmune diseases such as systemic lupus erythematosus could increase the reduced CD59 expression in order to maintain the homeostasis of the complement system [21]. Further research on the impact of 8-MOP and UVA treatment on the CD59 expression in RBCs seems to be required.

The RBC expression of CD147 indicated no influence of the 8-MOP and UVA treatment. However, we observed a tendency for reduced CD147 expression in the treatment group. Interestingly, CD147, a member of the immunoglobulin superfamily, seems to be a ligand for parasite invasion into mature RBCs [22]. Whether slightly reduced CD147 expression levels after ECP treatment could contribute to the prevention of other infectious diseases is unclear. If reduced CD147 expression would be linked to resistance against other infections, a potential reduction in CD147 expression after ECP could contribute to the effect that ECP is linked with low levels of opportunistic infections [8, 9].

CD147 also plays an important role in the re-entry of mature RBCs back from the spleen in the peripheral blood circulation and is, therefore, linked to anaemia. Our analysis of the RDW value showed no indication for anaemia induction in the treated in vitro cell culture samples, but in vivo there could maybe a small effect due to splenocytic RBC reduction. Further investigation is necessary to shed light on the potential involvement of CD147 in the ECP mechanisms.

The expression of platelet-associated glycoproteins is a basic test for platelet activation. Directly after 8-MOP and UVA treatment, the density of CD41, CD62P and CD63 glycoprotein was increased, which is a clear sign of platelet activation by the 8-MOP and UVA treatment. Several interactions of platelets with the immune system and especially with the T cells are described in the literature [23–25]. Gerdes et al. could show that platelets support the differentiation and cytokine production of CD4 T cells after CD3/CD28 stimulation [26]. These interactions could contribute to the modification of T cell subsets after ECP therapy.

The effect of the treatment on CD41, CD62P and CD63 expression seems to be reversible. After 24 h in culture, we barely found differences in the glycoprotein expression between the treated and the control group. In contrast to CD41, CD62P and CD63, the density of the glycoprotein CD42b was not significantly increased by the 8-MOP and UVA treatment. However, the CD42b expression is in general not elevated by the stimulus with ADP or TRAP, either. Other factors like the surface material of the irradiation cell culture flask and the centrifugation step could maybe contribute to the platelet activation, too. The surface of the plastic flasks we used in our experiments differs from the clinical apheresis bags from different companies such as Therakos and Macopharma. The surface of all these bags could be slightly different which could result in slightly different activation of the platelets. We did not observe additional activation potential of the platelets after stimulation with ADP or TRAP, which indicates that the platelets were already substantially activated by the 8-MOP and UVA treatment.

These results fit the findings of the Edelson group that the ECP procedure triggers the activation of the platelets in the apheresis bag [18]. They also suggest that the contact of the platelet with the plastic surfaces within the apheresis device is likely to also contribute to platelet activation.

RBCs are able to promote T cell proliferation and they are able to inhibit T cell apoptosis [27]. This interaction could be influenced by ROS-controlled damage or modification of the RBCs [28]. Our measurement of ROS release after 8-MOP and UVA treatment revealed no significant increase in ROS production. However, following PMA stimulation a tendency for higher ROS production in the 8-MOP and UVA-treated cell cultures was apparent. An increase in ROS could contribute to the ECP mechanism by inhibiting RBC-supported T cell proliferation and reducing the inhibitory effect of RBCs on T cell apoptosis. Although we found no significant RBC haemolysis or eryptosis after 8-MOP and UVA treatment, the effect of possible ROS induction by the 8-MOP and UVA treatment should be investigated further.

In summary, our study revealed high RBC robustness against 8-MOP and UVA treatment. Some immune-associated RBC antigens, especially CD59, may be affected by the 8-MOP and UVA treatment, which could contribute to a restoration of the altered complement homeostasis in autoimmune situations. In contrast to the RBCs, we found that platelets are significantly activated by the treatment. This platelet activation was previously seen in publications as a factor of dendritic cell maturation as an important mechanism of ECP therapy. Further studies on the less investigated RBCs and platelets could help to uncover further ECP-mediated immune mechanisms.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

# Development of an in-house bone marrow collection kit: The Catalan bone marrow transplantation group experience

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## Abstract

**Background and Objectives:** Bone marrow (BM) harvesting is one of the essential sources of stem cells for haematopoietic stem cell transplantation. In 2019, commercial BM collection kits became unavailable in Europe. Consequently, we created an in-house BM collection kit as an alternative.

**Materials and Methods:** We compared two groups of BM collections. The first collections were taken using an in-house kit from June 2022 through February 2023 and the second with a commercial kit from February 2021 through May 2022. These all took place at seven collection centres (CC). We analysed the harvest quality (cell blood count, CD34+ cells, viability, potency and sterility), the incidents occurring with each kit and the time to neutrophil and platelet engraftment in recipients.

**Results:** A total of 23 donors underwent BM harvesting with the in-house kit and 23 with the commercial one. Both cohorts were comparable regarding donor

  

characteristics, CC and time to procedure. No statistical differences were found in harvest quality between the in-house and commercial kits. A new transfusion set was required in three BM harvests (13%) with the in-house kit because of filter clogging. The median time to neutrophil and platelet engraftment was 21 days for both cohorts and 29 days (in-house) and 33 days (commercial), $p = 0.284$, respectively.

**Conclusion:** The in-house BM collection kit offers a real approach to solve the diminished supply of commercial kits. A higher risk of filter clogging was observed compared with commercial kits due to the lack of 850 and 500 μm filters.

## Keywords

bone, collection, commercial, in-house, kit, marrow

## Highlights

- The in-house bone marrow (BM) collection kit offers a real alternative to solving the diminished supply of commercial kits.
- The in-house BM collection kit guarantees quality control, sterility and engraftment.
- Higher risk of filter clogging was observed in comparison to the commercial BM collection kit.

## INTRODUCTION

A bone marrow (BM) stem cell source from fully HLA-matched donors is recommended in haematopoietic stem cell transplantation for BM failure syndromes, acute lymphoblastic leukaemia in children, selected inborn errors of metabolism and acquired severe aplastic anaemia in adult patients [1]. According to an activity survey conducted by the European Bone Marrow Transplantation in 2022, 2811 BMs were collected in 2020 in Europe [2]. Most collection centres (CC) have been harvesting BM with commercial medical devices, such as those manufactured by Fresenius Kabi. Such devices prevent the thrombosis associated with the BM infusion.

In 2018, the new Regulation (European Union [EU]) 2017/745 on medical devices (Medical Device Regulation, MDR [3]) meant that all suppliers of commercial BM kits in the European Union (EU) (mainly Fresenius Kabi with around 80% of the market share) lost their European Conformity (CE) mark and could no longer be used in Europe. In 2019, that company informed all their customers that they were leaving the European region and would only supply the kits they still had in stock, which would expire in 2022. In response to this, in summer 2022, the European Commission's Sector for Substances of Human Origin noted that, according to Article 59 of the MDR, a pathway was in place that allowed for regulatory flexibility at a national level in justified cases and in the interest of patient safety or public health. Thus, the different member states could ask for a derogation of the CE mark for the kits. However, at that time, Fresenius Kabi stated that they were unable to resume the supply in Europe. BioAccess, the other main provider, showed an interest in the market, but they were unable to meet the demand to supply the entire European region. Fortunately, article 5.5 of Regulation (EU) 2017/745 allows in-house manufacturing of BM collection kits in healthcare institutions as long as they meet the safety and functionality requirements described in Annex 1 of the regulation [3].

The aim of our study was to retrospectively analyse BM collections using an in-house kit created by the Catalan bone marrow transplantation group (CBMTG) and used by seven authorized BM centres in Catalonia, Spain and to compare them with those taken using the prior commercial kit.

## MATERIALS AND METHODS

### The Catalan bone marrow transplantation group

In April 2022, the CBMTG was set up with representatives from each of seven authorized BM CC and the central cell processing laboratory at Banc de Sang i Teixits (BST) located within 30-min drive away from CC, all JACIE accredited, in Catalonia, Spain. The main aim of this group was to develop an in-house BM collection kit as an alternative to the lack of supply of commercial BM collection kits and the imminent expiration of available commercial kits. In June 2022, we decided to start using the in-house kits in all the centres, having commercial kits available as a contingency plan with an expiration of 2 months. Regular meetings were held to evaluate the results and user satisfaction.

### Donors, BM products and patients

All donors and recipients provided written informed consent prior to collection and haematopoietic stem cell transplantation. The Vall d'Hebrón University Hospital ethics committee reviewed and approved the study (CEIC code PR(BS)284/2022). All consecutive BM

donors and the subsequent recipients between February 2021 and February 2023 were retrospectively included in the study. We compared two cohorts, one using our in-house kit (after May 2022) and the other using the commercial kit (before June 2022).

Demographic characteristics of the donors (age, sex and weight) and type of donor (related or unrelated) were recorded. For the harvest procedures, we obtained time until filtration, technical deviations and specific anticoagulation from the accompanying collection report. We calculated the volume of the BM product, from its weight, applying a density of 1.055 g/cm$^3$ (Sartorius PT 6, Gottingen, Germany). A sample underwent quality control procedures: complete cell blood count (CBC) using a haematology analyser (XN550, Sysmex, Kobe, Japan); phenotyping by flow cytometry (Navios, Beckman Coulter, Brea, USA); assessment of CD45+ and CD34+ expression by the ISHAGE protocol single-platform multigating strategy adjusting for forward side scatter, straight side scatter, CD45+ and CD34+ [4] and viability assessment using 7-aminoactinomycin D (7-AAD); potency was analysed with a cfu granulocyte-macrophage (GM) assay using Methocult 4434 (Stem Cell Technologies, Vancouver, Canada) supplemented with Iscove's Modified Dulbecco's Medium, BIOWEST, Nuaillé, France (IMDM). Sterility control was performed with BactAlert in aerobic and anaerobic culture at reception in cell processing laboratory and after BM was manipulated (Biomerieux, Ref. 704631 and 704628, Madrid, Spain). Samples were incubated for 14 days at 37°C. If the BM required minimal manipulation such as washing of buffy coat for major or minor ABO incompatibility, or plasma reduction, this was performed with Sepax (Cytiva, Eysins, Switzerland). In the case of BM cryopreservation, the freezing solution consisted of 10% dimethyl sulfoxide, 4% human serum albumin and 5% citrate dextrose solution, solution A (ACD-A) in Plasma-Lyte. A controlled-rate/programmable freezer CM2010 (Carburos Metálicos, Air Products, Barcelona, Spain) was used at <−5°C/min, and the samples were stored at <−150°C in the vapour phase of liquid nitrogen. We recorded the demographic characteristics of the recipients (age, sex, weight, diagnosis and time to haematology recovery).

## Kit components, production and operating instructions

The components of the in-house collection BM kit were: 1000 mL parenteral nutrition bag (Bexen, Ref. 502.10; CE 0318; blood compatibility tested on human blood, Gipuzkoa, Spain); safety valve connector (ABLE, Ref. 0102044; CE 0197, Foshan, China); and a transfusion set that included a 200 µm filter (Care Fusion, Ref. VH-92-E; CE 0123, San Diego, CA, USA), 1000 mL transfer bag (Fresenius Kabi, Ref. R6R2031; CE 0123, Bad Homburg, Germany) and tube clamp (Thermo Fisher, Ref. 10341444, Waltham, USA).

The in-house BM collection kit was produced by the cell processing laboratory at BST. The kit was assembled in a biological safety cabinet (class A) inside a classified area (class D); the 200 µm transfusion set filter was connected to the transfer bag with a sterile tubing welder (TSCD-II, Terumo BCT, Colorado, USA) to allow immediate BM filtration at collection sites. Thereafter, the filtration set was repackaged under the same environmental conditions. All the components described above were then packaged in a single plastic bag container and placed in a designated box before distribution. The secondary container was appropriately labelled and supplied through each local blood bank to the BM CC days before the harvest was scheduled.

The operating instructions for use of the in-house BM collection kit are shown in Data S1. Training for use was given by videoconference, and a specialist nurse supported the first BM collection in each participant centre.

## Harvest procedure

The target harvest volume was set based on the requested 3–4 × 10$^8$ total nucleated cells (TNC)/kg of recipient body weight (BW). We expected to reach this cell dose by drawing 20 mL of donor marrow/kg of recipient BW. The maximum volume of the harvest was usually set at 20 mL/kg of the donor BW. In the operating theatre, the harvest team had a supply of the in-house BM collection kit, 500 or 750 mL ACD-A, heparin 1% and Plasma-Lyte or Hanks' Balanced Salt Solution as a culture medium. Addition of heparin into the collection bag was carried out prior to the injection of the BM in all the CC. However, according to the centre's policy, the heparin concentration varied, ranging from 4 to 20 IU/mL. Time to add 10% of ACD-A to collection bag and the use of culture medium was also different between CC according to their policy. The technique used was based on the original method described by Thomas and Storb [5, 6]. The harvesting team consisted of at least two physicians, one anaesthetist and two assistant staff. The procedure was performed in the operating theatre of each BM CC. First, the anticoagulation solution was injected into the collection bag through the Luer-lock connection (see Data S1). The BM was aspirated in batches of 1–5 mL, each of which was then handed to assistant staff who injected it into the collection bag through the Luer-lock connection following the operating instructions (see Data S1). After each aspirated batch of marrow was placed into the collection bag, the syringes were serially flushed with anticoagulation solution to prevent clotting. The syringes were reused multiple times during the procedure. The collection bag was gently agitated by assistant staff during the entire procedure to prevent coagulation inside the bag. The volume of the harvested marrow was controlled by checking the collection bag volume lines (see Data S1). Once the maximum volume had been reached, the procedure was stopped. A final 10% ACD-A concentration was used by all CC. The collected bag was then connected to the 200 µm transfusion set filter through the spike and the harvest was filtered to a final 1000 mL transfer bag (see Data S1). Finally, the filtered BM product was labelled and shipped to the cell processing laboratory for quality control, manipulation (if applicable) and release for infusion.

## Definitions

BM contamination during collection was defined when a positive sterility test was detected at reception in the cell processing laboratory.

Neutrophil recovery was considered to occur on the first of three consecutive days with an absolute neutrophil count >500/μL after the post-transplantation nadir. Platelet recovery date was the first of three consecutive days with a platelet count >20,000/μL, in the absence of platelet transfusion for seven consecutive days [7]. Primary graft failure was considered when neutrophil and platelet engraftment was not achieved 28 days after transplantation, without evidence of disease relapse. Secondary graft failure was referred when a loss of a previously functioning graft was detected.

## Study endpoints

The primary study objective was to compare the harvest quality (CBC, CD34+ cells, viability, potency and sterility), any incidents that occurred and the time to neutrophil/platelet engraftment in recipients who used the in-house BM collection kit (between June 2022 and February 2023) versus those with the prior commercial kit (between February 2021 and May 2022).

## Statistical analysis

We analysed the two cohorts of BM collections, those using the in-house kit or the commercial one. Donor characteristics, harvest quality and BM procedure were compared between both cohorts using a comparison test (Mann–Whitney *U* test for quantitative variables and Chi-square or Fisher test for the categorical ones). Median and interquartile range (IQR) or frequency and percentage were used for quantitative and qualitative variables, respectively. Time to neutrophil/platelet engraftment was calculated using survival analyses. Kaplan–Meier curves and log-rank tests were used to compare the cohorts. A type-1 error of 5% was used in statistical analysis. Statistical analysis was performed with the software R Studio (Version 4.1.2).

# RESULTS

## Baseline characteristics of donors, grafts and recipients

A total of 46 donors underwent BM harvesting in the seven centres, 23 with the in-house kit and 23 with the commercial one, and all were included in the study. Fifteen (65%) and 14 (61%) donors were paediatric, respectively. All but one donor (in-house kit) were related to their recipients. No differences in sex, age and weight of the donors were found between the two cohorts (Table 1).

The median time for the BM harvest with both kits was 90 min. The heparin used with the in-house kit was 14 IU/mL versus 8.9 IU/mL in the commercial kit ($p = 0.395$). No statistical difference was found regarding TNC/mL, CD34+ cells/mL, 7-AAD CD45/CD34+ negative and cfu-GM/μL between the two kits (Table 1). In four (17%) BM collections using the in-house kit and in three (13%) with the commercial kit, the sterility test was positive at cell processing reception ($p = 1.000$) (Table 1). The isolated microorganisms were *Staphylococcus epidermidis* (3), *Cutinebacterium acnes* (2), *Staphylococcus lugdunensis* (1) and *Staphylococcus saccharolyticus* (1). All bacteria were detected before 7 days except *Cutinebacterium acnes*. No adverse reactions were recorded after infusion. BM manipulation, primarily cryopreservation which was recommended during COVID-19 pandemic, was required in 15 (65%) products collected with the commercial kit versus 8 (35%) with the in-house kit ($p = 0.077$) (Table 1).

Patients who received the BM collected with the in-house kit included more females (57% vs. 22%, $p = 0.034$) (Table 1). No differences were found regarding age, weight or diagnosis between the two kits.

The median TNC/kg in the in-house and commercial kits was $3.05 \times 10^8$ and $3.5 \times 10^8$, respectively (Table 1). The median time to neutrophil engraftment was 21 days in both cohorts. The median time to platelet engraftment was 29 days in the in-house group and 33 days in the commercial group, $p = 0.220$ (Table 1). In the commercial kit cohort, one patient with BM failure showed a primary graft failure despite having received a product with $2.8 \times 10^8$ TNC/kg, $2.3 \times 10^6$ CD34+ cells/kg, 95.8% and 98% viability and 17.6 cfu-GM/mL. Another patient in the same cohort did not achieve platelet engraftment 2 months after transplantation because of secondary graft failure, and one other patient died 2 days after transplantation because of septic shock despite no evidence of BM contamination. In the in-house kit cohort, the unrelated patient was still receiving platelet transfusions 84 days after transplantation as a result of septic thrombophlebitis of the brachial vein and the central catheter that require anticoagulation and an attempt to achieve platelet counts of more than 50,000/μL.

## Technical issues

The users of the in-house kit were satisfied with the collection system and did not report any problems with assembling the kit. In two harvests from the same BM CC, there was a significant difference between the volume shown in the collection bag and the final volume reported by the cell processing laboratory. This was due to the large amount of air in the collection bag, resulting in underestimation of the total BM collection volume. In three cases (13%), the 200 μm transfusion set filter connected to the final bag had to be replaced with a new one when BM was filtered because of filter clogging. BM products were successfully filtered and we found no impact on quality control. The total amount of heparin in the BM collected was 19 IU/mL in two cases and 4 IU/mL in the other. No procedural deviations occurred in these three cases.

# DISCUSSION

The CBMTG created an in-house BM collection kit for seven CC when a stoppage in the supply of commercial BM collection kits was

**TABLE 1** Baseline characteristics of bone marrow (BM) donors, grafts and recipients according to use of the in-house BM collection kit versus commercial kit.

| Characteristic | Kit | | *p* value |
|---|---|---|---|
| | In-house (*n* = 23) | Commercial (*n* = 23) | |
| Donors | | | |
| Age, years, median (IQR) | 14 (9–22) | 13 (7–22) | 0.545 |
| Sex, female, *n* (%) | 11 (48) | 9 (39) | 0.766 |
| Weight, kg, median (IQR) | 56.0 (29.5–68.6) | 59.2 (24.0–67.3) | 0.660 |
| BM grafts | | | |
| Time to procedure, minutes, median (IQR) | 90.0 (76.0–108.0) | 90.0 (72.5–100.0) | 0.466 |
| Volume, mL, median (IQR) | 504 (324–986) | 603 (446–987) | 0.517 |
| Heparin, IU/mL, median (IQR) | 14.0 (4.7–16.1) | 8.9 (4.0–15.0) | 0.395 |
| TNC, $10^6$/mL, median (IQR) | 18.8 (14.5–23.3) | 17.6 (13.4–21.3) | 0.668 |
| CD34+ cells, $10^3$/mL, median (IQR) | 106.0 (76.3–131.0) | 103.0 (78.0–156.0) | 0.947 |
| 7-AAD CD45+ negative, %, median (IQR) | 93.1 (91.0–95.8) | 93.0 (86.2–93.8) | 0.097 |
| 7-AAD CD34+ negative, %, median (IQR) | 96.4 (94.3–98.0) | 97.5 (92.8–98.7) | 0.658 |
| cfu-GM, /μL, median (IQR) | 14.9 (5.6–22.9) | 13.8 (9.4–18.3) | 0.818 |
| Microbiological contamination, *n* (%) | 4 (17.4) | 3 (13) | 1.000 |
| Cell processing manipulation, *n* (%) | 8 (35) | 15 (65) | 0.077 |
| Recipients | | | |
| Age, years, median (IQR) | 7 (3–17) | 8 (6–17) | 0.327 |
| Sex, female, *n* (%) | 5 (22) | 13 (57) | 0.034 |
| Weight, kg, median (IQR) | 33.0 (14.5–60.0) | 32.0 (22.9–51.5) | 0.496 |
| Diagnosis | | | 0.288 |
| Non-malignant disease[a], *n* (%) | 14 (61) | 14 (61) | |
| Malignant disease[b], *n* (%) | 9 (39) | 9 (39) | |
| TNC/kg, $10^8$, median (IQR) | 3.05 (2.41–4.65) | 3.52 (2.18–4.34) | 0.947 |
| CD34+ cells/kg, $10^6$, median (IQR) | 1.98 (1.48–2.78) | 1.80 (1.48–2.88) | 0.895 |
| Neutrophil recovery, days, median (IQR) | 21 (19–22) | 21 (19–23) | 0.548 |
| Platelet recovery, days, median (IQR) | 29 (19–37) | 33 (28–37) | 0.284 |

Abbreviations: 7-AAD, 7-aminoactinomycin D; ACD-A, citrate dextrose solution A; cfu-GM, granulocyte-macrophage colony-forming units; IQR, interquartile range; TNC, total nucleated cells.

[a]Inherited disorders (five and two), bone marrow failure (five and four) and haemoglobinopathy (four and eight) in the in-house versus commercial kit cohorts, respectively.

[b]Myelodysplastic syndrome (three and zero), acute lymphoblastic leukaemia (four and seven) and lymphoma (two and two) in the in-house and commercial kit cohorts, respectively.

notified. We studied the harvest quality (CBC, CD34+ cells, viability, potency and sterility), the BM collection procedure and the time to neutrophil and platelet engraftment in recipients, comparing our in-house BM collection kit (from June 2022 through February 2023) with the prior commercial kit (from February 2021 through May 2022).

The haematopoietic transplant community has been affected by numerous drug and device shortages over the past several years. One example was the recent worldwide shortage of fludarabine, which plays a central role in multiple conditioning regimens for both transplantation and immune effector cell therapies. Other shortages of reagents, devices and medicinal products are likely in the context of ever more stringent regulatory requirements that require higher investments that manufacturers may refuse in view of potentially small or shrinking markets and low revenues. This situation prompted our group to seek novel approaches to overcome supply chain disruption, including exploration of alternative therapies [8]. In 2019, Fresenius Kabi announced that they would stop supplying BM collection kits in Europe as it did not comply with Regulation (EU) 2017/745 on medical devices (MDR) [3]. As an alternative, the BM collection kit made by the company BioAccess Inc. (Baltimore, MD, USA) is available in some European countries through an exceptional national authorisation. However, this manufacturer is not expected to be able to supply all European countries, at least in the short term. This problem of the disruption in the supply of BM collection kits was widely reported through the Competent Spanish cell and tissue authority and

Spanish BM donor registry to AEMPS (Spanish Agency for Medicines and Medical Products). These procedures may vary from one national competent authority to another. Their response was not fast enough to meet clinical needs, with the result that CC started to investigate different approaches to solve this problem. In one approach, some CC started to collect BM into blood collection bags and then shipped them to their cell processing laboratory for filtering [9]. In our group, we decided to create an in-house BM collection kit as another approach. Interestingly, we found no published literature on in-house BM collection kits.

Our aim was to create a kit like the commercial kits, to guarantee minimal impact of the change. Because our cell processing laboratory is within 30 min by car from CC, we considered filtering essential at the collection site, as with the commercial kit, to minimize the risk of clumping during more extended storage [10]. Both cohorts had comparable donor characteristics. The median TNC/mL in the harvest products with both kits was approximately $18 \times 10^6$, as reported by the largest study [11]. We found no differences in CD34+ cells, viability, potency or time to neutrophil/platelet engraftment in the two cohorts. The amount of heparin in the harvest from the in-house kit was 14 IU/mL in contrast to 8.9 IU/mL in the commercial kit. This was intentionally higher to minimize the risk of final filter clogging due to the lack of 850 and 500 µm filters. These are in the commercial kits, but unobtainable for our in-house kits. Four (17%) in-house harvest products and three (13%) commercial were contaminated by normal skin flora, but no adverse reactions were reported. Our 14-day incubation period allows for the identification of slow growth bacteria. Both cohorts showed BM contamination by *C. acnes*. Some studies with no identification of slow growth bacteria did not give their incubation time; this could explain their low incidence of BM contamination of about 4.5% [12, 13]. In contrast, Vanneaux et al. reported 13.6% of initial BM bacterial contamination using the BathectAlert system and incubating 14 days, as in our study [14]. As for other technical issues, we found that air in the collection bag caused underestimation of the final collection volume. This was probably because the Fresenius Kabi kit has an open collection bag that releases air, which our kit does not [15]. Three harvests (13%) required the transfusion set connected to the final bag to be replaced by a new one because of clots. Commercial kits have at least three filters (850, 500 and 200 µm) and one pre-filter that allows for easy and uninterrupted filtration of the marrow during collection and reduces the likelihood of clogging [15]. Lacking this system, our kit has a higher risk of filter clogging despite the increased heparin. Nevertheless, we found no impact on quality control and all recipients engrafted in the in-house cohort. Since then, we recommend changing the 200 µm transfusion filter connected to the final bag before clogging occurs. This will be done two or three times during the harvest procedure, depending on the BM volume required.

Our study has limitations such as the sample size. It was also limited by the short recruitment period and the follow-up period was also necessarily brief. Therefore, more BM harvesting with the in-house kit and including other CC will be required to accumulate more experience.

In summary, to the best of our knowledge, this is the first study to assess an in-house BM collection kit. No differences were found regarding quality and sterility of the harvested product and its engraftment when using the in-house versus the commercial kit. Therefore, this offers a real approach to resolve the issue of diminished supply of commercial kits. A higher risk of filter clogging exists in the in-house kit due to a lack of 850 and 500 µm filters. Further experience is needed to confirm these results.

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## CONFLICT OF INTEREST STATEMENT

The authors state that they have no conflict of interest regarding this article.

## DATA AVAILABILITY STATEMENT

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

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

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

# Closed system production of serum eye drops using dockable saline

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## Abstract

**Background and Objectives:** A fully closed system solution to manufacture serum eye drops using diluted serum has remained elusive, necessitating production steps to mitigate bacterial contamination risks in a clean suite environment, hampering production efficiency amid growing demand. We describe our recent implementation of a fully closed manufacturing process at New Zealand Blood Service.

**Materials and Methods:** A dockable format for sterile saline manufactured to custom specifications configured with a 15-cm tubing to enable sterile connections was sourced from a local pharmaceutical manufacturer.

**Results:** From a total of 30,168 eye drop vials manufactured since implementation, the average production time was reduced by up to 45% performed in the general laboratory environment, attributed to eliminating processes performed in a clean suite. No bacterial contamination was observed, demonstrating robust sterile connections.

**Conclusion:** Dockable saline takes serum eye drops manufactured from a functionally closed system to a fully closed system, thereby enhancing patient safety, significantly reducing manufacturing time and cost and transforming production from a highly restrictive process into a portable workflow that is simple, practical and effective.

## Keywords

bacterial contamination, closed system, dockable saline, ocular surface disease, patient safety, serum eye drops

## Highlights

- Sourcing sterile saline in a dockable format enhances patient safety by reducing the risk of bacterial contamination by the introduction of a fully closed system for the manufacture of serum eye drops.
- The production of serum eye drops using a fully closed system has allowed the process to move from a clean suite facility to the general laboratory environment, significantly reducing cost and processing time.
- Manufacturing serum eye drops in a general laboratory environment allows New Zealand Blood Service laboratories without clean suite facilities to commence the manufacture of serum eye drops, providing essential contingency backup to the single site that currently produces them.

  

## INTRODUCTION

Autologous and allogeneic serum eye drops (SEDs) are well-established treatments for ocular surface disease when conventional eye drops are not efficacious [1]. Serum comprises a diverse range of epitheliotropic nutrients, including growth factors, glycoproteins, immunoglobulins, fibronectin and vitamins, promoting corneal healing. Safe and effective SEDs have been manufactured successfully by the New Zealand Blood Service (NZBS) using nationally standardized protocols for the past 20 years, with demand increasing year on year, notably a 45% increase between 2021 and 2022. Since 2007, allogeneic SEDs are provided specifically for patients who are infants or elderly, unable to donate blood due to anaemia, poor venous access, co-morbidities or unable to attend a collection venue [2]. Nonetheless, according to an international survey, only 29% of blood centres manufactured allogeneic SEDs worldwide [3].

Routinely, SEDs are manufactured in New Zealand using donor serum diluted to 25% with 0.9% sterile saline, transferred by spiking and filtering into a satellite transfer bag to create a diluted serum. However, the nature of spiking breaches the closed system and introduces the risk of bacterial contamination, necessitating production to be confined within a Class II biological safety cabinet in a Grade B clean suite environment. Fulfilling such fastidious manufacturing conditions is cumbersome, expensive, time-consuming, impedes production throughput and does not completely remove the risk of contamination. Additional filtration steps and sterility tests for contamination add further cost and operational complexity. A practical and effective method to achieve a fully closed system is to utilize sterile welding for bag-to-bag connections, but the current lack of saline packaging that could facilitate a sterile connection has been the key challenge preventing SED manufacture with a fully closed system.

In this short report, we describe our recent implementation of a fully closed manufacturing process for SEDs, facilitated by using a custom-made dockable saline packaging configuration to allow sterile connections with transfer bags.

## MATERIALS AND METHODS

### Preparation of autologous and allogeneic serum

Whole blood is collected in accordance with standard NZBS procedures from patients requiring autologous SEDs and from healthy group AB voluntary donors for allogeneic SEDs. Briefly, donations are collected into a dry bag (MRV502B Triple pack Macopharma, Mouvaux, France) and left to clot at room temperature for up to 6 h. Serum is prepared by centrifugation at 4303×*g* for 10 min, with acceleration 9 and brake 5 settings (Heraeus™ Cryofuge™ 6000*i*, Thermo Scientific, Waltham, MA, USA) and expressed into an empty dry satellite bag using a Fenwal manual plasma expresser (Baxter Healthcare, Zurich, Switzerland). The expressed serum is centrifuged and expressed again as above into the remaining dry satellite bag and sealed with at least 10 cm of tubing left to enable sterile connection to a transfer bag.

### Sourcing custom-made saline in dockable configuration for dilution

A dockable format for sterile saline conceived by the NZBS was manufactured to custom specifications using a U.S. FDA 510(k) cleared intravenous container (500 mL) made of ethylene-vinyl acetate (B. Braun Medical Inc., Allentown, PA, USA) [4], supplied with 0.9% saline by Biomed Ltd, Auckland. Biomed is a New Zealand-owned Good Manufacturing Practices-compliant pharmaceutical manufacturer specializing in intravenous and aseptic products, and as such are audited by Medsafe, the regulatory authority for therapeutic products in New Zealand.

### Preparation of diluted serum and eye drop vials

Dockable saline and prepared serum are transferred by gravity, respectively, to a sterile-connected (TSCD™-II, Terumo® Corporation, Tokyo, Japan) dry transfer bag (VSA6002Z 1000 mL or MRV502B 600 mL, Macopharma) (Figure 1, Steps 1 and 2) placed on a balance (New Classic Balance 3.2 L, Mettler Toledo, Columbus, OH, USA) to achieve the required weight for a 25% serum/75% saline solution upon docking and mixing (Figure 1, Step 3). The diluted serum is sterile connected and filled sequentially by gravity into a dispensing system comprised of 36 re-closable Poly-vinyl-chloride eye drop vials interconnected by short tubing, then heat-sealed into individual 1.5 mL eye drop applicators (TF36, Meise Medizintechhnik GmbH, Schalksmühle, Germany) (Figure 1, Step 4). All vials are labelled with product name, lot number, expiry and composition, packed into custom-made cardboard boxes (True North New Zealand Ltd, Auckland) each containing 30 applicators, stored at ≤−25°C with a shelf life of 6 months from date of manufacture.

### Sterility testing

All SED batches manufactured are tested for bacterial contamination qualitatively using an automated microbial detection system (BacT/ALERT® 3D bioMérieux, Marcy-l'Étoile, France). Samples of the residual diluted serum (5 mL) are collected from the vent bag using a SampLok® Sampling Kit (ITL BioMedical, Kingston, Canberra, Australia) and inoculated into aerobic and anaerobic culture bottles. SEDs are released to inventory if no bacterial growth is reported following 14 days of incubation.

## RESULTS

Since our implementation of the closed system for SEDs production, a total of 30,168 eye drop vials from 115 whole blood donations have been manufactured by NZBS, comprising 17,928 vials from 89 autologous blood donations and 12,240 vials from 26 whole blood donations for allogeneic use.

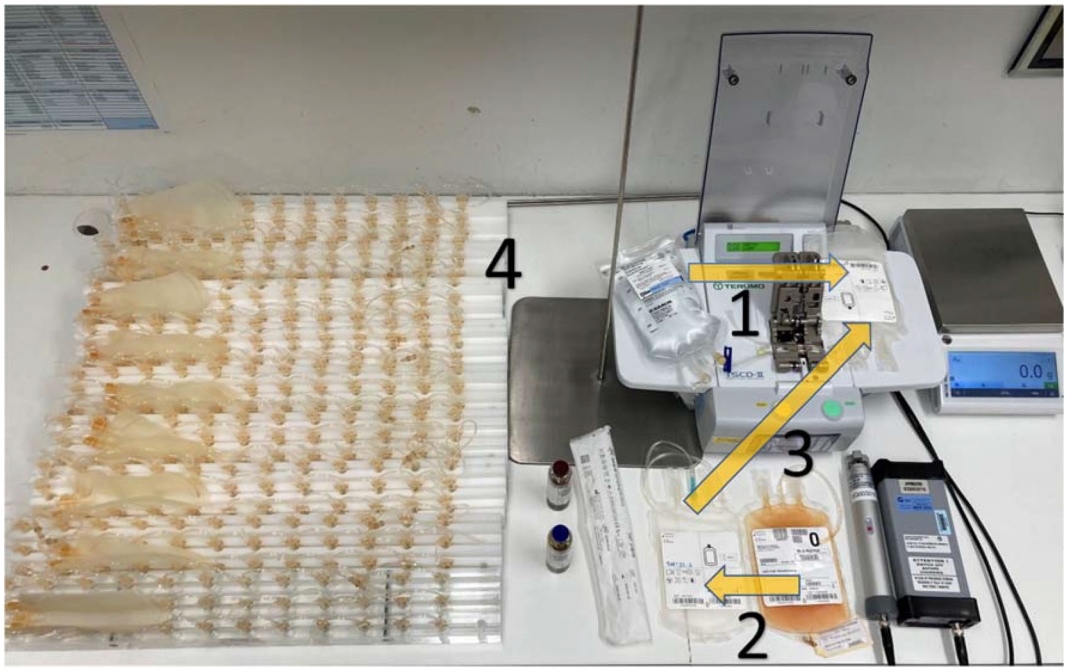


**FIGURE 1** Closed system preparation of diluted serum. Step 1: Connect saline via sterile docking to empty transfer bag, aliquot correct volume to transfer bag, heat seal and remove the primary saline bag. Step 2: Connect donor serum via sterile docking to empty transfer bag, aliquot correct volume to transfer bag, heat seal and remove primary serum bag. Step 3: Connect saline in transfer bag via sterile docking to serum in transfer bag, combine serum and saline into one bag, mix well, heat seal and remove empty transfer bag. Step 4: Connect diluted serum to Meise TF36 via sterile docking, hang the bag on a stand and allow gravity to fill vials.

**TABLE 1** Comparison of serum eye drop (SED) manufacturing characteristics between a functionally closed system and a fully closed system using dockable saline implemented at New Zealand Blood Service.

| SED manufacturing characteristics | Functionally closed | Fully closed |
|---|---|---|
| a. Production time (min) | | |
| Batch size: 180 vials | 263 | 146 [45% faster] |
| Difference in duration | 117 | |
| Batch size: 540 vials | 342 | 225 [34% faster] |
| Difference in duration | 117 | |
| b. Facilities and equipment requirements | | |
| Clean suite | Yes | No |
| Laminar flow cabinet | Yes | No |
| Particle counter | Yes | No |
| Saline spiking | Yes | No |
| Filtration | Yes | No |
| Pre-production sterility testing | Yes | No |
| Post-production sterility testing | Yes | Yes |

The average time taken to manufacture an autologous batch of 180 vials was shortened from 4 h and 23 min (263 min) to 2 h and 26 min (146 min) using the closed system, resulting in a time reduction of 117 min or 45% faster compared to the previous method using a functionally closed system (Table 1a). Similarly, for an allogeneic batch of 540 vials, the average time taken for manufacture was shortened from 5 h and 42 min (342 min) to 3 h and 45 min (225 min), which resulted in a time reduction of 117 min or 34% faster compared to the previous method (Table 1a). The significant reduction in manufacturing time was attributed to eliminating processes previously performed in a clean suite facility necessary for a functionally closed system (Table 1b).

All batches manufactured using the fully closed system were negative for bacterial contamination.

## DISCUSSION

A fully closed system for the manufacture of SEDs using diluted serum has not been described to date. SED manufacture has been limited to a functionally closed system since its inception at NZBS in 2003, which has impeded production efficiency amid growing demand. Here, we describe for the first time a fully closed manufacturing process for SEDs using a customized dockable saline configuration for serum dilution.

Currently, the dilution of serum for the preparation of SEDs is commonly incorporated into the manufacturing process among blood centres internationally [3], as the serum concentration of transforming growth factor-β (TGF-β) has been shown to be nearly five times higher compared to normal tears and is detrimental to the ocular surface due to anti-proliferative effects [5, 6]. With the exception of instances where SEDs are prepared using undiluted serum in a fully closed

system [7], the production of SEDs using diluted serum, despite requiring the use of clean rooms and laminar flow cabinets [8–10], has shown to be susceptible to bacterial contamination, including *Staphylococcus* spp., *Corynebacterium* spp., *Bacillus* spp., *Streptococcus* spp. and *Micrococcus* spp. [8–10]. Indeed, the change to a fully closed system for manufacturing SEDs represents an important reduction in the risk of bacterial contamination and therefore an important improvement in patient safety.

Additionally, a fully closed system simplifies production. Removing the requirement to spike a saline bag with an adaptor followed by filtration into a transfer bag has not only significantly reduced production time (Table 1a), it also negates the need to sample the transferred serum/saline mix for pre-production sterility testing (Table 1b), as well as avoiding the potential removal of growth factors during filtration due to the protein absorption capacity of filters [11]. The change has streamlined our workflow considerably by allowing production to move from a Class II biological safety cabinet in a Grade B clean suite into the general laboratory, facilitating additional NZBS manufacturing sites currently without a clean suite facility to commence the production of SEDs using basic laboratory equipment and procedures (Table 1b). This is an important benefit as it provides NZBS with contingency backup to ensure service continuity significantly reducing the risk of non-supply. By using fewer items of equipment in the simplified procedure (Table 1b), we are removing several pre-processing requirements to better standardize our product, making appreciable progress towards harmonization of SED manufacture that is much needed [12].

Given the rapid growth in demand for SEDs, it is imperative and prudent to substantially enhance our production efficiency and throughput to adequately address the urgent need. By using a customized dockable saline to establish a fully closed system, we have achieved important improvements in patient safety and successfully demonstrated significant reductions in production time and cost, transforming our manufacturing from a highly restrictive process into a portable workflow that is simple, practical and effective.

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All authors were involved in developing and implementing the new method; S.K. wrote the first draft of the manuscript; R.S., P.S.L.K. and A.T. reviewed and edited the manuscript. A.T. supervised product validations.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

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

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SHORT REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

# Serum anti-Spike immunoglobulin G levels in random blood donors in Italy: High-titre convalescent plasma is easier than ever to procure

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## Abstract

**Background and Objectives:** COVID-19 convalescent plasma (CCP) has retained potency and clinical efficacy against SARS-CoV-2 and is currently of utmost value for seronegative immunocompromised patients. Since most of the effect is due to the vaccine boost of infection-elicited antibodies, there is a theoretical concern that the frequency of suitable donors is declining.

**Materials and Methods:** In this single-institution serosurvey, we screened 599 consecutive donors attending our area in two different seasons (300 in November 2022 and 299 in February 2023) using the Abbott Alinity® anti-Spike immunoglobulin G assay.

**Results:** More than 80% of random donors qualify according to the FDA criteria for high-titre CCP (>4350 AU/mL), with a stable trend.

**Conclusion:** Despite reduced anti-Spike vaccine boost deployment in the general population, we have shown here that high-titre CCP units are easier than ever to procure. This finding also has implications for the derivation of standard immunoglobulins, which are finally approaching the potency of hyperimmune serum and could soon represent an alternative to CCP.

## Keywords

blood donors, convalescent plasma, COVID-19, SARS-CoV-2

## Highlights

- COVID-19 convalescent plasma (CCP) is retaining clinical utility for immunocompromised patients.
- More than 80% of random blood donors in Italy qualify as high-titre CCP donors according to their anti-Spike immunoglobulin G content.
- Lots of standard immunoglobulins deriving from current donations are likely to have high anti-Spike antibody content.

## INTRODUCTION

COVID-19 convalescent plasma (CCP) has been the main first-line treatment for the COVID-19 pandemic in spring 2020, based on the expected efficacy of anti-SARS-CoV-2 Spike-neutralizing antibodies (nAb). Most randomized controlled trials (RCTs) have failed when low-titre CCP units were delivered to late-stage hospitalized patients who were seropositive and immunocompetent [1], but it later emerged that, as per any other antiviral, efficacy is maximized when high-titre units are delivered to early outpatients [2]. Given the widespread

Daniele Focosi and Silvia Meschi contributed equally.

  

availability of oral small-molecule antivirals, interest in CCP for outpatients has almost disappeared. There is instead a growing interest in passive immunotherapies for immunocompromised patients who cannot mount a protective serological response after either vaccination or infection, for which full safety [3] and efficacy at reducing mortality have been established in RCTs [4]. These patients often have pharmacokinetic contraindications to small-molecule antivirals [5], and to date, none of the authorized anti-Spike monoclonal antibodies (mAbs) has retained in vitro efficacy [6–8]. Furthermore, mAbs come with a significant risk of treatment-emergent immune escape [9].

As such, there is an interest in both CCP (as an immediately available treatment with preserved efficacy against emerging strains [10, 11]) and standard polyclonal immunoglobulins. The latter is an industrial CCP derivative regularly administered to immunocompromised patients: while it comes with a 6-month delay between collections and marketing, the steady-state heterologous immunity granted by hybrid exposures (vaccinations + infection) provides opportunities for durable efficacy, hence equating hyperimmune serum [12].

In this study, we investigated the prevalence of high anti-Spike immunoglobulin G (IgG) titres (as defined by FDA standards [13] later adopted by the European Commission [14]) using high-throughput serology between two distinct groups of blood donors in two different time frames (end of 2022 and beginning of 2023).

## MATERIALS AND METHODS

### Donors and samples

Residual anonymized serum aliquots from 599 blood donors (300 from November 2022 and 299 from March 2023) attending one of the transfusion services afferents to the North-Western Tuscany Blood Bank (which would otherwise be disposed of after mandatory serological disease screening) were tested for anti-Spike IgG levels.

### Anti-Spike IgG testing

Briefly, 35 µL of serum from each aliquot were tested using the commercial chemiluminescence microparticle antibody assay SARS-CoV-2 IgG II Quant (Alinity I, Abbott Diagnostics, Chicago, IL, USA) according to the manufacturers' instructions. The assay is designed to detect IgG antibodies to the receptor binding domain of the S1 subunit of the Spike protein of SARS-CoV-2. Values ≥50 arbitrary units (AU)/mL were considered positive.

## RESULTS

Figure 1 reports the distribution of anti-Spike IgG levels in the 599 donors. Briefly, among the 300 donors from the November 2022 cohort, 8 (2.7%) were seronegative, 66 (22%) had levels >40,000 AU/mL and 11 (3.7%) had levels ≥80,000 AU/mL. Among the 299 donors from the March 2023 cohort 5 (1.7%) were seronegative, 48 (16.1%) had levels >40,000 AU/mL and 4 (1.3%) had levels ≥80,000 AU/mL. None of these parameters was statistically different ($p > 0.05$) between the two cohorts. Importantly, in the November 2022 cohort, 242 (80.7%) had levels >4350 AU/mL (a threshold shown to correlate with nAb titres >1:160 against wild-type SARS-CoV-2 and used by the FDA to define high-titre CCP), which remained stable at 249 (83.3%) in March 2023. Globally, 491 of 599 (82%) of random donors then would have qualified as high-titre CCP donors without any past medical history screening.

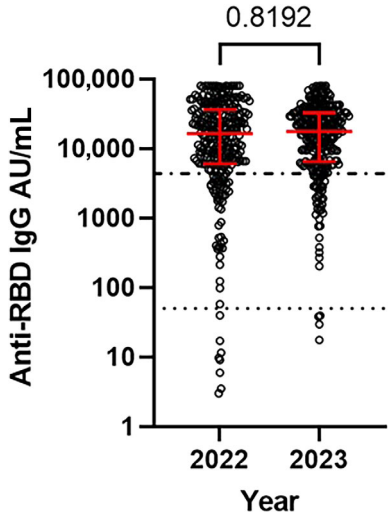


**FIGURE 1** Distribution of anti-Spike immunoglobulin G (IgG) levels in the two cohorts of blood donors.

## DISCUSSION

The recruitment of CCP donors is currently at very low volumes (mostly on-demand collections when infectious diseases wards have to manage some refractory immunocompromised COVID-19 patients). Identification of suitable donors is typically based on the collection of past medical histories, with most centres mandating documented exposures to both infection and vaccination, with at least one such event in the past 6 months. Since vaccine programmes are massively declining after most of the population has gotten the second boost (third dose), there are concerns that recruitment of qualified CCP donors could become more and more difficult over time.

We have shown here that the prevalence of high-titre CCP donors is still exceedingly high and steady. The main contributor to this evidence is likely the high-level circulation of SARS-CoV-2 after the removal of non-pharmaceutical interventions such as face masks or social distancing mandates: this translates into asymptomatic or pauci-symptomatic diseases that remain largely undiagnosed. This finding that more than 80% of plasma collections qualify as high-titre CCP has major consequences for CCP procurement and intravenous immunoglobulin (IVIG) qualification, respectively.

First, the criterion of identifying suitable CCP donors based on past medical history comes with the risk of excluding many suitable donors who had an asymptomatic and hence undocumented SARS-CoV-2 infection in the last 6 months. We then suggest relaxation of recruitment rules and avoidance of selection at all. An easier workflow would be to test random fresh plasma units that have not been initially intentionally collected as CCP, and relabel them as CCP whenever high-throughput serology satisfies the threshold mandated by local regulatory authorities. At the current stage, this approach seems cost-effective. An addendum to this consequence is that most hospitalized patients receiving random fresh plasma units for coagulation purposes are actually also receiving short-lasting COVID-19 prophylaxis, which is a welcome extra benefit.

Second, IVIG batches released from plasma manufacturers (made from pools of plasma units collected 6 months earlier) are currently approaching the anti-Spike IgG content of hyperimmune serum [12]: the latter product is much more complex to achieve, requiring dedicated manufacturing chains that no plasma manufacturer is currently willing to implement. Then the widespread availability of high-titre IVIG could represent a cheap, safe and effective pre-exposure prophylaxis (PEP) for immunocompromised patients, given the recent deauthorization of the Evusheld® mAb cocktail because of circulating resistant variants of concern [15, 16]. In summary, IVIG could soon represent a broad-spectrum PEP solution, embedding SARS-CoV-2, and saving money and time for both patients and the healthcare system. Despite RCTs of IVIG failing to show clinical benefit in hospitalized patients in the pre-vaccine era of the pandemic [17, 18], the heterologous immunity induced by hybrid exposure [10, 11] (vaccine + infection) has the potential to provide clinical benefit.

That said, it is important to note that the FDA threshold used here was based on correlation studies with viral neutralization assays employing the wild-type (Wuhan) SARS-CoV-2 lineage. Given reports of reduced neutralization by CCP against recent XBB.* sublineages compared with wild-type SARS-CoV-2 [10, 11], it is urgent for regulatory authorities to reassess high-throughput serology cut-offs correlating with high nAb titres specifically against XBB.*, or alternatively to rely on high-throughput serology employing XBB.* Spike antigens. With the recent WHO recommendation to remove wild-type SARS-CoV-2 and introduce XBB.* Spike into next COVID-19 vaccines, commercial development and marketing of XBB.* Spike-based high-throughput serology have become more likely to come.

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D.F. conceived the study and wrote the first draft; M.C.I. and M.L. provided donor samples; S.C. and S.M. performed laboratory tests; M.F. and F.M. revised the manuscript.

## CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflict of interest related to this manuscript.

## DATA AVAILABILITY STATEMENT

An anonymized dataset is available from the corresponding author upon reasonable request.

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REPORT

Vox Sanguinis ISBT International Society of Blood Transfusion

# Plasma collection and supply in Europe: Proceedings of an International Plasma and Fractionation Association and European Blood Alliance symposium

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## Abstract

At the symposium organized by the International Plasma and Fractionation Association and European Blood Alliance, experts presented their views and experiences showing that the public sector and its blood establishments may strengthen the collection and increase the supply of plasma using the right strategies in plasma donor recruitment, retention and protection, scaling-up collection by increasing the number of donors within improved/new infrastructure, supportive funding, policies and legislation as well as harmonization of clinical guidelines and the collaboration of all stakeholders. Such approaches should contribute to increased plasma collection in Europe to meet patients' needs for plasma-derived medicinal products, notably immunoglobulins and avoid shortages. Overall, presentations and discussions confirmed that European non-profit transfusion institutions are committed to increasing the collection of plasma for fractionation from unpaid donors through dedicated programmes as well as novel strategies and research.

## Keywords

fractionation, patients' needs, plasma, plasma-derived medicinal product, supply

## Highlights

- Plasma donation continues to be the only source from which to prepare plasma-derived medicines (immunoglobulins, albumin and coagulation factor concentrates).
- The supply of plasma-derived medicines in Europe depends on plasma collected both within Europe (approximately 63%) and outside Europe (approximately 37%).

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- Implementing best practice regarding voluntary unpaid donor recruitment and plasma collection, scaling-up collection capacity and providing a supportive legislative and financial framework are key parameters for achieving European strategic independence in plasma supply.

## INTRODUCTION

The International Plasma and Fractionation Association (IPFA) and the European Blood Alliance (EBA) organized a symposium 'Plasma Collection and Supply' on 15 and 16 March 2022 in Amsterdam, the Netherlands to discuss how the public sector and its blood establishments (BEs) can strengthen the collection and increase the supply of plasma to meet European patients' needs for plasma-derived medicinal products (PDMPs).

After the opening address by **Leni von Bonsdorff (IPFA), Pierre Tiberghien (EBA)** welcomed the participants and emphasized that reaching an adequate level of self-sufficiency in plasma for fractionation (PfF) to ensure European strategic independence is a key factor in sustaining the long-term supply of PDMPs to meet the needs of patients in Europe. The current dependence on PfF collected outside Europe is not sustainable and is associated with potential disruptions related to geopolitical or infectious threats and competition for the resource. During the waves of the COVID-19 pandemic, the high prevalence of the disease in the population, physical distancing measures and other restrictions on mobility led to a decline in PfF donations due to reduced donor availability and absenteeism of plasma collection staff. The increasing demand for convalescent plasma for COVID-19 for investigational therapies has posed an additional challenge to the plasma supply, particularly in the United States [1]. The PfF supply can be augmented by very frequent plasma donations per donor or by increasing the number of donors who donate plasma less frequently. High-frequency plasma donation, necessarily remunerated, relies on a narrow donor base and mostly on donor financial uncertainty and vulnerability. Such a donation may pose a risk to donor health, a risk of acute disruption in plasma collection in the presence of unforeseen adverse effects and a risk to the availability of blood products by erosion and/or fragmentation of a community-based donor population and competition for resources [2]. Lastly, plasma collected from high-frequency donors will be of lesser quality as it contains reduced protein concentrations including immunoglobulins (IGs). Low-frequency plasma donations, feasible without financial incentives/remuneration, rely on a large donor base with reduced individual donor burden and are more protective regarding health and ethics. It is based on a single integrated donor base supported by not-for-profit BEs across Europe, the same safety setting, management and donor haemovigilance.

## PLASMA COLLECTION AND FRACTIONATION IN EUROPE: PAST, PRESENT AND FUTURE

**Paul Strengers** asserted that most of the plasma collected in Europe until 1993 was obtained from whole blood donated by local voluntary non-remunerated blood donors and fractionated largely by public organizations. PDMPs produced from European plasma covered largely the patients' needs. Prior to 1989, PDMPs were exempted from medicinal regulations and were licensed and overseen only by national authorities. In 1989, PDMPs have become medicinal products and hence followed the related regulations and authorization systems. In 1993, 20 not-for-profit and 11 commercial fractionation plants were active in Europe. By 1993, cases of viral transmission via plasma and PDMPs increased necessitating improvements in their quality and safety. An open market for PDMPs was established, while plasma collection remained the responsibility of national authorities. The introduction of regulatory and quality requirements increased the cost of plasma fractionation and raised liability risks, leading to the closure of many public fractionation plants. In the period 1990–2000, only three public and six commercial fractionators could survive in Europe. The European Commission (EC) adopted directives governing blood and blood components including source plasma. In an open European Union (EU) market, PDMP producers faced financial pressure because some products were considered generic and could be marketed when market protection had expired, prices were reduced by competition, there was insufficient financial compensation due to limited product pipelines and there were high costs and risks of developing new products. Moreover, the use of PDMPs has been hampered due to increasing pressure on healthcare costs. Small companies could not compete with larger ones active in international markets. As a result, between 2000 and 2022, only one public and six private fractionation companies remained active in Europe.

Currently, PDMPs are unevenly distributed globally, and their prices are rising. Two products—IGs and albumin—determine the demand for plasma. In addition, there are also concerns about the lack of regulatory harmonization of plasma supply and PDMP manufacturing worldwide and finding ways to cover the 15%–20% plasma shortage due to the COVID-19 pandemic and to improve plasma wastage and the limited access to PDMPs for all patients.

The worldwide growth of the PDMP-dependent patient population and the development of new therapeutic options will dictate the evolution of PDMP treatment in the future. A new generation of known recombinant products with an extended shelf life and less immunogenicity, as well as new recombinant substitutes and products, are expected to be developed. Global markets for polyvalent IGs are expected to continue to grow, highlighting the need for harmonized and updated guidelines for their clinical use. Many guidelines [3–6] recommend the use of IGs based on the evidence of clinical effects in diseases with often unknown pathology where the mechanism of action of IGs is mostly unclear or hypothetical when it comes to immunomodulation. However, the relatively small

number of manufacturers might imply a future risk of PDMPs supply such as unforeseen production collapse, reduced availability of products, monopolistic behaviour, higher prices, less innovation in production technologies and the risk of non-introduction/withdrawals of PDMPs in selected markets. There is also a risk of inadequate management of plasma supply resulting from the increasing requirements and growing discrepancies between the supplies of recovered and source plasma. The impact of the COVID-19 pandemic has highlighted the need for a balanced plasma supply, which will increase significantly.

## MARKET LANDSCAPE FOR PLASMA AND IMMUNOGLOBULINS

As presented by **Matthew Hotchko**, the global plasma protein market has shown steady growth of 7.4% per year from $4.8 billion in 1996 to $26.6 billion in 2020. Sales of IGs achieved the fastest growth in the last 5 years in both volume and price, Albumin sales also grew, but at a slower pace, reflecting steady volume growth driven by China, although prices declined. Demand for plasma-derived Factor VIII and IX has decreased due to the presence of recombinant and non-factor therapies and growing competition showing a large decline in high-income countries but an increase in emerging markets. Sales also decreased for PDMPs with competition from recombinant or non-plasma-derived products but increased for those without competition or those prescribed for acute blood loss.

Since the 1990s, IGs have been the main driver of the global plasma market, so manufacturers balance plasma needs according to forecasts of IGs sales. The only way to increase (or decrease) their supply is to collect and fractionate more (or less) plasma. Albumin demand grew more slowly while the demand for IGs grew the fastest of all PDMPs. To meet the expected demand for IGs in the last decade, companies have been collecting plasma with an average growth of 8%–9% per year which was severely affected by the COVID-19 pandemic. Global plasma volume fell from 69 million litres in 2019 to 59 million litres in 2020, which is a 14.7% decrease.

However, the origin of plasma did not change globally. In 2020, most of the plasma (67%) was from North America (99% from the United States), 18% from Asia-Pacific (75% from China), 14% from Europe and the remaining 1% from Latin America, the Middle East and Africa. North America and Asia-Pacific produced enough plasma for their internal use [7]. In Europe, the need for additional amounts of IGs produced from PfP collected outside Europe, mainly in the United States, arises from an imbalance in which Europe consumes 25% of the world's IG supply but collects only 14% of the world's PfF. In 2020, a total of 8.3 million litres of plasma were collected in Europe which was 9.4% less compared to 2019, while IG use and demand continued to rise. Consequently, the self-sufficiency of Europe dropped by 8% from a deficit of 32% in 2019 to 40% in 2020. The required increase in plasma collections to meet the IG needs in Europe was 4.3 million litres in 2019 and grew to 5.6 million litres in 2020.

## DONOR RECRUITMENT, RETENTION AND PROTECTION

**Karin Magnussen** reported that donations of whole blood or plasma have some common but also different positive and negative effects elicited during the donation. From the perspective of both types of donors, the overall atmosphere with welcoming, kind, respectful and attentive professional staff acknowledging donors and their donations is perceived as positive.

Some plasma donors perceive plasma donation as being less physically demanding compared to whole blood donation and are associated with a lower likelihood of deferral due to certain aetiologies (e.g., travel history to malaria-endemic country). Donors understand that there is a need for plasma to help patients and sometimes the increased donation frequency helps to make donations a routine. Increased frequency also eases social interaction with staff and fellow donors. Donors appreciate easy accessibility to the donation facility with the possibility to park a car and access to drinks and snacks. The donors, however, do experience some negative effects, including time constraints due to the longer duration of plasmapheresis compared to whole blood donation. They also can experience adverse events such as citrate toxicity, needle injury and fainting. It is, therefore, crucial to take into account the donor's blood volume when assessing their suitability for plasma donation in order to prevent circulatory adverse reactions. As donors are expected to donate more often, they can sometimes feel exploited. Unavailable car parking is also indicated as a negative point. Donors enjoy being helpful and personalized handling of donors is recommended to improve the plasma donation experience.

**Christian Erikstrup** showed previous studies indicating that frequent plasma donation may be associated with decreased levels of proteins, IGs and changes in other biochemical parameters in the blood [8, 9]. There is, however, a lack of data on the clinical effects of plasma donation on the donor's health. One study concluded that long-term intensive plasmapheresis was safe when donors were monitored according to the study protocol, although 16% of donors were excluded when laboratory parameters fell below cut-off values [9]. It is unknown what constitutes an acceptable decrease in blood IG levels and whether a simple deferral of donors with low IG/protein levels is a sufficient measure. When analysing donor health in studies, one of the challenges is to mitigate the 'healthy donor effect' that can introduce selection bias due to the health criteria that donors must meet for plasma collection. Another study showed that a personalized approach to the collection of increased plasma volume does not increase the incidence of hypotensive adverse events [10].

Is it adequate to solely consider acute or subacute events, such as hypotensive reactions, in order to evaluate the safety of donor health [11]? Plasma donation may lead to long-term effects (e.g., increased risk of infection or cardiovascular disease) not captured by the standard monitoring of acute and subacute adverse events [12]. Adequate research is needed to determine the long-term effects of donating plasma on the health of the donor. To obtain relevant donor data for such analyses, the databases of blood and plasma

centres and digital health data in public health registries must be linked. The lack of comprehensive surveillance of donor health performed at the European level indicates a need to improve European plasma donor vigilance.

**Marloes Spekman** asserted that in 2020, Sanquin, the Netherlands opened a proof-of-concept plasma-only donation centre in Utrecht (the Powerbank), where they started recruiting donors directly for plasma donations. Unlike most traditional Dutch donation centres, the Powerbank is in a highly visible location (shopping mall) and is designed to create an efficient and hospitable experience for donors. A survey showed that both Powerbank's plasma donors and plasma donors from a nearby traditional donation centre were similar in terms of gender, education and household composition. However, Powerbank donors were slightly younger on average and had a higher proportion of working donors and fewer students and retirees. Results from qualitative focus group discussions showed that donors liked the look and atmosphere of the Powerbank, as well as its accessibility, service level, food, efficiency and loyalty programme. Preliminary conclusions are that the Powerbank attracts a population of donors similar to those in traditional blood centres, though with a higher proportion of the younger and actively working population. New generations of plasma donors could benefit from highly visible locations, efficiency (i.e., more donor-focused processes), high service levels and a non-clinical atmosphere at the donation centre.

## INFRASTRUCTURE AND SCALING-UP PLASMA COLLECTION

**Stuart Chesneau** indicated that **the Australian Red Cross Lifeblood** has set a fast-track donor panel target to achieve plasma collection targets of approximately 1300 tonnes by 2025 and to provide a cushion against the ongoing impacts of COVID-19. The panel growth target is from 502,000 to 600,000 donors in 2022–2023. Some existing blood collection sites have been refurbished or relocated, and a small number of fixed sites are new. Optimization goals at some locations are to increase both whole blood and plasma collections by extending business hours and increasing staffing and appointment availability. Regional sites will increase predominantly plasma collections and efficiency within existing staffing at fixed sites. Mobile and pop-up sites will help to increase whole blood donation. In terms, 50% of fixed sites in metropolitan and suburban areas will provide 70% of the collection with a donor panel that can support plasma growth. Donor awareness and marketing are improved with a strategy of 'brand behaviour' to reframe blood donation as a community activity. The plasma donation 'game-changer' campaign has been launched highlighting that donated plasma can help more people more often than donated whole blood. In Australia, a newly implemented programme aims to provide a personalized customer experience by focusing on 'the moments that matter'. This approach aims to make a significant impact on a donor's decision to donate again while prioritizing donor retention and reducing the number of lapsed donors. Tools based on machine learning and artificial intelligence are also evaluated to deliver communications via the donor's preferred channel and to propose a tailored approach to the donor journey/donation lifecycle stage. Currently, self-service makes up 70%+ of total appointments predominantly done via the mobile phone app.

**Judy Jones** showed that in 2019, the **Canadian Blood Services** (CBS) developed a strategic plan to 'Ensure a secure supply of Canadian plasma for immune globulin' [13]. At that time, the rate of usage for IG products had increased by 111% over the past 10 years, with the IG consumption rate of approximately 219 g per 1000 population. The COVID-19 pandemic has further elevated the risk of plasma supply sufficiency by exposing domestic vulnerabilities. In 2022, the Canadian plasma sufficiency rate for the demand of IG was 15%. The changing dynamics in Canada with the growth of commercial parties in a competitive market have added pressure to the system. Supply constraints in the early stages of the pandemic were mitigated through the pre-emptive purchase of additional inventory. With the elevated risk-increasing pressure on the Canadian security of IG supply, a refreshed risk-based decision-making exercise was conducted. Risk modelling suggests a target of at least a 50% level of self-sufficiency that balances the supply risk and ensures sufficient IG to meet all needs of critical patient groups. In that perspective, CBS started over 3 years ago a plasma division for stand-alone plasma locations. The objective is to establish 11 specialized locations for collecting source plasma by March 2024, with each site collecting a total of 20,000 L of plasma. Additionally, CBS plans to extend the collection capacity within the current mixed centres. To support these goals CBS developed a donor recruitment strategy based on recruiting whole blood donors to donate source plasma, recruiting directly to plasma donation, retaining source plasma donors and increasing the donation frequency.

According to **Bjarne K. Møller**, the challenges to attaining or enhancing self-sufficiency in the supply of PDMPs in **Denmark** stem from various factors. These include navigating through turbulent periods of heightened clinical demand and market economy, volunteer-based donor recruitment, ensuring a balanced geographical distribution of apheresis sites, managing the complexities of political decision-making in the development of national tenders for contract fractionation, as well as navigating political, legal and financial constraints. Therefore, it is essential to enhance the national structure and professional organization of blood centres, while also implementing 'professional recruitment practices'. Currently, volunteers are recruiting donors at blood centres that are not evenly geographically distributed over the country but are placed in five regions. There are only two plasma-only centres in Denmark. The national contracts for fractionation place political, economic and legal constraints on plasma collections. Right now, most of the Danish donors are between the ages of 30 and 40 and men, indicating the possibility and need to include donors from other age and sex groups in the plasma donor pool. In addition, focusing on the quality of plasma donation drives helps retain donors, whereby communication about why donations help patients is the key to success, the frequency of donations decreases. In the future, Denmark is planning to build more plasma-only centres and disperse them geographically.

**Peter Verheggen** indicated that **the Netherlands** has set up a target to increase plasma collections to 450,000 kg in 2025. To achieve this goal, it is necessary to reduce plasma production costs and bring them closer to selling prices. The collection has to increase to 20,000 donations in their plasma-only centre and to 15,000 donations in the hybrid centres per year. The demand for whole blood of about 400,000 units per year is expected to remain stable. The goal of the Powerbank (plasma-only centre) set up in 2020 was to reach a donation frequency of 5 annual donations per donor, by 4000 unpaid donors to achieve a total of 20,000 source plasma donations. Several process modifications have been implemented, such as conducting post-donation haemoglobin measurements and eliminating the determination of blood groups. Additionally, process automation has been introduced, including online appointment scheduling and questionnaire submission. Costs reduction is highly dependent on the number of donors and the donation frequency, as was also observed in the pilot Powerbank. It looks that recruiting new plasma-only donors and thereby increasing the number of donations is essential to lower the costs. During the pandemic, it was not possible to recruit the desired number of donors, only online recruitment was possible. The regular recruitment programme started again in 2022 as the online activity oriented to the recruitment of the non-Western local population. The loyalty programme enhances the plasma donation frequency (7.0 donations/year) when compared with donors who do not participate in this loyalty programme (2.6 donations/year).

**Gerard Gogarty** outlined how the National Health Service Blood and Transplant (NHSBT) in **England** built a completely new plasma operating system from scratch when it began collecting COVID-19 convalescent plasma (CCP) in 2020 to support trials of its use as a therapeutic option to treat the disease. To collect and process the CCP an infrastructure with more than 40 collection sites was built and the supply chain was updated. Some of these CCP collection sites started collecting PfF by NHSBT in 2021 when the United Kingdom lifted its ban on the collection of United Kingdom-source plasma for the preparation of PDMPs, particularly IGs. NHSBT is committed to delivering a world-class plasma service to support patients in England with an initial target of 20% IG self-sufficiency, building a foundation to meet the future ambition of 30% self-sufficiency and beyond. Today, the United Kingdom consumes a significant quantity of IGs, all of which are derived from plasma collected in Europe and the United States. As the United Kingdom builds self-sufficiency in IG medicine, it can reduce the amount it needs to purchase on the international market. The NHSBT, along with its European partners, holds the perspective that the United Kingdom's reintroduction of plasma for medicinal purposes can yield mutual benefits. It can enhance the United Kingdom's self-sufficiency in IG supply while simultaneously decreasing the reliance of European nations on plasma imports. NHSBT is aiming to collect around 1 million litres of plasma between 2022 and 2026, which will ease the pressure on the European plasma supply.

**Polonca Mali** said that **Slovenia** entered a contract for the fractionation of plasma with a manufacturer that performed viral inactivation of PDMPs in 1991. Since then, the national toll fractionation of mainly recovered plasma (95%) is ongoing. During 2004–2021, the number of plasmaphereses increased, but self-sufficiency remains challenging. IGs are the main driver of plasma supply, though evidence-based medical indications for Ig therapy are missing and the late COVID-19 effects on plasma supply can be expected. A capacity-building 2 years programme for BEs has recently been launched, which foresees among others: adjusting opening hours and ensuring effective collection and equipment utilization. This programme has been enabled through the Emergency Support Instrument (ESI) funds made available by the EU to support countries facing the COVID-19 crisis and to collect CCP [14].

According to **Mazen Elzaabi**, Laboratoire français du Fractionnement et des Biotechnologies (LFB) in **France** fractionates recovered and source plasma collected by the civilian and military transfusion public services (Etablissement Français du Sang—EFS and Centre de Transfusion Sanguine des Armées—CTSA, respectively). LFB distributes PDMPs produced from this plasma primarily on French territory. Although 75% of patients are treated with LFB PDMPs, the market share of LFB for IGs in France is 35%. During the COVID-19 pandemic, France also experienced a drop in plasma collection, resulting in a decrease in IGs production and supply to French hospitals (11% decrease in December 2021), followed by increased competition between EU Member States (MS) for IG supply. Due to the impact of production timelines linked to industrial constraints and multi-years hospital contracts, this situation is likely to persist. By opening a new fractionation plant LFB will triple its production capacities. However, there is an ongoing challenge to increasing PfF collections in France. To cope with supply tensions, the EFS and the French Ministry of Health are preparing a 'plasma plan'. This plan is likely to mark a major change in the institution's mission. Linked to this supply tension situation, the National Agency for the Safety of Medicines and Health Products has published a list prioritizing the immunoglobulin indications and has provided 'temporary import authorization' for unregistered IGs.

## POLICIES AND LEGISLATION FAVOURING PLASMA COLLECTION

**Vincenzo de Angelis** highlighted that the EU blood legislation, transposed into national blood laws, regulates the collection of plasma in BEs and plasma collection centres, while EU pharmaceutical legislation is covering the production of PDMPs. The standards within the European Directorate for the Quality of Medicines & HealthCare (EDQM) Guide for the preparation, use and quality assurance of blood components define the collection and quality of plasma, while its properties as source material are defined in the EU pharmacopoeia. Finally, Good Practice Guidelines should be followed during plasma collection and Good Manufacturing Practice during fractionation. The definition of 'voluntary non-remunerated donation' and the compensation for plasma donors remains to be delineated in the EU legislation. Twenty-five EU countries provide some form of compensation for donors covering expenses incurred and recognizing the

inconvenience related to donating. In four EU countries (Austria, Czech Republic, Germany and Hungary), private plasma centres apply for compensation as a fixed rate allowance. Key characteristics of national policies and laws in France, Spain, Germany, Portugal, the Netherlands, Belgium, Slovenia, Hungary, Poland and Italy, show a diversity also in the regulation of plasma collection and fractionation models, remuneration and self-sufficiency. In addition, a lack of infrastructure and cooperation between EU bodies and national authorities are obstacles to achieving strategic independence for plasma collection.

There is a need for supporting EU MS to set up plasmapheresis programmes and to better inform citizens of the critical importance of PDMPs and therefore the need for plasma donations. **Fabio Candura** presented the environment of decentralized, regional-based healthcare governance divided into 16 regions and five autonomous provinces in Italy. Each of them is responsible for the organization and delivery of health services at their local level. The national blood law has made 'national self-sufficiency' a key priority and has been constructed to facilitate the implementation of agreements between the regions and fractionation companies, by including agreement models and procedures. Since 2015, Italy has revisited the number of interregional agreements, moving from two to four agreements with a wider range of fractionators. This made it possible to achieve adequate volumes of PfF collected and further increase PDMP availability while remaining cost-effective. While Italy's plasma system is regarded as one of the most self-sufficient among the EU countries, the regional tendering process is very time-consuming but has resulted in a very significant decrease in costs for fractionating domestic plasma allowing savings that are reinvested in plasma collection.

**Françoise Rossi** pointed out that a significant amount of plasma collected worldwide, including in both the EU and the United States, is being discarded due to non-compliance with quality standards to enter the fractionation chain [15]. The shortage of PDMPs mostly impacts low- and middle-income countries (LMIC) due to insufficient domestic supply of PfF. Given PDMPs are considered essential medicines, PfF is valued and considered a strategic resource. Small EU MS facing a shortage of PDMPs may decide to either sell plasma to a fractionator or establish a contract for fractionating national plasma into PDMPs in return. Irrespective of the fractionation strategy, BEs should meet several conditions: minimum volume and quality requirements set by the authorities and fractionators and following the EDQM Good Practice Guidelines as the reference in Europe.

The WHO has developed the guidance intended to provide an overview and recommendations for actions to be taken by all stakeholders of the blood systems as a roadmap towards reducing the wastage of plasma and thus increasing access to PDMPs in LMIC [16]. The guidance further highlights the benefits for both the plasma collectors and fractionators of establishing national/regional plasma fractionation programmes. However, this requires, among other things, the engagement of governments, strengthening national/local capacity building and knowledge and close cooperation between plasma collectors and fractionators. Ways to avoid plasma wastage are to be explored, for instance, allowing small EU countries bilateral and regional cooperation in pooling plasma.

**Bernardo Rodrigues** said that the Services of General Economic Interest (SGEI) are defined as economic activities that public authorities identify as being of particular importance to citizens and that would not be supplied (or would be supplied under different conditions) if there were no public intervention. When the government of an EU MS designates plasma collection as an SGEI and provided it complies with EU competition law, public authorities can provide support to improve plasma collection activities, including dedicated public funding (under specific conditions) or other forms of support such as attributing exclusivity to specific operators. However, these options need to be individually evaluated as they may not be suitable for every EU MS. This applies to all EU MS although other European countries outside the EU may have similar regimes.

## MANUFACTURER'S APPROACH

**Ramune Sepetiene** presented an '**Abbott** Transfusion Health Institute' designed to support BEs and plasma centres in their practical and scientific activities. **Thomas Lenzen** emphasized that **Haemonetics** offers the integration of plasmapheresis devices with donation centre software to overcome risks of errors, non-compliance and safety associated with the manually completed documentation systems in blood and plasma centres. **Stephan Walsemann** presented the **Scinomed** offers of high-quality products and services to plasma collection facilities including plasmapheresis machines and disposables, supporting the validation of machines at customers' facilities and enhancing GMP compliance by connecting apheresis machines to BEs computer systems.

## SUPPLY AND USE OF IMMUNOGLOBULINS IN TIMES OF CRISES

The European Medicines Regulatory Network aims to minimize the impact of medicine shortages by working with pharmaceutical companies to resolve manufacturing and distribution issues, sharing information with international partners about alternative sources of supply, seeking input from patients and healthcare professionals on the impact of medicine shortages and supporting decision-making on taking measures to allow alternative medicines or suppliers to be used [17]. EMA publishes information on shortage when it affects more than one EU MS. The Heads of Medicines Agencies and EMA task force on the availability of authorized medicines are designed to function as a 'supply and availability hub' that is tracking progress on the medicine availability and shortage-related activities.

**Jean-Philippe Plançon** stressed that the shortages of PDMPs, particularly IGs, directly impact patients' quality of life through suboptimal medical care or, sometimes, instant treatment with second-line drugs (e.g., immunosuppressants or corticosteroids). For some patients, absence or delay in access to treatment can mean a loss of

autonomy that might be irreversible. The prioritization of indications for IG treatment places patients in competition with each other, as physicians choose to treat some patients and not others simply because treatment is unavailable.

There is no plasma programme in Romania, and access to PDMPs is also very limited. Patients' organizations in Spain have information on a shortage of IG starting from September 2020, causing delays in treatment, reduced dosages and transfer from IG therapy to plasma exchange (with difficulties in albumin access). The Immunoglobulin Observatory (OBSIG) survey conducted among French patients with autoimmune peripheral neuropathies treated with IGs in early 2022 by the French Association Against Peripheral Neuropathies showed that 30% of patients have problems satisfying their needs [18]. European Patient Organisation for Dysimmune and Inflammatory Neuropathies (EPODIN) believes that the rights and ethical principles of both the patients and the donors are equally important and that the protection of plasma donors guarantees the protection of patients using PDMPs.

**Cynthia So-Osman** presented the BEST# 160 study that started on 1 March 2022 and aims to create clinical awareness of Ig shortage and to gain insight into decision-making on how IGs are used within hospitals. This qualitative study involves the development of surveys targeted to hospital professionals, including conducting semi-structured in-depth interviews on the use of Ig in hospitals (demand side). The study seeks answers to the questions of which clinical specialties most often use IGs and in what way (appropriateness and cost aspects); whether changes in their use are observed; what are the possible causes for reduced IG use; and which strategies are used to reduce the use of Ig in inpatients and outpatients. Aiming for a representative sample from different settings and regions, the survey will interview hospital pharmacists/blood bank providers (Phase I), end users (clinicians [Phase II]) and in the semi-structured interviews (Phase III) selected key persons.

**Isabelle Durand-Zaleski** emphasized that currently, IGs are the most expensive pharmaceutical products utilized in hospitals, exhibiting an annual increase in usage of 10% in recent years [19]. Spain reported a pharmaceutical expenditure of €92 M in 2012 for IG-authorized indications only [20]. The retrospective survey from 2019 showed that the daily practice of French neurologists with IG treatment for multifocal motor neuropathy and chronic polyneuropathies followed the guidelines from 2010 [21]. In 2012, IG replacement therapy used for secondary immunodeficiency (SID) accounted for 18% of polyvalent IGs use in the Ile de France region with a total expenditure of €9.7 M out of a total of €54 M for all indications. Although the study population did not fully represent patients with all the SID indications, 75% of patients received IG treatment outside the EMA guidelines and the estimated cost of the misuse was €8.2 M for the entire region [22]. Data from Spanish hospitals show a huge variability of IG use for the same indications among the different regions [23]. In 2007, IGs administered in Belgian hospitals constituted 17% of hospital drug expenditure where 50%–60% of the IG use was associated with approved indications and 40%–50% was off-label, occurring in unspecified conditions (surgery, orthopaedics and oncology). The study demonstrated a rapid change in indication for investigational IG use, which may account for the high percentage of 'off-label' use and poor documentation on the decision process for compassionate use and obtaining informed consent [24]. Computerized decision support for IG prescriptions showed 74 unique indications included in the final list of appropriate use and the appropriate dosages for each indication were programmed into the final order set, allowing a reduction in dose deviation [25]. The use of IGs represents a typical condition where rapid access to a full Health Technology Assessment is applicable because all the elements of a crisis are present: limited supply, increasing demand, high variability in adherence and high costs [26].

## MECHANISMS TO SUPPORT EFFORTS TO INCREASE PLASMA COLLECTION

At the time of the symposium, the revision of the EU legislation on blood, tissues and cells was at the final stage. It was expected that the new EU legislation would strongly focus also on the better management of supply issues including donor protection as well as protecting EU patients from the risk of shortages or sudden supply disruption.

**Giuseppina Facco** presented that the European Support Instrument (ESI) Grant Project for 'Increasing Capacity for COVID-19 Convalescent Plasma Collection' financed 20 projects in 13 Member States and the United Kingdom, starting from 1 September 2020 and ending between 30 June and 15 October 2021. A total of 67 BEs received funding under this scheme. The overall objective of the granted projects was to improve the ability of public and non-governmental blood services in the EU to collect plasma by plasmapheresis from donors recovered from COVID-19. PDMP This had a secondary long-term benefit of creating conditions for the growth of PfF collection by increasing the pool of plasma donors, opening new collection sites and improving the quality of plasma storage that meets PDMP production standards.

**Daphne Thijssen-Timmer** stipulated that the COVID-19 pandemic demonstrated the need for coordinated EU-level action to respond to health emergencies and it revealed gaps in foresight, including demand/supply dimensions, preparedness and response tools. The main goal of such a response is to achieve European strategic independence in plasma supply by establishing an equitable, low-risk balance between source plasma collected in the EU by public suppliers and commercial non-EU suppliers to meet domestic demand for IGs and avoid shortages. EBA is working to raise awareness of the need for plasma and is organizing webinars for BEs on how to increase their plasma collection. Together with IPFA, which supports the public sector for plasma strategies internationally, EBA organizes symposia on plasma collection and supply in Europe. Finally, the EBA conducts regular research and exchange of best practices among transfusion institutions. A 2020 EBA internal survey showed that 66% (3%–100%) of the total fractionation plasma requirements for self-sufficiency in the EBA members is collected by BEs or other non-profit facilities. At the time of the symposium, the EBA submitted for EU funding for a project named SUPPLY to analyse the whole plasma

supply chain, aiming to increase non-paid plasma collection and strengthen the resilience of plasma collection by BEs throughout Europe to ensure optimal availability of PDMP's both in the current situation as well as in times of crises. To reach EU strategic independence, especially during times of crisis, all stakeholders including EC, BEs and National Competent Authorities for blood and blood components, need to work together.

We may conclude from the presentations and discussions at the symposium that European non-profit transfusion institutions are committed to increasing the collection of PfF from unpaid donors through dedicated programmes as well as novel strategies and research.

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## CONFLICT OF INTEREST STATEMENT

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LETTER TO THE EDITOR

Vox Sanguinis ISBT International Society of Blood Transfusion

# Can reactive donor screening results be presumed false positive following unstandardized testing procedures?

In their original article recently published in *Vox Sanguinis*, Lucey et al. [1] presented an evaluation of signal-to-cut-off ratios (S/CO) of an approved antibodies against hepatitis C virus (anti-HCV) screening assay (Architect Anti-HCV antibody assay; Abbott GmbH, Germany), along with an alternative antibody test (Standard Q HCV Ab test; SD Biosensor, Korea) and an HCV core antigen (HCV cAg) test (Architect HCV Ag; Abbott GmbH, Germany) in order to propose a blood donor screening algorithm containing supplemental testing using screened reactive HCV samples ($n = 470$). The authors present data, notably claiming an 84% false discovery rate. Combining these results with those of a rapid, qualitative HCV Ab assay and quantitative HCV cAg test, they report acute hepatitis C case positivity of 8.1%. We insist on highlighting certain critical aspects of this study which should encourage re-evaluation of the authors' conclusions and possible modifications for the benefit of readers.

There are considerable donor and recipient health safety implications of incorrect deployment of assays with suboptimal performance characteristics. The Architect Anti-HCV antibody assay reactive results in this study could not be categorized as false positive because they were not further confirmed by an approved, reference standard as defined in STARD [2] with equivalent or better performance and are, therefore, not adherent to global authorities' recommendations [3].

In the article text and the algorithm in fig. 1, the Architect Anti-HCV assay reactive samples that tested negative for HCV cAg and by the second HCV Ab assay are labelled as presumed false positive. This assumes that the SD Biosensor assay has adequate performance characteristics and the specification of a 'confirmatory' assay, which it does not according to the package insert of that assay. Positive results from a rapid, qualitative test cannot be considered equivalent to the S/CO values used to inform the qualitative results from the Architect Anti-HCV assay [4, 5]. Lucey et al. acknowledged that 'In three (3/38; 7.9%) cAg-positive samples, the SD Biosensor was negative, suggesting false-negative SD Biosensor results. The anti-HCV S/CO ratios of these three samples were 1.4, 3.1 and 17.5 with cAg values of 500, 5043 and 690 fmol/L, respectively'. Given the reactive Architect Anti-HCV results for these three samples and the known seroconversion period of HCV (6–8 weeks), the SD Biosensor assay's sensitivity (quoted sensitivity of 99.4% [96.6–100]) for chronic active HCV infections and use of this assay as a supplemental option is questionable.

The article also calls into question the specificity of the Architect Anti-HCV assay, but it does not clearly indicate the total number of donor samples collected during the study period to accurately discuss specificity. Given the lack of a valid, firm denominator, a discussion of specificity is questionable. Quantities such as false discovery rates or positive predictive values could not be computed since the subjects' condition status (as determined by a reference standard) was unknown [6]. The rate of active HCV infection among blood donors in Uganda has been recently reported as high as 7.8% [7], and the authors quote the National HCV seroprevalence rates for the general population as 2.7%. This highlights that the reactive rates (quoted 1.8%) observed by the Anti-HCV screening assay on Architect correlate well with the country's seroprevalence. A two-assay serological testing algorithm provides a cost-effective method of improving the positive predictive value of HCV screening, but it shall encompass the use of validated methods with adequate performance characteristics [8]. This is not the case with the published algorithm.

Another critical point to highlight is implementation of pre-analytical standard operating procedures in the interest of quality laboratory practice [9, 10]. Samples were held for up to 33 days after collection prior to the initial screen; this is outside the Anti-HCV assay's 'Instructions for Use' storage duration of 7 days. Delayed testing (significant difference in the proportion of samples with haemolysis in those that were processed after a delay [>2 days] [185/298; 62.1%] vs. those that were processed without delay [0–2 days] [77/157; 49.0%] [$p$ value = 0.0075]) needs for additional sample handling and retesting (104 [22.1%] of the screen-reactive samples became negative [S/CO ratio < 1.00] following retesting, with 73/104 [70.2%] of these occurring in samples from a centre where an extra centrifugation step had occurred), and haemolysis (57.2% of samples showed some level of visual haemolysis) are noted in the article. However, there is no description or report of variances related to primary sample storage condition and duration prior to centrifugation.

The lack of a valid confirmatory test that adheres to current global recommendations leaves the reader with no basis to assess the study results. It may be more appropriate to urge readers to view this article as an exploration of blood wastage reduction in challenging pre-analytical conditions. Pre-analytic variances and their impact on

testing results could be discussed, especially since lab quality is a global point of emphasis to the readers of this journal. Taken together, it is difficult to justify potential risk to donors and recipients by marginalizing the demonstrated contribution of pre-analytical challenges, proposing standardized acceptance of screen-reactive units and adding an additional layer of supplemental testing to offset quality and donor screening challenges.

## CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interests.

## DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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Vox Sanguinis ISBT International Society of Blood Transfusion

LETTER TO THE EDITOR

# UK donor plasma could have an immediate beneficial effect on the availability of plasma-derived medicinal products in Europe

We welcome the news that the European Medicines Agency (EMA) will, in 2023, review the *Committee for Medicinal Products for Human Use (CHMP) Position Statement on CJD and plasma-derived and urine derived medicinal products (EMA/CHMP/BWP/303353/2010)* [1]. This is in response to stakeholder requests that highlight the increased risk to immunoglobulin supply and the diminishing risk of variant Creutzfeldt–Jakob disease (vCJD). We have recently reviewed the safety profile of the United Kingdom (UK) plasma with respect to vCJD [2] and have concluded that it is a safe starting material for the manufacture of plasma-derived medicinal products (PDMPs).

From the perspective of European Member States, the UK's return to plasma fractionation will have two key benefits. First, by becoming more self-sufficient, the UK will source less of its immunoglobulin requirement from the international market. Given the significant volume of PDMPs already used in the UK, accepting plasma from UK donors is perhaps the biggest single action that any actor in Europe could take to immediately increase total plasma for medicines collection in Europe. This would reduce dependency on importation, which is primarily from the United States of America (USA) where plasma has recently been designated as a strategic resource, meaning that exports may be restricted [3]. We have previously reported that Europe (including the UK) requires 64 tons of immunoglobulin per annum for up to 350,000 patients [2] and demand is rising >6% per year [4]. Second, the acceptance of donors who have previously resided in the UK will also have beneficial effects in European countries where many currently deferred donors will become eligible to donate whole blood and plasma again.

Recent changes in donor acceptance criteria in some jurisdictions have led to immediate benefits in the supply of blood and plasma. For example, Australia lifted its UK geographical deferral on 25 July 2022, and data from Australian Red Cross Lifeblood reveal that in the 6-month period after removing the deferral, there were over 32,000 newly eligible UK donors who successfully gave just under 68,000 donations [5].

Using migration data from the UK Home Office, we estimate that of the 12.8 million people who have emigrated since 1980, 7.7 million were in the UK at some point between 1980 and 1996 [6]. Of those we estimate that 4.3 million have not since returned to the UK and of which 1 million are either in the USA or Australia (where they are now eligible to donate) and 1.8 million are in Europe. If the reviewed CHMP position statement were to allow these people to donate again, there would be an immediate benefit to the European plasma supply. Based on the proportion of the UK population who donate blood (1.53%, [7, 8]), Europe could immediately benefit from 26,000 donors who, if they donate at the average UK rate of 1.7 times per year (rather than the four times per year seen in newly eligible donors in Australia) could contribute at least 12,000 L of recovered plasma per year. If 4% become plasma donors and donate 600 mL five times per year, this could add a further 3000 L per year of source plasma [9].

It is vital to have an updated Europe-wide position to strengthen the total European plasma supply, as well as to clear the regulatory path for the many European Union (EU)-based fractionators who have expressed enthusiasm for working with UK plasma. From our conversations with the industry, it is clear that the current recommendations from both the EMA [10] and the European Centre for Disease Prevention and Control [11], for individual countries to conduct their own risk assessments, are unhelpful. To avoid a potentially large duplication of effort, many countries are awaiting a central recommendation from the EMA, and this guidance is needed to provide certainty to EU member states that plasma fractionators could obtain the necessary manufacturing licence to work with UK plasma. In the interim, given the challenge of operating two separate donation and processing streams, many blood services simply defer 'UK donors' from donation. Acceptance of UK donors would, therefore, enable countries to collect whole blood for the preparation of labile components and to recover plasma for fractionation from these donations. This would ensure the best use of all donations and maximum benefit to patients across Europe.

The UK is currently collecting around 300,000 L of recovered plasma per year and hopes to also direct a further 40,000 L of cryo-depleted plasma to fractionation. Together with the contribution from former UK residents in Europe, there is the potential for the immediate availability of 355,000 L per year of plasma to be available for fractionation. This is equivalent to the output of 18 mature and high-performing plasmapheresis centres and would relieve a significant amount of pressure on the supply of life-saving PDMPs to patients in Europe.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

  

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See also https://www.isbtweb.org/events/hvwebinars.html

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