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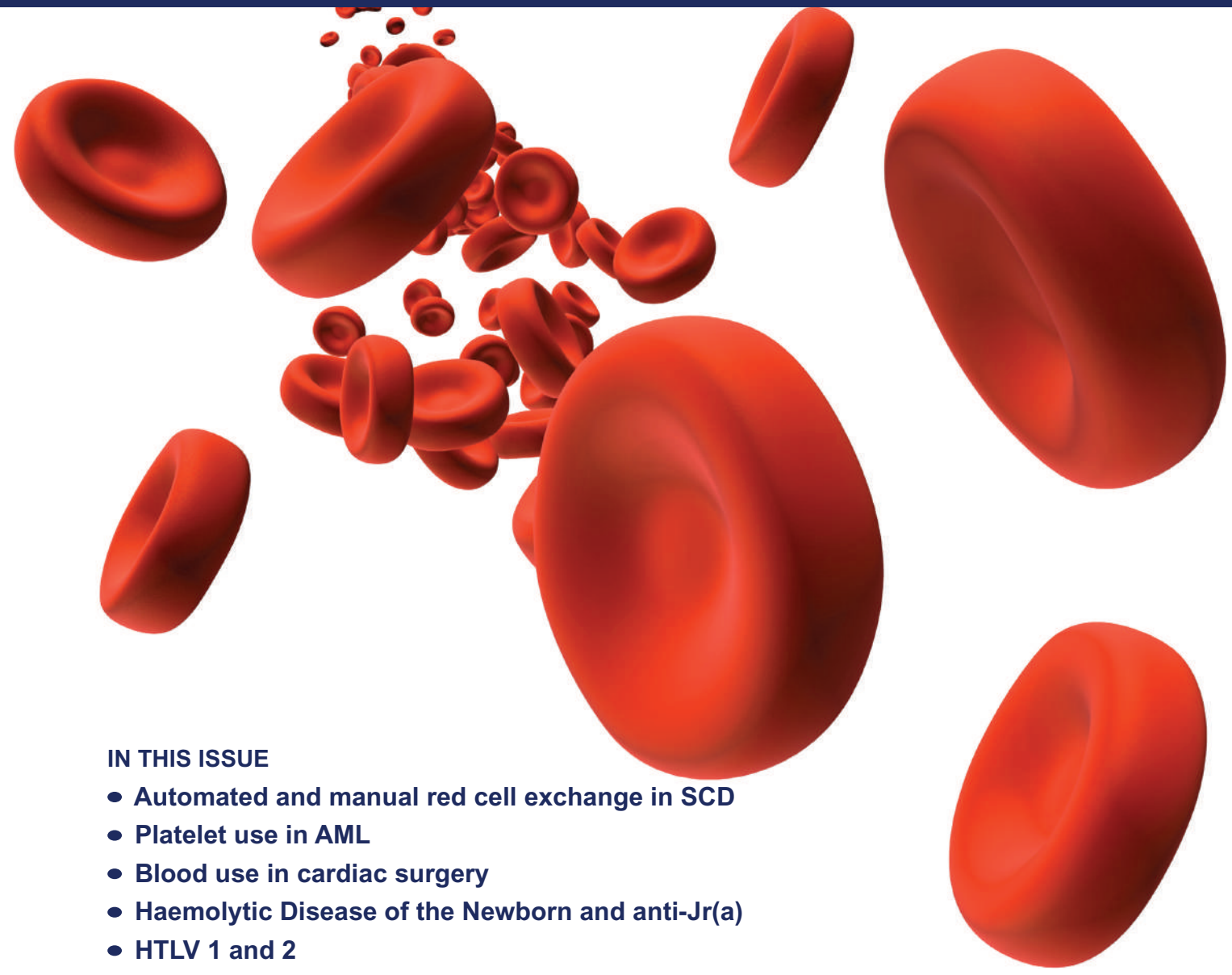
# TRANSFUSION MEDICINE

Official Journal of the British Blood Transfusion Society and the Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis



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# Transfusion Medicine

*An international journal published for the British Blood Transfusion Society*



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ORIGINAL ARTICLE

# Motivations for blood donation by HIV-positive individuals on antiretrovirals in South Africa: A qualitative study

Karin van den Berg[1,2,3] | Edward L. Murphy[4,5,6] | Vernon J. Louw[2] | Gary Maartens[7] | Shana D. Hughes[6]

[1]Medical Division, South African National Blood Service, Roodepoort, South Africa

[2]Division of Clinical Haematology, Department of Medicine, University of Cape Town, Cape Town, South Africa

[3]Division of Clinical Haematology, University of the Free State, Bloemfontein, South Africa

[4]Department of Laboratory Medicine, University of California, San Francisco, San Francisco, California, USA

[5]Department of Epidemiology/Biostatistics, University of California, San Francisco, San Francisco, California, USA

[6]Epidemiology and Bioinformatics Core, Vitalant Research Institute, San Francisco, California, USA

[7]Division of Clinical Pharmacology, Department of Medicine, University of Cape Town, Cape Town, South Africa

**Correspondence**
Karin van den Berg, Medical Division, South African National Blood Service, 1 Constantia Boulevard, Constantia Kloof, Roodepoort, Gauteng, 1715, South Africa.
Email: karin.vandenberg@sanbs.org.za

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## Abstract

**Objectives:** We performed a mixed-methods study to explore the motivations associated with blood donation by donors with known, but undisclosed HIV-positive status and ARV use (HIV+/ARV+), seeking potential strategies to reduce such donations and mitigate risk for blood recipients. Here, we report predominantly the qualitative component.

**Background:** A safe and sustainable blood supply is dependent in part, on effective pre-donation donor assessment. We previously described failure by HIV+/ARV+ blood donors to disclose their status. Such donations may lead to transfusion-transmitted HIV.

**Methods:** The social ecological model provided the conceptual framework for this study. Previously identified HIV+/ARV+ donors were invited to complete a survey (including a validated stigma scale) and qualitative interview, which underwent inductive and deductive thematic analysis.

**Results:** We uncovered two primary motivational paths to HIV+/ARV+ blood donations: privacy and altruism. The latter included a motivation not previously reported in the literature: donating specifically for other people living with HIV (PLWH). The other primary factor was a lack of privacy. These accounts often included donors encountering donation opportunities when accompanied by people to whom they had not and did not plan to disclose their HIV status. Most were highly confident their donations would be identified as HIV-positive and discarded.

**Conclusion:** We demonstrated a complex interaction between individual, social, cultural, and structural/policy factors in blood donations by PLWH who take ARV. Recommendations to limit HIV + ARV+ donations include: (1) Targeted communication strategies to increase knowledge among PLWH of their deferral from blood donation—without increasing stigma, and (2) development of procedures to assist those who feel unable to opt-out of donation due to privacy concerns.

KEYWORDS
anti-retroviral agents, blood donation, health status disclosure, HIV, motivations

## 1 | INTRODUCTION

The World Health Organization recently reaffirmed the global need for a safe, sustainable blood supply to support the effective delivery of health services and programs.[2] A key safety component is the reduction of the risk of transfusion-transmitted infections (TTI) such as HIV, Hepatitis B and C.[3] To achieve this, blood transfusion services employ a multi-pronged approach. While laboratory screening is conducted on all donated blood, donors must pass a combination of pre-donation education, donor assessment via a Donor History Questionnaire (DHQ) and potential deferral. Deferral is disqualification from donation, whether indefinitely (e.g. people living with certain infections, including HIV) or for a specified period (e.g. iron deficiency).[4] These efforts have been remarkably successful, decreasing the number of transfusion-related HIV transmissions in the United States from thousands in the early 1980s[5] to an estimated risk of <1 in 1.6 million donations in 2021.[6] Even in South Africa, with its generalised and growing HIV epidemic of more than 8 million people[7] of whom an estimated 70% are on treatment,[8] improved screening strategies reduced the risk of HIV transmission from an estimated 22 per million transfusions in 1994[9] to less than 13 per million transfusions in 2015.[10]

The effectiveness of pre-donation donor assessment and deferral, as a blood safety strategy, depends both on asking the right questions (those that address behaviours and health conditions that truly pose risk) and the willingness of would-be donors to disclose such personal information.[11–13] This can pose challenges. Studies in Europe,[11,14] North America,[15] Australia[16] and India[17] confirmed higher rates of nondisclosure of risk factors for HIV TTI among donors who tested positive for these than among donors who tested negative. Most studies investigating non-disclosure among blood donors focus on *risk behaviours* for HIV and other TTI. Non-disclosure of *known* HIV status or antiretroviral drug (ARV) use among blood donors has been less frequently explored.

Donations from donors with undisclosed, but known HIV-positive status and/or ARV use (HIV+/ARV+), even with undetectable viral loads, may pose a risk to the safety of the blood supply, especially in high HIV prevalence and ARV uptake settings such as South Africa. This is because when people living with HIV (PLWH) are not deferred prior to donation, detection of virus in their blood is dependent on serologic and molecular HIV assays, the efficacy of which may be compromised in persons with early initiation of ARV or those with pre-exposure prophylaxes breakthrough infections.[18–20] It should be noted that while an undetectable viral load is largely protective for sexual transmission of HIV, this is not necessarily true for transfusion-associated transmission, as demonstrated by modelling.[21] South Africa implemented a universal 'test and treat' strategy in September 2016,[22] so early ARV initiation should now be the norm. With a continued HIV incidence rate greater than 1%, the number of people treated (early) for HIV in the country grows annually,[7] and the risk of non-compliant blood donation resulting in HIV transmission to a blood recipient grows along with it.

Failure to disclose HIV+/ARV+ has recently been quantitatively described in both South Africa[23,24] and the United States.[25] Our group at the South African National Blood Service (SANBS) became aware of anecdotal reports of undisclosed HIV+/ARV+ among South African blood donors. Subsequent investigation revealed detectable ARV in two-thirds of donors who tested HIV antibody positive but negative by individual donation nucleic acid amplification testing, a result that suggests viral suppression from antiretroviral therapy in an HIV-positive person.[23] We found that almost 10% of all HIV-positive donors who donated at SANBS had demonstrable levels of ARV.[24] In the United States, Custer et al.[25] demonstrated undisclosed ARV use among 15% of HIV-positive blood donors and in 0.6% of all first-time male blood donors. To our knowledge, qualitative studies of this phenomenon are, as yet, non-existent. To further explore the phenomenon of blood donation by HIV+/ARV+ donors, we designed a mixed-methods study to explore the motivations associated with this behaviour. Here, we report predominantly the findings of the qualitative component.

## 2 | METHODS

Institutional review board approval was obtained from both SANBS and the University of Cape Town. This mixed-methods study was conducted at SANBS, a blood service that serves 8 of the 9 provinces in South Africa and collects approximately 900 000 units of blood from ~450 000 donors. From February to April 2019, eligible, consenting HIV+/ARV+ donors were invited to complete a survey, administered through audio computer-assisted structured interview (ACASI) technology, and an individual, in-depth qualitative interview ('interview'). The Social Ecological Model (SEM) provided the conceptual framework for this study. The SEM is frequently used in health research[26–28] and posits a complex interplay between multiple levels of influences, human behaviour and health outcomes. A version of the SEM adapted specifically to deal with HIV risk[1] guided the development of data collection instruments (Figure 1). Specifically, survey items and interview questions addressed influences at individual, social, cultural and policy levels, as well as potential donor motivations entertained by the research team during study conception (e.g. desire for incentives offered for donation, CD4 or viral load test seeking, donor belief they had been cured of HIV).

### 2.1 | Sampling and recruitment

The 122 HIV-positive donors who tested positive for ARV as part of the HIV+/ARV+ prevalence study[24] were eligible for enrolment. Eligibility criteria included: being aged 18 years or older, conversant in English, residing in the accessible areas of the Gauteng, KwaZulu Natal, Mpumalanga or Eastern Cape Provinces, able to complete data collection procedures and provide consent. Initial outreach followed routine SANBS procedures but was performed by trained research staff with prior experience in HIV counselling and study recruitment.

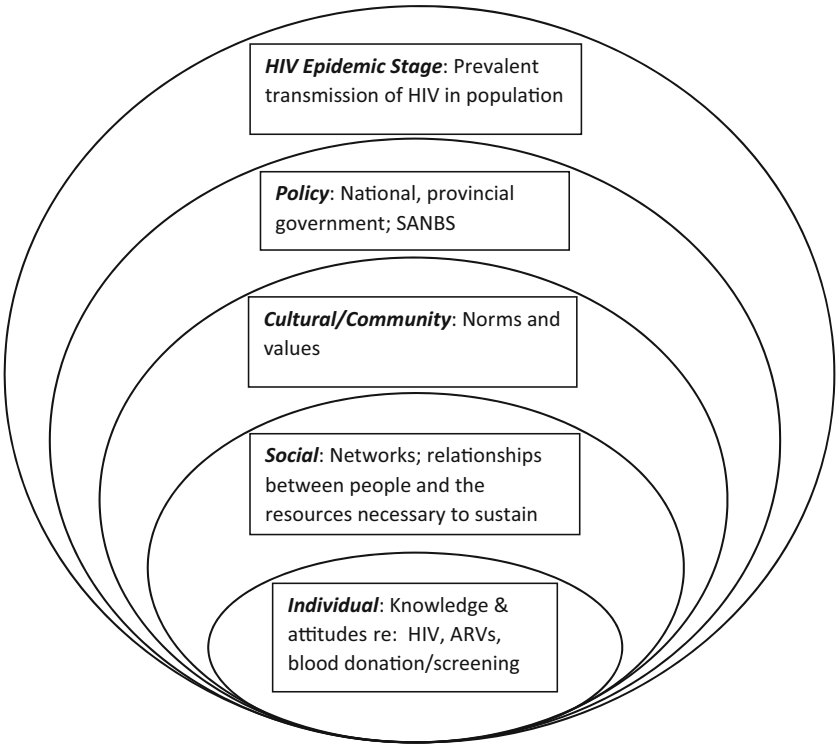


**FIGURE 1** SEM showing levels and potential influences on decision by HIV+/ARV+ individuals to donate blood (adapted from Baral et al. 2013[28]) used as conceptual framework for study development.

All eligible participants were approached for enrolment and invited to complete the ACASI and interview. Participants had the option to consent to either the ACASI or interview or to both procedures.

## 2.2 | Data collection

The ACASI surveys and interviews were administered in person, at a location mutually acceptable to study participants and staff. The survey instrument included, among other elements, a validated 12-item stigma scale.[29] (Table S1). The scale had four domains, each with three items: (1) personalised stigma, (2) disclosure concerns, (3) concerns with public attitudes and (4) negative self-image. All items were answered using a 1–4 point Likert scale, such that higher numbers indicated greater stigma.

The interviews occurred immediately after survey completion, lasted 45–90 min, and were audio-recorded with participant consent. These interviews examined the trajectory of HIV testing, diagnosis and treatment initiation; the impact of HIV; perceptions of HIV transmission risk; perceptions of blood donation and the health care system; timing and context of donation; and motivation to donate. Interview questions were open-ended and designed to produce detailed narratives through a story-telling approach, with interviewers probing as needed for full understanding.

## 2.3 | Data analysis

Survey data were extracted to Excel. Responses from stigma subscales two to four were summed to produce an overall score (subscale 1, which focuses on personalised stigma was of questionable utility within this sample. As noted by the scale's developers, high levels of secrecy surrounding serostatus, like those present in our sample, may negatively affect the reliability of questions on personalised stigma[30]). Using subscales two to four, the lowest possible score was 12 and the highest 36. Scores were averaged (19.1), and then dichotomized into high (above sample mean) or low (below sample mean) stigma categories. These categories were used to segment qualitative data and search for any patterns in motivation related to stigma.

De-identified verbatim transcripts from all interviews were uploaded to Dedoose (a cross-platform application for analysing qualitative and mixed methods data, including text and spreadsheet data)[31] and subjected to thematic analysis.[32] This included inductive, line-by-line analysis to identify themes that emerge from close reading of the text, as well as deductive analysis, which focused on pre-identified themes drawn from the interview guide and relevant academic literature.[33,34] A codebook was created following accepted procedures.[35] Data were compared across participant gender and stigma score.[34] In addition, we applied narrative analysis to interviewees' accounts of their study qualifying donations (SQDs) to better grasp their experience as a whole.[36]

All study procedures were approved by the SANBS and University of Cape Town institutional review boards.

# 3 | RESULTS

Contact details were available for 120 of the 122 potential participants, 62 (52%) of whom were unreachable (either due to incorrect

**TABLE I** Participant demographics, disclosure practices and stigma scores.

| | *N* (median) | % (IQR) |
|---|---|---|
| Total | 25 | 100 |
| Ethnicity | | |
| Black African | 23 | 92 |
| Coloured | 2 | 8 |
| Gender | | |
| Female | 18 | 72 |
| Male | 7 | 28 |
| Donor type | | |
| First time | 17 | 68 |
| Lapsed | 5 | 20 |
| Repeat | 3 | 12 |
| Age | | |
| Median | 32 | (24–39) |
| Province | | |
| Eastern Cape | 2 | 8 |
| Free State | 4 | 16 |
| Gauteng | 10 | 40 |
| KwaZulu Natal | 5 | 20 |
| Mpumalanga | 4 | 16 |
| Clinic type | | |
| Mobile | 22 | 88 |
| Fixed site | 3 | 12 |
| Disclosure practice | | |
| Extremely restricted | 3 | 12 |
| Very highly restricted | 5 | 20 |
| Highly restricted | 8 | 32 |
| Moderately restricted | 8 | 32 |
| Least restricted | 1 | 4 |
| Stigma scores | | |
| Overall | 22 | (16–29) |
| Disclosure concerns | 6 | (4–9) |
| Public attitude | 7 | (3–10) |
| Self-image | 4 | (3–6) |

*Note*: Extremely restricted = Disclosed to no one; very highly restricted = disclosed to one person; highly restricted = disclosed to 2–3 people; moderately restricted = disclosed to >3 people, but not outside family and friends; least restricted = disclosed to >3 people, including beyond family and friends.
Abbreviation: IQR, Inter quartile range.

details or not responding to calls); 12 (10%) were ineligible (11 were not conversant in English); 20 (17%) directly or indirectly refused participation; 1 participant enrolled only for the ACASI and was excluded from this manuscript. Hence, a survey and interview were collected from the 25 (21%) consenting participants. (Table I) Participants were predominantly Black African, female, in their 30s, residents of Gauteng province, and had donated at a mobile site. Eight donors (32%) had donated at least one previous donation. Only one-third of participants had ever disclosed their HIV status to more than three people. Eleven were classified as perceiving high stigma. Two interviewees (1101 M and 2202 M) insisted they had not knowingly donated while HIV+/ARV+.

Reviewing interviewees' reported motivations for their SQDs, we grouped responses into three themes: (1) altruism, expressed both as a general wish to 'save lives', and the specific intention of donating so that blood could be given to other PLWH; (2) a lack of privacy at the donation location, associated with a fear of status disclosure; and (3) other reasons. The latter category included disparate but largely secondary motivations, such as donation to manage a perceived superabundance of blood, or as a way to confirm HIV status. Here we focus on the first two themes, as they heavily predominated among interviewees' responses. Notably, very few accounts suggested any kind of test-seeking, only one mentioned incentives, and none provided evidence of interviewees believing they had been cured of HIV.

After stratifying interviewees by stigma scale score, and considering the narratives in their entirety, we did not find clear differences in reported motivations. For example, interviewees in both low and high stigma-perceiving categories mentioned: altruistic motivations, privacy-related motivations (including highly restricted serostatus disclosure practices outside of the donation context), and donating as a blood management practice. However, when segmenting interviewees by motivation, those who reported general altruism or privacy concerns as the predominant factor in their donation were evenly split in their stigma perceptions (2 high vs. 2 low; 5 high vs. 5 low, respectively); while those who reported donating blood for other PLWH were more likely to be classified as perceiving low stigma (6 low vs. 2 high). While the overall average stigma score in this data set was 19.2, the averages by motivation were 20.4 for donors concerned with privacy and 18 for those citing altruism. Given the small sample size, the above results are offered in a purely descriptive vein.

As the different stories told by these participants unfold largely along the lines of reported motivations, the following sections segment interviewees by their concern with altruism or privacy to explore the thematic findings in depth.

## 3.1 | Altruism-motivated donors

Overall, altruism was the most commonly reported motivation, mentioned by nearly half of the interviewees ($N = 12$). Frequently framed as the desire to 'save lives', this was reported by interviewees from Gauteng, Free State and Mpumalanga provinces, men and women (6 and 6, respectively), and from both stigma categories.

We identified two distinct sub-themes within these accounts. The first was a general wish to help others ($N = 4$). Some interviewees talked about friends or family members having previously needed or received a transfusion; others reported awareness of the general need for blood. For instance, one man explained, 'I wanted to donate blood because I knew my blood type was the most wanted one' and that traffic accidents had caused 'a need for blood' (2202 M). A woman

shared her wish to donate 'because my mother was sick and they donated blood for her. So, I thought if I could donate maybe I could help someone else just like they helped my mother' (4402 F).

The wish to engage in a more specific form of altruism was expressed by eight interviewees. They were motivated to donate blood so that it could be given to a recipient also living with HIV. Three other interviewees (1103, 4402 and 4403F) explicitly raised the possibility that a PLWH might donate for other PLWH, although that was not the primary motivation for their SQD. Talk of donating for other PLWH often drew on notions of 'matching', seeming to equate serostatus matching with the matching of blood types required for transfusion. For instance, a male interviewee discussed donating to help 'someone else who also has HIV and our blood codes are the same' (4406 M). Some interviewees spoke of themselves as being particularly suitable donors for other PLWH, attributing this to their overall health, serological indicators, and/or medication adherence. One interviewee, on ARVs since 2012, said of her blood, 'I think it is better than the people who have just found out that they are HIV positive…. because I am drinking the medications regularly and then my health is fine. It will help the people who are HIV positive, especially the ones with low CD4 count' (3306 F).

Interviewees' certainty about the feasibility of donating for another PLWH varied, though most were remarkably confident. Eleven of these 12 interviewees reported having been unaware of the deferral of PLWH at the time they donated and spoke of feeling confusion or remorse when they learned of their ineligibility. One interviewee had been explicitly told he was not eligible but continued to believe his donations could help other PLWH. He explained his reasoning: 'Blood is blood, whether infected or not. I still believe it can help other people in need' (3305 M). The idea that PLWH would be ineligible to donate rarely surfaced in interviewees' accounts of the decision-making that led to their donation, and donating was sometimes framed as a duty: 'If someone who is HIV-positive needs blood, … I have to [donate] so that I can help' (3302 M).

### 3.2 | Privacy-motivated donors

The other primary factor raised by interviewees as playing a meaningful role in their decision to donate was a lack of privacy. This was described by over one-third of interviewees ($n = 10$), across all provinces, and both stigma categories. Nine were female, one male. There was a mix of eligibility beliefs: some were aware of the deferral for HIV; others were uncertain or seemed not to have considered this possibility. In most cases, interviewees reported that when they encountered the opportunity to donate blood, frequently at school or workplace blood drives, they were with other people (co-workers, classmates, friends, romantic partners) to whom they had not planned to disclose their HIV status. The accounts featured a series of decision points, all experienced as threatening to interviewees' privacy. The first involved *presenting for donation*. Interviewees generally felt unable to opt-out of attempting to donate without prompting questions and raising suspicion. The second revolved around *discussing HIV status or donation eligibility* with SANBS staff. Most interviewees reported there was no private place to have such a conversation (though some had gone to the donation site with precisely this intent). The third decision point involved *answering the DHQ*. Interviewees felt unable to disclose their HIV status on the DHQ, either due to the proximity of co-workers and friends, or because they believed the confidentiality of their answers might be compromised. Overall, in comparison to narratives shared by altruism-motivated donors, these stories were more rooted in the donation context itself. They framed donation as the only safe way out and knowledge of donation eligibility was, in a practical sense, immaterial.

This is well-illustrated by 1106 F's account. She explained that SANBS ran a blood drive at her workplace and 'everyone, most of the people in the office, they were going to donate…and yah, so I didn't have much of an excuse as to why I shouldn't go' (*presenting for donation*). She thought that 'when I get there, I will [be] able to speak, maybe it will be in private… but it was in the boardroom and you know they had the beds and stuff so we all just filled in the forms in one table' (*discussing eligibility*). Regarding the screening questions, she 'didn't answer them truthfully' because her co-workers were close at hand (*answering the DHQ*). In addition, she observed that being deferred from donation attracted undue attention to her as the donor, which was exactly what she was trying to avoid: 'Everyone was looking at each other … it was like a joke because even those who had iron problems and [were] turned away… people were, like, talking like, 'Oh why have [they] been turned away?' Similar stories were shared by interviewees who had donated with classmates at school, a female donor who had donated with a boyfriend, and a domestic worker taken to donate by her employer.

Though a more private environment might have allowed some of these donors to reveal their HIV status to SANBS staff, for others, additional perceived risks would likely still have precluded disclosure. A total of 1107 M noted that even having a question about HIV status on the DHQ, 'is like, a violation of my privacy'. He elaborated, 'If [the form] fall on the wrong hands…my name is there, my ID is there, confidentiality is not there'. Thus, in these narratives, fear of inadvertent HIV status disclosure surfaced in multiple ways, and no interviewee reported disclosing their status on the DHQ. Most were untroubled by having withheld this information because they generally reported confidence that testing done by SANBS would identify their donation as being HIV-positive. One female repeat donor explained, 'I know how it works…the blood they are taking, it is going to go through these tests… You know hundred percent sure that this blood that I am giving…is not going to go anywhere, it is not going to be given to anyone' (1105 F).

## 4 | DISCUSSION

Qualitative research with South African HIV+/ARV+ blood donors revealed that their primary reported motivations were altruism and privacy concerns, with no discernable difference by stigma scores. Among those motivated by altruism, we identified both a general wish

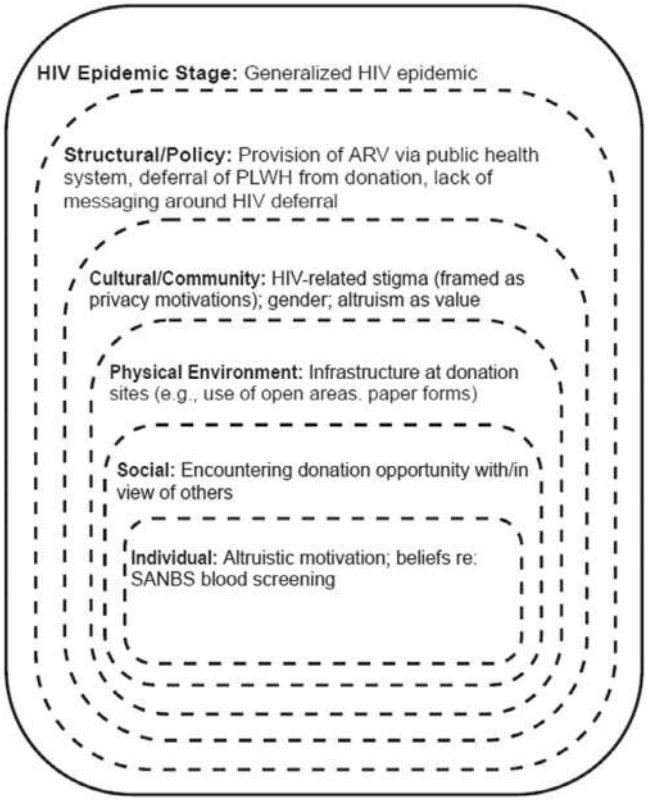


**FIGURE 2** SEM showing levels and influences, including newly identified influences, on decision by HIV+/ARV+ individuals to donate blood (Adapted from Baral et al. 2013[28]).

to 'save lives' and a motivation not previously reported in the literature: the specific desire to donate for other PLWH. Donors motivated by privacy concerns shared quite different accounts, highlighting various elements in the donation process they felt threatened the confidentiality of their HIV status. Far from offering multiple opportunities to exit the process, the experience was framed as a series of decision points in which, in each instance, donation was the only safe option. In this Section 4, we slot our research findings into an adapted version of the SEM that grounded our study, discuss the utility of the model, and offer recommendations for reducing the likelihood of future HIV +/ARV+ donation.

Despite the similar way altruism and privacy were described by interviewees (i.e. as 'the reason' for their donation), they are different. Altruistic motivations led interviewees to feel *drawn* to donate, whereas privacy concerns led interviewees to feel *pushed* to donate. This difference led us to locate these factors in different levels of the SEM.

We categorised altruistic motivations as an individual-level influence because they were always constructed as an expression of personal morality. We recognise, however, that all levels of the model are interconnected (hence the dotted lines around them in Figure 2). For example, individual altruistic impulses arise within, but are shaped by a cultural context that frames altruism as morally good. Similarly, individual, altruistic motivations may be influenced by blood service policies. For example, blood services implement a policy of minimising the likelihood of TTIs through multiple strategies. One strategy employed by SANBS is educating potential donors that the blood service should not be used as an HIV testing center, yet to date, however, SANBS has not explicitly disseminated messaging that PLWH are permanently deferred from donation. Considering the U = U (undetectable = untransmissible) campaigns[37] in the media, and successful kidney donations from HIV-positive persons with undetectable HIV viral loads to recipients living with HIV,[38] it should not be surprising that PLWH might consider the same to be possible for blood donation.

In contrast to individual-level altruism, we classified privacy motivations as a cultural factor. This was because interviewees only raised privacy concerns about their HIV status (vs. other personal information), and linked them to fear of unwanted disclosure given the reported lack of privacy and confidentiality encountered while donating. Privacy concerns are therefore best understood as indexing something beyond individually varying comfort levels: they are reactions to still-pervasive HIV-related stigma in donor communities. Supporting this, the privacy-motivated group had higher average stigma scores than those donating for altruistic reasons (20.4 vs 18.0), though high and low scores were present in both groups. Furthermore, the overwhelming majority of donors with privacy concerns were female. This is of particular importance in a strongly patriarchal South Africa with its predominantly heterosexual epidemic, which disproportionally affects younger, economically vulnerable, females.[39,40]

The cultural factor of stigma (expressed as privacy concerns) intersected with a social-level influence on interviewees' decision-making: HIV+/ARV+ donors often encountered donation opportunities in the presence of other people to whom they had not disclosed and did not wish to disclose, their status. These companions exerted pressure on interviewees' decision-making beyond the cultural influence of stigma because the imagined potential losses that status disclosure might cause were more concrete and had their own social implications (e.g. loss of a romantic partner, colleagues' esteem).

A further factor added to this complexity: the physical environment and infrastructure at donation locations, especially mobile blood drives. Many interviewees noted that elements such as the use of paper forms and open spaces lacking privacy constituted an obstacle to disclosure. We created a separate level in the model for such considerations (as done in other ecological work in which the physical environment played an important role[41,42]). Since interviewees reported experiencing them as highly proximate, but more fixed than social interactions, we placed this level between social and cultural levels. It is worth noting that SANBS policy actually stipulates that screening, even at mobile drives, be conducted in a private environment. Thus, the lack of privacy, in this case, is a question of *implementation* rather than policy per se.

Policy does influence HIV+/ARV+ blood donation, even if not explicit in the interview data. As mentioned, SANBS's education efforts, aimed at reducing TTI risk, may influence eligibility perceptions and motivations among donors. At a national level, health policies are catalysts for both the deferral of PLWH from donation and

the wide availability of ARVs in South Africa. All of the factors discussed heretofore operate within policy structures that grapple with the generalised South African HIV epidemic, which leaves significant proportions of the potential donor pool affected by HIV.[40] The need to balance supply and safety means deferrals of sub-populations perceived to be at-risk for TTI (e.g. men who have sex with men, as had been done elsewhere), is simply not possible in the South African context.

We accommodated factors this study found relevant for understanding HIV+/ARV+ blood donation in South Africa within the Social Ecological Model, adapting as necessary (Figure 2). We offer this as a heuristic upon which we will build (e.g. by incorporating findings related to eligibility beliefs and screening experiences), and that others may find useful for considering HIV+/ARV+ donation in other contexts.

This version of the SEM reveals a complex interaction between individual, social, cultural, and structural/policy factors and multiple pathways to donations by PLWH who take ARVs. Indeed, the nuances of the decision-making in interviewees' narratives cannot be adequately grasped, or responded to, without a multi-level model. In particular, the SEM is helpful in understanding that addressing factors related to HIV+/ARV+ donation at one level, such as individual motivation to help others, may not eliminate the behaviour, as influences at other levels (HIV-related stigma and privacy concerns) will still be operant if measures are not taken to mitigate them. For example, someone who was initially motivated to donate for other PLWH might learn this is not possible and no longer wish to donate, but still feel compelled to if encountering a blood drive at their workplace. Thus, an 'altruism-motivated' donor could 'transform' into a 'privacy-motivated' donor if blood collection infrastructure/procedures and HIV-related stigma have not changed. Furthermore, a more private environment and screening experience might have made a meaningful difference in the comfort with status disclosure for some HIV+/ARV+ donors, but others clearly stated little could be done to mitigate the perceived threat posed by potential status revelation in a broader context of HIV-related stigma.

The foregoing notwithstanding, we must mention a major conclusion of our analysis and note that reaching it required us to think beyond the adapted SEM that grounded this study. Adapting a model is typically seen as an appropriate way to attend to research context.[43,44] In this research, adapting the SEM allowed us to focus on HIV risk, which was both helpful and somewhat obfuscatory. This tailoring allowed us, for example, to consider the nature of South Africa's HIV epidemic, but it also led us to implicitly conceptualise 'HIV+/ARV+ donors' as unique, rather than prompting us to ask what they might share with other donors, or how their behaviour might be similar to that exhibited in other contexts. Despite this, commonalities emerged. For example, a large group of our interviewees reported altruistic motivations for donation. Activators for altruism were varied and included donating because of loved ones, knowledge of blood shortages, and a moral duty to donate, including specifically for other PLWH. Though donating for other PLWH is, as far as we know, a novel finding, the other reasons are indistinguishable from those offered by many donors *not* living with HIV, both in South Africa,[45,46] and elsewhere.[47] We came to realise that the expectation that HIV+/ARV+ donors' motivations *would* be different derived from assumptions that, in some cases, were not supported by data (e.g. HIV+/ARV+ donors know they are ineligible to donate; such donors would not consider their blood helpful to others). For those donors who donated prior to HIV acquisition, it makes little sense to expect their donation motivations, post-HIV diagnosis, and treatment initiation, to be different than they had been historically, especially given the messaging around 'U = U' and HIV being just 'another chronic disease'.[37,48]

In addition, though reports of HIV+/ARV+ blood donations were initially surprising, looking beyond the specific context of blood donation suggests perhaps they should not have been. Non-disclosure of health information, including HIV status and ARV use, in other medical settings, is well described. Failure to disclose general medication use, even upon direct questioning by their clinicians, was reported in up to 15% of patients in the USA.[49] Furthermore, non-disclosure of known HIV status and ARV use have been confirmed in several African household surveys[50,51] and in HIV and ARV research programs.[52–54] ARV denial was reported in as many as one in three participants in a study validating self-reported ARV use in rural South Africa.[55] These trends should have led us to *expect* status disclosure in a semi-public setting to be problematic, even though other HIV+/ARV+ donors have disclosed their status.[56,57] What may warrant more investigation is the conditions under which some would-be donors living with HIV do disclose their status.

From the discussions with the participants and the main themes identified in this study, certain recommendations to limit HIV+/ARV+ donations emerged. Specifically, these include: (1) Improve the likelihood that PLWH are aware of their permanent deferral from blood donation, as a way to reduce the potency of altruism as a motivator. Historical blood donor messaging relating to HIV centered on the risk of donation during the HIV 'window period' and donation sites not being used as HIV-testing sites. This should be augmented with clear communication on the ineligibility of PLWH as blood donors. It is crucial that this be conveyed in a manner that will not further stigmatise those living with the virus. Crafting effective messaging and identifying appropriate channels for dissemination should be done in collaboration with PLWH. (2) Develop procedures to assist those who feel unable to opt-out of donation due to peer pressure and privacy concerns. These could include systems for donors to confidentially withdraw their donations directly after donation or providing donors with a 'palatable' option to explain their potential deferral to those observing their donation process. The latter would still require improved privacy infrastructure conducive to confidential discussion at donation sites. Enforcement and compliance monitoring of existing privacy policies, especially at often-used facilities need to be further strengthened.

Our study had several limitations, including the potential for selection bias. We recruited participants from a relatively small pool of HIV+/ARV+, English conversant, South African blood donors. Those who were not interviewed may have had meaningfully different

experiences that are not represented here. Furthermore, we tried to reduce potential social desirability bias through assurance of anonymity, personal safety and the use of open-ended questions. While these measures might not have been entirely successful (two interviewees refused to acknowledge awareness of HIV+/ARV+ status at the time of donation), interviewees did recount behaviour often considered socially undesirable, suggesting they felt sufficiently comfortable at some level to share such responses.

To our knowledge, this is the first attempt at investigating the motivations driving donations by HIV+/ARV+ donors. As appropriate for highly exploratory, qualitative research, we make no claims of exhaustiveness and instead offer the significant convergence of themes we found around altruism and privacy as a starting point on which to build. Researchers and professionals should critically consider how these findings may apply in different contexts (national, cultural and types of epidemics). We believe our findings may well have utility in other settings, as HIV is a relatively stigmatised infection globally, blood donation requires fundamentally similar processes regardless of national context, and our findings dovetail with those from other research on disclosure of healthcare information in general.[49–53]

## 5 | CONCLUSION

The phenomenon of blood donation by HIV+/ARV+ has been documented in two contexts.[23,25] Though its global prevalence is unknown, there is little reason to assume that it is not occurring more widely. Our research uncovered complex, diverse motivations related to privacy and altruism leading to HIV+/ARV+ blood donations. The growing HIV+/ARV+ populations both in South Africa and elsewhere and the increasing uptake of pre-exposure HIV prophylaxis may well result in increasing numbers of such donations unless actively managed. To reduce such donations, we need a better understanding of why they are occurring. Here we have reported only on the main donor motivations associated with these non-compliant donations, which is but a small component of this complex phenomenon. We urge other scholars to assess the occurrence of this phenomenon in other settings and further elucidate the motivations and contexts leading or contributing thereto.

### AUTHOR CONTRIBUTION

SDH, KvdB, ELM, VJL and GM designedthe research study. KvdB and SDH performed the research and analysed the data.KvdB and SDH wrote the paper and ELM, VJL and GM reviewed it.

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### CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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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 COMMUNICATION

# Emergency red cell exchange for the management of acute complications in sickle cell disease: Automated versus manual

**Diana Mihalca [1,2] | Adeel Ahmad [1] | Dipal Mehta [1] | Dorothy Kyeyune [2] | Susan Acana Elaborot [2] | Dimitris A. Tsitsikas [1,2]**

[1]Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Leeds, UK

[2]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK

[3]Leeds Orthopaedic & Trauma Sciences, Leeds General Infirmary, University of Leeds, Leeds, UK

[4]Leeds Institute of Health Sciences, University of Leeds, Leeds, UK

[5]NIHR Leeds Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK

Correspondence
Michalis Panteli, Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Clarendon Wing, Level D, Great George Street, Leeds LS1 3EX, West Yorkshire, UK.

Email: di.ts@doctors.org.uk

**Abstract**

Background

Red blood cell exchange is the cornerstone of the management for acute complications of sickle cell disease. It improves anaemia and improvesperipheral tissue oxygen delivery while at the same time reduces the proportion of circulating sickle erythrocytes. Even though automated red cell exchange is very effective in rapidly lowering the Hb S level, 24-h availability is currently not feasible for most specialist centres including our own.

Methods

Eighty-six such episodes have been recorded between June 2011 and June 2022 comprising of 68 episodes of automated and 18 episodes of manual red cell exchange.

Results

The post procedure Hb S/S + C level was 18% after automated and 36% after manual red cell exchange. The platelet count dropped by 41% and 21% after automated and manual red cell exchange respectively. The clinical outcomes including need for organ support, duration of stay in the intensive care units.

Conclusion

In our experience, manual red cell exchange is a safe and effective alternative to an automated procedure that can be used while specialist centres are building up their capacity to offer automated red cell exchange for all patients requiring the intervention.

KEYWORDS

KAP, Blood, Transfusion, Donor, Medecine

## 1 | INTRODUCTION

The incidence of hip fractures continues to increase, along with the global expansion of aging population observed secondary to improved healthcare and quality of life.[1] Subtrochanteric fractures are defined as fractures encountered between the inferior border of lesser trochanter and 5 cm distal to it.[2] They represent a complex subset of injuries surrounding the hip, which are most commonly managed with

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

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

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

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

## 2 | METHODS

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

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

### 2.1 | Data

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

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

**TABLE 1** Grouping of variables into constructs for each model

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

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

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

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

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

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

## 2.2 | Statistical analysis

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

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

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

**TABLE 2** Distribution of explanatory variables by donor status and sex

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

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

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

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

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

## 3 | RESULTS

### 3.1 | Sample composition

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

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

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

## 3.2 | Model selection

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

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

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

## 3.3 | Parameter estimates

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

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

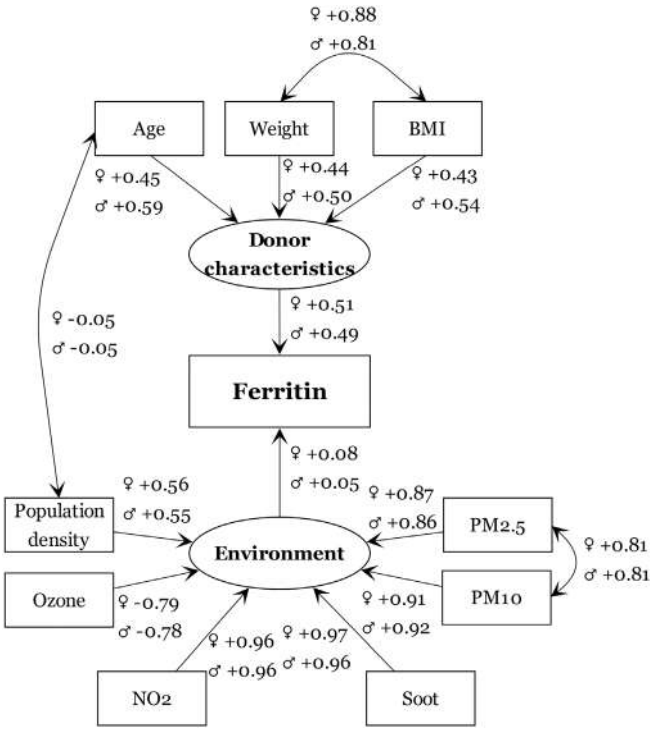


**FIGURE 1** Final structural equation model for ferritin determinants in new donors, with parameters estimated separately for men and women. All parameter estimates are standardised so that the variance of each observed variable and latent construct equals 1

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

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

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

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

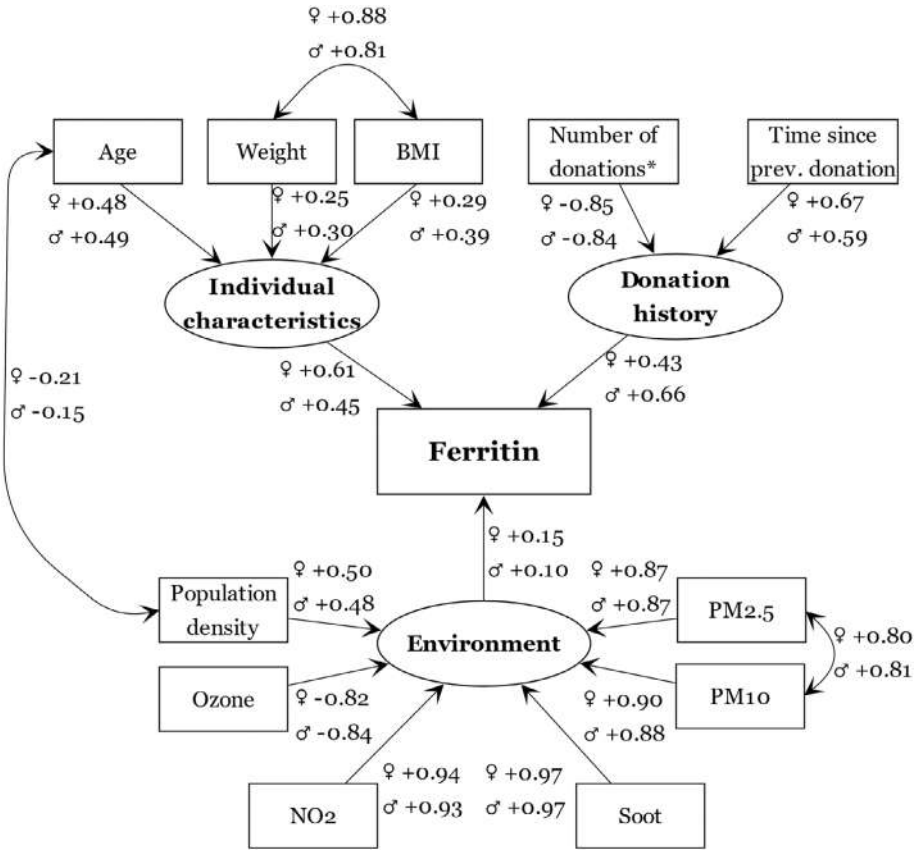


**FIGURE 2** Final structural equation model for ferritin determinants in active donors, with parameters estimated separately for men and women. All parameter estimates were standardised so that the variance of each observed variable and latent construct equals 1

## 4 | DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

## AUTHOR CONTRIBUTIONS

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

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## CONFLICT OF INTEREST

The authors have no competing interests.

## DATA AVAILABILITY STATEMENT

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

## ORCID

*Marieke Vinkenoog* https://orcid.org/0000-0001-5653-8078
*Mart Janssen* https://orcid.org/0000-0002-1682-7817

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## APPENDIX A

### A.1 | STRUCTURAL EQUATION MODELLING OVERVIEW

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

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

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

#### A.1.1. | Measurement model

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

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

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

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

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

#### A.1.2. | Structural model

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

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

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

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

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

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

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

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ORIGINAL ARTICLE

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# Optimising platelet usage during the induction therapy of acute myeloid leukaemia: Impact of physician education

**John Gnanaraj[1,2] | Abhishekh Basavarajegowda [1] | Dibyajyothi Sahoo [1] | Esha Toora [2] | Susan Biswajit Dubashi [2] | Prasanth Ganesan [1,2]**

[1]Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Leeds, UK

[2]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK

[3]Leeds Orthopaedic & Trauma Sciences, Leeds General Infirmary, University of Leeds, Leeds, UK

[4]Leeds Institute of Health Sciences, University of Leeds, Leeds, UK

[5]NIHR Leeds Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK

Correspondence
Michalis Panteli, Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Clarendon Wing, Level D, Great George Street, Leeds LS1 3EX, West Yorkshire, UK.

Email: prasanth.g@jipmer.ac.in

**Abstract**

Introduction

Platelet products are scarce and expensive resources to be used judiciously. However, inappropriate usage is common. Lack of physician awareness is an important issue.

Methods

Charts of patients with acute myeloid leukaemia (AML) treated between January 2020 and August 2020 was reviewed, and the mean platelet usage per patient per day was calculated. Physician education was implemented between September 2020 and December 2020 (2 PowerPoint lectures of 20 min each and weekly WhatsApp messages containing the guidelines). Data of patients treated between Jan 2021 and August 2021 was prospectively audited to understand platelet usage and the indications for transfusions.

Results

Group A (before physician education) consisted of 22 patients, and group B (after physician education) consisted of 23 patients. The 190 requests for platelet transfusion received during this period were classified as appropriate (157/190), which constituted 82.63% of the requests, or inappropriate (33/190), which accounted for 17.36%.

Conclusions

A short-duration education programme supplemented with weekly WhatsApp messages and an active feedback mechanism on the rationale of platelet transfusion by the treating physician and transfusion specialist

KEYWORDS

KAP, Blood, Transfusion, Donor, Medecine

## 1 | INTRODUCTION

The incidence of hip fractures continues to increase, along with the global expansion of aging population observed secondary to improved healthcare and quality of life.[1] Subtrochanteric fractures are defined as fractures encountered between the inferior border of lesser trochanter and 5 cm distal to it.[2] They represent a complex subset of injuries surrounding the hip, which are most commonly managed with

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

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

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

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

## 2 | METHODS

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

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

### 2.1 | Data

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

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

**TABLE 1** Grouping of variables into constructs for each model

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

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

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

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

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

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

## 2.2 | Statistical analysis

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

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

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

**TABLE 2** Distribution of explanatory variables by donor status and sex

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

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

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

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

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

## 3 | RESULTS

### 3.1 | Sample composition

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

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

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

## 3.2 | Model selection

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

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

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

## 3.3 | Parameter estimates

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

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

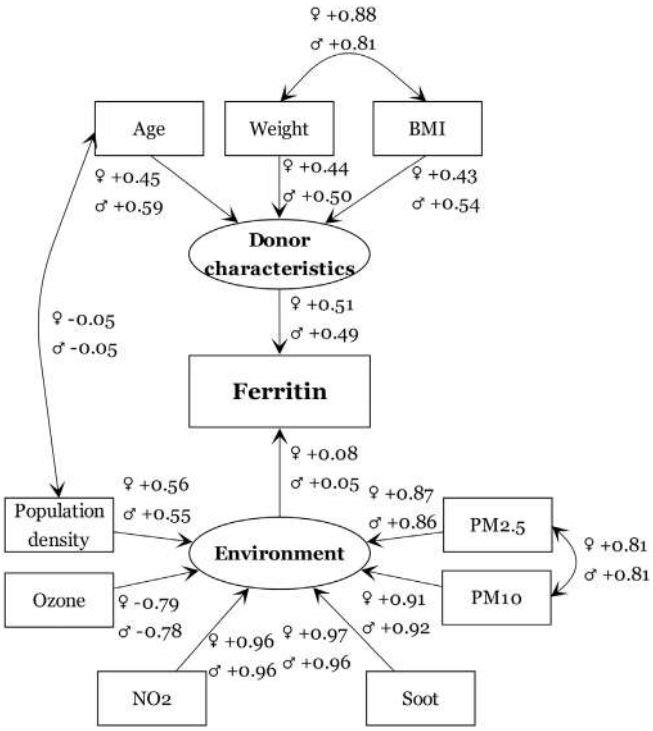


**FIGURE 1** Final structural equation model for ferritin determinants in new donors, with parameters estimated separately for men and women. All parameter estimates are standardised so that the variance of each observed variable and latent construct equals 1

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

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

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

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

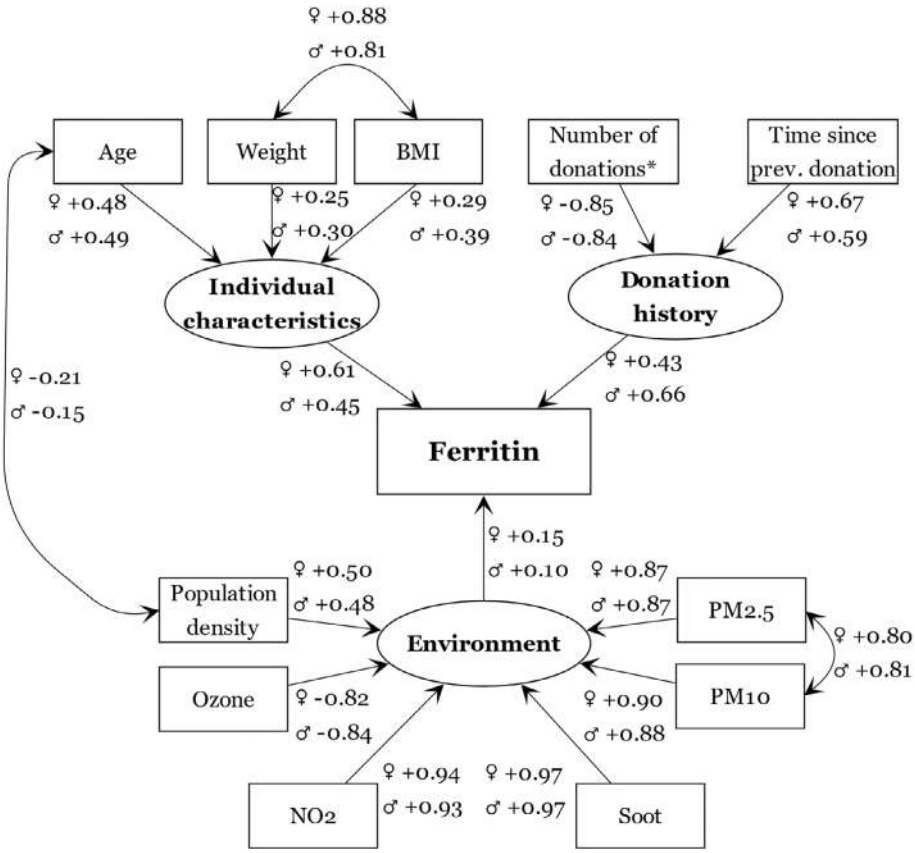


**FIGURE 2** Final structural equation model for ferritin determinants in active donors, with parameters estimated separately for men and women. All parameter estimates were standardised so that the variance of each observed variable and latent construct equals 1

## 4 | DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

## AUTHOR CONTRIBUTIONS

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

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## CONFLICT OF INTEREST

The authors have no competing interests.

## DATA AVAILABILITY STATEMENT

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

## ORCID

*Marieke Vinkenoog* https://orcid.org/0000-0001-5653-8078
*Mart Janssen* https://orcid.org/0000-0002-1682-7817

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## APPENDIX A

### A.1 | STRUCTURAL EQUATION MODELLING OVERVIEW

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

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

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

#### A.1.1. | Measurement model

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

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

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

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

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

#### A.1.2. | Structural model

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

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

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

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

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

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

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

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ORIGINAL ARTICLE

# Hemolytic disease of the newborn due to anti-Jra from a Chinese mother with one novel and one classic heterozygous mutation

**Jie Xiao [1,2] | Qixiu Yang [1] | Shouxi Gao [1] | Hui Song [2] | Fengyong Zhao [2] | Zhonghui Luyi Ye [1,2] | Dong Xiang [1,2]**

[1]Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Leeds, UK

[2]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK

[3]Leeds Orthopaedic & Trauma Sciences, Leeds General Infirmary, University of Leeds, Leeds, UK

[4]Leeds Institute of Health Sciences, University of Leeds, Leeds, UK

[5]NIHR Leeds Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK

Correspondence
Michalis Panteli, Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Clarendon Wing, Level D, Great George Street, Leeds LS1 3EX, West Yorkshire, UK.

Email: yeluyi@sbc.org.cn; xiangdong@sbc.org.cn

**Abstract**

Objective

Investigation of a Jr(a-)family samples, identification of the mutant and assessment of the differences of Jr antigen density of the Jr(a-) family members, random adult and newborn individuals' RBCs.

Background

The anti-Jra antibody is generated when a Jr(a-) individual pregnant or transfused with Jr(a+) blood unit, which can lead to mild-to-moderate hemolytic disease of the foetus and newborn (HDFN) or hemolytic transfusion reaction (HTR). Several mutations had been identified. The anti-Jra caused HDFN is not rare in East Asia, but due to the lack of antibody and molecular background, it is likely to lead missed detection.

Methods and Materials

One G4P1 woman had been detected as IAT positive during prenatal examination. Suspected as anti-Jra after the laboratory serological testing, the maternal sample was further assessed by molecular analysis. The antigen density was detected by flow cytometry after reacting with anti-Jra serum in family members and the normal individuals.

Results

One novel frameshift mutation c.717delC and one previously identified mutation c.706C > T in ABCG2 was identified on proband. The infant haemoglobin(Hb) and bilirubin increased significantly after exchange transfusion and the severe HDFN was relieved. Flow cytometry results showed that the Jra antigens on adult RBCs were significantly less than those on the infant.

Conclusion

The c.717delC mutation can lead to the shortening of protein ABCG2 in the site of p.Leu307Stop, result in the loss of Jra antigen. Breastfeeding may lead to slower recovery from HDFN.

K E Y W O R D S

Jr(a-)family, ABCG2, Blood, Transfusion, Donor, Medecine

## 1 | INTRODUCTION

Severe acute respiratory infections caused by strains of influenza or coronavirus often lead to hospitalisation and sometimes death. Symptomatic infection with SARS CoV-2 (COVID-19) has surpassed the annual global burden of death due to influenza or coronaviruses.[1] Although there are several effective vaccines for COVID-19 therapeutic treatments are still required. Patients particularly at risk are those with disorders that affect the immune system, for example, haematological malignancies or those receiving drugs that suppress an immune response, for example, after organ transplantation.[2,3]

Passive antibody therapies, including monoclonal antibody combinations have proven effective for COVID-19[4] However, the cost of these therapies is prohibitive[5] and new SARS-CoV variants may become resistant to anti-virals developed in response to previous variants.[6] Alternative and affordable responses to emerging strains of virus are needed.

Convalescent plasma (CP) is typically collected from donors with confirmed diagnosis of infection at least 2 weeks after recovery.[7] CP contains neutralising antibodies specific to the infectious agent but may also contain other immune modulators and clotting factors that can be fractionated out to produce hyperimmune-immunoglobulin (hIVIG).[8]

CP containing high titres of polyclonal antibody (Ab), has been used to treat patients hospitalised with respiratory syndromes caused by viral infections. Many studies have been poorly controlled but such series suggested decreased mortality in H1N1 Influenza infections in 1918–1920 and in 2009/2010, SARS-CoV-1 infections in 2003 and most recently COVID-19. Recent systematic reviews lacked data from RCTs and analysis did not consider the titre used within trials.[9] Moreover, there are concerns that CP may cause harm, potentially causing severe transfusion reactions such as transfusion-associated acute lung injury (TRALI) or antibody dependent enhancement of the viral infection.[10]

Prior to the COVID-19 pandemic, studies investigating the effectiveness of CP for viral infections varied in quality and the outcomes reported may not have reflected current international guidelines.[11,12]

## 2 | OBJECTIVE

To evaluate the evidence for the safety and effectiveness of using convalescent plasma (CP) or hyperimmune immunoglobulin (hIVIG) to treat severe respiratory disease caused by coronaviruses or influenza.

## 3 | METHODS

The protocol for this review was prospectively registered on PROSPERO (CRD42020176392), and the review was carried out in accordance with Cochrane methodology and reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13]

### 3.1 | Search strategy

We searched multiple electronic databases (MEDLINE, PubMed, The Cochrane Library, Embase, Epistemonikos), ClinicalTrials.gov and WHO International Clinical Trials Registry Platform for ongoing studies, without language restriction, for all publication types on 12th October 2020 (see Appendix A1 in Data S1). We updated our search on 28th June 2021, increasing the number of databases (Cochrane COVID-19 Study Register, Transfusion Evidence Library, Web of Science). We limited the update search to systematic reviews and RCTs due to the significant number of randomised trials available at this point. Ongoing studies identified in our searches were checked on 30th November 2021 and included if published in full (peer-reviewed) by this date. We hand searched reference lists of systematic reviews and included RCTs.[11]

### 3.2 | Selection criteria

For assessments of effectiveness, we included RCTs comparing transfusion of CP products to any control arm with participants of any age who were admitted to hospital with severe respiratory illness. For assessments of safety, we included all study designs where patients received CP or hIVIG.

Two reviewers (CK, AL, LJG, SV) independently screened title and abstract, and then full-text using Covidence.

Where a publication was in a non-English language, we used electronic translation tools and sought the help of native speakers where appropriate (Appendix A2 in Data S1).

### 3.3 | Data extraction

Two of four reviewers (CK, AL, LJG, JS) independently extracted data using Covidence and Excel. Reviewers who were involved with any original trials (AL, LE) were not involved in the data extraction for those trials.

Extracted data included: details of study participants (demographic and disease characteristics), details of interventions (including titre, volume, timing of CP/hIVIG), and outcomes.

Outcomes extracted: all-cause mortality up to 30 and 90 days; need for mechanical ventilation (MV) and non-invasive ventilation (NIV) at up to 30 days; duration of MV or NIV; length of hospital stay; length of intensive care unit (ICU) stay; duration of viral detection from admission up to 30 days; transfusion-related serious adverse events (SAEs).

In a deviation from our protocol, we also assessed SAEs up to 30 days due to substantial variability in the way that SAEs were reported. For papers from the 1918 to 1920 influenza pandemic, reporting style was substantially different and, if reported, there was no grading of AEs. We recorded any potential AE described in these publications.

Where data were not available for a particular timepoint, we extracted data to the nearest possible timepoint. We sought clarification from trial authors where necessary.

### 3.4 | Risk of bias assessment

Two review authors (CK, AL, LJG, JS) independently assessed all eligible studies for risk of bias (ROB), using the Cochrane ROB tools. ROB1 for RCTs[14] and ROBINS-I for observational studies according to the Cochrane Handbook for Systematic Reviews of Interventions.[15] Reviewers who had worked on a trial (AL, LE) did not participate in ROB assessments for those studies.

Observational studies assessed as having "critical" ROB were not included in quantitative analyses.

### 3.5 | Data analysis

Statistical analyses were undertaken in Review Manager 5.4,[16] R[17] and the *metafor* package in R.[18] For dichotomous outcomes, we used the Mantel–Haenszel method, or Peto OR for rare events. We calculated the pooled risk ratio (RR) with a 95% confidence interval (CI), using the random effects model in RevMan5.[16] We used Tau$^2$ and $I^2$ in the assessment of heterogeneity, according to the guidelines laid out in the Cochrane handbook.[19]

We have not combined RCTs and non-RCTs and so have reported the results separately.

We planned to analyse continuous outcomes using mean difference (MD) or standardised mean difference (SMD) where different scales had been used. Continuous outcomes reported as median (IQR/range) could not be meta-analysed or pooled and have been reported narratively within tables.

Information from observational studies was collated in tables and not meta-analysed. Certainty of the evidence (based on meta-analysable data only) was assessed using GRADEPro.[20]

#### 3.5.1 | Subgroup and sensitivity analysis

We subgrouped included trials by the type of respiratory infection.

We also subgrouped COVID-19 studies by their use of high titre or low titre/unselected plasma (see Appendix A3 in Data S1) in response to emerging research that highlighted the wide variability in CP titres used in practice.

We intended to undertake sensitivity analyses based on selection bias to examine evidence from 'low risk' studies only. However, this was not necessary for the RCTs as all included RCTs were assessed as low (or unclear) risk for mortality endpoints within this domain.

#### 3.5.2 | *Post hoc* analysis of seropositivity

We performed a *post hoc* analysis of trials where there were sufficient data to assess the impact of SARS-CoV-2 antibody status at baseline due to emerging evidence of greater effectiveness of passive antibody therapy (monoclonal antibodies) for patients who are antibody negative at baseline.[21] Meta-regression for *post hoc* analysis of seropositivity was performed using the metafor[18] package in R.

## 4 | RESULTS

Our search yielded 4826 references (Figure 1 PRISMA flow diagram; for excluded studies see Appendix A4 in Data S1).

### 4.1 | Study Characteristics

We identified 110 completed studies (Figure 1), including 30 RCTs (four for influenza, n = 578; and 26 for COVID-19 SARS-CoV-2, *n* = 18 204).[3,7,22–49] There were no RCTs or non-randomised controlled trials identified for MERS or SARS (SARS-CoV-1) (Appendix A Supplementary Table A1 in Data S1). We included 76 non-randomised studies (Appendix B in Data S1). Of these, eleven were controlled studies, of which only two were at less than "critical" ROB[50,51] (Appendix A Supplementary Table A2 in Data S1) We included 67 uncontrolled studies: 12 assessing influenza A; two on MERS-CoV; four on SARS-CoV, and 49 on COVID-19 (SARS-CoV-2).

We also identified 143 ongoing studies (Appendix C) which were either controlled trials or single arm studies, which listed at least one safety outcome in their intended primary or secondary outcomes.

Study size in the quantitative analyses ranged from 29 to 11 555 (34 to 308 for influenza).

Of the four RCTs assessing influenza: two included children (*n* = 24/236 < 18 years)[39,45]; three RCTs[39,45,47] included pregnant women (3/270 pregnant women).

Of the 26 RCTs and 2 non-randomised studies that assessed COVID-19: one RCT included children (*n* = 26/11558 < 18 years).[3] Three RCTs[29,34,44] did not report whether they included children. Three RCTs[3,29,35] included pregnant women (*n* = 36/12575 pregnant women). Eight RCTs[22,24,30–33,36,44] did not report whether they included pregnant women.

### 4.2 | Comparisons

We identified four comparisons within the data that could be combined in quantitative analysis:

(1) CP versus standard care (SoC) or biologically inactive placebo (saline) (20 RCTs): 19 RCTs compared CP to SoC,[3,7,22–25,27–31,33–36,38,39] one RCT[26] compared SoC with saline placebo, and two retrospective observational studies[50,51] compared CP patients with matched controls;

(2) CP versus biologically active control (FFP or IVIG) (6 RCTs): five RCTs compared CP to non-immune FFP,[40–43,45] and one compared CP with IVIG.[44]

(3) hIVIG versus control (3 RCTs) Of these, two compared hIVIG with SoC,[46,47] one compared hIVIG with saline placebo.[48]

(4) early CP versus deferred CP (1 RCT).[49]

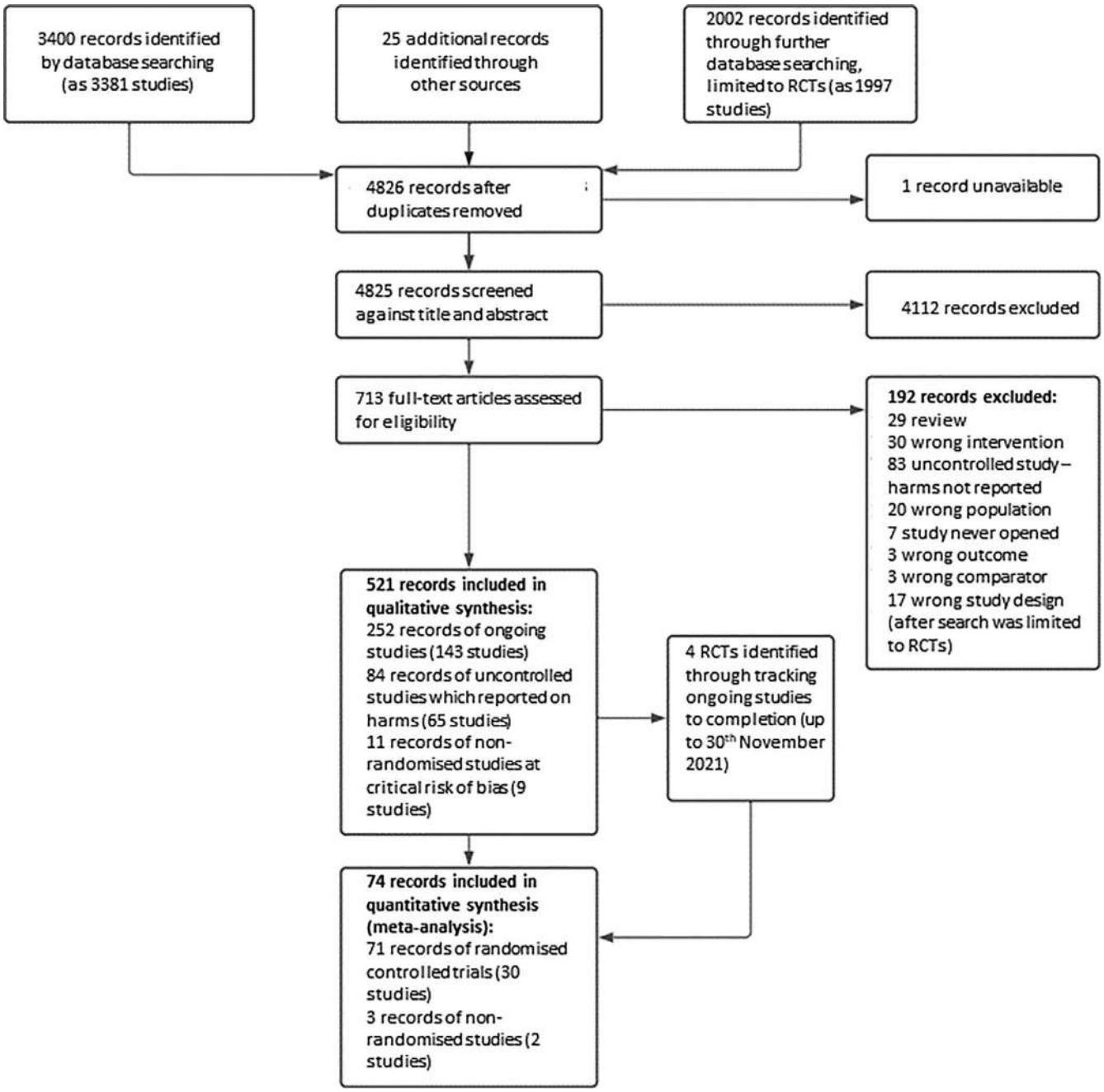


**FIGURE 1** PRISMA flow diagram. Caption: The reasons for exclusion at each stage are shown with arrows to the right.

The comparators and baseline characteristics of participants in each of the thirty RCTs and two non-RCTs (retrospective observational studies)[50,51] within meta-analyses are summarised in Appendix A Table A1 in Data S1.

## 4.3 | Outcomes

We could only extract sufficient data to meta-analyse mortality and serious adverse events. We have presented remaining data from controlled studies in tables (Appendix A, Tables A3–A6 in Data S1). A summary of all outcomes reported is available in Appendix A5.

Most trials did not describe any method for dealing with competing risks when reporting their results. A competing risk is one which prevents the event of interest from occurring. Death is a competing risk for both (time to) mechanical ventilation and (time to) discharge. Devos 2021[28] approached competing risks using competing events analysis[52] to obtain cause-specific hazard ratios (HR). REMAP-CAP[30] used ordinal logistic regression by assigning each participant a category labelled with the number of ventilator-free days up to 21 days, with people who died up to day 90 being assigned −1, people who were on MV at

**TABLE 1** Overview of meta-analysed results from patients hospitalised with severe respiratory infections

| Comparison | 30-day mortality | | 90-day mortality | Grade 3 or 4 transfusion related AEs | SAEs |
|---|---|---|---|---|---|
| **Comparison 1:** CP versus SoC or biologically inactive placebo (saline) | **All RCTs:** RR 0.99 (0.92 to 1.06) 15 RCTs[a], $n = 17\,266$ (37 children, 38 pregnant women) ⨁⨁⨁◯ $I^2 = 4\%$ $Tau^2 = 0.00$ | **High Titre subgroup:** RR 0.98 (0.93 to 1.04) 9 RCTs[b], $n = 15\,954$ (26 children, 33 pregnant women) ⨁⨁⨁⨁ $I^2 = 0\%$ $Tau^2 = 0.00$ | RR 0.92 (0.74 to 1.15) 6 RCTs[b], $n = 3210$ (8 pregnant women) ⨁⨁◯◯ $I^2 = 0\%$ $Tau^2 = 0.02$ | *No transfusion in control group; results in intervention group are summarised in table A12* | RR 1.14 (0.92 to 1.41) 13 RCTs[a], $n = 16\,730$ (37 children, 38 pregnant women) ⨁◯◯◯ $I^2 = 56\%$ $Tau^2 = 0.07$ |
| **Comparison 2:** CP versus biologically active control (FFP or IVIG) | RR 0.85 (0.56 to 1.29) 5 RCTs[a] $n = 700$ (13 children, 1 pregnant woman) ⨁◯◯◯ $I^2 = 33\%$ $Tau^2 = 0.07$ | | RR 0.99 (0.75 to 1.29) 2 RCTs[b], $n = 264$ ⨁◯◯◯ $I^2 = 0\%$ $Tau^2 = 0.00$ | POR 0.43 (0.14 to 1.33) 6 RCTs[a], $n = 716$ (13 children, 1 pregnant woman) ⨁◯◯◯ $I^2 = 4\%$ $Chi^2 = 4.18$ | RR 0.88 (0.65 to 1.19) 4 RCTs[b], $n = 523$ (13 children, 1 pregnant woman) ⨁⨁◯◯ $I^2 = 0\%$ $Tau^2 = 0.00$ |
| **Comparison 3:** hIVIG versus control | RR 0.77 (0.34 to 1.73) 3 RCTs[c] $n = 392$ ⨁◯◯◯ $I^2 = 50\%$ $Tau^2 = 0.26$ | | *No RCTs reported mortality at 90 days in this comparison* | RD 0.00 (−0.08 to 0.08) 2 RCTs[a] $n = 84$ ⨁◯◯◯ $I^2 = 0\%$ $Tau^2 = 0.00$ | RR 1.10 (0.76 to 1.58) 2 RCTs[a] $n = 342$ ⨁◯◯◯ $I^2 = n/a$ $Tau^2 = n/a$ |
| **Comparison 4:** Early CP versus deferred CP | RR 2.68 (0.56 to 12.71) 1 RCT[b], $n = 58$ ⨁◯◯◯ $I^2 = n/a$ $Tau^2 = n/a$ | | *No RCTs reported mortality at 90 days in this comparison* | *Transfusion-related AEs were only reported for patients receiving CP; results are summarised in table A12* | *No RCTs reported SAEs in this comparison* |

*Note*: Key: ⨁◯◯◯ very-low certainty evidence; ⨁⨁◯◯ low certainty evidence; ⨁⨁⨁◯ moderate certainty evidence; ⨁⨁⨁⨁ high certainty evidence.
Abbreviations: POR, Peto odds ratio; RD, risk difference; RR, risk ratio.
[a]Includes 1 RCT in influenza.
[b]All COVID-19.
[c]Includes 2 RCTs in influenza.

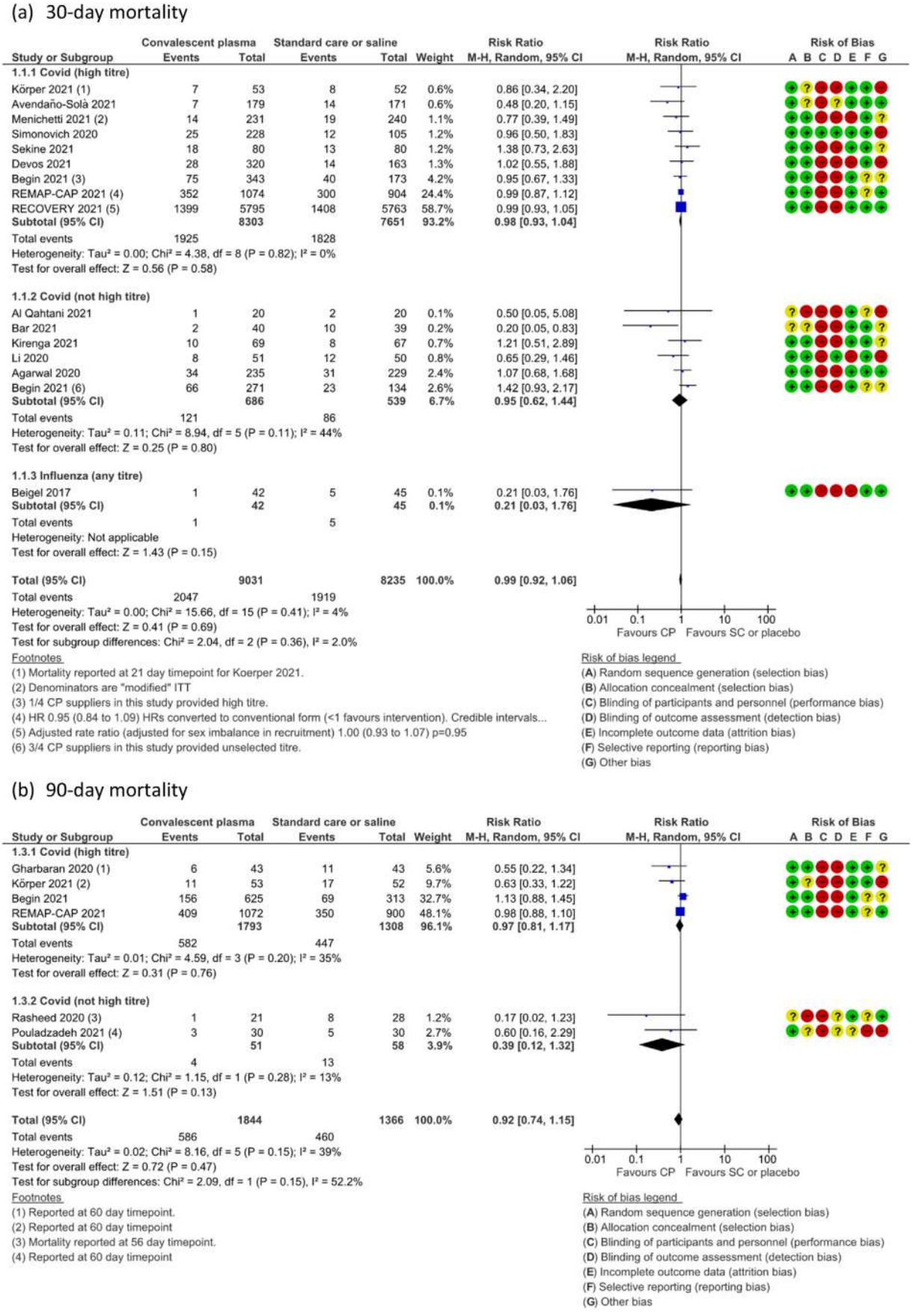


**FIGURE 2** Forest plot of all-cause mortality, for comparison 1 (CP compared to SoC or a biologically inactive placebo) at up to (A) 30 days, and (B) 90 days

randomisation being assigned 0, and people who remained ventilator-free beyond day 21 being assigned 22. This is a useful way to compare the two groups while accounting for the very different possible outcomes but the resulting odds ratio (OR) and medians are difficult to interpret. No other trials used these methods and so we cannot combine the results but instead report the summary within Table A4 in Data S1.

Duration of viral detection was expressed as time (median IQR) to first negative test (2 RCTs).[23,36] One study,[25] reported the number of patients who had had two consecutive negative tests by day 30. See table A5 for viral detection data and table A6 for details of changes in viral loads.

## 4.4 | ROB in included studies

### 4.4.1 | RCTs (using Cochrane ROB1)

Nineteen RCTs were open-label, comparing CP to SoC, and were therefore assessed as having a high ROB for all outcomes except mortality, as knowledge of treatment allocation may have affected clinical decision-making. A summary of ROB judgements is available in Table A7 and Figure A1 in Data S1.

### 4.4.2 | Non-RCTs (using ROBINS-I)

Two non-RCTs[50,51] were assessed at serious RoB for selection bias and confounding at baseline. The remaining 9 studies[53–61] were at critical ROB due to baseline confounding or selection bias and were therefore not meta-analysed.

## 4.5 | Certainty of the evidence (GRADE)

Certainty of the evidence was GRADEd as very-low to high; primary reasons for downgrading were ROB and imprecision (wide confidence intervals and small sample size) (Tables A8–A11 in Data S1). We assessed publication bias through the generation of a funnel plot (Figure A2 in Data S1) for 30-day mortality in comparison 1, which suggests that some small studies have not been published. However, this was not significant enough to downgrade the certainty of the evidence because the analysis is dominated by two large, high-quality, and RCTs.

## 4.6 | Effect of the Intervention

See Table 1 for an overview of meta-analysed results.

### 4.6.1 | Comparison 1: CP versus SoC or biologically inactive placebo

Twenty RCTs and two retrospective studies assessed CP compared with SoC or a biologically inactive placebo.

*All-cause mortality*

30-day mortality data were available from 15 RCTs (30 days, 5 RCTs; 28 days, 9 RCTs; 21 days, 1 RCT) (Figure 2a); 90-day mortality data were available from 6 RCTs (56 days, 1 RCT; 60 days, 3 RCTs; 90 days, 2 RCTs) (Figure 2b).

Overall, CP did not reduce 30-day mortality (15 RCTs, n = 17 266; moderate-to-high certainty of evidence [Table A8 and footnotes in Data S1]) and there may be no effect on 90-day mortality (6 RCTs $n = 3210$; low certainty of evidence [Table A8]).

Two non-RCTs reported in-hospital mortality, and showed results consistent with the randomised evidence (2 studies, $n = 436$; very-low certainty evidence) (Figure A3A Table A8 in Data S1).

*Improvement of clinical symptoms*

Duration of NIV was reported in 4 studies (2 RCTs),[3,24,50,51] and duration of MV was reported by 11 studies (9 RCTs).[3,24,25,28–30,35,38,39,50,51] Two RCTs[27,31] reported any ventilatory support, but did not differentiate between MV, NIV, and passive oxygen support. One RCT[29] reported any ventilation, but also reported separately a composite outcome of patients who progressed to MV or death. Most studies reported the data as duration of support, either median (IQR) or mean (SD) (Table A4 in Data S1).

These outcomes were very variably reported, and many did not fully account for competing events, or report methods of analysis in sufficient detail. Based on what was reported, there was no apparent difference in duration of MV, NIV or ECMO support between the two groups.

*Length of stay (LOS): hospital and ICU*

Length of hospital stay was reported by 16 RCTs[7,23,25–28,30,31,38,39,42–47] and 1 non-RCT,[51] and length of ICU stay was reported by 9 RCTs[23,26,28,29,33,39,43,45,47] (Table A3 in Data S1). There was no evidence of an effect in length of hospital stay or length of ICU stay (Table A3 in Data S1).

*Duration of viral detection from admission up to 30 days (viraemia, nasopharyngeal swabs, bronchoalveolar lavage, stool)*

The 3 RCTs which reported time to negative test do not suggest any evidence of an effect (Table A5 in Data S1).

*Adverse events*

AEs due to transfusion were reported in 15 RCTs[3,7,22–39] (Table S10 in Data S1).

Seven RCTs reported no Grade 3 or 4 AEs due to transfusion.[22,24,26,27,31,35,39] Both non-RCTs reported AEs due to transfusion. All but one RCT[26] had SoC comparators, and therefore no transfusion-related SAEs are reported for the control group. Group comparison was not possible; results are summarised in Table A12 of in Data S1.

There was no evidence of an effect on reported SAEs[3,23–31,35,36,39] (13 RCTs, $n = 16\,730$, very-low certainty of evidence) (Figure A3B).

Data were not available on SAEs in seven RCTs.[7,22,32–34,37,38]

See forest plots Figure A3 in Data S1 and GRADE profile Table A8 in Data S1 for further detail.

### 4.6.2 | Comparison 2: CP versus biologically active control (FFP or IVIG)

RCTS assessed CP compared to FFP[40–43,45] or IVIG[44]

*All-cause mortality*

There was insufficient evidence to say whether or not there is a difference between groups in all-cause mortality at up to 30 days (5 RCTs $n = 700$; very-low certainty evidence, Figure A4A in Data S1), or at up to 90 days (2 RCTs, $n = 264$; very-low certainty evidence Figure A4B in Data S1). See forest plots Figures A4A and A4B in Data S1 and GRADE profile Table A9 in Data S1 for further detail.

*Adverse events*

Six RCTs reported transfusion-related Grade 3 or 4 AEs.[40–45] Events were rare (~2%) with no clear evidence of a difference (6 RCTs, $n = 716$; very-low certainty evidence. [Figure A4C in Data S1]). Four RCTs[40–42,45] reported SAEs up to 30 days, showing no evidence of an effect, although the rate of SAEs seems very low, given the severity of disease in hospitalised individuals (4 RCTs, $n = 523$; low certainty evidence, Figure A4D in Data S1). See forest plots Figure A4 and GRADE profile Table A9 in Data S1 for further detail.

*Improvement of clinical symptoms*

Duration of MV[40,43,45] and any ventilatory support[41] were reported as median (IQR) or mean (SD). Given the difficulties of dealing with competing events, and the small number of patients involved, it is very unclear if CP therapy had any effect on the duration of MV, NIV or ECMO support between the two groups. We have presented the data in Table A4 in Data S1 as reported by the individual studies.

Data were not available for LOS (hospital or ICU), and duration of viral load.

### 4.6.3 | Comparison 3: hyperimmune immunoglobulin versus control

Three assessed hIVIG compared with SoC or a biologically inactive placebo.

*All-cause mortality*

There was insufficient evidence to say whether or not there is an effect on mortality compared to control at up to 30 days (3 RCTs $n = 392$; very-low certainty evidence) (Table 1, Figure A5A, Table A10 in Data S1). There were no data for 90-day mortality.

*Adverse events*

Two RCTs reported transfusion-related AEs; neither reported any AEs due to transfusion in either group (2 RCTs, $n = 84$; very-low certainty evidence, Figure A5B in Data S1). Two RCTs reported SAES (2 RCTs $n = 342$; very-low certainty evidence. [Figure A5C in Data S1]). See forest plots Figure A5 and GRADE profile Table A10 in Data S1 for further detail.

*Improvement of clinical symptoms*

One RCT in influenza[48] reported on duration of MV and NIV. However, the data were presented using an ordinal scale that was not mappable to our outcomes or other trial results, and we were unable to extract the data.

Data were not available for LOS (hospital or ICU), and duration of viral load.

### 4.6.4 | Comparison 4: early CP versus deferred CP

One RCT assessed early CP compared to deferred CP.

*All-cause mortality*

There was insufficient evidence to say whether there is a difference in 30-day mortality between early CP and deferred CP (1 RCT $n = 58$; very-low certainty of evidence) (Figure A6 in Data S1). There were no data for 90-day mortality. See forest plots Figure A6 and GRADE profile Table A11 in Data S1 for further detail.

*Adverse events*

There were three Grade 3 or 4 transfusion-related AEs within 24 h, all in the early CP group: (1 RCT $n = 58$, very-low certainty evidence) (Table A12 in Data S1). SAEs were not reported. See forest plots and GRADE profile Table A11 in Data S1 for further detail.

*Improvement of clinical symptoms*

Duration of MV and NIV was reported as median (IQR). We have presented the data in Table A4 in Data S1 as reported by the RCT. Both groups had similar duration of ventilatory support. It is unclear if the authors accounted for competing events.

Data were not available for LOS (hospital or ICU), and duration of viral load.

## 4.7 | Results from uncontrolled studies (for safety only)

We identified 73 non-randomised or uncontrolled studies [49 case reports or case series] that assessed the use of CP or hIVIG in respiratory viral infection and reported AEs: 12 in influenza A, 2 in MERS-CoV, and 4 in SARS-CoV-1, and 67 in SARS-CoV-2 (COVID-19). Of the influenza studies, 10 were from the 1918 to 1920 pandemic. Fifty-one studies reported that no AEs were observed (37/49 case reports or case series). Eighteen studies reported transfusion-related AEs, and four studies reported other SAEs. These data are presented in Appendix B in Data S1.

## 4.8 | Post hoc subgroup analysis: seropositivity at baseline

Three RCTs,[3,30,62] including the two largest, reported 30-day mortality for subgroups defined by seropositivity at baseline. These results are shown in Figure 3.

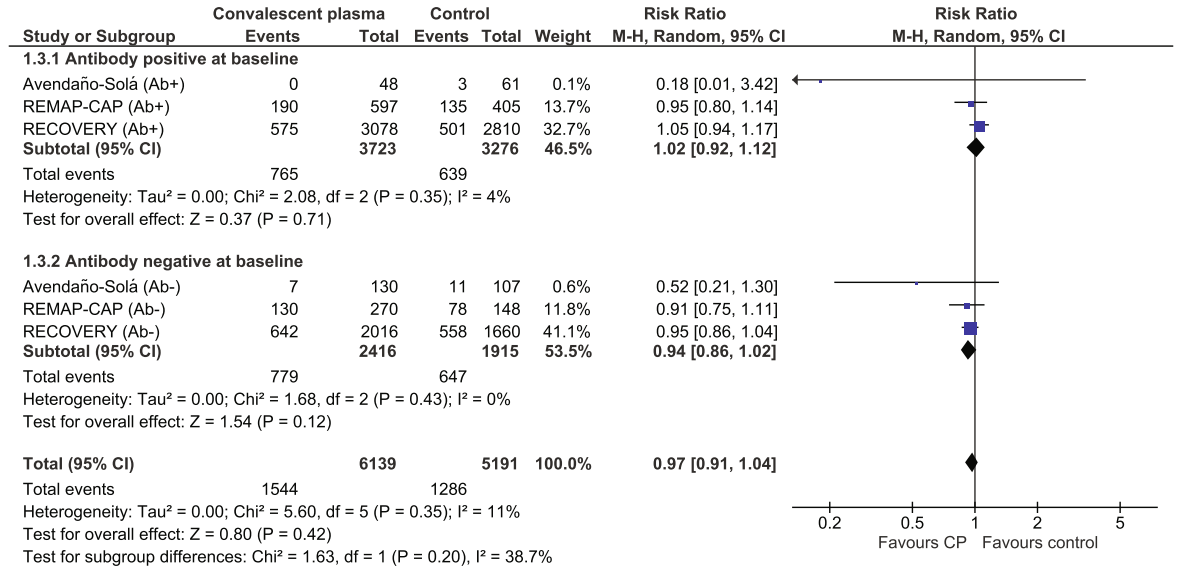


**FIGURE 3** Subgrouped by seropositivity at baseline: RCTs reporting 30-day mortality for comparison 1 (CP compared to SoC or a biologically inactive placebo)

With almost all the information coming from the two large, high-quality RCTs,[3,30] the pooled estimates from these three RCTs are: RR 1.02 (0.92 to 1.12) for people who are seropositive at baseline and 0.94 (0.86 to 1.02) for those who are seronegative. The test for interaction (subgroup difference) gives a *p*-value of 0.20 with very little heterogeneity either within or between groups.

We explored this further using meta-regression on the group of trials comparing high titre CP with SoC which reported the proportion seropositive at baseline and 30-day mortality. This analysis produced near identical results with an estimated RR at 0% seropositivity of 0.93 (0.85, 1.01) and 1.02 (0.93, 1.12) at 100% seropositivity (See Appendix A6 in Data S1. Mortality results are summarised in Table A14 in Data S1).

## 5 | DISCUSSION

The objective of this review was to determine the safety and effectiveness of CP or hIVIG from CP to treat patients with serious respiratory disease due to influenza or coronavirus infection. In order to increase the relevance of our findings to the COVID-19 pandemic we used the core outcome set[63] for assessing treatments for patients infected with SARS-CoV-2. We aimed to use high-quality evidence from RCTs to assess safety and effectiveness. We also used all other study designs to describe serious harms reported following transfusion with CP or hIVIG.

### 5.1 | Main findings

We were able to meta-analyse 32 studies for our primary outcome of 30-day mortality (30 RCTs and 2 non-RCTs). We found little evidence of any difference between the groups in either benefits or harms for patients hospitalised with a severe viral respiratory infection requiring hospital admission. Most evidence was of low or very-low certainty. The only high-certainty evidence was for the COVID high-titre subgroup in the outcome all-cause mortality at up to 30 days in CP versus SoC (Table 1).

Adverse events were variably reported. No RCTs reported a high number of transfusion-related AEs (proportion 0% to 5.67%[22–24,26,27,31,35,38,39,43,44,46,47]) (very-low to low certainty evidence). There was no evidence of an increase in harms compared with standard plasma.

### 5.2 | Quality (certainty) of the evidence

Where meta-analysis was possible, we used GRADE to assess our certainty in the result (Table 1). Certainty in the evidence was assessed as very-low to low certainty for all outcomes apart from mortality data in the comparison CP versus standard care.

Evidence was downgraded for serious ROB (lack of blinding, baseline imbalance, randomisation processes, missing data and unclear reporting of outcomes) and imprecision (wide confidence intervals around the effect estimate, and small sample sizes for the outcome of interest). Some of the sources of potential bias (such as patient and personnel blinding) would be hard to overcome in future trials due to the issues in finding an ethical control infusion: even saline is problematic, with the risk of volume overload, and ease with which it can be differentiated from plasma.

SAEs were also downgraded for inconsistency as the heterogeneity was significant between studies, this is likely to be due to the variation in reporting of the SAEs. This may be in part due to differing regulatory environments and different classifications of CP, requiring

varying levels of AE reporting including the need to use a grading system (e.g., MedDRA[64]).

We included lower-level evidence for the assessment of safety outcomes. However, we were unable to perform quantitative analyses, and so have only presented these data as reported in Appendix B in Data S1.

There were very few endpoints reported consistently enough for meta-analysis. The difficulty in defining endpoints, especially time-to-event endpoints,[65] is discussed further in Appendix A6 in Data S1.

## 5.3 | Strengths and Limitations of this review

We have attempted to minimise potential bias in the review process, using Cochrane methods and PRISMA guidelines for reporting. We conducted a comprehensive search: searching data sources to ensure that all relevant studies would be captured, using multiple databases and reference lists of included studies. We included conference proceedings and included a search of clinical trial registries. We also attempted to contact authors for additional data and for clarification of their data.

There were no restrictions for the language in which the paper was originally published. We pre-specified outcomes prior to analysis and have explained the rationale for including one additional outcome (any SAEs).

We undertook duplicate screening, data extraction, and assessment of bias. Additionally, the clinical advisor (LE) was consulted for disagreements, or need for clarification.

The limitations of this review mostly arose due to gaps in the evidence base, which are discussed more fully in the next section.

## 5.4 | Interpretation and context

A recent analysis of individual patient data (IPD) pooled from eight RCTs[9] IPD reported an OR for mortality of 0.85 at day 28 (95% credible interval, 0.62 to 1.18; posterior probability of OR <1 of 84%). These results are broadly comparable and in agreement with our own aggregate analyses for 30-day mortality. However, it should be noted that the IPD analysis included two RCTs[66,67] published after our 30th November 2021 cut-off, but did not include the two largest RCTs of CP RECOVERY[3] and REMAP-CAP[30] which we have analysed, and which together contribute 83% of sample size contributing to our analysis of 30-day mortality for CP versus SoC.

A limitation of the current evidence base is that of the 30 RCTs and two non-randomised studies included in our meta-analysis, 26 studies (24 RCTs) excluded children and 16 RCTs excluded pregnant women, with 1 RCT[39] admitting pregnant women only on the second round of recruitment. Given that children and pregnant women are both considered to be at increased risk of serious disease and death from many severe respiratory viral infections, their exclusion from trials is concerning. Of the 144 ongoing studies we identified, most trials will exclude children and pregnant women. Many ongoing studies have an upper age cut-off (of 65, 70 or 80 years), despite older age being one of the biggest risk factors for COVID-19.

The precision of our meta-analysis was affected by the different titres of CP-neutralising antibodies between trials (Table A1 in Data S1). We tried to address this by subgrouping studies based on the CP-titre reported, and whether it was considered high enough according to FDA criteria (see Appendix A3 in Data S1). However, several studies used local assays that could not be correlated with an FDA reference method. Since we conducted our first search, several variants of SARS-CoV-2 have arisen worldwide and may require much higher antibody titres measured using ELISA assays.[68] Much higher titre CP, from vaccinated convalescent donors, may be active against future variants[69] indicating that new COVID CP trials should aim to use very high titre CP standardised using internationally recognised methods.

Similarly, between trials, there was heterogeneity of patient groups and severity of illness on admission to hospital (Table 1). The RCTs in COVID may not have used the same criteria to categorise trial participants at enrolment and trials designed to treat different patient groups based on comorbidities and immune states were absent. Several COVID-19 studies reported clinical improvement using the WHO ordinal scale. However, the scale was revised several times over the course of 2020–2021, going from an 8-point scale[70] to a 10-point scale at its latest revision[71] which have made comparisons between trials difficult.

The results of our post hoc subgroup analysis by seropositivity at baseline are very similar to the results reported by RECOVERY alone. We have not found stronger evidence of this potential interaction than that reported by RECOVERY (with a similar trend also reported by REMAP-CAP, especially for organ support-free days) but similarly, we have not found any reason to discount the possibility that there is a small but important interaction, with immunocompromised individuals potentially benefitting more. This hypothesis is consistent with the REGN-COV2 RECOVERY trial,[21] which has shown no benefit of monoclonal antibodies for seropositive patients who either have advanced disease or who are immunocompetent. The very high baseline risk of immunocompromised individuals might translate very small relative risks into substantial absolute risk differences. REMAP-CAP has recently reopened for immunocompromised people to test this hypothesis.[72]

## 5.5 | Implications for research and practice

There is currently no evidence for a benefit of CP in an unselected population of patients hospitalised with coronaviruses or influenza. It is likely that the titre of the CP and the immune response of the recipient may both be important factors affecting response to treatment.

Studies should use CP of a high enough titre to elicit a biological response, and report the actual titre used as well as the minimum as described in the protocol. Matching variants between donor and recipient may not be feasible, but viral variants circulating at the time of collection of plasma and during the study should be recorded.

Studies should assess and publish antibody status (seropositivity) at baseline in both intervention and control groups, and identify and

report immunocompromised patients separately, to establish whether certain groups of patients are more likely to benefit from this intervention.

There are difficulties in designing truly blinded RCTs of CP or hIVIG (see Reference 73 for review). There are ethical problems with using a placebo which is assumed to have no clinical benefit, but has known harms.[74] One RCT[26] used a saline placebo, with potential concerns about volume overload, and six RCTs used a biologically active control, (FFP in 5 RCTs,[40–43,45] and IVIG in one[44]) which raises additional concerns about transfusion reactions.

Unless reported explicitly by investigators, it was difficult to distinguish the AEs experienced following transfusion from the symptoms of severe respiratory disease.[75] This limited the number of RCTs that we could include in our meta-analysis of AEs due to transfusion. There was also substantial variability in the way that AEs were recorded and reported in these studies. It was not always possible to determine the severity of AEs, and different studies used different criteria for SAEs. In some cases, it was hard to determine if SAE reporting was per event or per patient, making it extremely difficult to compare rates of AEs between studies. Blood components in the UK are not classified as medicines and so require a different grading system for reporting AEs to countries that classify CP as a medicine, e.g. Germany. A consensus on how AEs associated with blood products are reported in RCTs would help to address this problem.

## 6 | CONCLUSION

This review has highlighted several issues regarding study design and reporting which should be addressed in current and future research. A minimum titre should be established and ensured for a positive biological response to the therapy. Further research on the impact of CP/hIVIG in patients who have not produced antibodies to the virus prior to hospital admission or who are immunocompromised would be useful to target therapies at groups who will potentially benefit the most.

## AUTHOR CONTRIBUTIONS

**Catherine Kimber:** screening and full text assessment, retrieved full text publications, data extraction, risk of bias assessment, entered data into RevMan and undertook subgroup analyses, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Abigail A. Lamikanra:** screening and full text assessment, retrieved full text publications, data extraction, risk of bias assessment, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Louise J. Geneen:** screening and full text assessment, retrieved full text publications, data extraction, risk of bias assessment, entered data into RevMan and undertook subgroup analyses, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Josie Sandercock:** data extraction, risk of bias assessment, and undertook all metaregression analyses, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Carolyn Doree:** developed and performed all search strategies and de-duplication, retrieved full text publications, contributed to the development of the manuscript. **Sarah J. Valk:** screening and full text assessment, retrieved full text publications, contributed to the development of the manuscript. **Lise J. Estcourt:** developed the initial idea of the review, developed, wrote, and registered the protocol, interpreted the results, and contributed to the development of the manuscript.

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## ORCID

*Catherine Kimber* https://orcid.org/0000-0002-7796-9931
*Abigail A. Lamikanra* https://orcid.org/0000-0002-9287-2177
*Louise J. Geneen* https://orcid.org/0000-0001-6759-0437
*Josie Sandercock* https://orcid.org/0000-0001-7337-8635
*Carolyn Dorée* https://orcid.org/0000-0001-6955-7497
*Sarah J. Valk* https://orcid.org/0000-0003-3964-2505
*Lise J. Estcourt* https://orcid.org/0000-0003-4309-9162

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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

# Prevalence and specificity of red blood cell antibodies in patients transfused in tertiary hospitals in Burkina Faso

**Salam Sawadogo** [1,2] | **Koumpingnin Nébié** [1] | **Catherine Traoré** [1] | **Yérémadé Juste Bonzi, Mariam Boro** [2] | **Dieudonné Yonli** [2] | **Joseph Yaméogo** [2]

[1]Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Leeds, UK

[2]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK

[3]Leeds Orthopaedic & Trauma Sciences, Leeds General Infirmary, University of Leeds, Leeds, UK

[4]Leeds Institute of Health Sciences, University of Leeds, Leeds, UK

[5]NIHR Leeds Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK

Correspondence
Michalis Panteli, Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Clarendon Wing, Level D, Great George Street, Leeds LS1 3EX, West Yorkshire, UK.

Email: salemserein@hotmail.com ;

## Abstract

### Background

Sub-Saharan African countries face the challenge of immunological transfusion safety that puts many patients at risk of post-transfusion hemolytic reactions. This is because pre-transfusion testing for irregular/unexpected antibodies that helps to prevent these risks are neither universally available nor accessible. The aim of our study was to determine the prevalence of red blood cell alloantibodies and their specificity in patients transfused in Burkina Faso.

### Materials and Methods

This was a cross-sectional study including patients who had received at least one blood transfusion. Indirect antiglobulin testing using LISS-enhanced medium gel column agglutination technique was used for antibodies screening and identification. Enzymatic technique with papain-treated red cell reagent was performed in attempt to solve some difficulties if necessary as well as auto-control test and RH-KEL phenotyping when possible to help antibodies identification.

### Results

A total of 832 patients were included, 51.6% of whom were female, and the median (IQR) age was 34 (20-49) years. The median (IQR) number of immunisation episodes (blood transfusion and pregnancies) was 3 (2-6) with the median (IQR) number of blood units received per patient of 2 (1-5). The proportion of patients with RBCs antibodies was 6.4% (53/832), with mainly anti-Rh antibodies. A combination of 2 antibodies was found in 7 patients and a combination of 3 antibodies in one patient. Antibodies of unknown specificity (AUS) were encountered in 29%. Independent factors associated with antibody positivity were age (OR = 1.02; p = 0.026), sickle cell disease (OR = 3.23; p = 0.017) and receiving more than 10 blood units (OR = 7.33; p = 0.01).

### Conclusion

In this study, the proportion of patients with RBC antibodies was quite similar to that observed in Sub-Saharan African countries. However, the availability and accessibility of pre-transfusion compatibility tests as well as the quality of methods used should be improved to ensure the safety of blood transfusions..

K E Y W O R D S

RH-KEL phenotyping, IQR, Blood, Transfusion, Donor, Medecine

## 1 | INTRODUCTION

Severe acute respiratory infections caused by strains of influenza or coronavirus often lead to hospitalisation and sometimes death. Symptomatic infection with SARS CoV-2 (COVID-19) has surpassed the annual global burden of death due to influenza or coronaviruses.[1] Although there are several effective vaccines for COVID-19 therapeutic treatments are still required. Patients particularly at risk are those with disorders that affect the immune system, for example, haematological malignancies or those receiving drugs that suppress an immune response, for example, after organ transplantation.[2,3]

Passive antibody therapies, including monoclonal antibody combinations have proven effective for COVID-19[4] However, the cost of these therapies is prohibitive[5] and new SARS-CoV variants may become resistant to anti-virals developed in response to previous variants.[6] Alternative and affordable responses to emerging strains of virus are needed.

Convalescent plasma (CP) is typically collected from donors with confirmed diagnosis of infection at least 2 weeks after recovery.[7] CP contains neutralising antibodies specific to the infectious agent but may also contain other immune modulators and clotting factors that can be fractionated out to produce hyperimmune-immunoglobulin (hIVIG).[8]

CP containing high titres of polyclonal antibody (Ab), has been used to treat patients hospitalised with respiratory syndromes caused by viral infections. Many studies have been poorly controlled but such series suggested decreased mortality in H1N1 Influenza infections in 1918–1920 and in 2009/2010, SARS-CoV-1 infections in 2003 and most recently COVID-19. Recent systematic reviews lacked data from RCTs and analysis did not consider the titre used within trials.[9] Moreover, there are concerns that CP may cause harm, potentially causing severe transfusion reactions such as transfusion-associated acute lung injury (TRALI) or antibody dependent enhancement of the viral infection.[10]

Prior to the COVID-19 pandemic, studies investigating the effectiveness of CP for viral infections varied in quality and the outcomes reported may not have reflected current international guidelines.[11,12]

## 2 | OBJECTIVE

To evaluate the evidence for the safety and effectiveness of using convalescent plasma (CP) or hyperimmune immunoglobulin (hIVIG) to treat severe respiratory disease caused by coronaviruses or influenza.

## 3 | METHODS

The protocol for this review was prospectively registered on PROSPERO (CRD42020176392), and the review was carried out in accordance with Cochrane methodology and reported using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[13]

### 3.1 | Search strategy

We searched multiple electronic databases (MEDLINE, PubMed, The Cochrane Library, Embase, Epistemonikos), ClinicalTrials.gov and WHO International Clinical Trials Registry Platform for ongoing studies, without language restriction, for all publication types on 12th October 2020 (see Appendix A1 in Data S1). We updated our search on 28th June 2021, increasing the number of databases (Cochrane COVID-19 Study Register, Transfusion Evidence Library, Web of Science). We limited the update search to systematic reviews and RCTs due to the significant number of randomised trials available at this point. Ongoing studies identified in our searches were checked on 30th November 2021 and included if published in full (peer-reviewed) by this date. We hand searched reference lists of systematic reviews and included RCTs.[11]

### 3.2 | Selection criteria

For assessments of effectiveness, we included RCTs comparing transfusion of CP products to any control arm with participants of any age who were admitted to hospital with severe respiratory illness. For assessments of safety, we included all study designs where patients received CP or hIVIG.

Two reviewers (CK, AL, LJG, SV) independently screened title and abstract, and then full-text using Covidence.

Where a publication was in a non-English language, we used electronic translation tools and sought the help of native speakers where appropriate (Appendix A2 in Data S1).

### 3.3 | Data extraction

Two of four reviewers (CK, AL, LJG, JS) independently extracted data using Covidence and Excel. Reviewers who were involved with any original trials (AL, LE) were not involved in the data extraction for those trials.

Extracted data included: details of study participants (demographic and disease characteristics), details of interventions (including titre, volume, timing of CP/hIVIG), and outcomes.

Outcomes extracted: all-cause mortality up to 30 and 90 days; need for mechanical ventilation (MV) and non-invasive ventilation (NIV) at up to 30 days; duration of MV or NIV; length of hospital stay; length of intensive care unit (ICU) stay; duration of viral detection from admission up to 30 days; transfusion-related serious adverse events (SAEs).

In a deviation from our protocol, we also assessed SAEs up to 30 days due to substantial variability in the way that SAEs were reported. For papers from the 1918 to 1920 influenza pandemic, reporting style was substantially different and, if reported, there was no grading of AEs. We recorded any potential AE described in these publications.

Where data were not available for a particular timepoint, we extracted data to the nearest possible timepoint. We sought clarification from trial authors where necessary.

### 3.4 | Risk of bias assessment

Two review authors (CK, AL, LJG, JS) independently assessed all eligible studies for risk of bias (ROB), using the Cochrane ROB tools. ROB1 for RCTs[14] and ROBINS-I for observational studies according to the Cochrane Handbook for Systematic Reviews of Interventions.[15] Reviewers who had worked on a trial (AL, LE) did not participate in ROB assessments for those studies.

Observational studies assessed as having "critical" ROB were not included in quantitative analyses.

### 3.5 | Data analysis

Statistical analyses were undertaken in Review Manager 5.4,[16] R[17] and the *metafor* package in R.[18] For dichotomous outcomes, we used the Mantel–Haenszel method, or Peto OR for rare events. We calculated the pooled risk ratio (RR) with a 95% confidence interval (CI), using the random effects model in RevMan5.[16] We used Tau$^2$ and $I^2$ in the assessment of heterogeneity, according to the guidelines laid out in the Cochrane handbook.[19]

We have not combined RCTs and non-RCTs and so have reported the results separately.

We planned to analyse continuous outcomes using mean difference (MD) or standardised mean difference (SMD) where different scales had been used. Continuous outcomes reported as median (IQR/range) could not be meta-analysed or pooled and have been reported narratively within tables.

Information from observational studies was collated in tables and not meta-analysed. Certainty of the evidence (based on meta-analysable data only) was assessed using GRADEPro.[20]

#### 3.5.1 | Subgroup and sensitivity analysis

We subgrouped included trials by the type of respiratory infection.

We also subgrouped COVID-19 studies by their use of high titre or low titre/unselected plasma (see Appendix A3 in Data S1) in response to emerging research that highlighted the wide variability in CP titres used in practice.

We intended to undertake sensitivity analyses based on selection bias to examine evidence from 'low risk' studies only. However, this was not necessary for the RCTs as all included RCTs were assessed as low (or unclear) risk for mortality endpoints within this domain.

#### 3.5.2 | *Post hoc* analysis of seropositivity

We performed a *post hoc* analysis of trials where there were sufficient data to assess the impact of SARS-CoV-2 antibody status at baseline due to emerging evidence of greater effectiveness of passive antibody therapy (monoclonal antibodies) for patients who are antibody negative at baseline.[21] Meta-regression for *post hoc* analysis of seropositivity was performed using the metafor[18] package in R.

## 4 | RESULTS

Our search yielded 4826 references (Figure 1 PRISMA flow diagram; for excluded studies see Appendix A4 in Data S1).

### 4.1 | Study Characteristics

We identified 110 completed studies (Figure 1), including 30 RCTs (four for influenza, n = 578; and 26 for COVID-19 SARS-CoV-2, *n* = 18 204).[3,7,22–49] There were no RCTs or non-randomised controlled trials identified for MERS or SARS (SARS-CoV-1) (Appendix A Supplementary Table A1 in Data S1). We included 76 non-randomised studies (Appendix B in Data S1). Of these, eleven were controlled studies, of which only two were at less than "critical" ROB[50,51] (Appendix A Supplementary Table A2 in Data S1) We included 67 uncontrolled studies: 12 assessing influenza A; two on MERS-CoV; four on SARS-CoV, and 49 on COVID-19 (SARS-CoV-2).

We also identified 143 ongoing studies (Appendix C) which were either controlled trials or single arm studies, which listed at least one safety outcome in their intended primary or secondary outcomes.

Study size in the quantitative analyses ranged from 29 to 11 555 (34 to 308 for influenza).

Of the four RCTs assessing influenza: two included children (*n* = 24/236 < 18 years)[39,45]; three RCTs[39,45,47] included pregnant women (3/270 pregnant women).

Of the 26 RCTs and 2 non-randomised studies that assessed COVID-19: one RCT included children (*n* = 26/11558 < 18 years).[3] Three RCTs[29,34,44] did not report whether they included children. Three RCTs[3,29,35] included pregnant women (*n* = 36/12575 pregnant women). Eight RCTs[22,24,30–33,36,44] did not report whether they included pregnant women.

### 4.2 | Comparisons

We identified four comparisons within the data that could be combined in quantitative analysis:

(1) CP versus standard care (SoC) or biologically inactive placebo (saline) (20 RCTs): 19 RCTs compared CP to SoC,[3,7,22–25,27–31,33–36,38,39] one RCT[26] compared SoC with saline placebo, and two retrospective observational studies[50,51] compared CP patients with matched controls;

(2) CP versus biologically active control (FFP or IVIG) (6 RCTs): five RCTs compared CP to non-immune FFP,[40–43,45] and one compared CP with IVIG.[44]

(3) hIVIG versus control (3 RCTs) Of these, two compared hIVIG with SoC,[46,47] one compared hIVIG with saline placebo.[48]

(4) early CP versus deferred CP (1 RCT).[49]

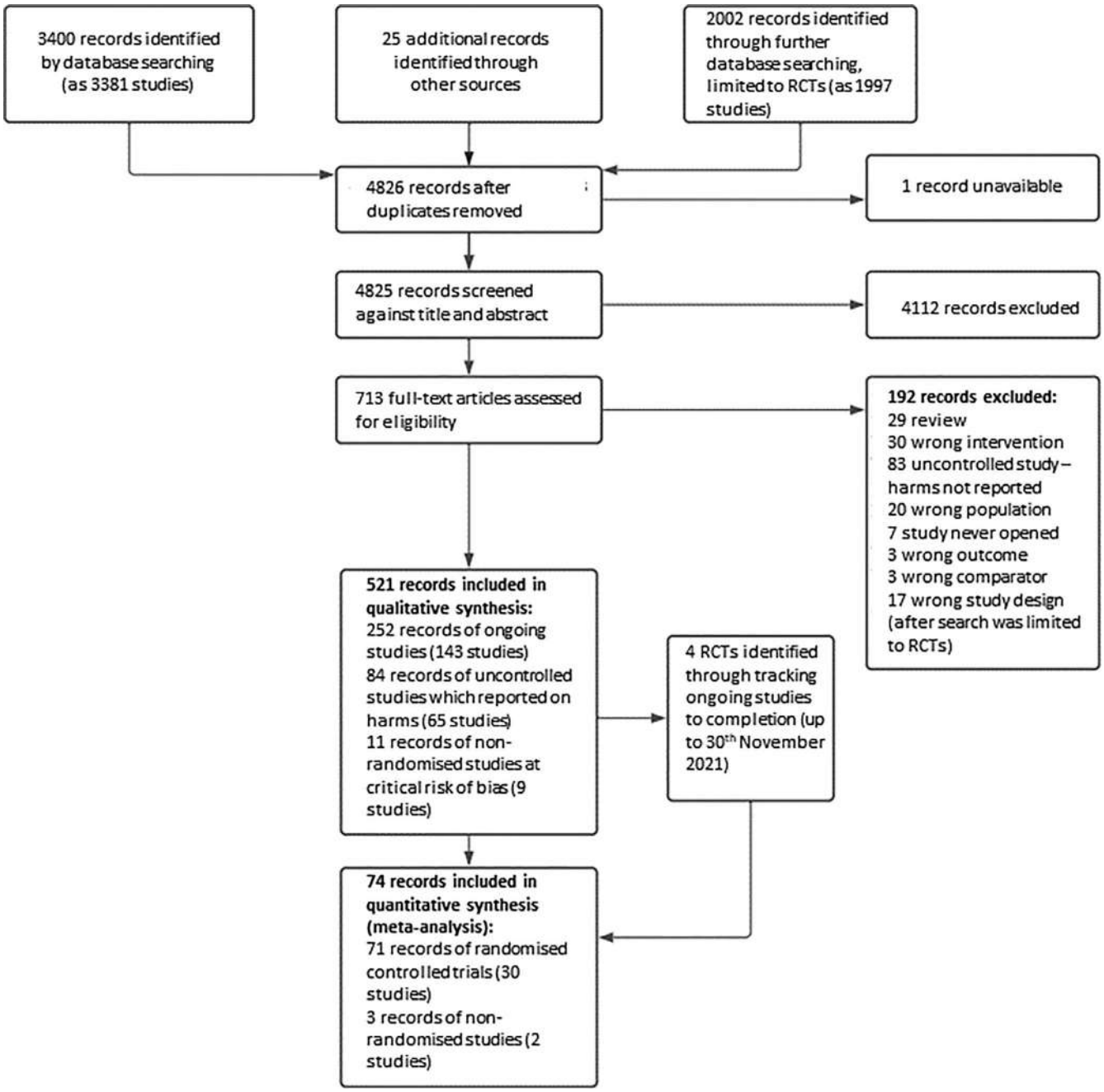


**FIGURE 1** PRISMA flow diagram. Caption: The reasons for exclusion at each stage are shown with arrows to the right.

The comparators and baseline characteristics of participants in each of the thirty RCTs and two non-RCTs (retrospective observational studies)[50,51] within meta-analyses are summarised in Appendix A Table A1 in Data S1.

## 4.3 | Outcomes

We could only extract sufficient data to meta-analyse mortality and serious adverse events. We have presented remaining data from controlled studies in tables (Appendix A, Tables A3–A6 in Data S1). A summary of all outcomes reported is available in Appendix A5.

Most trials did not describe any method for dealing with competing risks when reporting their results. A competing risk is one which prevents the event of interest from occurring. Death is a competing risk for both (time to) mechanical ventilation and (time to) discharge. Devos 2021[28] approached competing risks using competing events analysis[52] to obtain cause-specific hazard ratios (HR). REMAP-CAP[30] used ordinal logistic regression by assigning each participant a category labelled with the number of ventilator-free days up to 21 days, with people who died up to day 90 being assigned −1, people who were on MV at

**TABLE 1** Overview of meta-analysed results from patients hospitalised with severe respiratory infections

| Comparison | 30-day mortality | | 90-day mortality | Grade 3 or 4 transfusion related AEs | SAEs |
|---|---|---|---|---|---|
| **Comparison 1:** CP versus SoC or biologically inactive placebo (saline) | **All RCTs:** RR 0.99 (0.92 to 1.06) 15 RCTs[a], $n = 17\,266$ (37 children, 38 pregnant women) ⨁⨁⨁◯ $I^2 = 4\%$ $Tau^2 = 0.00$ | **High Titre subgroup:** RR 0.98 (0.93 to 1.04) 9 RCTs[b], $n = 15\,954$ (26 children, 33 pregnant women) ⨁⨁⨁⨁ $I^2 = 0\%$ $Tau^2 = 0.00$ | RR 0.92 (0.74 to 1.15) 6 RCTs[b], $n = 3210$ (8 pregnant women) ⨁⨁◯◯ $I^2 = 0\%$ $Tau^2 = 0.02$ | *No transfusion in control group; results in intervention group are summarised in table A12* | RR 1.14 (0.92 to 1.41) 13 RCTs[a], $n = 16\,730$ (37 children, 38 pregnant women) ⨁◯◯◯ $I^2 = 56\%$ $Tau^2 = 0.07$ |
| **Comparison 2:** CP versus biologically active control (FFP or IVIG) | RR 0.85 (0.56 to 1.29) 5 RCTs[a] $n = 700$ (13 children, 1 pregnant woman) ⨁◯◯◯ $I^2 = 33\%$ $Tau^2 = 0.07$ | | RR 0.99 (0.75 to 1.29) 2 RCTs[b], $n = 264$ ⨁◯◯◯ $I^2 = 0\%$ $Tau^2 = 0.00$ | POR 0.43 (0.14 to 1.33) 6 RCTs[a], $n = 716$ (13 children, 1 pregnant woman) ⨁◯◯◯ $I^2 = 4\%$ $Chi^2 = 4.18$ | RR 0.88 (0.65 to 1.19) 4 RCTs[b], $n = 523$ (13 children, 1 pregnant woman) ⨁⨁◯◯ $I^2 = 0\%$ $Tau^2 = 0.00$ |
| **Comparison 3:** hIVIG versus control | RR 0.77 (0.34 to 1.73) 3 RCTs[c] $n = 392$ ⨁◯◯◯ $I^2 = 50\%$ $Tau^2 = 0.26$ | | *No RCTs reported mortality at 90 days in this comparison* | RD 0.00 (−0.08 to 0.08) 2 RCTs[a] $n = 84$ ⨁◯◯◯ $I^2 = 0\%$ $Tau^2 = 0.00$ | RR 1.10 (0.76 to 1.58) 2 RCTs[a] $n = 342$ ⨁◯◯◯ $I^2 = n/a$ $Tau^2 = n/a$ |
| **Comparison 4:** Early CP versus deferred CP | RR 2.68 (0.56 to 12.71) 1 RCT[b], $n = 58$ ⨁◯◯◯ $I^2 = n/a$ $Tau^2 = n/a$ | | *No RCTs reported mortality at 90 days in this comparison* | *Transfusion-related AEs were only reported for patients receiving CP; results are summarised in table A12* | *No RCTs reported SAEs in this comparison* |

*Note*: Key: ⨁◯◯◯ very-low certainty evidence; ⨁⨁◯◯ low certainty evidence; ⨁⨁⨁◯ moderate certainty evidence; ⨁⨁⨁⨁ high certainty evidence.
Abbreviations: POR, Peto odds ratio; RD, risk difference; RR, risk ratio.
[a]Includes 1 RCT in influenza.
[b]All COVID-19.
[c]Includes 2 RCTs in influenza.

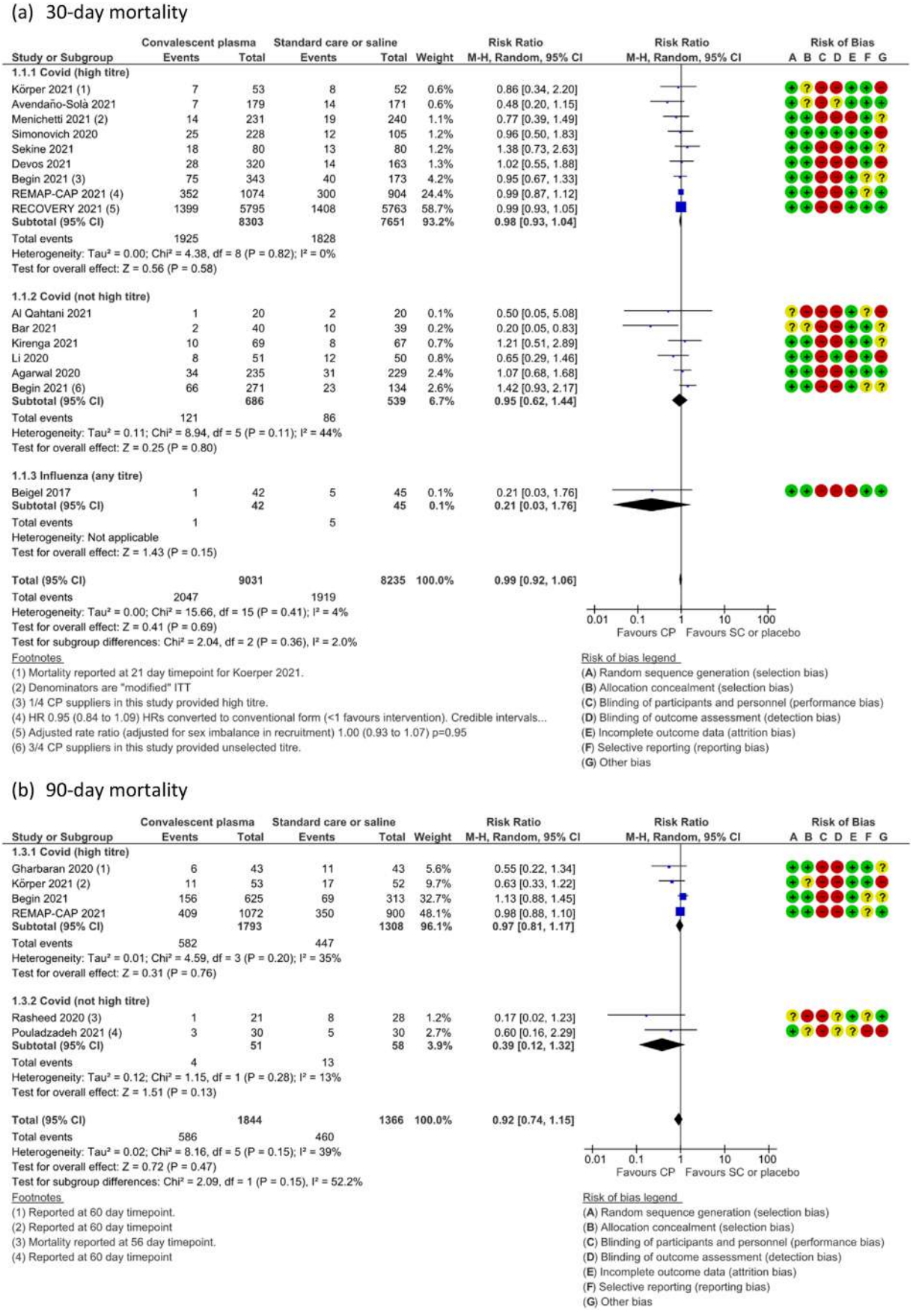


**FIGURE 2** Forest plot of all-cause mortality, for comparison 1 (CP compared to SoC or a biologically inactive placebo) at up to (A) 30 days, and (B) 90 days

randomisation being assigned 0, and people who remained ventilator-free beyond day 21 being assigned 22. This is a useful way to compare the two groups while accounting for the very different possible outcomes but the resulting odds ratio (OR) and medians are difficult to interpret. No other trials used these methods and so we cannot combine the results but instead report the summary within Table A4 in Data S1.

Duration of viral detection was expressed as time (median IQR) to first negative test (2 RCTs).[23,36] One study,[25] reported the number of patients who had had two consecutive negative tests by day 30. See table A5 for viral detection data and table A6 for details of changes in viral loads.

## 4.4 | ROB in included studies

### 4.4.1 | RCTs (using Cochrane ROB1)

Nineteen RCTs were open-label, comparing CP to SoC, and were therefore assessed as having a high ROB for all outcomes except mortality, as knowledge of treatment allocation may have affected clinical decision-making. A summary of ROB judgements is available in Table A7 and Figure A1 in Data S1.

### 4.4.2 | Non-RCTs (using ROBINS-I)

Two non-RCTs[50,51] were assessed at serious RoB for selection bias and confounding at baseline. The remaining 9 studies[53–61] were at critical ROB due to baseline confounding or selection bias and were therefore not meta-analysed.

## 4.5 | Certainty of the evidence (GRADE)

Certainty of the evidence was GRADEd as very-low to high; primary reasons for downgrading were ROB and imprecision (wide confidence intervals and small sample size) (Tables A8–A11 in Data S1). We assessed publication bias through the generation of a funnel plot (Figure A2 in Data S1) for 30-day mortality in comparison 1, which suggests that some small studies have not been published. However, this was not significant enough to downgrade the certainty of the evidence because the analysis is dominated by two large, high-quality, and RCTs.

## 4.6 | Effect of the Intervention

See Table 1 for an overview of meta-analysed results.

### 4.6.1 | Comparison 1: CP versus SoC or biologically inactive placebo

Twenty RCTs and two retrospective studies assessed CP compared with SoC or a biologically inactive placebo.

*All-cause mortality*

30-day mortality data were available from 15 RCTs (30 days, 5 RCTs; 28 days, 9 RCTs; 21 days, 1 RCT) (Figure 2a); 90-day mortality data were available from 6 RCTs (56 days, 1 RCT; 60 days, 3 RCTs; 90 days, 2 RCTs) (Figure 2b).

Overall, CP did not reduce 30-day mortality (15 RCTs, n = 17 266; moderate-to-high certainty of evidence [Table A8 and footnotes in Data S1]) and there may be no effect on 90-day mortality (6 RCTs $n = 3210$; low certainty of evidence [Table A8]).

Two non-RCTs reported in-hospital mortality, and showed results consistent with the randomised evidence (2 studies, $n = 436$; very-low certainty evidence) (Figure A3A Table A8 in Data S1).

*Improvement of clinical symptoms*

Duration of NIV was reported in 4 studies (2 RCTs),[3,24,50,51] and duration of MV was reported by 11 studies (9 RCTs).[3,24,25,28–30,35,38,39,50,51] Two RCTs[27,31] reported any ventilatory support, but did not differentiate between MV, NIV, and passive oxygen support. One RCT[29] reported any ventilation, but also reported separately a composite outcome of patients who progressed to MV or death. Most studies reported the data as duration of support, either median (IQR) or mean (SD) (Table A4 in Data S1).

These outcomes were very variably reported, and many did not fully account for competing events, or report methods of analysis in sufficient detail. Based on what was reported, there was no apparent difference in duration of MV, NIV or ECMO support between the two groups.

*Length of stay (LOS): hospital and ICU*

Length of hospital stay was reported by 16 RCTs[7,23,25–28,30,31,38,39,42–47] and 1 non-RCT,[51] and length of ICU stay was reported by 9 RCTs[23,26,28,29,33,39,43,45,47] (Table A3 in Data S1). There was no evidence of an effect in length of hospital stay or length of ICU stay (Table A3 in Data S1).

*Duration of viral detection from admission up to 30 days (viraemia, nasopharyngeal swabs, bronchoalveolar lavage, stool)*

The 3 RCTs which reported time to negative test do not suggest any evidence of an effect (Table A5 in Data S1).

*Adverse events*

AEs due to transfusion were reported in 15 RCTs[3,7,22–39] (Table S10 in Data S1).

Seven RCTs reported no Grade 3 or 4 AEs due to transfusion.[22,24,26,27,31,35,39] Both non-RCTs reported AEs due to transfusion. All but one RCT[26] had SoC comparators, and therefore no transfusion-related SAEs are reported for the control group. Group comparison was not possible; results are summarised in Table A12 of in Data S1.

There was no evidence of an effect on reported SAEs[3,23–31,35,36,39] (13 RCTs, $n = 16\,730$, very-low certainty of evidence) (Figure A3B).

Data were not available on SAEs in seven RCTs.[7,22,32–34,37,38]

See forest plots Figure A3 in Data S1 and GRADE profile Table A8 in Data S1 for further detail.

### 4.6.2 | Comparison 2: CP versus biologically active control (FFP or IVIG)

RCTS assessed CP compared to FFP[40–43,45] or IVIG[44]

*All-cause mortality*

There was insufficient evidence to say whether or not there is a difference between groups in all-cause mortality at up to 30 days (5 RCTs $n = 700$; very-low certainty evidence, Figure A4A in Data S1), or at up to 90 days (2 RCTs, $n = 264$; very-low certainty evidence Figure A4B in Data S1). See forest plots Figures A4A and A4B in Data S1 and GRADE profile Table A9 in Data S1 for further detail.

*Adverse events*

Six RCTs reported transfusion-related Grade 3 or 4 AEs.[40–45] Events were rare (~2%) with no clear evidence of a difference (6 RCTs, $n = 716$; very-low certainty evidence. [Figure A4C in Data S1]). Four RCTs[40–42,45] reported SAEs up to 30 days, showing no evidence of an effect, although the rate of SAEs seems very low, given the severity of disease in hospitalised individuals (4 RCTs, $n = 523$; low certainty evidence, Figure A4D in Data S1). See forest plots Figure A4 and GRADE profile Table A9 in Data S1 for further detail.

*Improvement of clinical symptoms*

Duration of MV[40,43,45] and any ventilatory support[41] were reported as median (IQR) or mean (SD). Given the difficulties of dealing with competing events, and the small number of patients involved, it is very unclear if CP therapy had any effect on the duration of MV, NIV or ECMO support between the two groups. We have presented the data in Table A4 in Data S1 as reported by the individual studies.

Data were not available for LOS (hospital or ICU), and duration of viral load.

### 4.6.3 | Comparison 3: hyperimmune immunoglobulin versus control

Three assessed hIVIG compared with SoC or a biologically inactive placebo.

*All-cause mortality*

There was insufficient evidence to say whether or not there is an effect on mortality compared to control at up to 30 days (3 RCTs $n = 392$; very-low certainty evidence) (Table 1, Figure A5A, Table A10 in Data S1). There were no data for 90-day mortality.

*Adverse events*

Two RCTs reported transfusion-related AEs; neither reported any AEs due to transfusion in either group (2 RCTs, $n = 84$; very-low certainty evidence, Figure A5B in Data S1). Two RCTs reported SAES (2 RCTs $n = 342$; very-low certainty evidence. [Figure A5C in Data S1]). See forest plots Figure A5 and GRADE profile Table A10 in Data S1 for further detail.

*Improvement of clinical symptoms*

One RCT in influenza[48] reported on duration of MV and NIV. However, the data were presented using an ordinal scale that was not mappable to our outcomes or other trial results, and we were unable to extract the data.

Data were not available for LOS (hospital or ICU), and duration of viral load.

### 4.6.4 | Comparison 4: early CP versus deferred CP

One RCT assessed early CP compared to deferred CP.

*All-cause mortality*

There was insufficient evidence to say whether there is a difference in 30-day mortality between early CP and deferred CP (1 RCT $n = 58$; very-low certainty of evidence) (Figure A6 in Data S1). There were no data for 90-day mortality. See forest plots Figure A6 and GRADE profile Table A11 in Data S1 for further detail.

*Adverse events*

There were three Grade 3 or 4 transfusion-related AEs within 24 h, all in the early CP group: (1 RCT $n = 58$, very-low certainty evidence) (Table A12 in Data S1). SAEs were not reported. See forest plots and GRADE profile Table A11 in Data S1 for further detail.

*Improvement of clinical symptoms*

Duration of MV and NIV was reported as median (IQR). We have presented the data in Table A4 in Data S1 as reported by the RCT. Both groups had similar duration of ventilatory support. It is unclear if the authors accounted for competing events.

Data were not available for LOS (hospital or ICU), and duration of viral load.

## 4.7 | Results from uncontrolled studies (for safety only)

We identified 73 non-randomised or uncontrolled studies [49 case reports or case series] that assessed the use of CP or hIVIG in respiratory viral infection and reported AEs: 12 in influenza A, 2 in MERS-CoV, and 4 in SARS-CoV-1, and 67 in SARS-CoV-2 (COVID-19). Of the influenza studies, 10 were from the 1918 to 1920 pandemic. Fifty-one studies reported that no AEs were observed (37/49 case reports or case series). Eighteen studies reported transfusion-related AEs, and four studies reported other SAEs. These data are presented in Appendix B in Data S1.

## 4.8 | Post hoc subgroup analysis: seropositivity at baseline

Three RCTs,[3,30,62] including the two largest, reported 30-day mortality for subgroups defined by seropositivity at baseline. These results are shown in Figure 3.

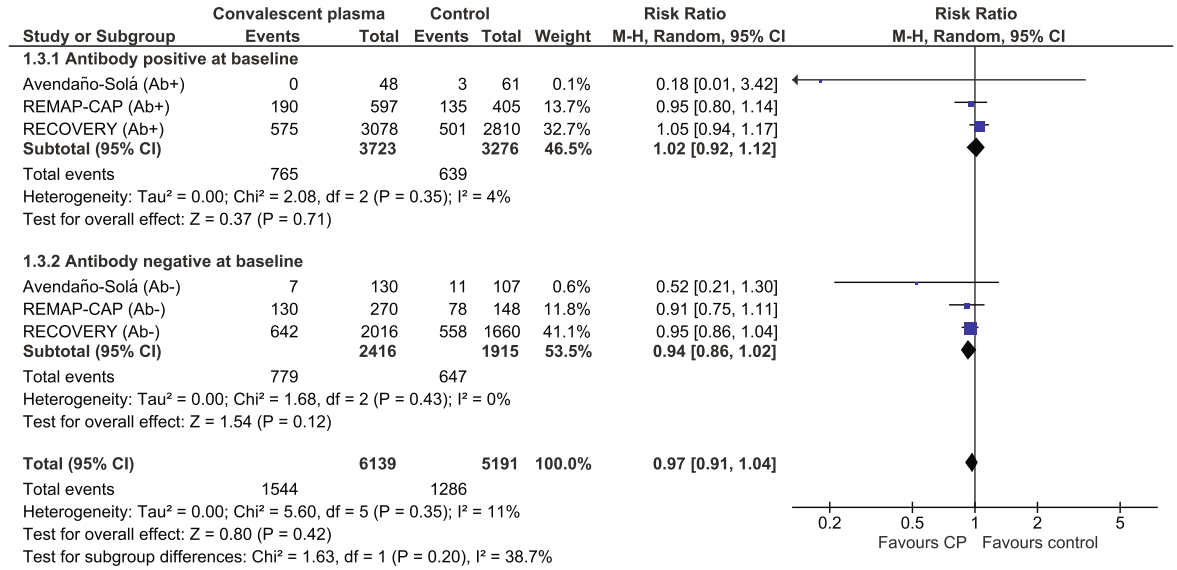


**FIGURE 3** Subgrouped by seropositivity at baseline: RCTs reporting 30-day mortality for comparison 1 (CP compared to SoC or a biologically inactive placebo)

With almost all the information coming from the two large, high-quality RCTs,[3,30] the pooled estimates from these three RCTs are: RR 1.02 (0.92 to 1.12) for people who are seropositive at baseline and 0.94 (0.86 to 1.02) for those who are seronegative. The test for interaction (subgroup difference) gives a *p*-value of 0.20 with very little heterogeneity either within or between groups.

We explored this further using meta-regression on the group of trials comparing high titre CP with SoC which reported the proportion seropositive at baseline and 30-day mortality. This analysis produced near identical results with an estimated RR at 0% seropositivity of 0.93 (0.85, 1.01) and 1.02 (0.93, 1.12) at 100% seropositivity (See Appendix A6 in Data S1. Mortality results are summarised in Table A14 in Data S1).

## 5 | DISCUSSION

The objective of this review was to determine the safety and effectiveness of CP or hIVIG from CP to treat patients with serious respiratory disease due to influenza or coronavirus infection. In order to increase the relevance of our findings to the COVID-19 pandemic we used the core outcome set[63] for assessing treatments for patients infected with SARS-CoV-2. We aimed to use high-quality evidence from RCTs to assess safety and effectiveness. We also used all other study designs to describe serious harms reported following transfusion with CP or hIVIG.

### 5.1 | Main findings

We were able to meta-analyse 32 studies for our primary outcome of 30-day mortality (30 RCTs and 2 non-RCTs). We found little evidence of any difference between the groups in either benefits or harms for patients hospitalised with a severe viral respiratory infection requiring hospital admission. Most evidence was of low or very-low certainty. The only high-certainty evidence was for the COVID high-titre subgroup in the outcome all-cause mortality at up to 30 days in CP versus SoC (Table 1).

Adverse events were variably reported. No RCTs reported a high number of transfusion-related AEs (proportion 0% to 5.67%[22–24,26,27,31,35,38,39,43,44,46,47]) (very-low to low certainty evidence). There was no evidence of an increase in harms compared with standard plasma.

### 5.2 | Quality (certainty) of the evidence

Where meta-analysis was possible, we used GRADE to assess our certainty in the result (Table 1). Certainty in the evidence was assessed as very-low to low certainty for all outcomes apart from mortality data in the comparison CP versus standard care.

Evidence was downgraded for serious ROB (lack of blinding, baseline imbalance, randomisation processes, missing data and unclear reporting of outcomes) and imprecision (wide confidence intervals around the effect estimate, and small sample sizes for the outcome of interest). Some of the sources of potential bias (such as patient and personnel blinding) would be hard to overcome in future trials due to the issues in finding an ethical control infusion: even saline is problematic, with the risk of volume overload, and ease with which it can be differentiated from plasma.

SAEs were also downgraded for inconsistency as the heterogeneity was significant between studies, this is likely to be due to the variation in reporting of the SAEs. This may be in part due to differing regulatory environments and different classifications of CP, requiring

varying levels of AE reporting including the need to use a grading system (e.g., MedDRA[64]).

We included lower-level evidence for the assessment of safety outcomes. However, we were unable to perform quantitative analyses, and so have only presented these data as reported in Appendix B in Data S1.

There were very few endpoints reported consistently enough for meta-analysis. The difficulty in defining endpoints, especially time-to-event endpoints,[65] is discussed further in Appendix A6 in Data S1.

## 5.3 | Strengths and Limitations of this review

We have attempted to minimise potential bias in the review process, using Cochrane methods and PRISMA guidelines for reporting. We conducted a comprehensive search: searching data sources to ensure that all relevant studies would be captured, using multiple databases and reference lists of included studies. We included conference proceedings and included a search of clinical trial registries. We also attempted to contact authors for additional data and for clarification of their data.

There were no restrictions for the language in which the paper was originally published. We pre-specified outcomes prior to analysis and have explained the rationale for including one additional outcome (any SAEs).

We undertook duplicate screening, data extraction, and assessment of bias. Additionally, the clinical advisor (LE) was consulted for disagreements, or need for clarification.

The limitations of this review mostly arose due to gaps in the evidence base, which are discussed more fully in the next section.

## 5.4 | Interpretation and context

A recent analysis of individual patient data (IPD) pooled from eight RCTs[9] IPD reported an OR for mortality of 0.85 at day 28 (95% credible interval, 0.62 to 1.18; posterior probability of OR <1 of 84%). These results are broadly comparable and in agreement with our own aggregate analyses for 30-day mortality. However, it should be noted that the IPD analysis included two RCTs[66,67] published after our 30th November 2021 cut-off, but did not include the two largest RCTs of CP RECOVERY[3] and REMAP-CAP[30] which we have analysed, and which together contribute 83% of sample size contributing to our analysis of 30-day mortality for CP versus SoC.

A limitation of the current evidence base is that of the 30 RCTs and two non-randomised studies included in our meta-analysis, 26 studies (24 RCTs) excluded children and 16 RCTs excluded pregnant women, with 1 RCT[39] admitting pregnant women only on the second round of recruitment. Given that children and pregnant women are both considered to be at increased risk of serious disease and death from many severe respiratory viral infections, their exclusion from trials is concerning. Of the 144 ongoing studies we identified, most trials will exclude children and pregnant women. Many ongoing studies have an upper age cut-off (of 65, 70 or 80 years), despite older age being one of the biggest risk factors for COVID-19.

The precision of our meta-analysis was affected by the different titres of CP-neutralising antibodies between trials (Table A1 in Data S1). We tried to address this by subgrouping studies based on the CP-titre reported, and whether it was considered high enough according to FDA criteria (see Appendix A3 in Data S1). However, several studies used local assays that could not be correlated with an FDA reference method. Since we conducted our first search, several variants of SARS-CoV-2 have arisen worldwide and may require much higher antibody titres measured using ELISA assays.[68] Much higher titre CP, from vaccinated convalescent donors, may be active against future variants[69] indicating that new COVID CP trials should aim to use very high titre CP standardised using internationally recognised methods.

Similarly, between trials, there was heterogeneity of patient groups and severity of illness on admission to hospital (Table 1). The RCTs in COVID may not have used the same criteria to categorise trial participants at enrolment and trials designed to treat different patient groups based on comorbidities and immune states were absent. Several COVID-19 studies reported clinical improvement using the WHO ordinal scale. However, the scale was revised several times over the course of 2020–2021, going from an 8-point scale[70] to a 10-point scale at its latest revision[71] which have made comparisons between trials difficult.

The results of our post hoc subgroup analysis by seropositivity at baseline are very similar to the results reported by RECOVERY alone. We have not found stronger evidence of this potential interaction than that reported by RECOVERY (with a similar trend also reported by REMAP-CAP, especially for organ support-free days) but similarly, we have not found any reason to discount the possibility that there is a small but important interaction, with immunocompromised individuals potentially benefitting more. This hypothesis is consistent with the REGN-COV2 RECOVERY trial,[21] which has shown no benefit of monoclonal antibodies for seropositive patients who either have advanced disease or who are immunocompetent. The very high baseline risk of immunocompromised individuals might translate very small relative risks into substantial absolute risk differences. REMAP-CAP has recently reopened for immunocompromised people to test this hypothesis.[72]

## 5.5 | Implications for research and practice

There is currently no evidence for a benefit of CP in an unselected population of patients hospitalised with coronaviruses or influenza. It is likely that the titre of the CP and the immune response of the recipient may both be important factors affecting response to treatment.

Studies should use CP of a high enough titre to elicit a biological response, and report the actual titre used as well as the minimum as described in the protocol. Matching variants between donor and recipient may not be feasible, but viral variants circulating at the time of collection of plasma and during the study should be recorded.

Studies should assess and publish antibody status (seropositivity) at baseline in both intervention and control groups, and identify and

report immunocompromised patients separately, to establish whether certain groups of patients are more likely to benefit from this intervention.

There are difficulties in designing truly blinded RCTs of CP or hIVIG (see Reference 73 for review). There are ethical problems with using a placebo which is assumed to have no clinical benefit, but has known harms.[74] One RCT[26] used a saline placebo, with potential concerns about volume overload, and six RCTs used a biologically active control, (FFP in 5 RCTs,[40–43,45] and IVIG in one[44]) which raises additional concerns about transfusion reactions.

Unless reported explicitly by investigators, it was difficult to distinguish the AEs experienced following transfusion from the symptoms of severe respiratory disease.[75] This limited the number of RCTs that we could include in our meta-analysis of AEs due to transfusion. There was also substantial variability in the way that AEs were recorded and reported in these studies. It was not always possible to determine the severity of AEs, and different studies used different criteria for SAEs. In some cases, it was hard to determine if SAE reporting was per event or per patient, making it extremely difficult to compare rates of AEs between studies. Blood components in the UK are not classified as medicines and so require a different grading system for reporting AEs to countries that classify CP as a medicine, e.g. Germany. A consensus on how AEs associated with blood products are reported in RCTs would help to address this problem.

## 6 | CONCLUSION

This review has highlighted several issues regarding study design and reporting which should be addressed in current and future research. A minimum titre should be established and ensured for a positive biological response to the therapy. Further research on the impact of CP/hIVIG in patients who have not produced antibodies to the virus prior to hospital admission or who are immunocompromised would be useful to target therapies at groups who will potentially benefit the most.

## AUTHOR CONTRIBUTIONS

**Catherine Kimber:** screening and full text assessment, retrieved full text publications, data extraction, risk of bias assessment, entered data into RevMan and undertook subgroup analyses, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Abigail A. Lamikanra:** screening and full text assessment, retrieved full text publications, data extraction, risk of bias assessment, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Louise J. Geneen:** screening and full text assessment, retrieved full text publications, data extraction, risk of bias assessment, entered data into RevMan and undertook subgroup analyses, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Josie Sandercock:** data extraction, risk of bias assessment, and undertook all metaregression analyses, performed GRADE assessments, interpreted the results, contributed to the development of the manuscript. **Carolyn Doree:** developed and performed all search strategies and de-duplication, retrieved full text publications, contributed to the development of the manuscript. **Sarah J. Valk:** screening and full text assessment, retrieved full text publications, contributed to the development of the manuscript. **Lise J. Estcourt:** developed the initial idea of the review, developed, wrote, and registered the protocol, interpreted the results, and contributed to the development of the manuscript.

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## ORCID

*Catherine Kimber* https://orcid.org/0000-0002-7796-9931
*Abigail A. Lamikanra* https://orcid.org/0000-0002-9287-2177
*Louise J. Geneen* https://orcid.org/0000-0001-6759-0437
*Josie Sandercock* https://orcid.org/0000-0001-7337-8635
*Carolyn Dorée* https://orcid.org/0000-0001-6955-7497
*Sarah J. Valk* https://orcid.org/0000-0003-3964-2505
*Lise J. Estcourt* https://orcid.org/0000-0003-4309-9162

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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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# Effects of storage on quality and function of acid-treated platelets with reduced HLA Class I surface expression

Mohammad Reza Mirlashari[1] | Annette Vetlesen[1] | Lise Sofie H. Nissen-Meyer[1] | Geir Hetland[1,2]

[1]Department of Immunology and Transfusion Medicine, Oslo University Hospital, Oslo, Norway

[2]Institute of Clinical Medicine, University of Oslo, Oslo, Norway

**Correspondence**
Mohammad Reza Mirlashari, Department of Immunology and Transfusion Medicine, Oslo University Hospital, 0407 Oslo, Norway.
Email: uxmoir@ous-hf.no

**Funding information**
University of Oslo and Oslo University Hospital

## Abstract

**Background:** Refractory patients need to be provided with HLA-matched platelets (PLTs), which require time-consuming cross-matching. Treatment of PLTs with citric acid leads to denaturation of the HLA Class I complexes without significant damage to the PLTs. HLA Class I depleted PLTs could alternatively be used to HLA-matched PLTs for transfusion. These PLTs have verified normal function up to 4–6 h after acid treatment.

**Materials and Methods:** Buffy coat (BC) PLT concentrates were depleted of HLA Class I complexes by incubation in citric acid. The days after acid-treatment, surface expression of HLA Class I complexes, CD62P and CD63 were determined by flow cytometry, in addition to viability and mitochondrial membrane potential (MMP). Thromboelastography (TEG) tested PLT functionality.

**Results:** Expression of HLA Class I complexes was reduced by 70%–75% in acid-treated PLTs compared to untreated PLTs from day 1 through day 7. Controls and acid-treated PLTs showed insignificant loss of MMP stored for 4 days. Analysis of the residual PLT activation and viability showed no significant differences for 4 days of storage. However, the residual PLT activation potential and viability were significantly decreased in acid-treated PLTs and control PLTs after 7 days of storage. Acid treatment caused a significant decrease in the TEG variable, reaction time (R time), for acid-treated PLTs as compared to control PLTs from days 1 through day 3.

**Conclusion:** Our data suggest that extended storage of acid-treated PLTs is possible and will improve flexibility when planning for transfusion of patients with alloimmune PLT refractoriness caused by anti-HLA-antibodies.

KEYWORDS
HLA Class I, immune thrombocytopenia, platelet transfusion, platelets

## 1 | INTRODUCTION

PLT transfusion refractoriness is a common challenge in PLT transfusion-dependent patients.[1,2] The current common approach to overcome alloimmune PLT refractoriness caused by anti-HLA-antibodies is transfusion with HLA-matched PLTs,[1] which is complicated, expensive, time-consuming and logistically demanding,[1] especially in cases of emergency.

An alternative approach to handle PLT refractoriness is to deplete or denature HLA Class I molecules on PLTs before transfusion.[3–6] Treatment with citric acid (pH 2.9–3.0) leads to denaturation of their HLA Class I complexes without significant damage to the PLTs.[5,6]

These recent in vitro findings suggest that acid-treated PLTs could be used as an alternative to HLA-matched PLT transfusions. Normal function has been verified in such PLTs up to 4–6 h after acid treatment, which is considered a minimum time needed for all practical procedures before acid-treated PLTs can reach the patient.[5,6] We have also determined that the changes in PLT proteins after citric acid treatment are quite small and without functional significance.[7]

A longer post-treatment storage study would be useful to extend the shelf-life of the modified product and thus increase the flexibility of acid treatment in relation to the planned transfusion time point. Thus, we have investigated the effect of storage on acid-treated PLTs with regard to reduction in HLA class I antigens and $\beta_2$-microglobulin expression, residual PLT activation potential after thrombin receptor agonist peptide (TRAP) stimulation, viability and functionality function as determined by mitochondrial membrane potential (MMP) and thromboelastography (TEG).

## 2 | MATERIALS AND METHODS

### 2.1 | Automated preparation of BC-PLTs

The Terumo Automated Centrifuge & Separator Integration (TACSI) system (Terumo BCT) was used for the automatic processing of PCs with the TACSI PL kit (Terumo BCT). Processing was carried out following the manufacturer's instructions. Five ABO-matched BCs (24 h after collection) were placed in the TACSIWB system and were automatically pooled by adding 300 mL of PLT additive solution (T-PAS+, 40855, Terumo, Belgium). All tubes were automatically sealed. After mixing and centrifugation of the ring-shaped container, the PLT-rich supernatant was transferred via the integrated leukoreduction filter to the centrally located PLT storage bag made of *n*-butyryl tri-*n*-hexyl citrate (BTHC) plasticized PVC, and the tubes were sealed. The PCs rested for 1 h before they were moved to flatbed agitators at 22 ± 2°C for storage.

### 2.2 | Reagents

Thrombin receptor activator peptide 6 (TRAP6, T1573) and the tetrapeptide Arg-Gly-Asp-Ser (RGDS, A9041) were from Sigma–Aldrich (St. Louis, Missouri). Phosphate-buffered saline (PBS) was from Gibco Life Technologies, (10010056; Rockville, Maryland), and paraformaldehyde (PFA), was from Merck (8.18175, Darmstadt, Germany). The citric acid buffer (equal volumes of 263 mmol/L citric acid and 123 mmol/L $Na_2HPO_4$, resulting in pH 2.9–3.0) was aseptically produced at the pharmacy at Oslo University Hospital.

### 2.3 | Acid treatment of BC-PLTs

Protocol for the acid treatment was from Mirlashari et al.[6] Briefly, BC-PCs (420–500 mL and 1–1.5 × $10^9$ PLTs/mL) were split in two and transferred to two transfer bags (600 mL, Compoflex R6R2021; Terumo BCT, Lakewood, Colorado) 24 h after processing and centrifugated (1506 × *g*, 10 min, accel 8, decel 2, temperature 22°C). One half of the initial product was acid-treated, the other served as control. The PLT poor plasma was transferred to new bags by an automatic press (Optipress, Fenwal). To avoid transfer of untreated PLTs to the final product, the satellite bags with PLT-poor plasma were re-centrifuged (2524 × *g*, 14 min, accel 9, decel 5, at 22°C) to obtain PLT-free plasma, for later resuspension of the acid-treated PLTs and the control PLTs, respectively. The PLT pellets were gently blended with the rest of the plasma to dissolve PLT aggregates. The pellets were pre-cooled in ice-water for 2 min. To one pellet kept in ice-water, 30 mL of ice-cold citric acid buffer (equal volumes of 263 mmol/L citric acid and 123 mmol/L $Na_2HPO_4$, resulting in pH 2.9–3.0) was added by syringe in a sterile safety cabinet (model NU-201-430e, NuAire Lab Equipment, Plymouth, Minnesota), and the content was mixed gently thrice. After 5 min. of incubation in ice-water, the acid was neutralised in excess volume (20-fold) of PLT additive solution (T-PAS+, 18359006; Terumo BCT). PLTs were centrifuged, the supernatant was removed, the pellets were dissolved by gently mixing, and the PLTs were re-suspended in their original, now PLT-free plasma. Control PLTs were treated by the exact same procedure, except for the acid treatment. As sample processing of sensitive PLTs may lead to some artefacts of limited physiological and clinical relevance, control and acid treated PLT concentrates were kept unagitated for 30 min. followed by 30 min. on a horizontal flatbed PLT agitator (Helmer Laboratories, Inc., Noblesville, Indiana), allowing any reversible changes to reverse. Before splitting BC-PCs, 50 mL were kept in original bag and used as untreated control. Sampling was performed on days 1 (first day after acid treatment), 2, 3, 4 and 7 for analysis.

### 2.4 | Analysis of pH, platelet count, MPV and metabolic parameters

PLT suspension (1.5 mL) was collected into a safePICO (blood gas syringe (956620) Radiometer, Copenhagen, Denmark) and pH (at 37°C), glucose (mmol/L) and lactate (mmol/L) were measured using routine blood gas equipment (Cobas b221, Radiometer, Copenhagen, Denmark). PLT count and MPV were performed on a standard haematology analyser (ABX Pentra XL80, HORIBA Diagnostics, Kyoto, Japan). To estimate loss of PLTs during treatment, the percentage PLT recovery was calculated as PLT count in acid-treated PLTs or control PLTs divided with PLT count before acid treatment × 100.

### 2.5 | Antibodies to cell surface molecules

For flow cytometry analysis these monoclonal antibodies from BioLegend (San Diego, California) were used: Fluorescein isothiocyanate (FITC)-conjugated mouse anti-human HLA-A, -B, and -C, clone W6/32

(311404); and phycoerythrin (PE)-conjugated mouse anti-human β2-microglobulin, clone 2M2 (316306). Adequate isotype controls were FITC-conjugated mouse anti-mouse NK1.1, clone PK136 (553164), and PE-conjugated mouse IgG1, κ (555750), respectively. For activation studies we used PE-Cy5-conjugated mouse anti-human CD62P clone AK-4 (556020), isotype control PE-Cy5-conjugated mouse IgG1 κ, and PE-conjugated mouse anti-human CD63 clone H5C6 (551142) with isotype control PE-conjugated mouse IgG1, κ as (555750) (BD PharMingen, San Diego, California). Expression of cell surface molecules was analysed on a flow cytometer (Gallios, Beckman Coulter, Inc., Fullerton, California).

## 2.6 | Cell surface staining of HLA Class I molecules and β2-microglobulin

PLTs were diluted 1:50 with PBS. 5 μL of FITC-conjugated mouse anti-human HLA-A, -B, and -C or PE-conjugated mouse anti-human β2-microglobulin clone 2 M2 were added to a 100-μL cell suspension. Isotype controls were run in parallel. Samples were incubated for 30 min. at room temperature in the dark, re-suspended in 400 μL of PBS, and analysed by flow cytometry within 1 h. For the calculation of percent reduction of HLA Class I molecules and β2-microglobulin expression levels, the median fluorescence intensity (MFI) of samples incubated with the isotype control was subtracted from the MFI of PLTs stained with anti-human HLA-A, -B, and -C and anti-human β2-microglobulin and the corrected MFIs of treated sample were divided by the MFI of the corresponding untreated sample × 100.

## 2.7 | Cell surface staining of activation markers

We examined the surface expression of CD62P and CD63 on resting PLTs to evaluate spontaneous PLT activation. Furthermore we stimulated PLTs with TRAP to measure the residual PLT activation potential as described by Vetlesen et al.[8] Briefly, tetrapeptide RGDS (4.6 mmol/L final concentration) was added to the PLTs and incubated for 10 min. at 20°C to prevent aggregation before stimulation with 0.4 mmol/L (final concentration) of TRAP6 for 20 min. at 20°C. Stimulated and resting PLTs were fixed with 0.5% (final concentration) PFA in PBS, placed on ice for 20 min., and diluted 1:10 with PBS with 5.6 mmol/L glucose and 3.5 g/L bovine serum albumin. Suspensions of resting and stimulated PFA-fixed PLTs (25 μL) were then incubated 20 min. in the dark at 20°C with either 10 μL of PE-Cy5 mouse anti-human CD62P or PE-Cy5 mouse IgG1 κ isotype control, PE mouse anti-human CD63, or PE mouse IgG1, κ isotype control. After incubation, 500 μL of ice-cold 1% PFA were added for fixation, and flow cytometric analysis was performed within 30 min.

## 2.8 | Measurement of mitochondrial membrane potential by flow cytometry

Loss of MMP is an indicator of pro-apoptotic events in damaged PLTs.[9] The MMP was measured using a mitochondrial permeability transition detection kit (MitoProbe JC-1 Assay Kit, M34152, Thermo Fisher Scientific, USA), as described.[10] In brief, samples were stained with JC-1 (5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethyl-benzamidazolocarbocyanin chloride), a green fluorescent dye that forms red fluorescent aggregates inside intact mitochondria. After incubation at 37°C for 15 min. analysis were performed using flow cytometry (Gallios). Depolarized mitochondria (positive control) were prepared by incubating PLTs with 5 μmol/L carbonylcyanide *m*-chlorophenylhydrazone for 30 min at 37°C.

## 2.9 | TEG test

A haemostasis system (TEG Haemostasis System, model 5000; Haemonetics Corp., Braintree, Massachusetts) and auxiliary reagents were used to evaluate coagulation function according to the manufacturer's instruction. BC-PLTs were diluted 1:10 in AB Octaplas. Briefly, 1 mL of the diluted PLTs was added to a pre-warmed vial with kaolin (TEG Haemostasis System, Kaolin Reagent 6300), and mixed by inversion. Immediately, 340 μL of this suspension was pipetted into a 37°C pre-warmed analyser cup containing 20 μL 0.2 M $CaCl_2$ and the analysis was initialized. The test measures thrombin-activated clotting and was run until the following parameters were defined: Reaction time (R-time), the period from the sample was placed in the instrument until the first fibrin is formed, reflects the level of coagulation factors present. Clot kinetics (K time), reflects the time until the clot reaches a fixed strength. α angle gives steepness of the curve and reflects the speed of fibrin accumulation and level of fibrinogen. Max amplitude (MA) is the maximum distance between the curve lines and reflects number and function of the PLTs, level of fibrinogen, and strength of the formed clot.

## 2.10 | Statistical analysis

Data are presented as mean ± standard deviation (SD). Repeated-measures analysis of variance (ANOVA, MS Excel 2010) was used to compare groups (control and acid-treated) over time. Paired t-test was used for comparison between two groups (same treatment different days or different treatments same day). Differences with $p$ values < 0.05 were considered significant.

# 3 | RESULTS

## 3.1 | Kinetics of HLA class I reduction in acid-treated BC-PLTs

Our results show that the acid treatment reduced the expression of HLA class I molecules by 72.4% (±2.1) and $\beta_2$-microglobulin by 76.8% (±3.3) on day 1, and by 69.8% (±2.1), and 73.5% (±1.3) on day 7 as compared to controls, respectively (Figure 1A,B). Thus, PLT storage does not significantly affect the reduction of HLA class I complexes during 7 days.

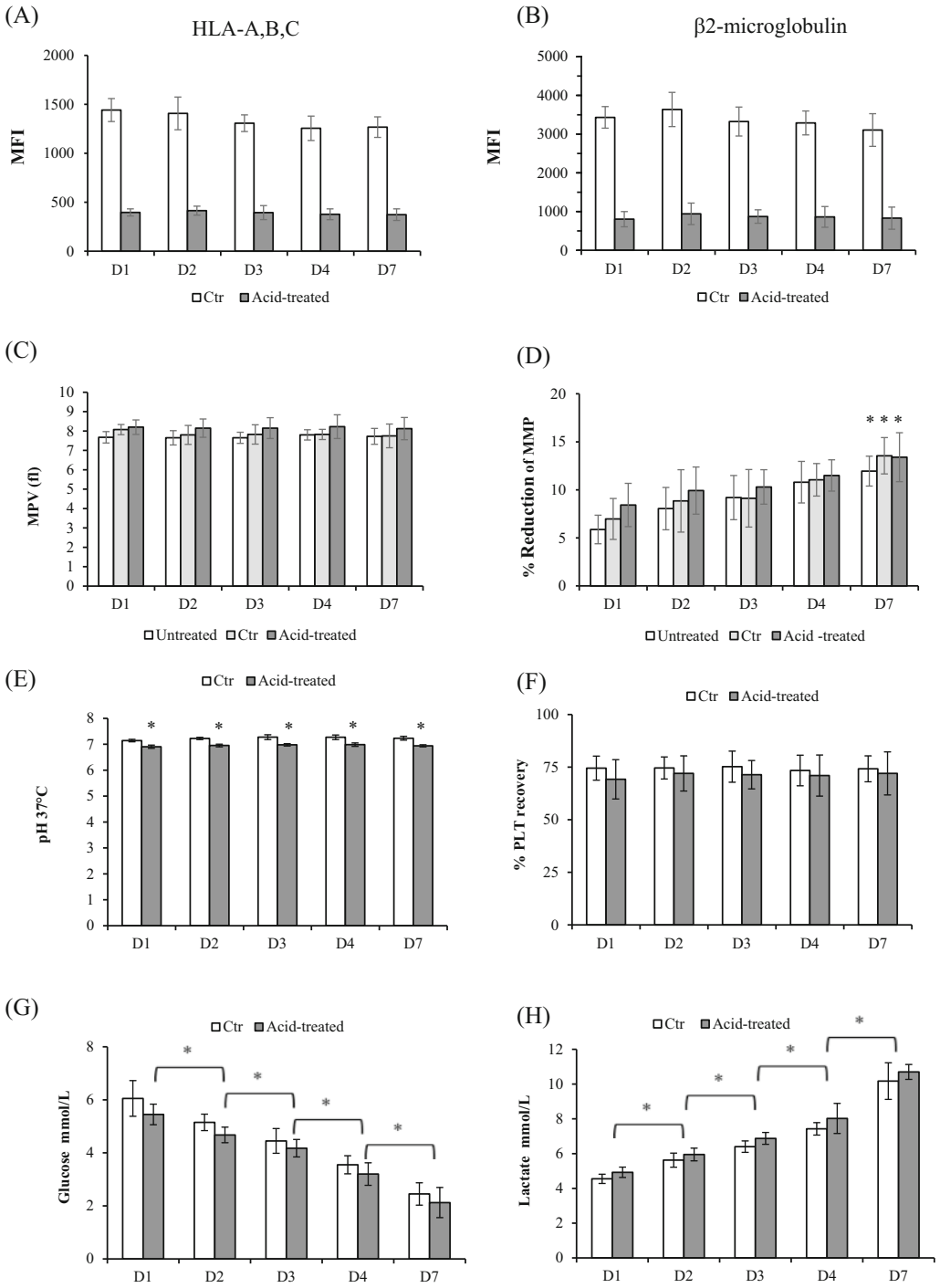


**FIGURE 1** Storage effects on acid-treated PLTs measured as: (A) HLA-A, B, C (B) β2-microglobulin expression, (C) MPV, (D) MMP, (E) pH, (F) %PLT recovery, (G) glucose concentration and (H) lactate concentration. The denaturation/reduction of HLA-A, -B, -C and β2-microglobulin is measured as reduction of median fluorescence intensity (MFI) of acid treated PLTs comparing to control PLTs (Ctr) when measured by flow cytometry. Results are given as mean ± SD ($n = 4$). ANOVA was used to compare within group (Untreated, Ctr or acid-treated) over time. Paired students t-test was used to evaluate the statistical significance between groups. Results with $p$-values < 0.05. *$p$ in comparison Ctr with acid treatment and ⌐*¬ daily comparison between groups.

## 3.2 | Storage of acid-treated BC-PLTs and effect on MPV and PLT viability

Acid treatment did not significantly change MPV during 7 days of storage (Figure 1C). Untreated control, control and acid-treated PLTs did not show any significant loss of MMP stored for 4 days, but loss of MMP increased significantly on day 7 compared to day 1 in both controls and acid-treated PLTs (Figure 1D). This indicates that acid treatment does not have negative effect on PLT viability for 4 days.

### 3.3 | Storage of acid-treated BC-PLTs and effect on pH, PLT count and metabolic parameters

The pH value did not change significantly in controls and acid-treated PLTs throughout storage for 7 days. However, the pH value was significantly lower on day 1 in acid-treated PLTs (6.91 (±0.06)) compared to the control PLTs (7.15 (±0.05)), and also on day 7 (pH 6.94 (±0.04)) in acid-treated PLTs versus (7.23 (±0.07)) in controls (Figure 1E). The percentage of PLT recovery remained stable in the control and acid-treated PLTs during 7 days of storage (Figure 1F). PLT metabolism was evaluated by measuring glucose and lactate. Glucose decreased and lactate increased daily in both control and acid-treated PLTs, with significant differences during 7 days of storage (Figure 1G,H).

### 3.4 | Storage of acid-treated BC-PLTs and effect on platelet activation

We examined the surface expression of CD62P and CD63 on acid-treated PLTs to evaluate spontaneous PLT activation after 1, 2, 3, 4 and 7 days.

CD62P surface expression increased evenly, but not significantly, from day 1 to 4 of storage, in both acid-treated PLTs and control PLTs, but the difference between the two groups was significant in this period. Day 1: control PLTs MFI =70.3 (±21.5) versus acid-treated PLTs MFI =145.3 (±30). Day 2: control PLTs 72 (±14.1) versus acid-treated PLTs MFI = 141 (±38.2). Day 3: control PLTs MFI = 106 (±17) versus acid-treated PLTs MFI = 165 (±31.3). Day 4: control PLTs MFI = 147(±25) versus acid-treated PLTs MFI = 190 (±21.8) versus However, on day 7, CD62P expression increased significantly compared to day 4 in both control PLTs, MFI = 272 (±28.8) and acid-treated PLTs, MFI = 279 (±22.6) (Figure 2A).

The same tendency was seen concerning the CD63 expression, evenly but not significant increase from day 1 to day 4 in the two groups, but between the groups, the difference was significant. Day 1: control PLTs MFI = 36.3 (±6.5) versus acid-treated PLTs MFI = 63.1 (±12.5). Day 2: control PLTs MFI = 34.3 (±10.1) versus acid-treated PLTs MFI = 69.7 (±13.3). Day 3: control PLTs MFI = 40 (±13.1) versus acid-treated PLTs MFI = 71.7 (±13.6). Day 4: control PLTs MFI = 47.7 (±15.1) versus acid-treated PLTs MFI = 79.6 (±10.7). On day 7, the CD63 expression increased significantly compared to day 4 in both control PLTs, MFI = 93 (±16.4) and acid-treated PLTs, MFI = 115 (±10.3), (Figure 2B).

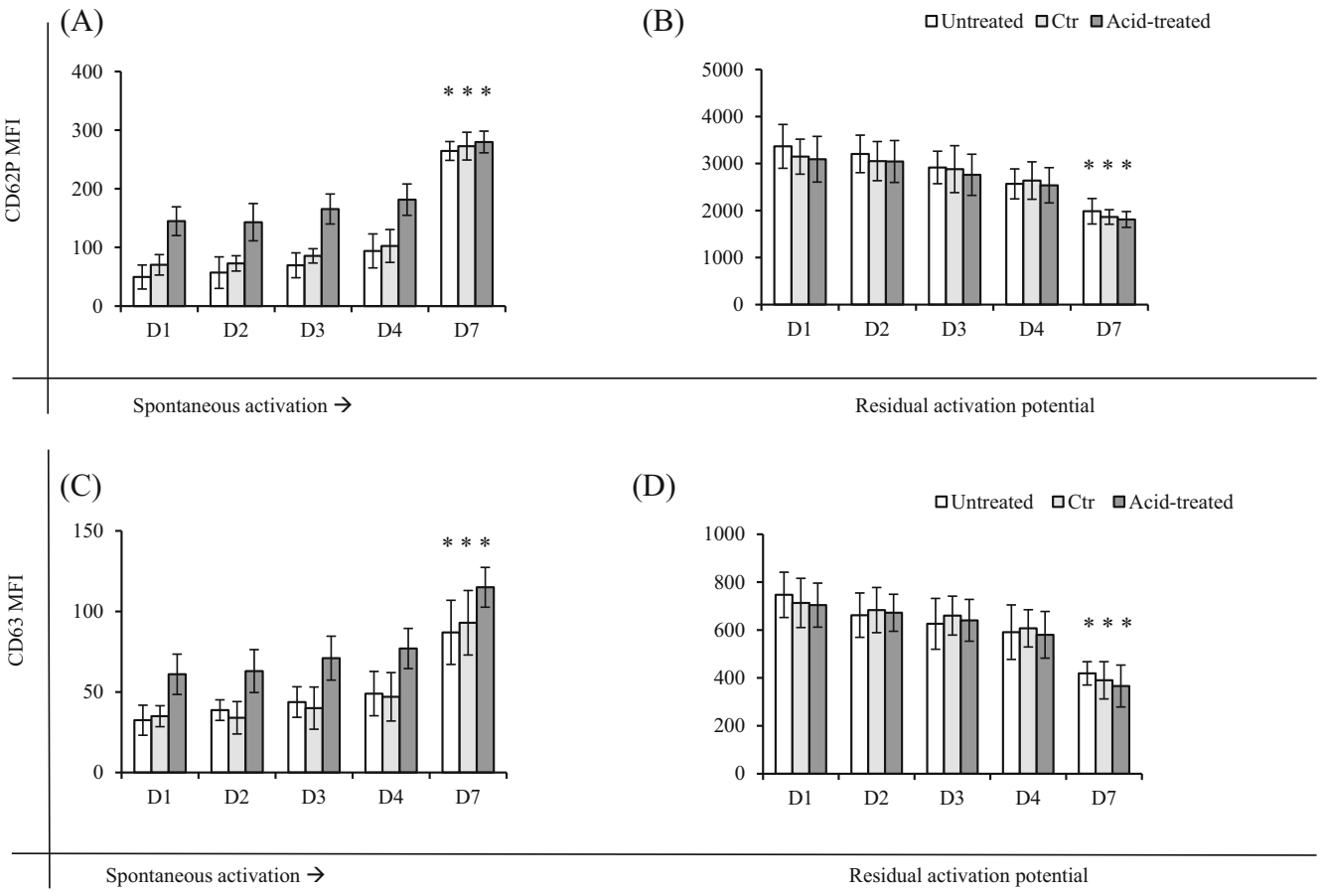


**FIGURE 2** Storage effects on acid treated PLTs measured as: spontaneous activation (A, C) and residual activation potential (B, D) of CD62P (A, B) and CD63 (C, D). Median fluorescence intensity (MFI) is measured by flow cytometry. Results are given as mean ± SD ($n = 4$). ANOVA was used to compare within group (Untreated, Ctr or acid-treated) over time. Paired Students t-test was used to evaluate the statistical significance between groups. Paired students t-test was used to evaluate the statistical significance of the results with $p$-values < 0.05. *$p$ in comparison Ctr PLTs (day 1 with 7) and **in comparison acid-treated PLTs (day 1 with day 7).

Analysis of the residual PLT activation potential (CD62P and CD63 expression) after TRAP stimulation showed no significant differences during 4 days of storage, neither in the two groups nor between the groups. However, on day 7 the residual PLT activation potential had significantly decreased in both controls, CD62P MFI = 1863 (±168), CD63 MFI = 390 (±89) and acid-treated PLTs, CD62P MFI = 1810 (±155), CD63 MFI = 390 (±89) and compared to day 4 (Figure 2C,D).

### 3.5 | Storage of acid-treated BC-PLTs and effect on PLT functionality

Acid treatment caused a significant decrease in R time for acid-treated PLTs compared to control PLTs from day 1 to day 3; controls day 1: 7.4 min. (±0.6) versus acid-treated 5.7 min. (±0.5), day 2: controls 7.4 min. (±0.3) versus acid-treated 6.3 min. (±0.5), day 3: controls 7.6 min (±0.2) versus acid-treated 6.5 min (±0.3). However, differences were not found in R time on days 4 and 7. In addition, no differences were found on day 1 concerning K-time: acid-treated PLTs 1.2 min. (±0.2) versus controls 1.5 min.(±0.3), α angle: acid-treated PLTs 65.7° (±10.4) versus controls 68.5° (±4.8), and MA: acid-treated PLTs 62.5 mm (±1.3) versus controls 60.3 mm (±2.4). From days 2 to 7 of storage these TEG variables did not change significantly (Figure 3A,D).

## 4 | DISCUSSION

Storage of PLT concentrates results in PLT storage lesions, including change in metabolism, surface receptor expression and activation response.[11,12] Recently, we and Meinke et al. reported that a short treatment of PLT concentrates with citric acid leads to denaturation of the HLA Class I complexes without significant damage to the PLTs.[5,6] Thus acid-treated PLTs could be used as an alternative to HLA-matched PLT transfusions to refractory patients. The treatment prevented binding of patient anti-HLA Class I antibodies to the PLTs, antibody-mediated complement activation, and reduced antibody-mediated phagocytosis. It was found that the physiological functions of the PLTs remained intact after acid treatment for at least 4–6 h.[5,6] However, for practical reasons a longer storage time of HLA Class I depleted PLTs is needed for the production and logistics in relation to the planned transfusion time point, especially when the product has to be sent to another hospital. Since recent studies have shown that there was no difference on effect of acid treatment on BC-PLTs and apheresis PLTs on day 1,[5,6] we used the more readily available BC-PLTs in the present study.

In the current study, we investigated the storage effects on acid-treated BC-PLTs for up to 7 days. Acid treatment was found to remove or denature >70% of HLA Class I molecules and >74% of

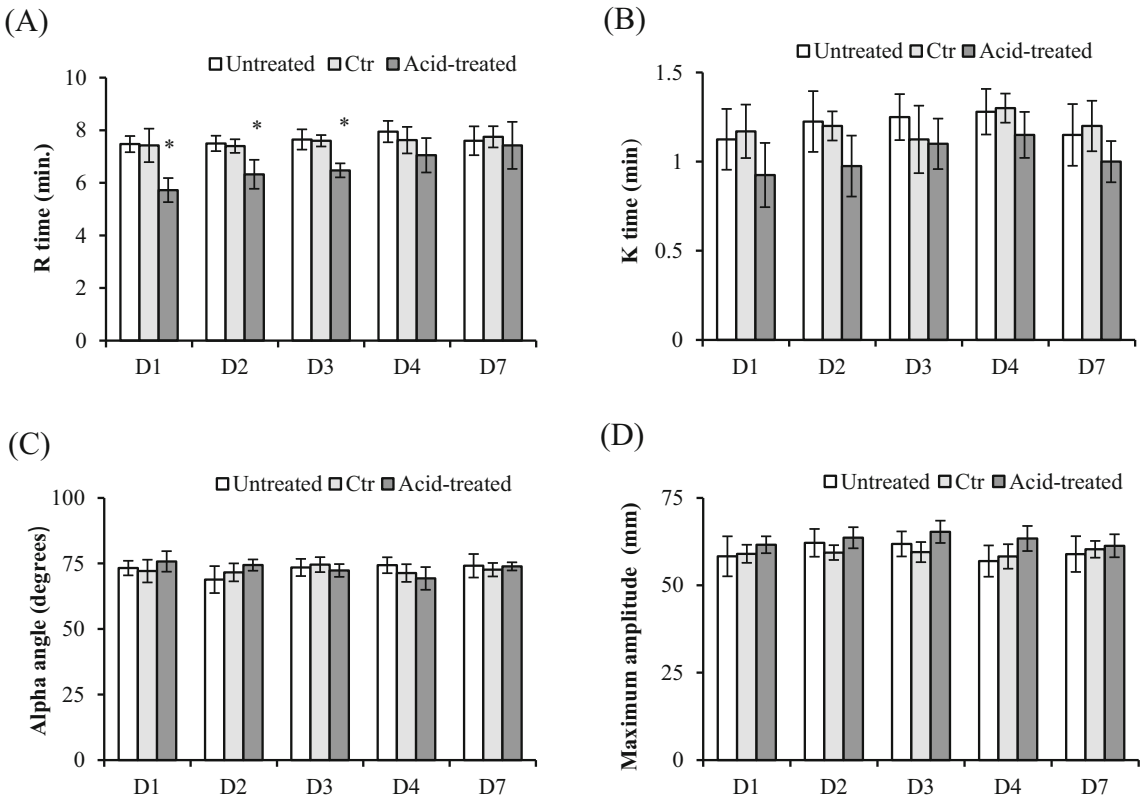


**FIGURE 3** Storage effects on acid-treated PLTs measured as the TEG parameters: R-time = reaction time, K-time = clot kinetics, α angle = clot strengthening (how steep the curve is rising), Max amplitude (MA) = max distance between the curve lines. Results are given as mean ± SD ($n = 4$). ANOVA was used to compare within group (Untreated, Ctr or acid-treated) over time. Paired Students t-test was used to evaluate the statistical significance between groups. Paired students t-test was used to evaluate the statistical significance of the results with $p$-values < 0.05. *$p$ in comparison Ctr PLTs with acid-treated.

$\beta_2$-microglobulin molecules, which was maintained throughout from day 1 to day 7 (Figure 1A,B).

PLT apoptosis and activation are two phenomena that mostly contribute to the PLT storage lesion during conventional and extended storage of PLTs.[13] The mitochondrial permeability transition is an important step in the induction of cellular apoptosis. During this process, the electrochemical gradient across the mitochondrial membrane collapses. Our results show that acid-treatment did not induce significantly increased depolarization of the MMP from day 1 to 4 compared to controls. However, on day 7 of storage reduction of MMP significantly increased when comparing with day 1 in both control and acid-treated PLTs (Figure 1D). Thus, acid treatment did not induce any pro-apoptotic events, as indicated by an intact MMP after 4 days of storage. Further, our results show that pH level in acid-treated and control PLTs remains within an acceptable range of 6.4–7.4[14] for 7 days of storage (Figure 1E).

We examined the surface expression of CD62P and CD63 on acid treated PLTs to evaluate spontaneous PLT activation until day 7. Our results show an increase in CD62P and CD63 expression on acid-treated PLTs as compared to control PLTs from day 1 to day 4 (Figure 2A,C), which most likely is caused by the exposure to acid. This is in accordance with earlier results.[5,6] However, no significant changes in CD62P and CD63 expression on control and acid-treated PLTs were seen on days 1 to 4, but on day 7 both CD62P and CD63 expression increased significantly in both groups compared to day 4. The residual activation potential of CD62P and CD63 was measured in response to stimulation with TRAP6. These activation markers were strongly upregulated on both control and acid-treated PLTs for 4 days (Figure 2B,D), but the upregulation decreased significantly on day 7. In this study, the residual activation potential was measured in the presence of a high dose of TRAP6, 0.4 mM. Based on our previous work,[8] this concentration was used to maximise the residual activation potential of the PLTs. Although, Meinke et al.[5] used lower concentration of TRAP6, the pattern of activation was similar to our results.[6]

The lack of significant difference between the residual activation potential of acid-treated and control PLTs from days 1 to 4, indicates that the acid-treated PLTs are as efficient as control PLTs in initiating and stabilising haemostasis/coagulation for 4 days. This is in agreement with the findings after proteomics analysis of such acid-treated PLTs, which showed that the majority of the key proteins involved in coagulation and haemostasis were not affected significantly by acid treatment.[7]

We used TEG to evaluate the haemostatic function of acid-treated PLTs after 7 days. Acid-treated PLTs showed significantly shorter R-time on days 1 to 3, indicating some increased acceleration of fibrin clot formation. The acid-treated PLTs had a shorter reaction time before starting to form the fibrin clot in the TEG test, probably because they were more pre-activated due to the acid treatment. This is also in agreement with and could possibly be related to the finding of upregulation of GPVI collagen receptor, which induces an increased aggregation response.[5,7,15] However, our results demonstrated that K-time, $\alpha$ angle and MA values were not significantly different in acid-treated PLTs relative to control PLTs from days 1 to 7 (Figure 3B–D). This again corresponds with the general findings after our proteomics analysis of acid-treated PLTs.[7] However, further research is needed to see functional assays using collagen, or even any GPVI specific agonist to improve the insights in platelet reactivity after acid treatment.

Our in vitro results suggest that the quantity and quality of acid-treated PLTs are comparable to control PLTs for 4 days and that they can be used as an alternative to HLA-matched PLT transfusions. Extended post-treatment storage would be useful to indicate flexibility in terms of when acid treatment can be done in relation to the planned transfusion time point for patients with alloimmune PLT refractoriness caused by anti-HLA-antibodies.

## AUTHOR CONTRIBUTIONS

Mohammad Reza Mirlashari, Annette Vetlesen, and Geir Hetland designed the study. Lise Sofie H. Nissen-Meyer provided blood donors. Mohammad Reza Mirlashari and Annette Vetlesen performed the research, data analyses, and drafted the manuscript. Geir Hetland and Lise Sofie H. Nissen-Meyer provided critical feedback. All authors read and approved the final version of article.

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## CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

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CASE STUDY

# Hyperhaemolysis caused by anti-HI antibodies in a patient with myelodysplastic syndrome following a first ever red cell transfusion

**Richard Hinton** [1,2] | **Ruby Haji** [1] | **Richard Kaczmarski** [1] | **Mark Layton** [2] | **Akila Danga** [2]

[1]Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Leeds, UK

[2]Leeds Institute of Rheumatic and Musculoskeletal Medicine, University of Leeds, Leeds, UK

[3]Leeds Orthopaedic & Trauma Sciences, Leeds General Infirmary, University of Leeds, Leeds, UK

[4]Leeds Institute of Health Sciences, University of Leeds, Leeds, UK

[5]NIHR Leeds Biomedical Research Unit, Chapel Allerton Hospital, Leeds, UK

Correspondence
Michalis Panteli, Academic Department of Trauma & Orthopaedics, School of Medicine, University of Leeds, Clarendon Wing, Level D, Great George Street, Leeds LS1 3EX, West Yorkshire, UK.

Email: richard.hinton1@nhs.net

**Abstract**

Background

Hyperhaemolysis is a rare and life-threatening delayed haemolytic transfusion reaction characterised by complement-mediated destruction of both host and transfused red cells. It is well recognised as a complication of transfusion in patients with haemoglobinopathies and has occasionally been described in haematological malignancy and anaemia of chronic disease. Anti-HI antibodies are usually clinically insignificant but have rarely been associated with haemolytic transfusion reactions, including cases of hyperhaemolysis in sickle cell disease.

Methods and materials

Here, we describe a novel case of a patient with myelodysplastic syndrome developing hyperhaemolysis as a result of an anti-HI alloantibody following their first-ever transfusion. The patient required multiple lines of treatment, including erythropoietin, haematinic supplementation, corticosteroids, intravenous immunoglobulin and rituximab.

Results

Following treatment, steady-state haemoglobin was achieved with quiescent haemolysis, and complement inhibition with eculizumab was considered but ultimately not required.

Conclusion

This is the first known report of hyperhaemolysis with an anti-HI antibody in a non-haemoglobinopathy patient. The treatment of hyperhaemolysis is evolving, and future commissioning needs to consider the role of complement inhibition in non-haemoglobinopathy patients.

KEYWORDS

Hyperhaemolysis; anti-HI antibodies; Blood, Donor, Transfusion

## 1 | INTRODUCTION

The incidence of hip fractures continues to increase, along with the global expansion of aging population observed secondary to improved healthcare and quality of life.[1] Subtrochanteric fractures are defined as fractures encountered between the inferior border of lesser trochanter and 5 cm distal to it.[2] They represent a complex subset of injuries surrounding the hip, which are most commonly managed with

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

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

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

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

## 2 | METHODS

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

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

### 2.1 | Data

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

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

**TABLE 1** Grouping of variables into constructs for each model

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

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

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

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

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

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

## 2.2 | Statistical analysis

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

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

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

**TABLE 2** Distribution of explanatory variables by donor status and sex

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

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

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

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

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

## 3 | RESULTS

### 3.1 | Sample composition

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

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

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

## 3.2 | Model selection

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

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

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

## 3.3 | Parameter estimates

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

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

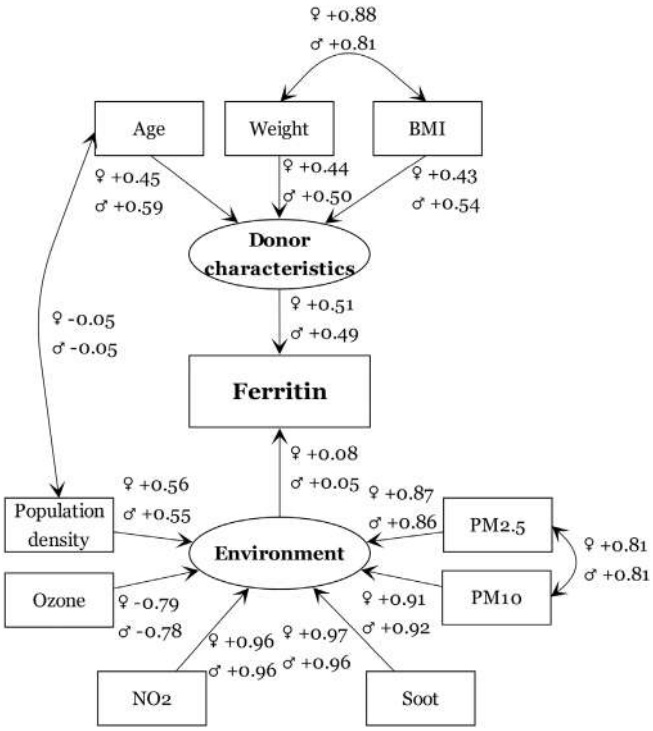


**FIGURE 1** Final structural equation model for ferritin determinants in new donors, with parameters estimated separately for men and women. All parameter estimates are standardised so that the variance of each observed variable and latent construct equals 1

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

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

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

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

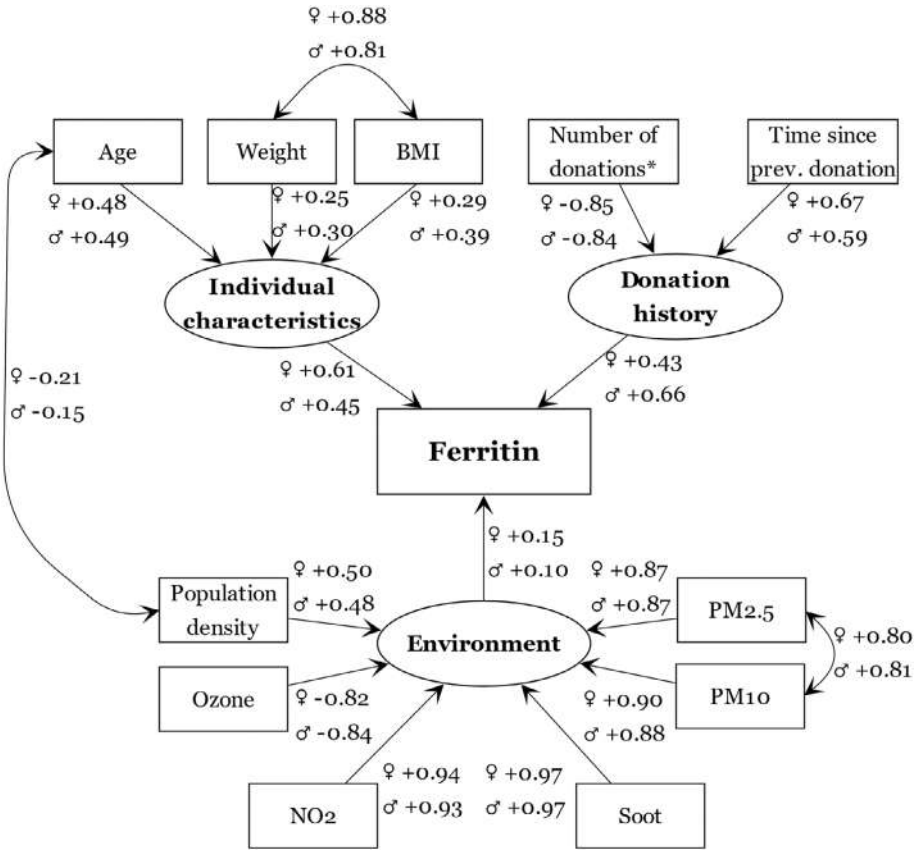


**FIGURE 2** Final structural equation model for ferritin determinants in active donors, with parameters estimated separately for men and women. All parameter estimates were standardised so that the variance of each observed variable and latent construct equals 1

## 4 | DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

## AUTHOR CONTRIBUTIONS

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

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## CONFLICT OF INTEREST

The authors have no competing interests.

## DATA AVAILABILITY STATEMENT

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

## ORCID

*Marieke Vinkenoog* https://orcid.org/0000-0001-5653-8078
*Mart Janssen* https://orcid.org/0000-0002-1682-7817

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## APPENDIX A

### A.1 | STRUCTURAL EQUATION MODELLING OVERVIEW

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

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

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

#### A.1.1. | Measurement model

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

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

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

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

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

#### A.1.2. | Structural model

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

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

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

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

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

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

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