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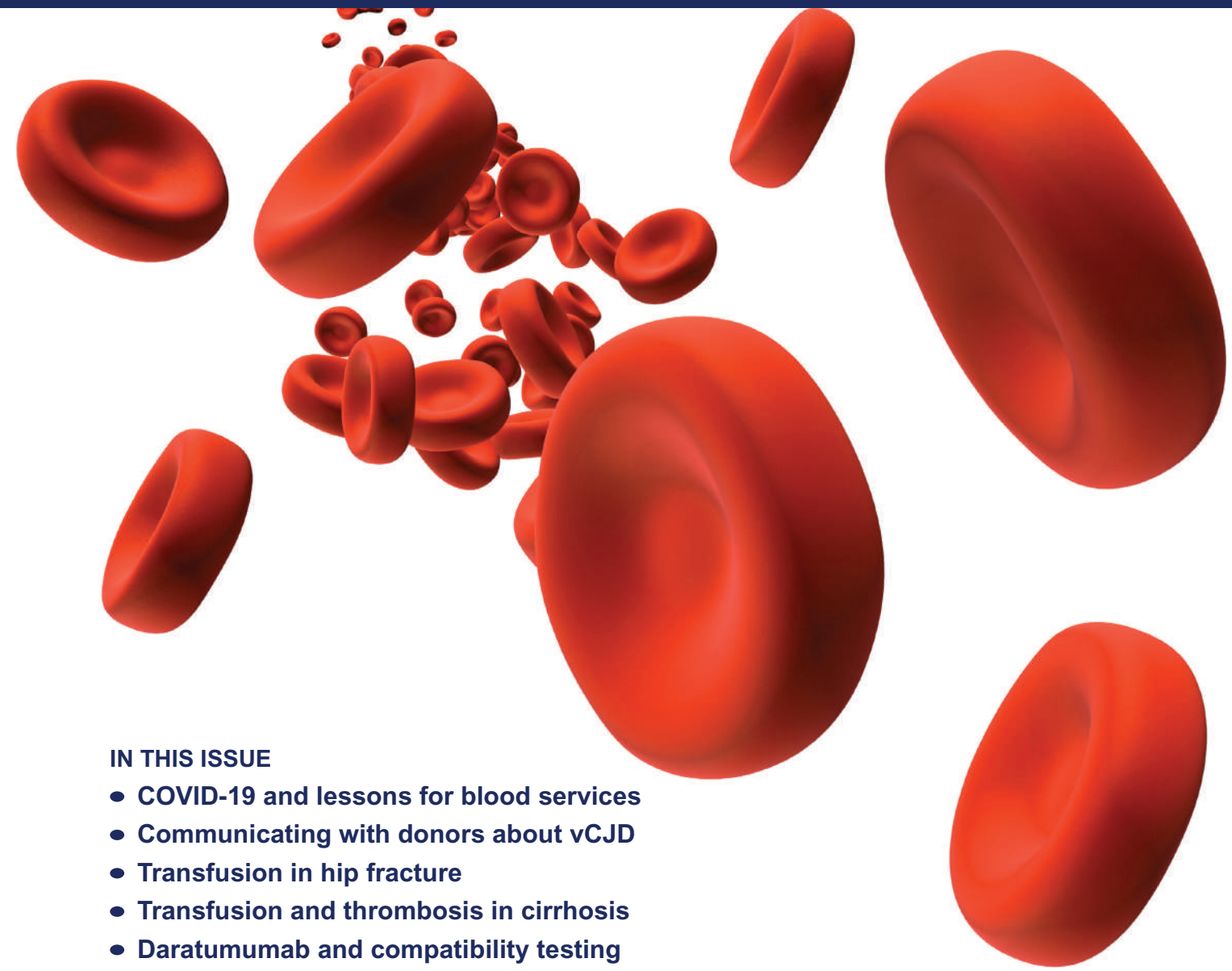

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EDITORIAL

TRANSFUSION MEDICINE WILEY

Blood services, COVID-19 and lessons being learnt: the past pandemic is not over, it's not even past

Although Rh immune globulin (RhIG) has been in use clinically for over 50 years, pregnant patients continue to become alloimmunised to the Rh D antigen due to inadequate perinatal care or RhIG failure.[1-3] In order to identify patients at risk for Haemolytic Disease of the Fetus and Newborn (HDFN), blood banks generally screen for anti-D using a qualitative assay. Once identified, further tests are used to quantify anti-D, with levels used to estimate the risk for HDFN. Although most blood banks throughout the world use the saline indirect antiglobulin test (SIAT; also known as tube test) to determine anti-D titre levels, the British Society of Haematology (BSH) revised guidelines recommend using continuous flow analysis (CFA), which yields a concentration of anti-D measured in international units/mL (IU/mL).

The level of anti-D is critical as it is used to guide patient care. Patients with levels above an accepted threshold are considered to be at high risk for HDFN and must be closely monitored by the obstetric service. Conversely, very low concentrations of anti-D can be categorised as low risk for HDFN, although correlation with clinical history is needed to distinguish between alloimmunisation, which carries the potential for HDFN, and passive antibodies from RhIG treatment. As a result, the test used to quantify antibody levels should be easy to use, allow for reasonable turnaround time and be reproducible.

Based on these requirements, the CFA and tube test both have critical drawbacks. The CFA test requires expensive equipment and specially trained technicians. As a result, the majority of labs in the United Kingdom must send their samples to a reference lab, creating potential delays in critical antenatal care. Also, significant inter-laboratory variability has been reported for the CFA.[4] Conversely, titre levels obtained by tube testing are inexpensive but are time intensive and prone to variability. A third alternative uses automated platforms to run column agglutination technology (CAT) or solid phase technology (SPT). CAT and SPT are affordable test options with decreased variability in methodology and interpretation, as recently demonstrated in evaluation of isohaemagglutinins.[5,6]

Automated platforms are commonplace within modern hospital laboratories, enabling improved workflow for blood typing and antibody screening. Automated platforms may also improve consistency of results, though this has not been extensively demonstrated. However, assessment of antibody levels by automated titration has lagged behind tube testing, as clinically actionable anti-D levels were previously defined by manual tube methods.[7] As studies have shown increased sensitivity in CAT and solid phase when compared to tube, there is concern that these modalities may result in relatively higher titres, which may lead to unwarranted testing as well as undue stress for the patient.

Prior evaluation by Mikesell et al showed that gel testing for RhIG with CAT was more sensitive than SIAT but less sensitive than when using SPT.[3] This group also showed that passive D reactivity can persist for up to 3.5 to 4.5 months after administration with expected variation among different commercially available formulations.[3] As most half-lives range between 20 and 30 days, with more sensitive testing, persistence of antibodies can become problematic as 5 to 6 half-lives are required for drug clearance.[8-11] As RhIG may be detected for long periods of time after prenatal administration, it is critical to delineate the true nature of an antibody and categorise it as passive and benign vs immunogenic with a concomitant risk of HDFN.

As such, it is with great interest that we read Evans and colleagues' work evaluating antibody titre scores by automated CAT vs CFA in the assessment of immune and passive anti-D antibodies. Herein, they describe their experience using the ORTHO VISION automated CAT platform to evaluate nearly 200 anti-D samples in five separate UK hospital transfusion laboratories. This study builds on the work of Bruce et al that initially compared anti-c and anti-D titre scores by manual CAT vs CFA, showing increasing manual CAT titre scores with higher concentrations by CFA.[4] A titre score is a value assigned to assess an antibody's level and avidity. It is calculated using the strength of reactivity at each titration with levels of reactivity assigned with scores (4+ 12, 3+ 10, 2+ 8, 1+ 5, ± 3, 0 0).[4] Evans et al expand on this work with a larger cohort and application of the titre score in conjunction with clinical history of RhIG administration.

The group shows automated CAT testing can effectively distinguish between high and low antibody levels. These low levels defined by titre scores align with currently in use definitions of high and low by CFA (low likely passive<0.4 IU/mL < high likely immune). This would make automated CAT testing an appropriate screening test for the identification of true immune anti-D antibodies vs passive antibodies not requiring CFA. Likewise, they suggest a testing algorithm in which patients could be screened out using titre score. Based on this schema, in which indeterminate results would be reflexed to CFA, all patients would have received appropriate testing in their study.

As current UK standards dictate quantification of anti-D to rule out alloimmunisation, availability of testing is a key factor.[12] This pilot study shows promising results and may represent a solution to the problem of anti-D level assessment. Moreover, this important work helps establish a correlation between automated CAT titre scores and absolute levels by CFA. Though no linear correlation was

demonstrated, understanding this correlation is key for the management of HDFN and ensuring appropriate perinatal care.

Anti-D antibody titre scores are a reasonable starting point for assessment of automated CAT as an antenatal testing modality, as Rh-alloimmunisation represents the prototypic cause of HDFN. Evaluation of maternal antibodies to other blood group systems is a key area of future investigation and is necessary for generalisation of results. This is a pilot study and the group intend to continue their work adding additional clinical correlation and interlaboratory comparison in further studies. Whether labs will internationally adopt this testing is unclear; however, the results point to automated CAT testing as an attractive possibility. Currently, automated CAT titre scores represent a practical screening test for passive anti-D antibody identification.

CONFLICT OF INTEREST

The authors declare no competing interests.

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REVIEW

TRANSFUSION MEDICINE WILEY

Beyond COVID-19 and lessons learned in the United States

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Abstract

The COVID-19 pandemic severely tested the resilience of the US blood supply with wild fluctuations in blood donation and utilisation rates as community donation opportunities ebbed and hospitals post-poned elective surgery. Key stakeholders in transfusion services, blood centres, supply chains and manufacturers reviewed their experiences during the SARS-CoV-2 pandemic as well as available literature to describe successes, opportunities for improvement and lessons learned. The blood community found itself in uncharted territory responding to restriction of its access to donors (approximately 20% decrease) and some supplies; environmental adjustments to address staff and donor concerns about coronavirus transmission; and the development of a new product (COVID-19 convalescent plasma [CCP]). In assuring that the needs of the patients were paramount, the donation process was safe, that clinicians had access to CCP, and vendor relationships aligned, the blood banking community relearned its primary focus: improving patient outcomes.

1 | INTRODUCTION

The COVID-19 pandemic tested the resilience of the US blood supply as it experienced wild fluctuations in blood donations following closure of community donation venues and post-ponement of hospital elective admissions. Shortages of supplies, reagents and personal protective equipment impacted blood centres at a time when they were initiating production of an unproven, novel therapeutic, COVID-19 convalescent plasma (CCP). These problems were unparalleled but not unforeseen: most hospitals and blood centres had decades old pandemic plans designed for influenza.[1] In this light, the COVID-19 pandemic offers an opportunity to consolidate lessons learned and plan for future disasters.

The US blood supply is dependent on a complex supply chain that converges on blood centres as the ultimate suppliers of blood components.[2] Donor recruitment, phlebotomy, testing, manufacturing and distribution must operate synchronously to ensure that the right blood is available for the right patient at the right time. Disruptions impact all stages of the process. Agencies such as the AABB Interorganizational Task Force on Domestic Disasters and Acts of Terrorism, arising out of the 9/11/2001 terrorist attacks, coordinate local and federal responses following environmental disasters and massive trauma situations that overwhelm local and regional resources. Pandemic influenza plans made a decade ago inadequately address the epidemiology of COVID-19 pandemic, but provided an indispensable blueprint.[3]

While large scale disruptions of the blood supply in the United States occur infrequently, the 9/11 disaster, Hurricane Katrina, West Nile virus, severe acute respiratory syndrome (SARS), the 2009 flu pandemic, Ebola and Zika epidemics serve as a prologue to COVID-19. Assuming the occurrence of another pandemic, we seek prescient lessons from the current episode to inform preparations for the next.

2 | BLOOD DONATION- ESSENTIAL SERVICES AND APPOINTMENTS

Several routes exist for engaging donors and raising awareness of the need for blood donations. During disasters, governmental officials and professional societies offer assistance. From the blood center

perspective, coordinated media and public relations campaigns including an integrated social media appeal provide the broadest visibility. Sustained messaging that aligns with anticipated needs serves as the key element so that all render the same script obviating confusion or conflicting information.

In this regard, the AABB Interorganizational Task Force on Domestic Disasters and Acts of Terrorism virtually assembles all stakeholders, untangles conflicting communications and disseminates information about all aspects of emergent needs and regulatory compliance issues.[3,4]

Messaging to the public is challenging at the best of times, but during the pandemic, when prospective donors faced multiple social contact and lockdown concerns and safety messages evolve, reducing noise and motivating individuals to action required unprecedented diligence. In March 2020, as the new virus' airborne transmission threat virus became clear, six US blood centres constituting 67.9% of whole blood collections noted blood drive cancellations.[5] Understanding the growing deficit and risk to health system security, Dr. Jerome Adams, the incumbent US Surgeon General, used the daily coronavirus press briefing platform to urge young donors to donate. As with many of his communications, Dr. Adams highlighted the need hospitals have for an adequate blood supply while reassuring the public that measures were in place to make the donation process safe for everyone.[6]

This created a surge of donations (at least in some geographic areas) for approximately 2 weeks (personal communications A. Hess [ImpactLIFE Blood Services], March 2, 2021, and D. Borge [American Red Cross], March 15, 2021), but not necessarily from the targeted audience. Decreases in donors under age 30 were seen from March to June 2020 compared to 2019.[7–9] This resulted, at least to some degree, from high schools and universities closures where the majority of blood donations from younger donors occurred.

Incentives relevant to the current situation such as antibody testing for SARS-CoV-2 significantly increased donations at some centres. In May 2020 the mean daily donations rose from 2759 to 3476 before and after offering the test ($p = 0.001$) (Figure 1).[7]

Appeals and incentives induced lapsed donors (established donors absent for ≥2 years) to present for donation. In general, blood centres now focus efforts on keeping these donors engaged and returning more regularly via digital marketing strategies and "personalised" messaging. As such, illustrating the need for a greater understanding about motivations, particularly those of the disaster donor and the younger generation to assure daily blood supplies and embedded resilience against unforeseen hazards.[10]

3 | DATA AND ANALYSIS

The dramatic decrease in blood use combined with substantial reductions in blood donation associated with the COVID-19 pandemic required new data analytical approaches for aligning transfusion demand with the donated blood supply.[11] One survey quantified the impact of COVID-19 on blood utilisation and discards among 72 hospitals. RBC and platelet utilisation declined, _9.9% ($p < 0.001$) and 13.6% ($p = 0.014$), respectively. Discards increased for RBCs (30.2%, $p = 0.047$) and platelets (60.4%, $p = 0.002$). The study concluded that because the pandemic led to delaying of elective surgical procedures, blood utilisation declined substantially while blood discards increased, resulting in substantial wastage of blood products.[12]

Previously acceptable data lags between hospital and blood center inventory levels lost utility as the blood supply exceeded demand for several months only to be replaced with blood shortages following resumption of routine hospital practices in late Spring 2020. Blood

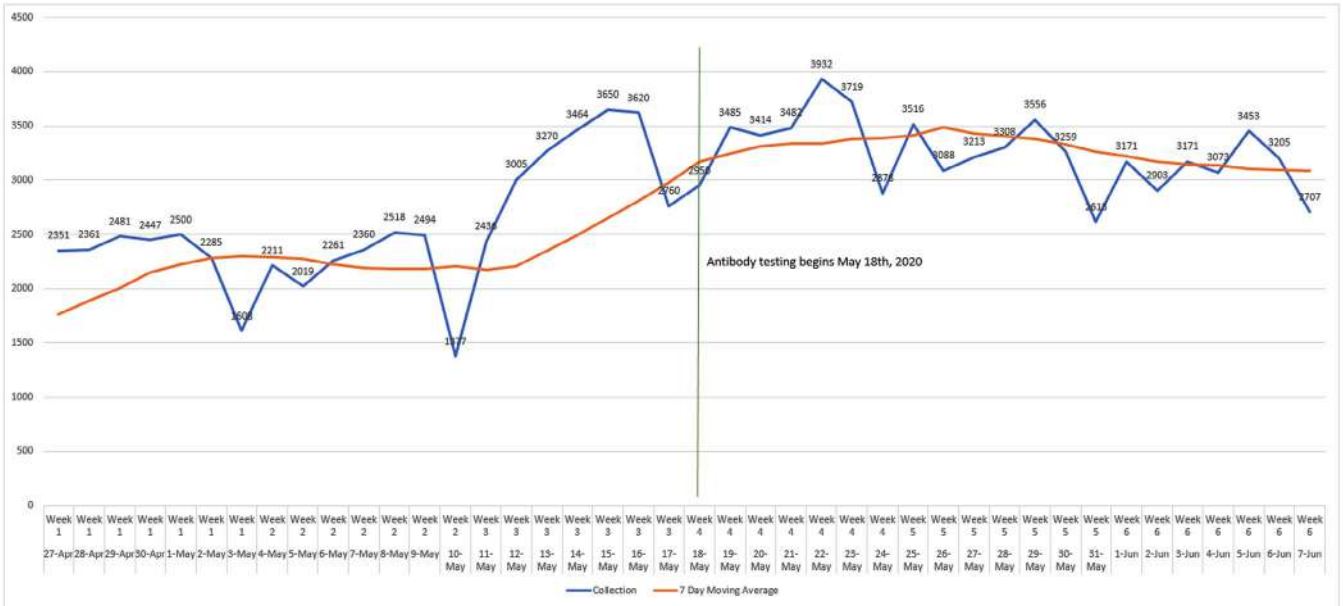

FIGURE 1 Mean daily donations were significantly increased from 2759 daily prior to implementation of anti-SARS-CoV-2 testing for all donors to 3476 post-implementation ($p = 0.001$). (Provided by Kelly Counts-OneBlood).

centres explored new business and computational analyses to mesh legacy utilisation data with current hospital demand to predict blood use and donation rates in real time.[11]

Previous heuristics proved useful during routine times but were inadequate for the agile responses required during the pandemic. For example, one center found, 30% of hospitals utilised 70% of blood collections. Through real-time communication with these hospitals, the blood center's IT department calculated three-day moving averages of blood utilisation. As blood donation venues changed from 80% off-site or mobile collections to less than 20%, it linked these data in designing algorithms for aligning blood demand and supply. This approach optimised product use and minimised wastage. In addition, blood centres revised approaches for obtaining antigen-negative units previously donated by demographically diverse donors who had historically given blood at mobile blood drives away from fixed sites.[11]

Change detection is a statistical method of identifying when current data points have diverged from the normal distribution with high levels of sensitivity. When COVID emerged and altered hospital demand, a blood center used this method to understand increasing and decreasing demand trends. A change comparison with pre-COVID demand was calculated by comparing the same day demand to the previous four same day pre-COVID averages. A leading demand indicator was calculated by comparing current demand to the four previous same day demand averages. The combination of these two metrics allowed for sensitive understanding of the shifts in demand relative to the demand decrease caused by COVID. If the leading indicator decreased, the demand relative to COVID will decrease. When the leading demand indicator increased, the demand relative to COVID would increase. It is important to note that the leading indicator precedes the COVID change in most instances; therefore, the change in demand relative to COVID will lag behind the leading indicator with general trends (Figure 2).[11]

Likewise, CCP collections and distribution demanded development of new relationships and associated data management involving hospital or healthcare provider-identified patients who were recovering from COVID-19 and soliciting plasma donations from them to maintain adequate and changing CCP utilisation patterns. Subsequently, the data fields were expanded to include changing anti-SARS CoV-2 antibody titers. Thus, real-time data availability and agile data management highlight tools and approaches needed for current and future pandemic preparedness responses (Figure 3).

4 | CONVALESCENT PLASMA

4.1 | Plasma collections

CCP donor recruitment challenges were mainly attributable to the blood centres' lack of access to patients who qualified as CCP donors early in the pandemic. This resulted from regulations intended to protect patient privacy (i.e., US Health Insurance Portability and Accountability Act- HIPPA) having the unintended consequence of inhibiting the hospitals as well as state and county health departments from

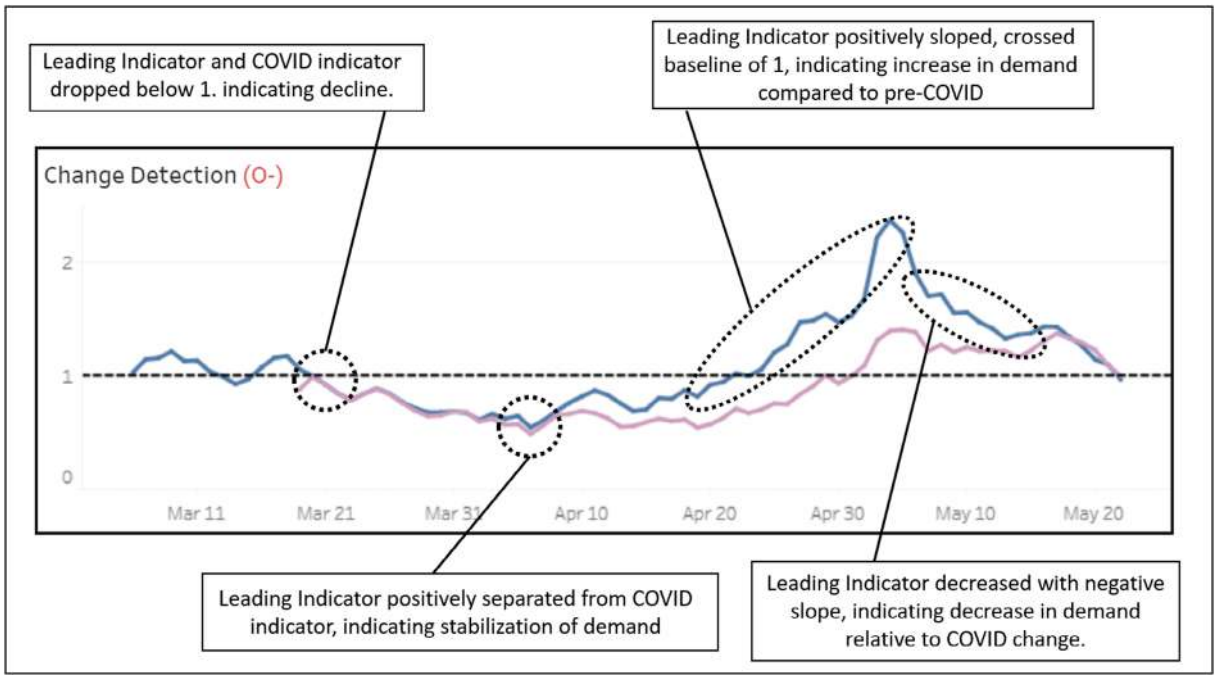

FIGURE 2 The change comparison of demand to pre-COVID demand was calculated by comparing the same day demand to the previous four same day pre-COVID averages (purple line). The leading demand indictor was calculated by comparing the current demand to the four previous same day demand averages (blue line). The combination of these two metrics is beneficial because it allows for sensitive understanding of the sifts in demand relative to the demand decrease caused by COVID. (Provided by Kelly Counts-OneBlood).

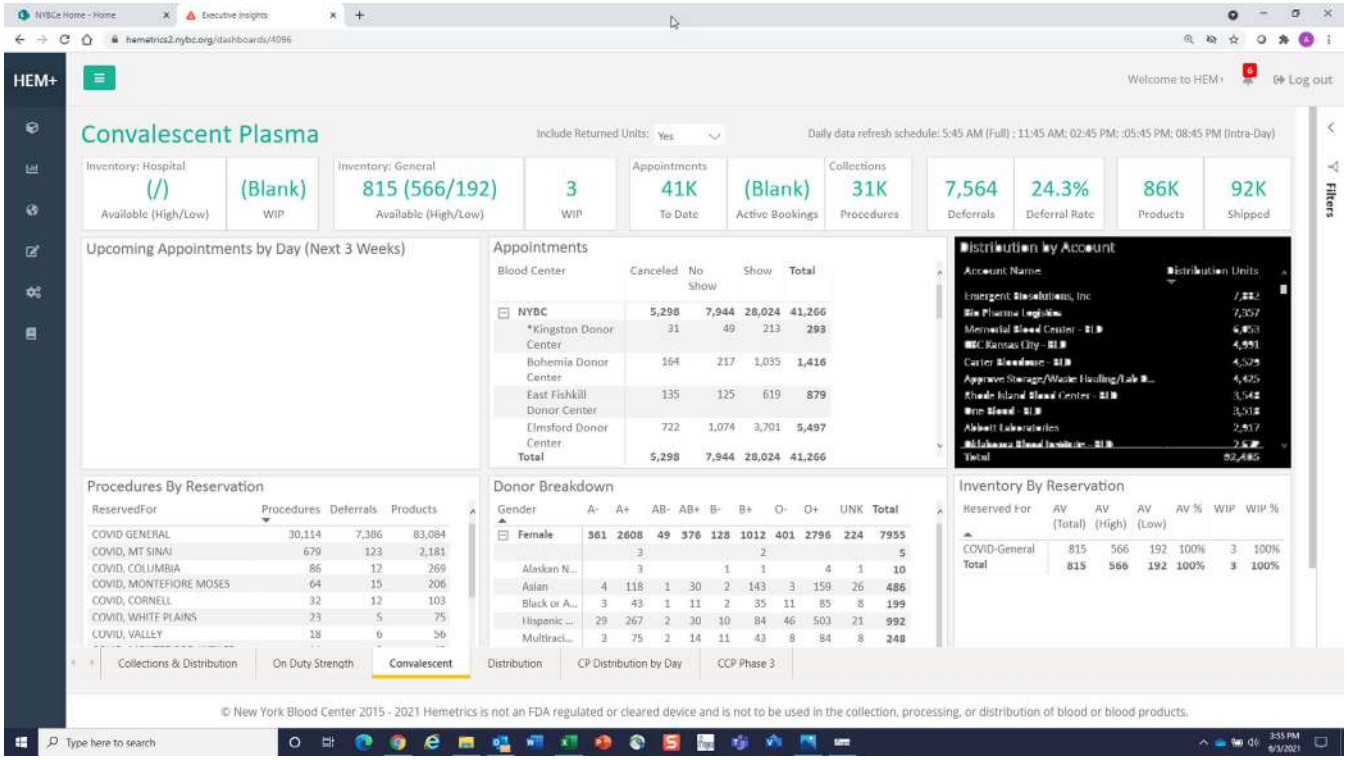

FIGURE 3 An example of real time data availability and agile data management highlight tools and approaches needed for robust pandemic and disaster preparedness responses. (Provided by author D. S.).

sharing needed information about COVID recovered patients.[13] Cooperation by blood centres with their hospitals overcame this barrier.[10]

For example, a blood center's communications team gathered testimonials from early CCP donors, posted them on social and traditional news media, and created behind-the-scenes videos to show the efforts taking place to collect, test, and distribute CCP. Public service announcements aired on local television stations and cable outlets that brought additional awareness to the need for more CCP donors.[13]

A strong pre-existing support structure for implementation f new initiatives such as a project management office, in-house information technology (IT) and business intelligence (BI) units and a business continuity team greatly enhanced blood center responsiveness to CCP collection challenges critical to disaster management at the pandemic onset. The IT/BI team was instrumental in streamlining and automating process intake and distribution. The BI team tracked and transformed complex data into highly functional dashboards and reports that allowed real-time assessment and strategy development (Figure 4).[13]

The continuity team performed daily horizon scanning on a global level keeping leadership apprised of the progression of the pandemic and additional threats. They gathered the CCP implementation team together daily for updates to facilitate and maintain communication in an extremely fluid environment, including frequent changes in food and drug administration (FDA) requirements.[13]

4.2 | COVID-19 convalescent plasma

Passive immunotherapy for infectious diseases has a long history in modern medicine. Early, uncontrolled reports from China suggested therapeutic benefit from CCP as early as February 2020.[14,15]FDA issued the first guidance for industry on collection and use of investigational CCP issued by FDA in March 2020[16] with multiple updates since, revising donor eligibility and later on requiring exclusive distribution of "high-titre" plasma. On August 23, 2020, a major shift in the transfusion of CCP occurred with issuance of the emergency use authorisation (EUA) lowering the barrier for transfusion, based on the "totality of the evidence" that suggested benefits would outweigh risk.[17,18]

4.3 | Hospitals and clinicians

Though unproven, the promise of the safety and effectiveness of CCP in the absence of other therapeutic modalities for COVID-19 resulted in high demand despite uncertainty about optimal use of the product. This necessitated ongoing communication between clinicians and blood center physicians.[13]

A relatively user-friendly expanded access protocol (EAP) under a single eIND facilitated access to CCP by hospitals, clinicians and patients who were unfamiliar with complex clinical research imperatives. Early issues of coordination and preparation at the blood center

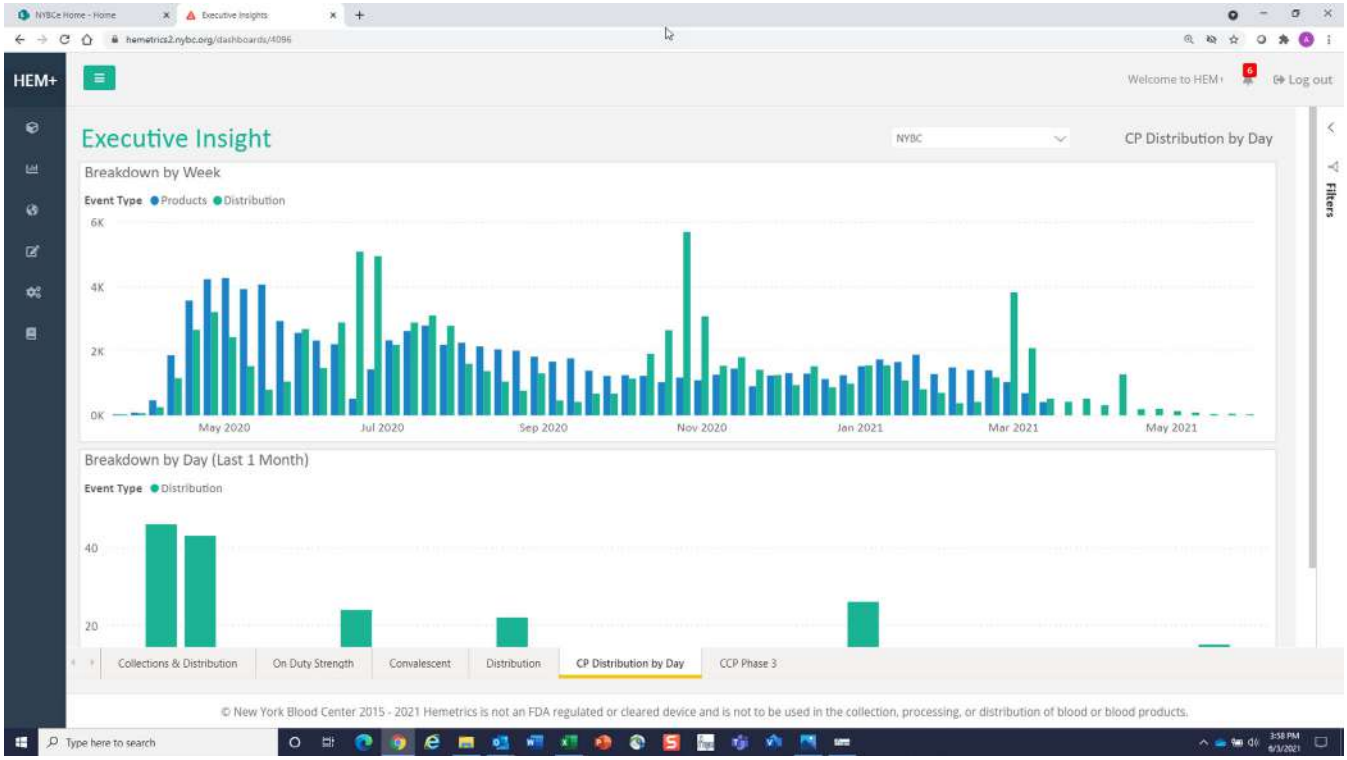

FIGURE 4 Example of a dashboard provided by BI. It displays blood products available in inventory and those distributed. The top half is a breakdown by week and the bottom is by month. (Provided by author D. S.).

and hospital levels for the national programs (eIND and EAP) and the need for use of manual systems caused delays and frustration and led to public relations issues when CCP was not immediately available. Ordering physicians required education on the use of plasma generally and ABO discordant products specifically, highlighting the generic need for improved transfusion medicine education.[13]

4.4 | Randomised controlled trials

The RCT designs for CCP have varied significantly; some were double blind trials in which CCP was compared to a control (placebo or standard plasma) and some were open label trials with the comparison arm being an evolving standard of care. The patients ranged from outpatients with post-exposure prophylaxis and outpatients/emergency room patients with clinically mild COVID, to inpatients with moderate, severe, or life-threatening illness with ranges of oxygen requirements including critically-ill patients on extracorporeal membrane oxygenation. The Randomised, Embedded, Multifactorial, Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP) showed that among critically ill adults with confirmed COVID-19, treatment with two units of high-titre, ABO-compatible convalescent plasma (CP) had a low likelihood of providing improvement in the number of organ support–free days.[19]

While some open label, uncontrolled and/or case-retrospective control studies showed a reduction in disease progression or mortality in patients treated[20]they were clearly insufficient to establish the role of CCP in clinical practice. Available data from non-randomised clinical studies to date preclude the development of clinical guidelines based on disease duration, severity or risk status. Some trials have been terminated pre-maturely due to futility, either related to lack of an efficacy signal at an interim analysis or because ebbing of a local infection surge precluded meeting enrollment targets prior to meeting clinical endpoints.[21,22] These represent ongoing issues for current and future trials.

In addition, several meta-analyses of these studies were performed.[23–27]One, compiling 10 studies concluded with a low to moderate certainty that CCP compared with placebo or standard of care was not significantly associated with a decrease in all-cause mortality or with benefit for clinical outcomes including length of hospital stay, clinical improvement, clinical deterioration, mechanical ventilation use, and serious adverse events.[28]However, it is important to recognise that some sample sizes in the included studies were quite small, the characteristics of the study CCP were heterogeneous, the serostatus of recipients was not well characterised, and the timing of infusion relative to disease onset was highly variable and often quite long.[29] Overall, an outpatient trial using well-characterised plasma very early after symptom onset is convincing evidence that CCP may have a role in the armamentarium, and is consistent with data that resulted in the EUA for monoclonal antibodies in the United States, an analogous form of passive immunotherapy.[19] A large randomised controlled trial conducted in outpatients with COVID-19, most of whom were unvaccinated, demonstrated that the administration of CCP within 9 days after the onset of symptoms reduced the risk of disease progression

leading to hospitalisation.[30] A review of 30 available RCTs demonstrated that signals of efficacy (including reductions in mortality) were more likely if the CCP neutralising titre was >160 and the time to randomization was less than 9 days.[31]It is important to interpret the results of clinical trials conducted amid a pandemic with caution.[32]

In retrospect, several issues are obvious. The impetus to deploy CCP for severely ill patients without other treatment options rapidly and early on during the pandemic was completely understandable. It entailed uncertainty regarding the optimal timing of use and characteristics of CCP for clinical use. In a majority of studies, the CCP SARS-CoV-2 antibody titers in donor plasma were not determined prior to clinical use or were introduced while the study was ongoing. Some studies administered CCP with completely unknown titre while in others the analysis of neutralising antibody titers was performed post-hoc. Likewise, where this information is available, the diverse assays used make direct comparison of studies difficult, especially in the absence of consensus correlates of protection.

Another variable that data from RCTs can address with greater rigour is the effect of concurrent therapies patients with COVID-19 receive. These therapies varied by center and evolved over time as treatment protocols were implemented and often changed by the week, particularly at the onset of the pandemic. Without randomization, factors such as age, severity of illness, the role of recipient antibodies prior to transfusion, and concurrent therapies cannot be adequately controlled. In summary, at present, the effectiveness of CCP in reducing severity of COVID-19 illness and mortality in different patient groups, for example general populations versus those with compromised humoral immunity or unable to respond to immunisation, is uncertain, particularly in those with longer durations of illness.[33,34]

4.5 | Role of Emerging Variants and new questions for evaluation by RCT's

SARS-CoV-2 variants were reported as early as late spring and summer during 2020 and have replaced "wild type" virus due to increased transmissibility. It can be hypothesised that CCP obtained from donors infected with earlier strains may be less efficacious for neutralisation against newer SARS-CoV-2 variants. This important question will need to be addressed in a timely fashion in upcoming trials. Early data suggests that boosting serologic responses of recovered COVID-19 patients using authorised mRNA vaccines may be able to provide cross-neutralisation of these variants of concern which may be useful to determine selection of CCP donors in the future.[35,36]

4.6 | Data harmonisation

The recruitment challenges as well as the range of study designs led to the design and launch of a study, "Continuous Monitoring of Pooled International Trials of Convalescent Plasma for COVID-19 Hospitalised Patients (COMPILE)" to pool deidentified patient level data from ongoing and discontinued RCTs with a goal to reach a consolidated answer on CCP efficacy. COMPILE will analyse data from 100s of patients in the United States and internationally using novel statistical methods to determine the effect of CCP on clinical status as the primary outcome and the effect of covariates, including CCP titre and concomitant medications in secondary analyses.[37] The COMPILE effort aimed at pooling individual results may provide a platform to meaningfully merge data from some of the national and international clinical trials.

5 | THE IMPORTANCE OF THE SUPPLY CHAIN

5.1 | United States

Prior to the pandemic, collection facilities rarely used masks, but, disposable or cloth ear loop mask, face shields, visors and various ancillary items quickly became critical items essential for daily operations. This lack of prior purchasing from suppliers presented a challenge since many suppliers could only commit available product to their "existing customers" and would not take on new business.

Routine supplies frequently purchased by blood centres such as exam gloves, surface disinfectants, hand sanitiser and disposable apparel (e.g., lab jackets, gowns) suddenly became increasingly difficult to source as global demand for these items reached unprecedented levels. As manufacturing production capability fell further; many had to close for a period of time due to COVID outbreaks in their facilities; these difficulties were compounded by raw material shortages. Products produced outside of the United States became difficult to obtain as their home countries prioritised supplies of critical equipment for their population or as borders closed and international shipping was delayed or came to a halt.

To overcome these challenges, the group purchasing organisations worked with suppliers and regulators to lobby for the critical importance of the US blood supply, including CCP, in maintaining a functional healthcare system, thus, convincing suppliers and regulators to prioritise shipments of available supplies to collection facilities. Other successful strategies included working with the manufacturers to develop a monthly allocation of products, and allowing group purchasing organisations to leverage blood centres' combined volume against extended purchasing commitments. This greatly helped to alleviate shortages of critical supplies (Table 1).

5.2 | Outside of the United States

An international survey from 42 countries, including 24 low- and middle income countries, was analysed and found similar challenges to those faced in the United States. Decreases in blood donations occurred in 70.6% of collecting facilities. Despite safety measures and recruitment strategies, donor fear and refusal of institutions to host blood drives were major contributing factors. Almost half of

TABLE 1 Critical PPE items.

Mask (disposable and reusable ear loop mask)
N95 respirator style mask
Face shields/visors
Exam gloves
Surface disinfectants/wipes
Hand sanitizer
No touch or "touch less" thermometers
Disposable apparel (lab jackets, GOWNS)

Abbreviations: PPE, personal protective equipment.

respondents working at transfusion medicine services were from large hospitals with over 10 000 red cell transfusions per year, and 76.8% of those hospitals.

experienced blood shortages. Practices varied in accepting donors for blood or CCP donations after a history of COVID-19 infection, CCP transfusion, or vaccination. Operational challenges included loss of staff, increased workloads and delays in reagent supplies.[38]

6 | NEW PARTNERSHIPS

COVID-19 created opportunities to strengthen current blood center relationships and create new ones. The pandemic and supplying CCP as a first-line therapy forced many blood centres to engage regulators in a new, more collaborative way. Officials in many jurisdictions, with little prior understanding of the blood system, were engaged to support the needs of blood centres and CCP programs.

Another positive benefit was that blood centres in the United States had to work more closely, sharing capacity with one another where this had not been done before. Centres that had CCP would send to those who did not, as with red blood cells (RBC) and other components. Centres became more familiar with hospital customers by not only dealing with their transfusion services but with their administration, treating physicians and public relations personnel. Many worked with plasma fractionators to provide plasma for the development of hyperimmune globulin to treat and prevent COVID-19. Researchers who had not previously worked with blood organisations now had opportunities to collaborate on providing access to sample tubes and components from large and fairly representative populations.

New opportunities for public health collaboration arose including testing, seroprevalence studies and finally some providing SARS-CoV-2 vaccinations to blood center employees. New collection/donor advocacy groups and funding partners formed with which many blood centres collaborating with Blood Centres of America and America's Blood Centres (Table 2).

7 | THE CHANGING NATURE OF REGULATORY COMPLIANCE

Early in the pandemic, the FDA communicated the critical need for a continuous blood supply and moved quickly to augment inventories by liberalising recommendations that previously made some donor populations ineligible.

TABLE 2 Some new collection/donor advocacy groups and funding partners of blood centres.

Survivor corps
Refuah health/orthodox Jewish community-chaim lebovits
Archdiocese
Big 10 network
Microsoft (The fight is in United States)
Department of defence
Operations warp speed
BARDA

Abbreviation: BARDA, biomedical advanced research and development authority.

Although issued for immediate implementation, the public was unaware of the complexities of executing such changes in the highly-regulated blood center environment that requires updates to blood establishment computer systems, revisions to procedures, staff training, and proper notification to the donating public.[39,40]The associated weeks-to-months lag between FDA rule changes and blood center implementation caused frustration among donors and the media. Some stories about failed attempts to donate received national attention, especially when the involved persons were otherwise eligible to give the much-coveted CCP.[41]Understanding and addressing this disconnect is a necessary component for positive donor and community engagement.

As it is likely that there will be future outbreaks of viruses that may require treatment with CP or other novel blood products, the interpandemic period provides an opportunity for developing protocol templates and rigourously evaluating them (e.g., RCTs) to minimise the time required to move from theory to clear guidance on their clinical value and optimal use. In the event of a pandemic caused by an agent that is transfusion transmissible, regulatory options include mandating pathogen reduction of the entire blood supply is required. Current platforms are insufficient to accomplish this. Decision making parameters and funding for this capacity must be a top priority of governmental policy makers.[13]

8 | PREPARATION FOR FUTURE PANDEMICS AND OTHER WIDESPREAD DISASTERS

The US Department of Health and Human Services (HHS) released a report to Congress on the adequacy of the US national blood supply calling out several vulnerabilities including the ageing of the donor base, the centralisation of laboratories, and the deteriorating profitability of centres that has limited innovation.[42] While these issues are managed daily, a disaster or pandemic could overwhelm and debilitate the system similar to a storm surge that breaches an inadequate levee. Given the unknown nature of emerging pathogens, there is no "one

size fits all" plan for preparedness. However, while we cannot predict exactly when or in what form the next threat to our health system will appear, we need to be broad and creative in setting up systems that will promote rapid response resiliency or face the consequences of inadequate supply or inability to utilise available blood.

Elements of preparedness include horizon scanning and formal surveillance for early detection, identifying vulnerabilities among staff and donors to facilitate necessary protections, defining and recognising pandemic phases and staged responses, risk management principles and resource allocation. These elements intersect and complement each other. Depending on the given circumstances, certain activities will take precedence at certain times, but all of them require forethought and a structure (i.e., policies and personnel) on which to perform when needed.[43]

Planning is not a process with a beginning and an end. Just as the blood community has embraced continuous quality improvement, so should it view preparedness as continuous and iterative. COVID-19 has given us a clearer understanding of what is essential in many areas of life and work. Coordination with hospital partners, public health and disaster response organisations will prove invaluable, especially to avoid well intentioned public messaging that can threaten the blood supply. Good communication underpins good relationships. These need to be cultivated to permit rapid decisions makings and access to resources as a catastrophe evolves; just knowing who to call for help in a crisis can save valuable time. In the meantime, securing "essential worker" status for employees and ensuring blood center inclusion with public health planning venues could be a lynchpin for healthcare continuity.

9 | DISCUSSION

There are important lessons in the COVID-19 experience that should inform a blueprint for the inevitable next pandemic. In regard to CCP, this is not a critique of the truly impressive on-the-fly implementation of CCP collection programs, compliant with current good manufacturing practices in the midst of a pandemic that stressed the blood supply in many ways. Rather, it is an important "after action" responsibility in the context of disaster preparedness.

The EAP effort in the United States was biassed toward treatment of severe illness. The long history of passive immunotherapy suggests that, for acute pathogens, very early use (even pre-exposure) was likely to be more effective. For the next pathogen we need to address the issue of early versus later treatment explicitly and in advance of being called upon to design both expanded access and high-quality clinical investigations.[44]

There is a need for a prospective plan for systematically banking, locally and nationally, an appropriate range of donation specimens, anticipating the early scarcity of effective assays, both for diagnosis and characterisation of convalescent therapeutics, even if that characterisation will occur after the fact.

Another consideration is at a minimum, a realistically accessible set of objective recipient demographics and clinical outcomes to be collected from the very beginning. Examples might include elevation of the level of care, mortality, length of stay among others. Details requiring expert adjudication should be avoided. These must be suited to the assessment of what can be provided from the blood community, but also other aspects of care such as additional therapeutics, risk stratification and assessment. This may require federal action and funding to maximise the ability to collect and share the data, for example harmonisation of minimum regulatory requirements of electronic medical records. Facilitated data sharing that respects privacy interests must be included.

Many questions remain unanswered. What are the elements of the process required to decide which donors are safe sources of a convalescent product and when? Does uncertainty about the transfusion transmissibility of a future pathogen impose an affirmative responsibility for blood and plasma collectors to be able to apply available pathogen reduction to a convalescent product, even if that is not the standard-of-care for routine collections? Do we need to consider emergency authority from the regulatory authorities (e.g., FDA) to pool products from multiple recovered donors to increase the probability that a convalescent product will, in fact, contain reasonable levels of the antibodies we think may be clinically useful? If the answer is "yes" that sets a task for the regulator now, and then for the blood community to design and validate processes and have the capacity to implement them, either before or on short notice when they are needed.

While blood centres have become efficient at controlling costs and inventory under normal circumstances, there are lessons to be learnt to consider moving forward beyond the COVID-19 pandemic. (Table 3).

TABLE 3 Lessons learnt

Consider dual and multi-sourcing directly with manufacturers and distributors.
Develop product prioritisation approvals with each supplier in preparation of the next disaster.
Develop a broader contract portfolio of domestic-based suppliers to provide more control of access to critical products when international supplies may not be reliable.
Re-evaluate just-in time inventory management levels. Increase the critical items' supply-on-hand in the event of a disaster for both suppliers and blood center.
Address resistance at the local blood center level to funding the expense of maintaining inventories of supplies in excess of immediate need.
Establish a strategic stockpile of PPE and other supplies designated as critical that is prepositioned and managed by an appropriate entity and supported by HHS or other governmental agencies to accelerate capability.
Create and access the national stockpile of PPE products as needed such as with the EU model[45,46]
Consider the potential value of pathogen reduction of blood products as technologies become available. Future emerging infections may be transfusion transmitted and, even if this is not the case, pathogen reduction technologies would provide assurance during the inevitable delay between the onset of the threat and definitive discernment of the transfusion risk.

Abbreviations: EU, emergency use; HHS, health and human services; PPE, personal protective equipment.

New partnerships as a result of the COVID-19 pandemic assisted the industry during this event and are expected to provide similar assistance in future disasters. Maintaining and enhancing such partnerships facilitates new treatment development involving blood products and blood derivatives for example, encouraging investigators to envision blood centres and transfusion services as research material sources This requires in place donor/patient consents that meet contemporaneous requirements and allow immediate use of available materials and subsequent follow- up consent from donors/patients that obviates the current frustrating process and loss of potentially willing, eligible study participants.

10 | CONCLUSIONS

Our traditional assertion that blood donations "save lives" is our organising principle for selecting our lessons learned. By assuring the needs of the patients were paramount, that the donation process was safe to sustain inventory adequacy, that new products (CCP) might improve outcomes, and that vendor relationships align with these principles, the blood community "relearned" their mission focuses on improving patient outcomes.

At the time of submission of this manuscript, the pandemic is not over, nor is the involvement of the blood community in the response. The list above will grow as we have more time to reflect on what was done and what could have been improved, leaving us better prepared for both the next wave and the next pathogen.

Ultimately, we will not know if our work toward resilience was successful until the crisis has come and gone. When we ever find ourselves saying, "we should have done more" it is impossible to go back in time and make corrections, but we should aim to do better the next time.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REVIEW

TRANSFUSION MEDICINE WILEY

Convalescent plasma and COVID-19: Time for a second—second look?

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Abstract

In this short narrative, we highlight some of our experiences leading the US Convalescent Plasma Program at the beginning of the pandemic in the spring and summer of 2020. This includes a brief summary of how the program emerged and high-level lessons we learned. We also share our impressions about why convalescent plasma was used at scale in the United States, early in the pandemic and share ideas that might inform the use of convalescent plasma in future outbreaks of novel infectious diseases.

KEYWORDS
convalescent plasma, pandemic preparedness, real-world evidence

Convalescent plasma (CP) has been used worldwide to treat COVID-19 since the start of the pandemic. In the United States alone, more than 500 000 hospitalised COVID-19 patients have received CP since March 2020.[1] CP use in the United States started as compassionate use treatments for individual patients who were severely ill in the intensive care unit (ICU). The plasma was obtained by blood centres across the United States, who used approaches based on their experience with recruitment techniques and consistent with Food and Drug Administration (FDA) guidelines to capture CP donors. Officials at the US FDA quickly realised a single patient approach to treatment would lead to hundreds of individual emergency use IND applications (e-IND) with little organisation, safety oversight or coordination. Leadership at the US FDA contacted Mayo Clinic scientists to organise a national expanded access program (EAP), which became the US Convalescent Plasma Program (USCPP). The USCPP utilised an expanded access regulatory mechanism coupled with a research design that enabled the systematic collection of safety and high order outcome data on all patients enrolled into the expanded access program. The US Convalescent Plasma study was an analysis of data extracted from the USCPP organised as a real-world evidence (RWE) approach.[2] The goals were to evaluate safety directly and efficacy through several potential mechanisms including outcomes by neutralising antibody titre, timing of administration and electronic health record studies (to add a control group of patients not treated with convalescent plasma). These study elements were designed in close collaboration with the US FDA. The use of a randomised clinical trial mechanism was not endorsed by the FDA at the start of the USCPP in April 2020, so a RCT was not an authorised design option despite the criticisms post facto by many for not choosing a RCT Design.[3] The USCPP was facilitated by initial funding from Mayo Clinic and later by full funding from the US Biomedical Advanced Research and Development Authority (BARDA). There were substantial non-financial contributions from the public and private sector, numerous individuals, and media programs to promote donor awareness campaigns. We also acknowledge substantial contributions by specific community partners and the US Blood Banking network. For those interested, a more detailed narrative history is available here.[4]

The FDA expanded access mechanism was ideal for CP and the COVID-19 pandemic because it allowed the use of an experimental therapy in a life-threatening situation where limited treatment options existed.[5] This regulatory mechanism also provided access to CP for hospitalised patients with COVID-19 who were being treated at non-academic, non-research facilities with minimal regulatory barriers to initiating the protocol across the country. The original vision for the

USCPP was a modest sized demonstration project, expecting at most 5000 patients with a focus on providing broad access and understanding if CP was safe. Safety was defined primarily by the incidence of transfusion-related adverse effects within 4 h of transfusion. To monitor safety, a real-time data analytic workstream was established to generate weekly Data and Safety Monitoring Board reports. These safety reports were reviewed by a physician panel that included experts from critical care, transfusion medicine and clinical trials. Historical incidence of transfusion-related adverse effects were used to frame comparisons regarding the incidence observed in the treatment of COVID-19.

From a regulatory perspective, the study design allowed the evaluation to be conducted with a bioethical regulatory framework, with safety, ethical and regulatory oversight. All participants were enrolled using a standard informed consent process approved by the central IRB without substantial local modifications. All physicians administering CP had to be registered as local physician investigators with a valid medical licence, and all hospital and acute care sites where CP was administered had to utilise the study's central IRB. Sites with their own IRBs deferred to the central IRB. The acquisition of CP and its delivery were not part of the research process. The national standards of care and existing medical system processes for blood products were followed with close collaboration by national and local leaders in the blood banking community, again designed to maximise the safe and expedited collection and distribution of CP.

Utilisation of CP tracked the disease incidence across much of the country.[6] It is presumed that the high utilisation was largely due to a lack of other COVID-19 evidence-based life-saving therapies and broad, non-restrictive inclusion criteria coupled with the published safety data on CP. Enrolment ultimately included over 105 000 consented participants with over 94 000 receiving CP prior to September 2020. Additionally, hundreds of thousands more patients were treated with CP when the USCPP transitioned to an Emergency Use Authorization (EUA) in late August of 2020.[7] The sudden and rapid growth of CP use in the United States significantly beyond initial expectations occurred for multiple reasons including (1) lack of any established therapies which reduced mortality in patients who were perceived to be at high risk for disease progression and/or death, (2) the history of CP as an effective therapy in patients with acute respiratory illnesses associated with pandemics,[8] (3) anecdotal reports by physicians, families and the media of efficacy, (4) an intense desire by the health care community and the public writ large to offer all potentially efficacious treatments with minimal safety concerns, (5) the rapid spread of the infection which outstripped other available therapies, and (6) coverage of the cost of collection and production of CP at blood centres allowed the product to be provided to hospitals at no cost.

The FDA charge to evaluate efficacy with the USCPP included assimilation of data from large, non-public datasets and analyses using neutralising antibody titres (nAb) from donated plasma, when such diagnostic tests were available on stored residual aliquots from the donated CP. It was expected that we would explore efficacy in late 2020 or into 2021 (Marks P, US FDA personal communication). In the meantime, as 2020 progressed, matched control studies, some early small RCTs, and case reports in patients who could not generate endogenous antibodies to COVID-19 infection began to appear and generally showed at least some signals of efficacy.[9,10] The massive scale of the USCPP also increased pressure for us to see what efficacy signals might be embedded in our growing registry even though it lacked a "traditional" control arm. There was also significant interest from policy makers about any potential efficacy given the substantial public funding supporting the program. In this context, after in this context collaboration with officials at the US FDA, we hypothesized that 'early' treatment of patients with 'higher nAb plasma' would be associated with reduced rates of mortality. All the CP utilised in the USCPP was distributed to sites and patients without any prior knowledge of the nAb titre value. Thus, the distribution of CP without a prior knowledge led to random distribution of 'high titer nAb' plasma, 'low titer nAb' plasma and 'intermediate titer nAb'. Since neither the distributor of plasma nor the treating clinicians had knowledge about nAb titre levels within the CCP being administered. All of us were blinded as to the treatment allocated since the nAb titres had not been defined prior to administration of CCP. Because antibody testing of CP units was not available early in the pandemic, none of the treatment decisions were based on nAb titre knowledge. Thus, we were able to compare mortality across these titres comparing low titre to high titre nAb where we found a positive benefit from the high-titre nAb CP as hypothesized.[11]

By mid-summer 2020, early signals of efficacy were emerging from these efforts. Prior to elaborating further on those, it is helpful to understand some terminology. The US Convalescent Plasma study was conducted on the "expanded access" regulatory pathway. This pathway allows for compassionate use of investigational products outside of standard clinical trials. Like other clinical studies, expanded access protocols require standard research principles (e.g. consent, presumed favourable risk to benefit ratio, collection of and monitoring for safety and adverse events). An emergency use authorization (EUA) is a higher level of regulatory approval, which can be granted during a public health emergency.[12] In an emerging pandemic such as the COVID-19 pandemic, very few treatments can be fully tested and approved using the regular regulatory pathways for drugs and devices. To avoid catastrophic loss of life while the evidence is accumulated, the EUA pathway allows for products where it is 'reasonable to believe the product may be effective' and that have 'known and potential benefits [that likely] outweigh the known and potential risks' to be approved. FDA's issuance of the EUA for CP in August 2020 was based on, in part, the data that was rapidly gathered by the US Convalescent Plasma study.[13]

We suggest that those who identified limitations in our study design and analysis recognise the randomised aspect of the analysis of high-titre nAb CP, as well as the constraints we faced early in the pandemic of gathering a limited dataset from extraordinarily busy health care providers caring for critically ill patients during a global crisis. We were not afforded the customary approach to a RCT, with time for site training, investigator meetings, GCP training/certification and availability of trained monitors who could travel for site training and site monitoring. Many of our site PIs were working in conditions of

extreme duress—at a time when the vast majority of the world's population was sheltering in place. These front-line providers were facing an uncertain future with a virus that was incompletely understood with regard to risk, spread and mitigation. We captured the essential safety, demographic and vital status data to answer the primary goal—an assessment of safety with the finding that CP was safe to use. Efficacy was pre-planned but was a secondary goal with our study. As we look back on how and when these and other signals of efficacy for CP emerged and reflect about what we have learned for any potential 'next time' or simply to improve large scale trials, it is also important to address the shortcomings and criticisms related to how the CP story evolved in the United States.

The first lesson is that CP will almost always be the first antibody therapy available to treat an outbreak of a novel infectious disease. In this context, optimal use of CP should follow the historical principles of successful antibody therapy that have emerged during the late 19th century and prior to World War II.[14] Namely that (1) early treatment with plasma (2) that has sufficient antibodies specific for the pathogen is essential. Since the initiation of the USCPP, several important RCTs have been conducted for CP as a treatment for COVID-19 with a number of 'negative' studies that showed no harm but no benefit and a number of studies that found reduced mortality.[15,16,17,18] A careful analysis of all studies reveals that even so-called 'negative' trials show signals of efficacy when data are analysed using high titre plasma that is used early in the course of disease.[19,20,21] Some of these studies evaluated CP in a RCT in patients that we saw no signal of efficacy and contrary to all historical evidence. One must rhetorically ask why ethical boards approved such experimental plans despite the likely result of futility? An extension of this lesson is that one must be mindful of the source of CP that is used in the treatment. Matching CP with the circulating regional variant, something many initial clinical trials were not able to do, was later shown to be important.[22]

The second lesson is that assay systems are necessary to understand the properties of CP and other elements of the immune response generated against a novel infectious agent. In this context, if assay systems were developed against a suite of "model" viruses it should be possible to have adaptable tools that could be quickly modified by experts in assay development. These tools could then be used at scale to quantify the immune response to the novel agent, and antibody titres in CP from recovered patients. Basic research on the CP itself may reveal individuals that develop the optimal protective immune response (antibody and other factors) versus those that are more susceptible to disease progression, so that targeted immune strategies can be developed to prevent severe disease and death. If a nationwide expanded access program for CP is used in a future pandemic it may relatively quickly reveal differences in susceptibility across populations that could be used to inform strategies that reduce infection. Thus, platform technology needs to be developed for diagnostic testing and assay systems as well as vaccines in anticipation of CP use the *next time*.

The third lesson or lessons all relate to questions about what it takes to conduct randomised controlled trials in a pandemic. Beyond obvious regulatory issues like streamlined and coordinated approaches to IRB oversight, informed consent and data management—at least 11 other important questions need to be considered:

1. What is the use case under study—prophylactic, or early versus late disease?
2. What simple endpoints might give definitive answers in a chaotic situation with maximally stressed hospitals and staff?
3. What would be the right comparator?
4. In a rapidly changing treatment landscape, is it reasonable to hold all treatments identical except for the randomization of comparator versus plasma?
5. How do you characterise the plasma and know what dose to give?
6. How should (or even should) CP be prepositioned ahead of time—especially when regional variation in plasma may impact local efficacy?
7. As the disease erupts, waxes and wanes regionally, how do you anticipate where the sites will be ahead of time? What site selection criteria are essential for initial screening since ultimate participation will depend on disease incidence?
8. How do you train the study staff, especially when travel is suspended?
9. How do you monitor trial, after conduct especially when travel is suspended?
10. How do you overcome ethical and perceptual issues about randomization with a deadly illness, especially in scenarios where the preliminary data are returning some signals of efficacy?
11. In a huge country with diffuse approaches to medical care, what do you tell patients, loved ones and dedicated physicians seeking promising therapies in non-research settings?

All the considerations and caveats above relate to larger questions concerning 'did the US jump the gun on plasma use early in the COVID-19 pandemic before doing trials' instead of deploying CP in a more systematic way? In this context, the early establishment of CP safety by the EAP (later confirmed by large RCTs) demonstrated that CP did no harm.[23,24] Next, when the mosaic of data from all sources is considered, signals of efficacy consistent with the principles of antibody therapy noted earlier are clearly present for CP and COVID-19.9,10 So-called 'Real World Data' indicates that tens of thousands or even more than 100 000 lives were saved.[1] Additionally, there is strong observational evidence for CP efficacy in patients with conditions that limit their ability to generate endogenous antibodies.[25,26] Evidence for this use case may never have occurred or occurred later in the pandemic if only classical trials of CP for COVID-19 had been conducted. We also note that non-academic treating physicians seemed to have figured out the early use case for CP because treatment of mechanically ventilated patients dropped dramatically in the first several months of the USCCP prior to studies establishing the likely futility in late disease.[27] Finally, nothing prevented large academic centres and

networks from *not* participating in the USCCP and EUA for CP, and nothing prevented them from conducting traditional trials—in fact several did—there were certainly enough patients to sustain both. In fact, as 2021 progressed numerous RCTs, as highlighted by the Johns Hopkins' early outpatient treatment trial, demonstrated efficacy with early administration or high titre CP.[28] Finally, during the second and third years of the pandemic the issue of "escape" by newer variants has rendered many monoclonal antibody therapies ineffective. In this context, CP from donors that have been both vaccinated and recovered from infection generates a very high titre and may be especially useful as replacement therapy in the immune suppressed.[29,30] However, the cost of production and reimbursement issues remain a concern in the United States.

So, when there is a *next time* will it be possible to provide broad based access to a promising therapy like CP and at the same time study it in a controlled fashion? The short answer is yes, but the longer answer is not without some planning and intentionality of design. If we had waited the months required to set up a "proper" series of trials, tens of thousands of additional deaths or more would have occurred. If CP treatment had been focussed on early use from the outset and the CP administered had been better characterised, perhaps even more lives could have been saved. The good news is that we showed that it is possible to launch a successful nationwide program to make CP available during a crisis and from the lessons we have learned improvements can be made.

AUTHOR CONTRIBUTIONS

MJJ drafted the manuscript with subsequent detailed input and feedback from the co-authors.

CONFLICT OF INTEREST

The authors have no financial conflicts.

DATA AVAILABILITY STATEMENT

This is a brief review/commentary no original data included.

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ORIGINAL ARTICLE

A systematic review of the safety and efficacy of convalescent plasma or immunoglobulin treatment for people with severe respiratory viral infections due to coronaviruses or influenza

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Abstract

Objective: Evaluate the safety and effectiveness of convalescent plasma (CP) or hyperimmune immunoglobulin (hIVIG) in severe respiratory disease caused by coronaviruses or influenza, in patients of all ages requiring hospital admission.

Methods: We searched multiple electronic databases for all publications to 12th October 2020, and RCTs only to 28th June 2021. Two reviewers screened, extracted, and analysed data. We used Cochrane ROB (Risk of Bias)1 for RCTs, ROBINS-I for non-RCTs, and GRADE to assess the certainty of the evidence.

Results: Data from 30 RCTs and 2 non-RCTs showed no overall difference between groups for all-cause mortality and adverse events in four comparisons. Certainty of the evidence was downgraded for high ROB and imprecision. (1) CP versus standard care (SoC) (20 RCTS, 2 non-RCTs, very-low to moderate-high certainty); (2) CP versus biologically active control (6 RCTs, very-low certainty); (3) hIVIG versus SoC (3 RCTs, very-low certainty); (4) early CP versus deferred CP (1 RCT, very-low certainty). Subgrouping by titre improved precision in one outcome (30-day mortality) for the 'COVID high-titre' category in Comparison 1 (no difference, high certainty) and Comparison 2 (favours CP, very-low certainty). *Post hoc* analysis suggests a possible benefit of CP in patients testing negative for antibodies at baseline, compared with those testing positive.

Conclusion: 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 yet produced antibodies to the virus would be useful to target therapies at groups who will potentially benefit the most.

KEYWORDS
convalescent, hyper-immune immunoglobulin, infection, plasma, respiratory, safety

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]

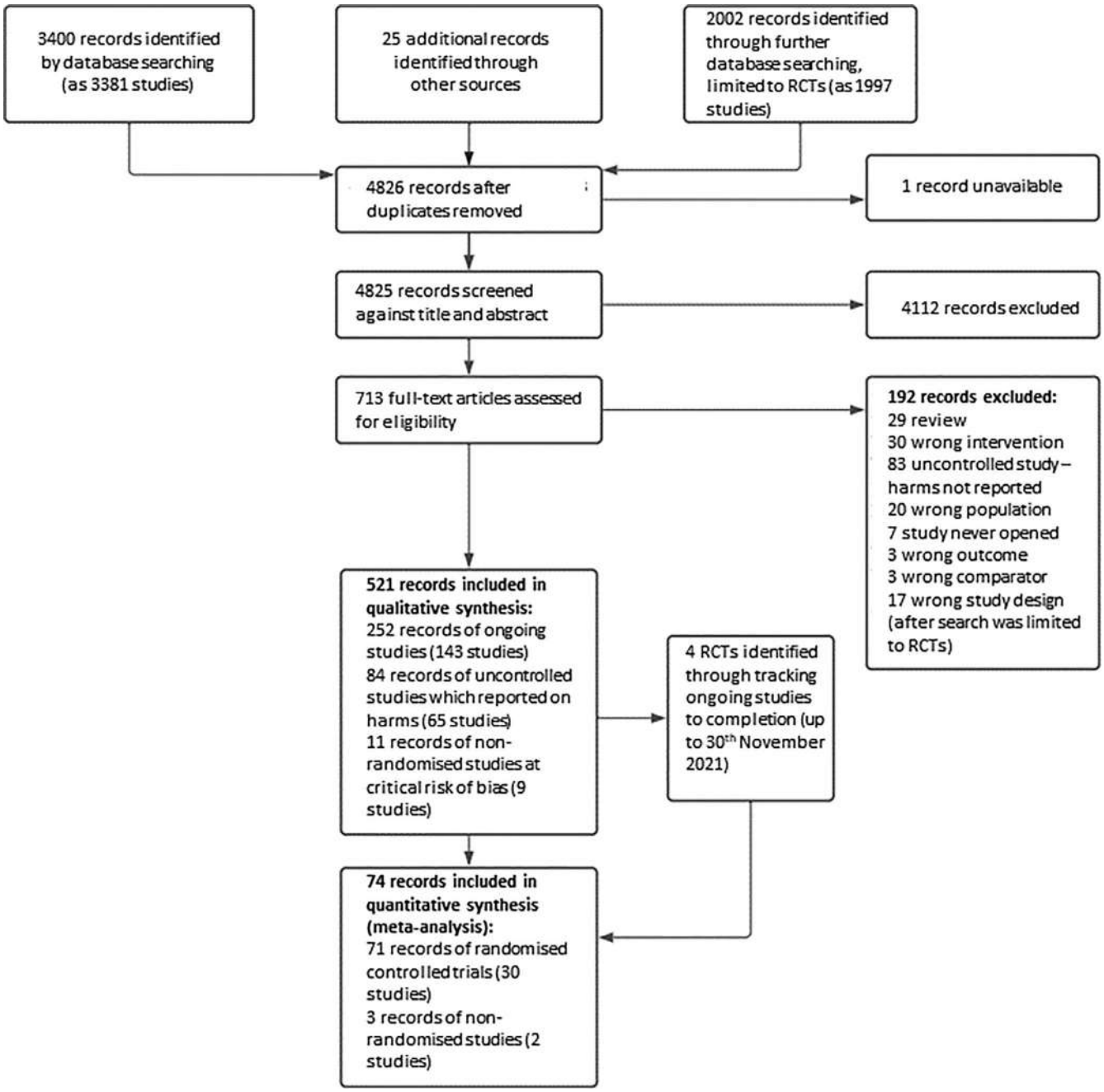

FIGURE 1 PRISMA flow diagram. Caption: The reasons for exclusion at each stage are shown with arrows to the right.

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.

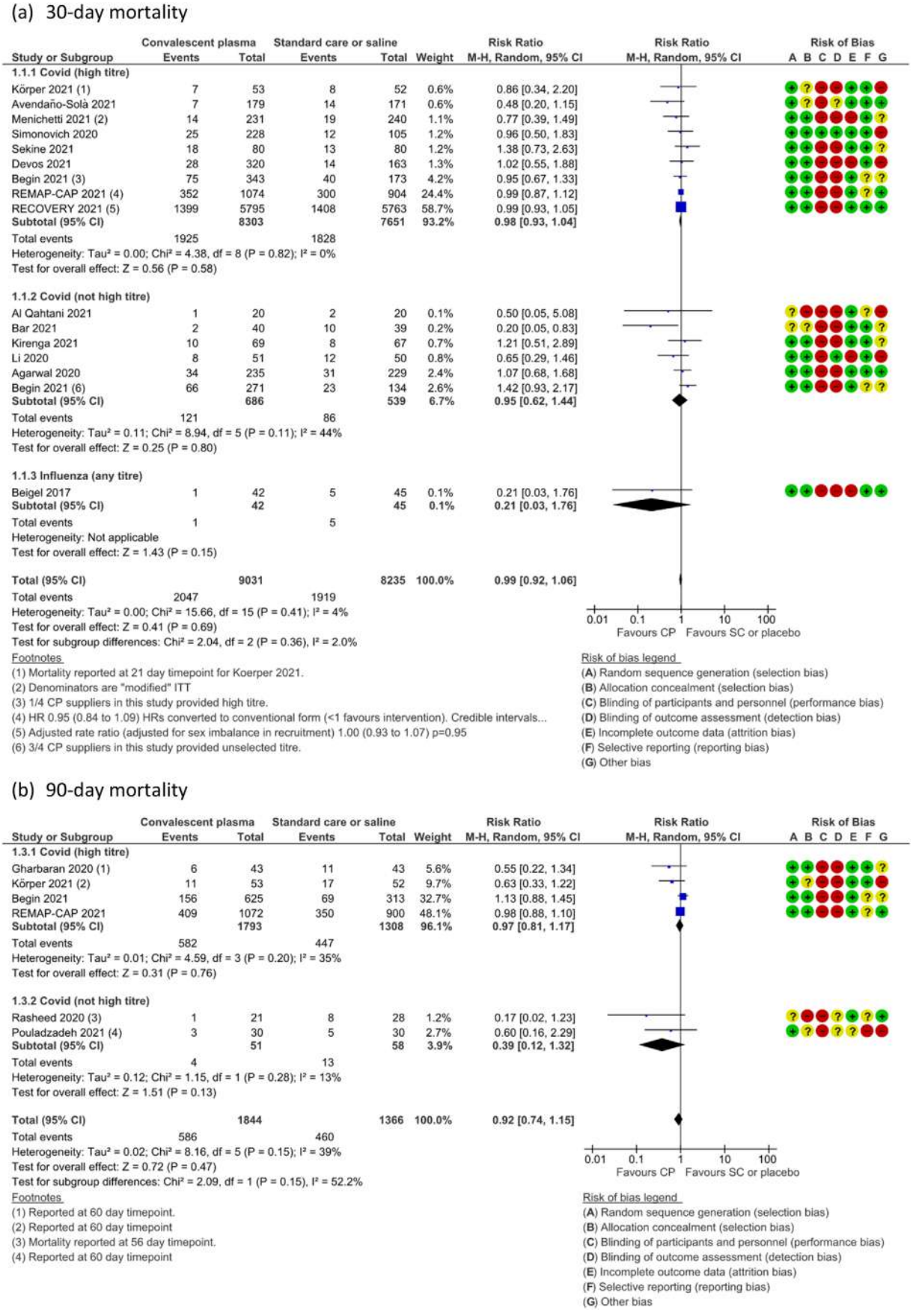

FIGURE 2 Forest plot of all-cause mortality, for comparison 1 (CP compared to SoC or a biologically inactive placebo) at up to (A) 30 days, and (B) 90 days

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.

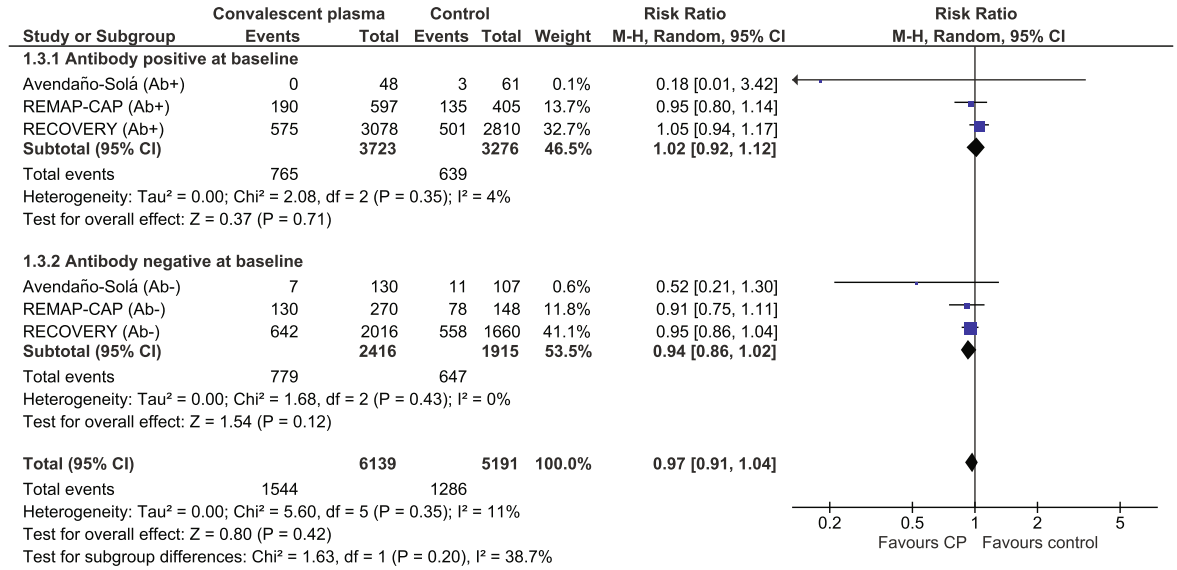

FIGURE 3 Subgrouped by seropositivity at baseline: RCTs reporting 30-day mortality for comparison 1 (CP compared to SoC or a biologically inactive placebo)

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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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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Blood loss and transfusion risk in intramedullary nailing for subtrochanteric fractures

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Abstract

Background: The incidence of hip fractures and subtrochanteric fractures in particular is increasing, along with the globally expanding aging population. Intramedullary nailing remains the 'gold standard' of their treatment. Blood loss can be a result of the original trauma, but also secondary to the subsequent surgical insult, especially during the reaming of the intramedullary canal.

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

Methods: Following institutional board approval, 431 consecutive patients (131 males; age: 79.03 years old, SD 13.68 years) presenting in a Level 1 Trauma Centre with a subtrochanteric fracture treated with an intramedullary nail were retrospectively identified, over an 8-year period. Exclusion criteria included patients with high energy injuries, pathological fractures, primary operations at other institutions and patients lost to follow-up. To identify risk factors leading to increased risk of transfusion, we first compared patients requiring intra-operative transfusion or transfusion during the first 48 h post-operatively against those who did not require transfusion. This was then followed by multivariate regression analysis adjusted for confounding factors to identify the most important risk factors associated with need for transfusion within the first 48 h post-operatively.

Results: Incidence of blood transfusion was 6.0% pre-operatively, compared to 62.7% post-operatively. A total of 230 patients (52.3%) required either intra-operative transfusion or transfusion during the first 48 h following surgery. Patients having a transfusion within the first 48 h post-operatively had a higher incidence of escalation in their care ($p = 0.050$), LOS ($p = 0.015$), 30-day ($p = 0.033$) and one-year mortality ($p = 0.004$). Multivariate regression analysis adjusted for confounding

All work was performed at Leeds General Infirmary and University of Leeds.

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

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

Level of Evidence: III.

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

1 | INTRODUCTION

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

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

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

2 | METHODS

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

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

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

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

2.1 | Statistical analysis

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

3 | RESULTS

3.1 | Demographics, mechanism of injury and transfusion requirements

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

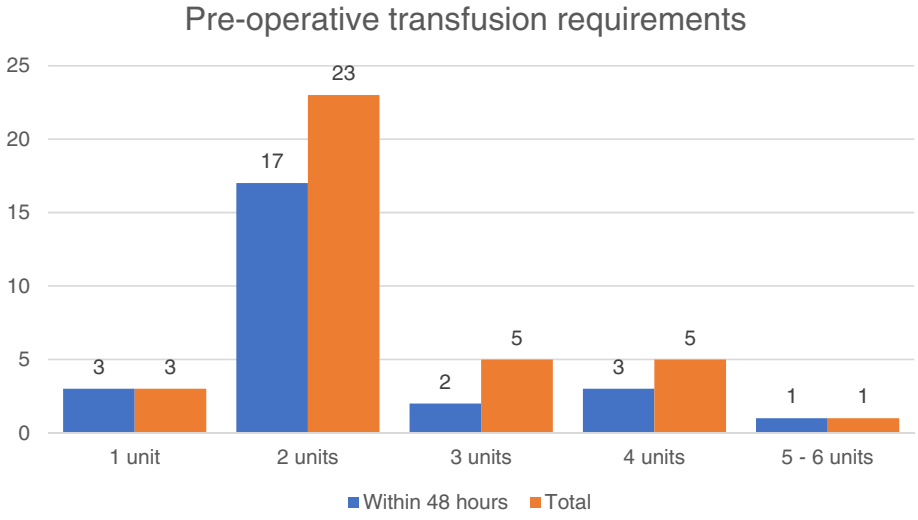

FIGURE 1 Transfusion requirements (within 48 h pre-operatively versus total pre-operative transfusion) of patients presenting to our institution with a subtrochanteric fracture

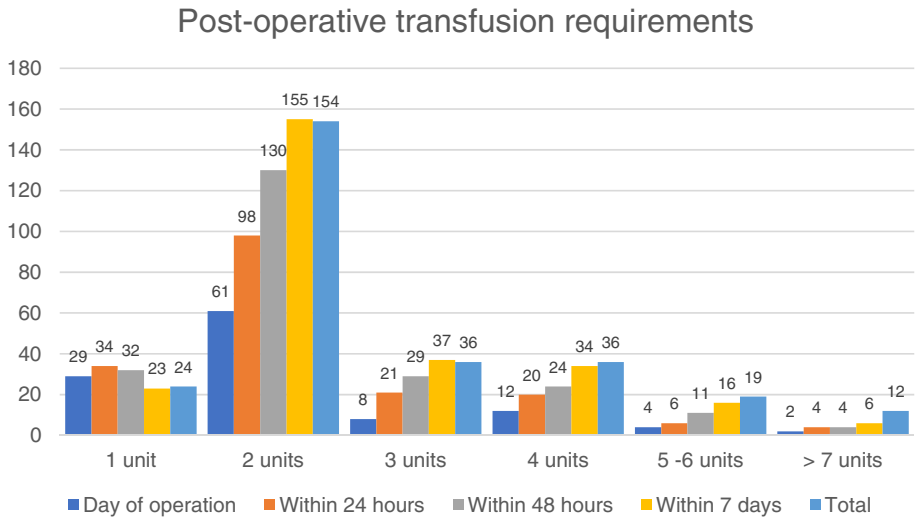

FIGURE 2 Transfusion requirements (within 48 h post-operatively versus total post-operative transfusion) of patients presenting to our institution with a subtrochanteric fracture

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

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

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

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

3.2 | Factors associated with risk of transfusion

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

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

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

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

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

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

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

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

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

(Continues)

TABLE 4 (Continued)

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

TABLE 4 (Continued)

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

(Continues)

TABLE 4 (Continued)

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

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

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

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

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

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

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

4 | DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5 | CONCLUSION

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

FUNDING INFORMATION

No funding was received for the completion of this project.

CONFLICT OF INTEREST

The authors have no competing interests.

DATA AVAILABILITY STATEMENT

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

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ORIGINAL ARTICLE

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An accurate genetic assay to identify human neutrophil antigen 2 deficiency

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Abstract

Objective: We aimed to develop accurate and user-friendly genetic assays to identify the inherited neutrophil antigen-2 (HNA-2) deficiency in humans.

Background: HNA-2 is one of the most important neutrophil antigens implicated in a number of human disorders. HNA-2 deficiency or HNA-2 null is a common phenotype observed in 3%–5% Americans. HNA-2 null individuals are at risk to produce isoantibodies (or alloantibodies) that play important roles in transfusion-related acute lung injury, immune neutropenia, and bone marrow graft failure. We previously demonstrated that the *CD177* coding SNP 787A > T (c.787A > T) is the most important genetic determinant for HNA-2 deficiency. However, reliable genetic assays are not available for routine clinical laboratory application up to now.

Study Design and Methods: A novel polymerase chain reaction (PCR) strategy was used to determine genotypes of the *CD177* SNP c.787A > T. In the simplified PCR assay, all allele specific primers and internal control primers were included in the same reaction, which ensures reliability of the assay. In addition, a novel high-throughput nested TaqMan assay was developed to determine genotypes of c.787A > T for large population genetic analysis of HNA-2 deficiency.

Results: *CD177* SNP c787A > T genotypes of 396 subjects were 100% concordant among the single PCR reaction method, the nested TaqMan assay, and Sanger Sequencing analysis. Out of 396 subjects, all 18 donors with the *CD177* STP homozygous genotype were HNA-2 null.

Conclusion: The novel PCR-based genotyping assay is accurate to identify HNA-2 deficient individuals and is suitable for clinical laboratories. In addition, the innovative high-throughput nested TaqMan assay will be useful for large-scale population screens and genetic studies of HNA-2 deficiency.

KEYWORDS
CD177, genetic assay, HNA-2 deficiency, polymorphisms

1 | INTRODUCTION

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

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

2 | MATERIALS AND METHODS

2.1 | Study subjects

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

2.2 | Nucleic acid isolation

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

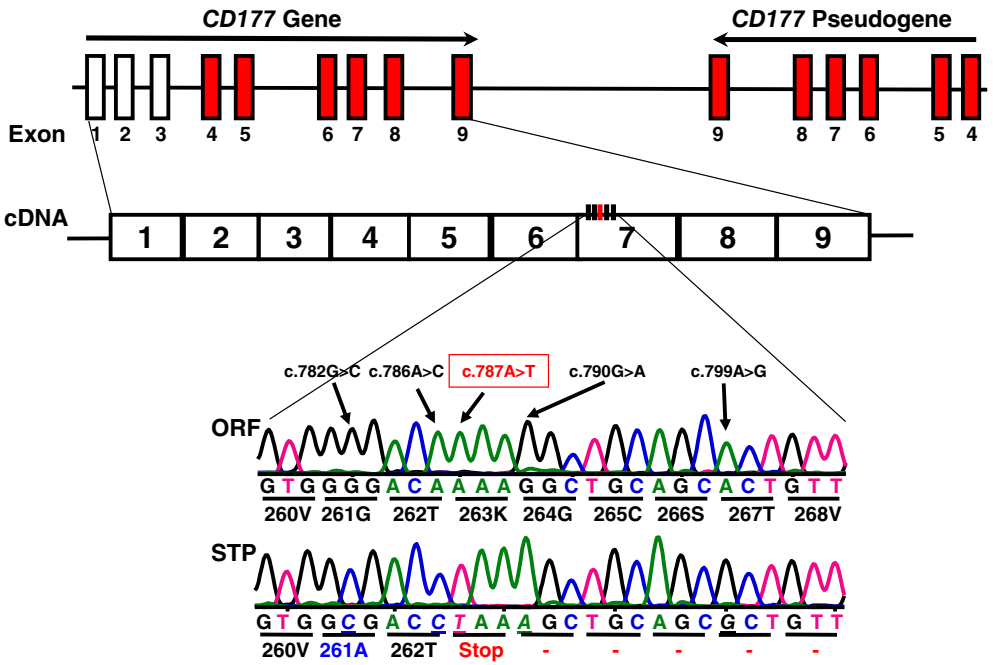

FIGURE 1 *CD177* SNPs responsible for HNA-2 expression deficiency. *CD177* gene contains nine exons and the *CD177* exon 4, 5, 6, 7, 8, and 9 are highly homologous to those of *CD177* pseudogene (upper panel). Locations of *CD177* SNPs responsible for HNA-2 expression deficiency are marked as vertical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

2.3 | Assessment of HNA-2 expressions on neutrophil

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

2.4 | PCR-based *CD177* SNP genotyping assays

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

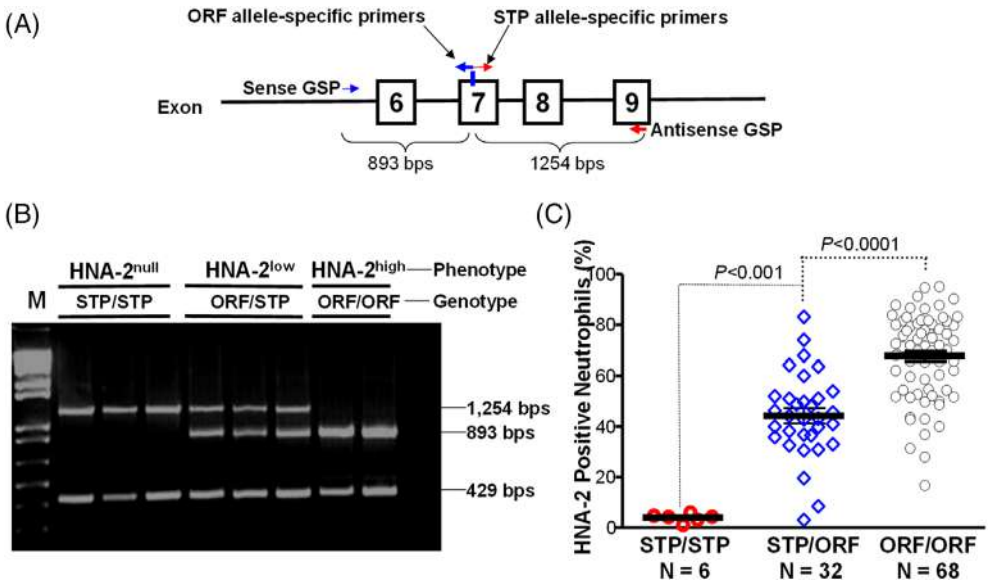

FIGURE 2 Polymerase chain reaction (PCR)-based assay to determine ORF/STP genotypes. (A) Primer locations of PCR-based assay. A sense *CD177* gene-specific primer (Sense GSP) was paired with an antisense *CD177* ORF allele-specific primer and a sense *CD177* STP allele-specific primer was paired with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

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

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

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

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

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

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

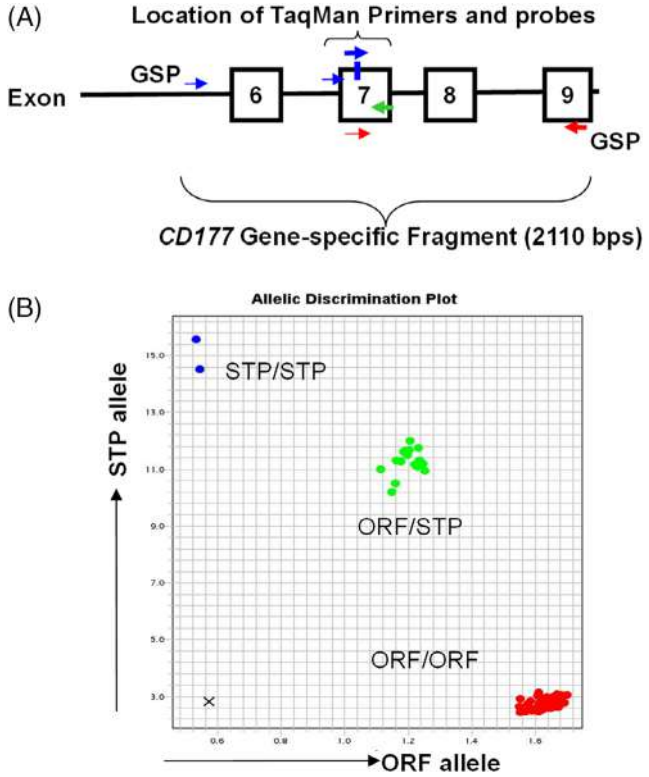

FIGURE 3 High-throughput *CD177* ORF/STP TaqMan genotyping assay. (A) Primer locations of TaqMan assays to determine the genotypes of the *CD177* SNP ORF or STP haplotypes. The *CD177* gene-specific PCR products (2110 bps) were amplified with gene-specific primers (GSP) and subsequently used as TaqMan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

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

2.5 | *CD177* TaqMan SNP assay

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

2.6 | Statistical analysis

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

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

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

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

3 | RESULTS

3.1 | PCR-based ORF/STP genotyping assay

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

3.2 | TaqMan *CD177* ORF/STP genotyping assay

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

4 | DISCUSSION

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

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

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

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

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

AUTHOR CONTRIBUTIONS

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

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CONFLICT OF INTEREST

The authors have no competing interests.

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CORRIGENDUM

Correction to Abstracts of the Annual Scientific Meeting of the British Blood Transfusion Society, 13–15 September 2022[1] | SS8: TP SESSION.

The author's name for SS8: TP SESSION | Data analysis for the management of the electronic BloodTrack bedside device was corrected to 'Raluca Candrea'. The name in the Author Index should appear as Candrea, R., SS8.

We apologise for this error.

REFERENCE

1. Abstracts of the Annual Scientific Meeting of the British Blood Transfusion Society, 13–15 September 2022. *Transfus Med*. 2022;32:S2.

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CORRIGENDUM

In Reference 1, under the subheading of Secondary Outcomes in the Results section, the manuscript stated: 'The rate of severe surgery-related complications (Clavien-Dindo grade IIIa and higher) was 1.2% in the control group versus 3.9% in the POTTS group ($p = 0.010$)'. The two numbers (1.2% and 3.9%) are incorrectly placed. The correct statement should be: 'The rate of severe surgery-related complications (Clavien-Dindo grade IIIa and higher) was 3.9% in the control group versus 1.2% in the POTTS group ($p = 0.010$)'.

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