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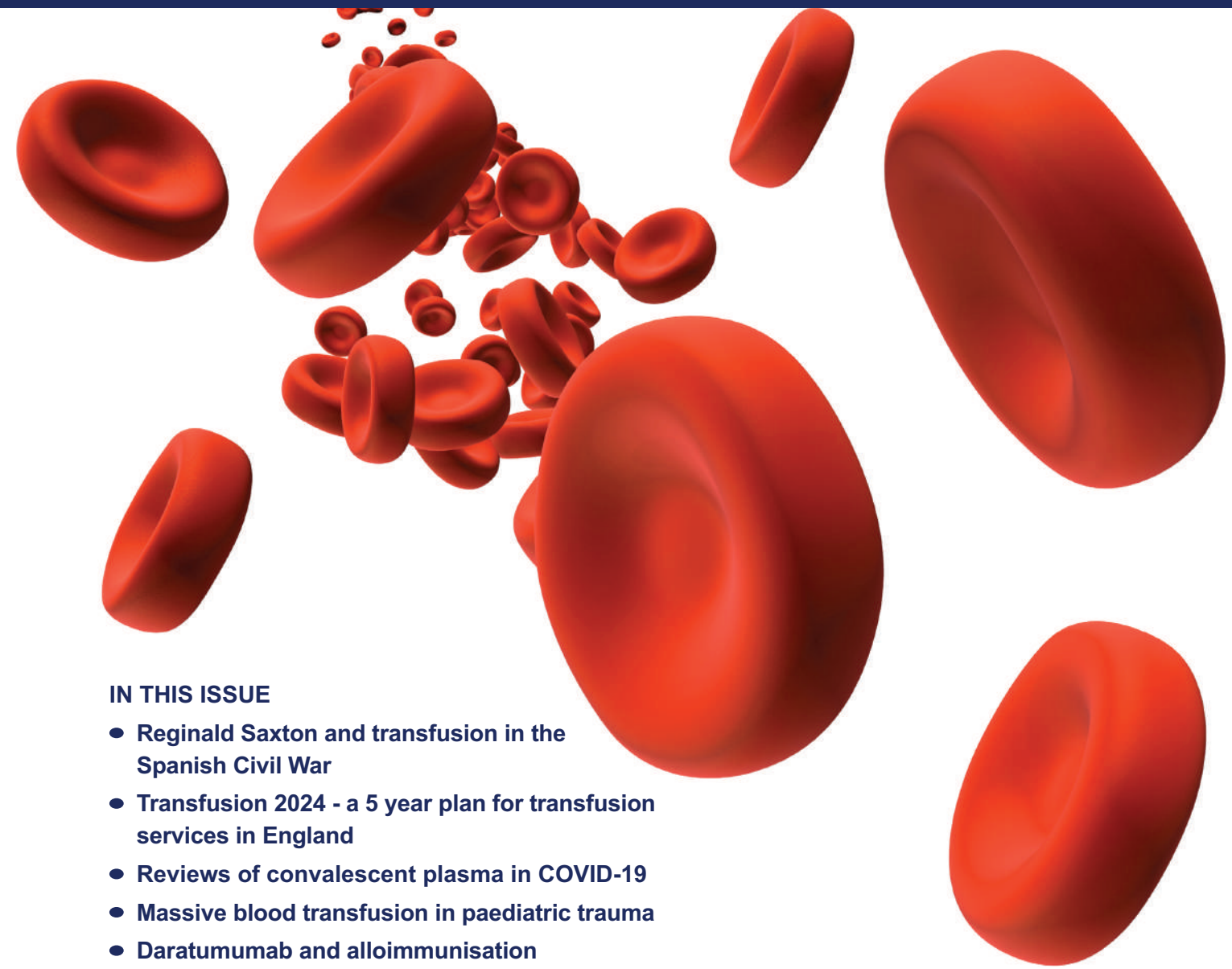

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Transfusion Medicine

An international journal published for the British Blood Transfusion Society

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EDITORIAL

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2021: That was the year that was

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

Reginald Saxton: An unlikely pioneer of battleground blood transfusion

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The development of blood transfusion services during the Spanish Civil War represented an intermediate step between the practices in the First World War (WW1, 1914–1918) and the more highly organised arrangements established in anticipation of the Second World War (WW2, 1939–1945).[1]

At the start of WW1, in Britain, blood transfusion in civilian clinical practice was an uncommon event and the resuscitation policies of the Royal Army Medical Corps emphasised the use of saline and colloid. With the arrival of Canadian, and later American, Army Medical Officers working at Casualty Clearing Stations and also closer to the Front, this policy was supplemented or replaced at first mainly with direct arm to arm whole blood transfusion, and later with the wider adoption of pre-donated citrated 'universal donor' whole blood.[2-4] By the end of the war, however, transfusions were being administered at advanced locations nearer the front.[5] There was no systematic policy for the supply of blood for transfusion, which was usually obtained locally by Army Medical Officers from healthy or only mildly disabled military personnel, as available.

In anticipation of WW2, the British Military Authorities devised a comprehensive system involving a civilian blood donor capacity based at home, with transport to supply stored blood for transfusion in military operations, including field transfusion units near battlefronts as well as military hospitals.[6,7]

The Spanish Civil War (July 1936–April 1939) began with an armed uprising of forces representing conservative military and religious Nationalist interests, backed by German and Italian Fascist elements, intent on removing the duly elected Republican Government, supported by Communist and Socialist interests. From the start, the War was bitter and deeply involved both the military and the civilian population, with the Republican side widely reinforced by volunteers from many nations who formed the 'International Brigades'. The medical history of the Spanish Civil War, summarised briefly by Coni,[8] has an extensive bibliography.[9] This discussion is concerned only with aspects of transfusion on the Republican side in the War.

Shortly after the beginning of the Civil War an extensive civilian based blood donor system was instituted in Barcelona by Frederic Duran Jorda, supplying both civilian hospitals and armies in the field with citrated blood for transfusion[10,11]; later, Norman Bethune established his *Instituto Hispano-Canadiene de Transfusion de Sangre*[12,13] incorporating into it the operations of a service previously associated with the University in Madrid. Between these two Institutes and with smaller contributions from elsewhere, it is estimated that about 12 000 L of blood were made available during the war, providing about 32 000 units (of between 300 and 500 mL, and mostly group O) for transfusion.[1] This development of a substantial civilian stored blood donation capacity for the support of civilian and military medical practice was a major advance on the situation at the end of WW1.

By contrast, there is little information on the clinical use and disposal of the large amounts of blood distributed for transfusion to casualties, civilian or military. The absence of systematic records of use is perhaps not surprising in view of the urgency with which the system of distribution and actual transfusion of units of blood was developed, combined with the appalling circumstances of conflict, military movement, environment and weather imposed upon those physicians, nurses and others working in 'hospitals' treating the wounded. Rather than being hospitals in a modern sense, their functions were frequently established ad hoc at short notice in any locally available buildings or other locations, including caves and railway tunnels, wherever possible concealed from air attack by the Nationalist and their allied forces, and were liable to have to be quickly abandoned in a move to safer ground.[9]

Members of the International Brigades, as individual volunteers without much prior organisation and formal training, had frequently to

turn their hands to unaccustomed tasks in unfamiliar circumstances, often complicated by language barriers. So it was with Reginald Soames Saxton, a recently qualified English physician, who found himself responsible for practical blood transfusion; although aware of the basics of the major blood groups, he had only hitherto administered a single transfusion.[14] His accounts of his service in Spain, however patchy, give a unique picture of the evolution of a function as a 'Blood Transfusion Officer' from a neophyte to a thoughtful organiser of battlefield blood transfusion in support of surgical care of the wounded.

Saxton was born in 1911 in South Africa, son of an academic botanist, and educated in India and at a traditional English Public School, where he was uncomfortable with the intimidating culture, and developed a sense of social justice. In 1929, he went to study medicine at Cambridge, and completed his training at St. Bartholomew's Hospital (Bart's) in 1935. There he would likely have been exposed to the enthusiastic advocacy of Geoffrey Keynes as a protagonist of a better understanding of the role of blood transfusion.[15,16]

While at Cambridge, his political leanings towards socialism crystallised (influenced by the then prevailing socialist atmosphere there, 'the era of notorious people like Philby') and in 1935 he joined the Communist Party of Great Britain.[14] In the summer of 1936, he attended a meeting of the organisation (Spanish Medical Aid Committee or SMAC) formed to support the Republican cause in the Civil War which began on 7 July, volunteering to serve in Spain. Among those present and also volunteering was Archie Cochrane ('sits in judgement above other people'[14]), a senior medical student, later to become the inspiration for the Cochrane Collaboration. Perhaps imbued by a spirit of naïve enthusiasm, Saxton visited the Soviet Union before departing for Spain in September, and after a short sojourn functioning more in a role of general practitioner in northern Spain, he joined the XIVth (French-Belgian) International Brigade, becoming involved in medical support at the battle of Jarama (6–27 February 1937), in defence of Madrid. His group set up its 'hospital' in the town hall with 60 beds; there were four physicians, three surgeons and Saxton with essentially no surgical training. It was a case of 'Saxton, you will do the transfusions' among other non-surgical responsibilities. Medical students were doing the anaesthetic duties.[14,17,18]

Within 2 days, the wounded were arriving and at this point, there appeared a visitor (Norman Bethune) bringing from his Madrid Institute stored blood for transfusion and a refrigerator with capacity for up to 20 half litre bottles. Absent any better alternative, Saxton cut down on veins and transfused the blood through a funnel, rubber tube and cannula, sterilising the equipment by boiling in water between transfusions. On a subsequent visit, along with blood, Bethune delivered blood grouping sera and Jube transfusion devices with a two-way syringe (Figure 1),[19] allowing the capacity for arm to arm direct transfusion from local donors as well as facilitating transfusion of stored donor blood.[14] Saxton was then able to determine blood groups of staff and local residents in anticipation of the need for supplementation of stored blood supplies. Although he kept no records of his transfusion episodes at this makeshift hospital, he indicates he

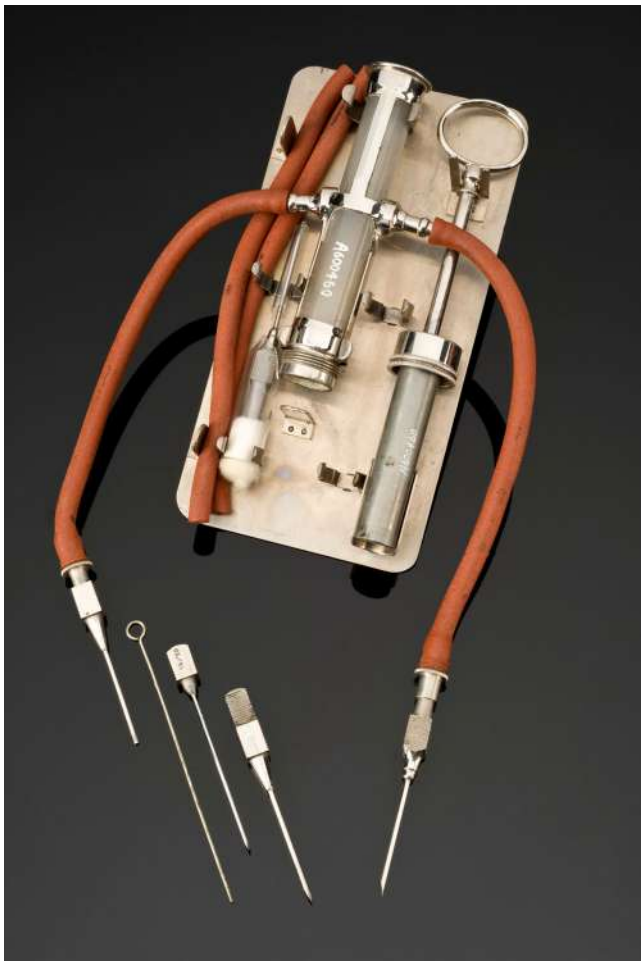

FIGURE 1 Jube transfusion device for transfusion of blood directly from donor to recipient using a syringe with a two-way mechanism allowing alternate aspiration of blood from the donor and transfer to the recipient. A detailed description of the operation of the Jube device is given by Brewer.[19] Jube-type Blood Transfusion Apparatus, Paris, France, 1900–1901. Credit: Science Museum, London. Attribution 4.0 International (CC BY 4.0). wellcomecollection.org. Accessed February 12, 2021 [Color figure can be viewed at wileyonlinelibrary.com]

treated about 20 cases using both direct transfusion and stored blood, with 10 the most transfusions in any one day and about half using cut down and cannula.[14,18,20] He notes 'probably a few lives were saved this way. Some were only prolonged for a few hours…'[17]

On 7 March, Saxton visited the Madrid Institute[20,21] and his notes from that visit not only detail information on the processes and techniques of blood collection, but also comment on the views of Institute staff on such practical matters as using blood of different groups during a transfusion and the risks of citrate toxicity. It is reasonable to infer that at least part of Saxton's purpose in visiting was to improve his general understanding of the proper conduct of transfusion practice. Following that visit, he had a brief period of home leave during which time he acquired additional Jube syringes (spares to be kept sterilised ready for use) and a then novel Henri–Jouvelet device (Figure 2)[22] with a metered rotary pump to enhance infusion and also

FIGURE 2 Henri–Jouvelet Transfusion Apparatus, 1934, for the direct transfusion of blood from donor to recipient with a rotary pump and metered flow for enhanced transfusion rates, diminished risk of clotting and accurate measurement of transfused volume. For more detail, see Cazalaa.[22] Rocchini Dumas Collection. Virtual Museum of Ancient Medical Instruments. Blood Transfusion. www.amber-ambre-inclusions.info/it-strumenti_medici.htm. Accessed 12 February 2021 [Color figure can be viewed at wileyonlinelibrary.com]

measure the volume transfused, particularly useful in direct arm to arm transfusion.[14,18,20]

The next military engagement in which Saxton was involved was an indecisive offensive by the Republican forces at Brunete just west of Madrid (6–27 July 1937). He notes that there were intermittent spells of high demand in which supplies of stored blood from Madrid had to be supplemented by using staff on the spot as donors; in bringing in these donors, it is apparent he was adding some screening for disease at least by questioning, as well as grouping donors in advance. He was also realising that 'shock', the pathogenesis of which was then still a matter of considerable academic debate, was mainly due, 'almost 100%', to loss of blood from wounding.[14] It is unclear how many transfusions he actually performed as he claims to have done '70–80 direct from staff, but mostly bottled blood from Madrid'[23]; he reports on 99 cases receiving blood, of whom 61 died including 11 of 12 who received more than 1 transfusion (mortality of about 60%) but that at least 90% mortality would have been expected without transfusion, and reasoning that transfusion might thus have been life-saving in 30 cases.[20] He also touches on the question of improving outcomes by selection of transfusion candidates on the basis of the likelihood of survival.

In the early summer of 1937, Republican surgeons, particularly Moises Broggi, pressed successfully for the development of mobile teams with capacity to set up, equip and supply surgical operating units, a revival of the WW1 French concept of the '*autochir*' (derived from '*automobile*' and '*chirurgerie*'), to facilitate movement of field medical/surgical operations in response to movement of battlefronts and in time these became widely deployed.[24]

The next battle where Saxton was actively involved was at Teruel in north-eastern Spain in the winter of 1937–1938. In the interval between Brunete and Teruel, Saxton was engaged in running a small hospital and managing an outbreak of typhoid. At the same time, he procured a large damaged ambulance in which the frame and engine remained intact; with the help of a Californian volunteer (Henry Rubin), who had some biological and laboratory background, and local tradesmen, Saxton had it rebuilt to provide for a mobile transfusion service and laboratory. The result was a self-contained unit equipped with refrigerator, autoclave, incubator, steriliser, water tank, and basic storage and supplies for blood grouping, cross-matching and simple laboratory testing,[25] able to move in conjunction with the surgical teams. He appears to have been reflecting also on his transfusion experience as he records some observations—'There was really no brand new discovery used in the blood transfusion work I was doing… what was new was its use on such a scale'[23] and 'a litre of blood is a small transfusion and in fact often not enough'.[25] He was also speculating on plans for a 'shock ward where will be kept together all the special cases requiring transfusions…always available stocks of stored blood and salines'.[25]

The battle at Teruel ran for almost 2 months from late December, and was fought in severe winter conditions at 1200 M altitude. The Republican forces at first held the town but were eventually forced out; a critical outcome was the division of the Republican-held territory in two. Saxton used the opportunity to take supplies and equipment, including a microscope, for his mobile unit from local premises. He does not provide any actual records of blood transfusions, but it is clear that supplies from civilian sources were short and conditions more than usually difficult. Electrical failures compromised his stored blood supply, necessitating recourse to direct transfusion from volunteer donors—'the donor just sits on the floor beside the patient's stretcher' with lighting by 'petrol lamps' and candles[26]—'I have quite a number of blood transfusions to do…severely wounded brought in at their last gasp…mortality is…quite high'.[27] At this point, he was actively considering the possibility of using cadaver blood and, indeed, experimented with the technique of collection.[28]

The final battle was a protracted and ferocious struggle over the summer of 1938, begun by the Republican forces in an attempt to reunite the two divided areas of their territories. Saxton's mobile laboratory and his small team of assistants (including Rubin and Paco, the driver) moved back and forth with the tide of battle, across the River Ebro and back again, supporting surgical teams (particularly that headed by Douglas Jolly with whom Saxton had worked before at Brunete) operating in caves and a railway tunnel as shelter from air attack. During part of this encounter, his mobile laboratory was concealed from the air in an olive grove, camouflaged under tree branches (Figure 3). Although, again, Saxton himself does not provide an account of transfusions given in the Ebro battle, contemporary accounts indicate a heavy demand for blood transfusion support.[29] He did, however, note frequent 'rigors' associated with transfusions given at the Ebro conflict, which he attributed to infection.[30]The practical difficulties under which he was carrying out his transfusions can be imagined from a rare illustration of the conditions under which he was working (Figure 4).

In the Fall of 1938, as part of the pursuit of a political solution to the Spanish conflict, the International Brigades were withdrawn and

FIGURE 3 Reginald Saxton (left) and Henry (Hank) Rubin (top) using tree branches to camouflage the mobile laboratory in an olive grove near the Cave Field Hospital, during the battle at the Ebro River, 1938. Reproduced with permission

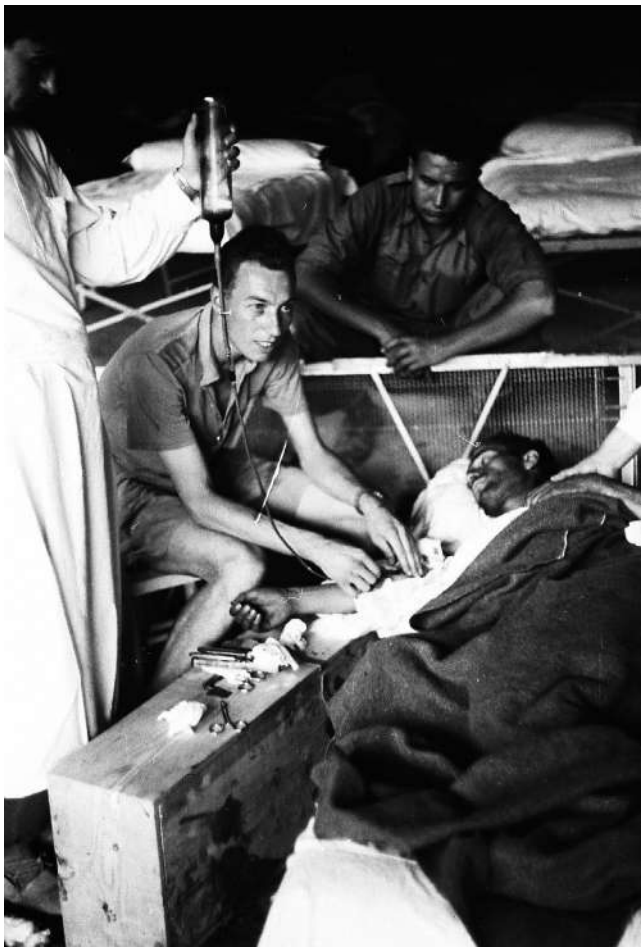

FIGURE 4 Reginald Saxton at a Field Hospital, during the battle at the Ebro River 1938, administering a blood transfusion to a severely wounded volunteer, a Welsh miner and Commissar in the British Brigade, who did not survive. Reproduced with permission

repatriated, along with many Republican Spaniards escaping retribution from the victorious Nationalist regime.

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AUTHOR CONTRIBUTIONS

PHP is the sole author. PHP designed, drafted and submitted the manuscript, corrected all versions, read the proofs and released them for publication.

CONFLICT OF INTEREST

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REVIEW

TRANSFUSION MEDICINE WILEY

Transfusion 2024: A 5-year plan for clinical and laboratory transfusion in England

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The development of blood transfusion services during the Spanish Civil War represented an intermediate step between the practices in the First World War (WW1, 1914–1918) and the more highly organised arrangements established in anticipation of the Second World War (WW2, 1939–1945).[1]

At the start of WW1, in Britain, blood transfusion in civilian clinical practice was an uncommon event and the resuscitation policies of the Royal Army Medical Corps emphasised the use of saline and colloid. With the arrival of Canadian, and later American, Army Medical Officers working at Casualty Clearing Stations and also closer to the Front, this policy was supplemented or replaced at first mainly with direct arm to arm whole blood transfusion, and later with the wider adoption of pre-donated citrated 'universal donor' whole blood.[2-4] By the end of the war, however, transfusions were being administered at advanced locations nearer the front.[5] There was no systematic policy for the supply of blood for transfusion, which was usually obtained locally by Army Medical Officers from healthy or only mildly disabled military personnel, as available.

In anticipation of WW2, the British Military Authorities devised a comprehensive system involving a civilian blood donor capacity based at home, with transport to supply stored blood for transfusion in military operations, including field transfusion units near battlefronts as well as military hospitals.[6,7]

The Spanish Civil War (July 1936–April 1939) began with an armed uprising of forces representing conservative military and religious Nationalist interests, backed by German and Italian Fascist elements, intent on removing the duly elected Republican Government, supported by Communist and Socialist interests. From the start, the War was bitter and deeply involved both the military and the civilian population, with the Republican side widely reinforced by volunteers from many nations who formed the 'International Brigades'. The medical history of the Spanish Civil War, summarised briefly by Coni,[8] has an extensive bibliography.[9] This discussion is concerned only with aspects of transfusion on the Republican side in the War.

Shortly after the beginning of the Civil War an extensive civilian based blood donor system was instituted in Barcelona by Frederic Duran Jorda, supplying both civilian hospitals and armies in the field with citrated blood for transfusion[10,11]; later, Norman Bethune established his *Instituto Hispano-Canadiene de Transfusion de Sangre*[12,13] incorporating into it the operations of a service previously associated with the University in Madrid. Between these two Institutes and with smaller contributions from elsewhere, it is estimated that about 12 000 L of blood were made available during the war, providing about 32 000 units (of between 300 and 500 mL, and mostly group O) for transfusion.[1] This development of a substantial civilian stored blood donation capacity for the support of civilian and military medical practice was a major advance on the situation at the end of WW1.

By contrast, there is little information on the clinical use and disposal of the large amounts of blood distributed for transfusion to casualties, civilian or military. The absence of systematic records of use is perhaps not surprising in view of the urgency with which the system of distribution and actual transfusion of units of blood was developed, combined with the appalling circumstances of conflict, military movement, environment and weather imposed upon those physicians, nurses and others working in 'hospitals' treating the wounded. Rather than being hospitals in a modern sense, their functions were frequently established ad hoc at short notice in any locally available buildings or other locations, including caves and railway tunnels, wherever possible concealed from air attack by the Nationalist and their allied forces, and were liable to have to be quickly abandoned in a move to safer ground.[9]

Members of the International Brigades, as individual volunteers without much prior organisation and formal training, had frequently to

turn their hands to unaccustomed tasks in unfamiliar circumstances, often complicated by language barriers. So it was with Reginald Soames Saxton, a recently qualified English physician, who found himself responsible for practical blood transfusion; although aware of the basics of the major blood groups, he had only hitherto administered a single transfusion.[14] His accounts of his service in Spain, however patchy, give a unique picture of the evolution of a function as a 'Blood Transfusion Officer' from a neophyte to a thoughtful organiser of battlefield blood transfusion in support of surgical care of the wounded.

Saxton was born in 1911 in South Africa, son of an academic botanist, and educated in India and at a traditional English Public School, where he was uncomfortable with the intimidating culture, and developed a sense of social justice. In 1929, he went to study medicine at Cambridge, and completed his training at St. Bartholomew's Hospital (Bart's) in 1935. There he would likely have been exposed to the enthusiastic advocacy of Geoffrey Keynes as a protagonist of a better understanding of the role of blood transfusion.[15,16]

While at Cambridge, his political leanings towards socialism crystallised (influenced by the then prevailing socialist atmosphere there, 'the era of notorious people like Philby') and in 1935 he joined the Communist Party of Great Britain.[14] In the summer of 1936, he attended a meeting of the organisation (Spanish Medical Aid Committee or SMAC) formed to support the Republican cause in the Civil War which began on 7 July, volunteering to serve in Spain. Among those present and also volunteering was Archie Cochrane ('sits in judgement above other people'[14]), a senior medical student, later to become the inspiration for the Cochrane Collaboration. Perhaps imbued by a spirit of naïve enthusiasm, Saxton visited the Soviet Union before departing for Spain in September, and after a short sojourn functioning more in a role of general practitioner in northern Spain, he joined the XIVth (French-Belgian) International Brigade, becoming involved in medical support at the battle of Jarama (6–27 February 1937), in defence of Madrid. His group set up its 'hospital' in the town hall with 60 beds; there were four physicians, three surgeons and Saxton with essentially no surgical training. It was a case of 'Saxton, you will do the transfusions' among other non-surgical responsibilities. Medical students were doing the anaesthetic duties.[14,17,18]

Within 2 days, the wounded were arriving and at this point, there appeared a visitor (Norman Bethune) bringing from his Madrid Institute stored blood for transfusion and a refrigerator with capacity for up to 20 half litre bottles. Absent any better alternative, Saxton cut down on veins and transfused the blood through a funnel, rubber tube and cannula, sterilising the equipment by boiling in water between transfusions. On a subsequent visit, along with blood, Bethune delivered blood grouping sera and Jube transfusion devices with a two-way syringe (Figure 1),[19] allowing the capacity for arm to arm direct transfusion from local donors as well as facilitating transfusion of stored donor blood.[14] Saxton was then able to determine blood groups of staff and local residents in anticipation of the need for supplementation of stored blood supplies. Although he kept no records of his transfusion episodes at this makeshift hospital, he indicates he

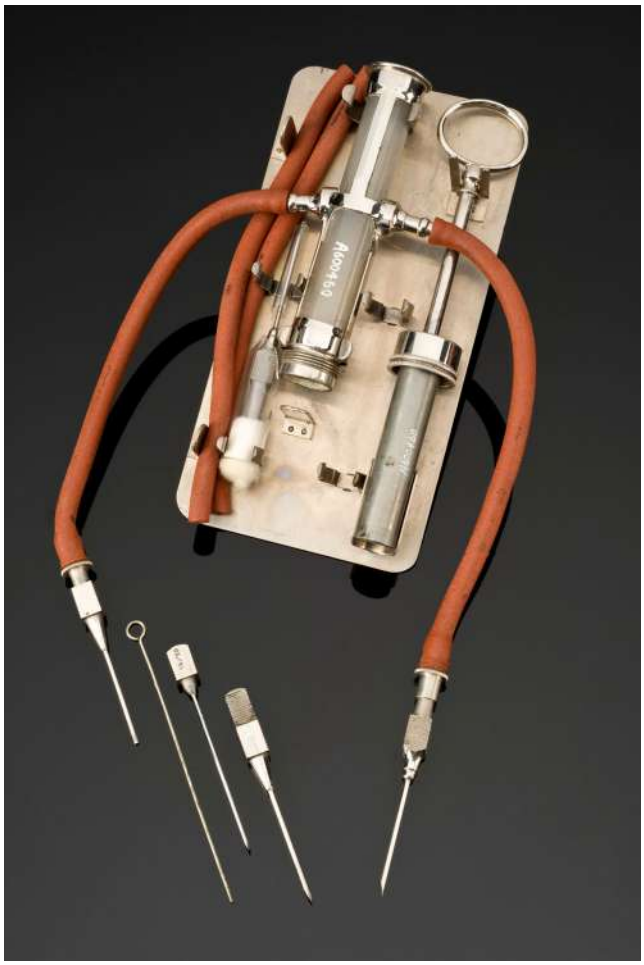


FIGURE 1 Jube transfusion device for transfusion of blood directly from donor to recipient using a syringe with a two-way mechanism allowing alternate aspiration of blood from the donor and transfer to the recipient. A detailed description of the operation of the Jube device is given by Brewer.[19] Jube-type Blood Transfusion Apparatus, Paris, France, 1900–1901. Credit: Science Museum, London. Attribution 4.0 International (CC BY 4.0). wellcomecollection.org. Accessed February 12, 2021 [Color figure can be viewed at wileyonlinelibrary.com]

treated about 20 cases using both direct transfusion and stored blood, with 10 the most transfusions in any one day and about half using cut down and cannula.[14,18,20] He notes 'probably a few lives were saved this way. Some were only prolonged for a few hours…'[17]

On 7 March, Saxton visited the Madrid Institute[20,21] and his notes from that visit not only detail information on the processes and techniques of blood collection, but also comment on the views of Institute staff on such practical matters as using blood of different groups during a transfusion and the risks of citrate toxicity. It is reasonable to infer that at least part of Saxton's purpose in visiting was to improve his general understanding of the proper conduct of transfusion practice. Following that visit, he had a brief period of home leave during which time he acquired additional Jube syringes (spares to be kept sterilised ready for use) and a then novel Henri–Jouvelet device (Figure 2)[22] with a metered rotary pump to enhance infusion and also

FIGURE 2 Henri–Jouvelet Transfusion Apparatus, 1934, for the direct transfusion of blood from donor to recipient with a rotary pump and metered flow for enhanced transfusion rates, diminished risk of clotting and accurate measurement of transfused volume. For more detail, see Cazalaa.[22] Rocchini Dumas Collection. Virtual Museum of Ancient Medical Instruments. Blood Transfusion. www.amber-ambre-inclusions.info/it-strumenti_medici.htm. Accessed 12 February 2021 [Color figure can be viewed at wileyonlinelibrary.com]

measure the volume transfused, particularly useful in direct arm to arm transfusion.[14,18,20]

The next military engagement in which Saxton was involved was an indecisive offensive by the Republican forces at Brunete just west of Madrid (6–27 July 1937). He notes that there were intermittent spells of high demand in which supplies of stored blood from Madrid had to be supplemented by using staff on the spot as donors; in bringing in these donors, it is apparent he was adding some screening for disease at least by questioning, as well as grouping donors in advance. He was also realising that 'shock', the pathogenesis of which was then still a matter of considerable academic debate, was mainly due, 'almost 100%', to loss of blood from wounding.[14] It is unclear how many transfusions he actually performed as he claims to have done '70–80 direct from staff, but mostly bottled blood from Madrid'[23]; he reports on 99 cases receiving blood, of whom 61 died including 11 of 12 who received more than 1 transfusion (mortality of about 60%) but that at least 90% mortality would have been expected without transfusion, and reasoning that transfusion might thus have been life-saving in 30 cases.[20] He also touches on the question of improving outcomes by selection of transfusion candidates on the basis of the likelihood of survival.

In the early summer of 1937, Republican surgeons, particularly Moises Broggi, pressed successfully for the development of mobile teams with capacity to set up, equip and supply surgical operating units, a revival of the WW1 French concept of the '*autochir*' (derived from '*automobile*' and '*chirurgerie*'), to facilitate movement of field medical/surgical operations in response to movement of battlefronts and in time these became widely deployed.[24]

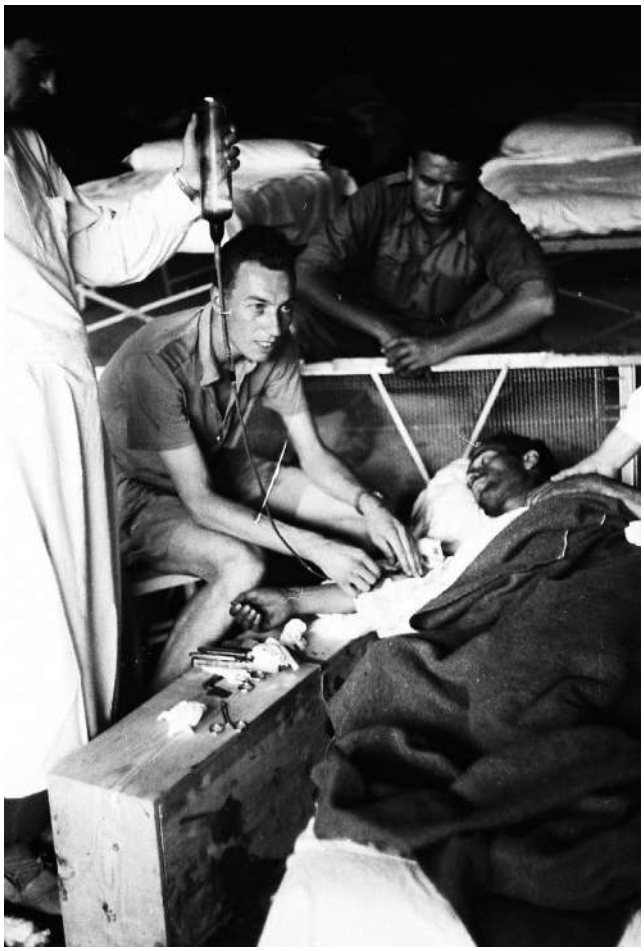
The next battle where Saxton was actively involved was at Teruel in north-eastern Spain in the winter of 1937–1938. In the interval between Brunete and Teruel, Saxton was engaged in running a small hospital and managing an outbreak of typhoid. At the same time, he procured a large damaged ambulance in which the frame and engine remained intact; with the help of a Californian volunteer (Henry Rubin), who had some biological and laboratory background, and local tradesmen, Saxton had it rebuilt to provide for a mobile transfusion service and laboratory. The result was a self-contained unit equipped with refrigerator, autoclave, incubator, steriliser, water tank, and basic storage and supplies for blood grouping, cross-matching and simple laboratory testing,[25] able to move in conjunction with the surgical teams. He appears to have been reflecting also on his transfusion experience as he records some observations—'There was really no brand new discovery used in the blood transfusion work I was doing… what was new was its use on such a scale'[23] and 'a litre of blood is a small transfusion and in fact often not enough'.[25] He was also speculating on plans for a 'shock ward where will be kept together all the special cases requiring transfusions…always available stocks of stored blood and salines'.[25]

The battle at Teruel ran for almost 2 months from late December, and was fought in severe winter conditions at 1200 M altitude. The Republican forces at first held the town but were eventually forced out; a critical outcome was the division of the Republican-held territory in two. Saxton used the opportunity to take supplies and equipment, including a microscope, for his mobile unit from local premises. He does not provide any actual records of blood transfusions, but it is clear that supplies from civilian sources were short and conditions more than usually difficult. Electrical failures compromised his stored blood supply, necessitating recourse to direct transfusion from volunteer donors—'the donor just sits on the floor beside the patient's stretcher' with lighting by 'petrol lamps' and candles[26]—'I have quite a number of blood transfusions to do…severely wounded brought in at their last gasp…mortality is…quite high'.[27] At this point, he was actively considering the possibility of using cadaver blood and, indeed, experimented with the technique of collection.[28]

The final battle was a protracted and ferocious struggle over the summer of 1938, begun by the Republican forces in an attempt to reunite the two divided areas of their territories. Saxton's mobile laboratory and his small team of assistants (including Rubin and Paco, the driver) moved back and forth with the tide of battle, across the River Ebro and back again, supporting surgical teams (particularly that headed by Douglas Jolly with whom Saxton had worked before at Brunete) operating in caves and a railway tunnel as shelter from air attack. During part of this encounter, his mobile laboratory was concealed from the air in an olive grove, camouflaged under tree branches (Figure 3). Although, again, Saxton himself does not provide an account of transfusions given in the Ebro battle, contemporary accounts indicate a heavy demand for blood transfusion support.[29] He did, however, note frequent 'rigors' associated with transfusions given at the Ebro conflict, which he attributed to infection.[30]The practical difficulties under which he was carrying out his transfusions can be imagined from a rare illustration of the conditions under which he was working (Figure 4).

In the Fall of 1938, as part of the pursuit of a political solution to the Spanish conflict, the International Brigades were withdrawn and

FIGURE 3 Reginald Saxton (left) and Henry (Hank) Rubin (top) using tree branches to camouflage the mobile laboratory in an olive grove near the Cave Field Hospital, during the battle at the Ebro River, 1938. Reproduced with permission



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AUTHOR CONTRIBUTIONS

PHP is the sole author. PHP designed, drafted and submitted the manuscript, corrected all versions, read the proofs and released them for publication.

CONFLICT OF INTEREST

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REVIEW

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Systematic review and meta-analysis of randomised controlled trials testing the safety and efficacy of convalescent plasma in the treatment of coronavirus disease 2019 (COVID-19): Evidence-base for practise and implications for research

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Abstract

Background: Despite scientific advances, there is no effective medical therapy for coronavirus disease 2019 (COVID-19). This systematic review and meta-analysis aimed to evaluate the safety and efficacy of convalescent plasma therapy in COVID-19.

Methods: This review was carried out in accordance with Cochrane methodology including risk of bias assessment and grading of the quality of evidence. Only prospective clinical trials randomly assigning COVID-19 patients to convalescent plasma plus standard of care therapy (test arm) versus placebo/standard of care (control arm) were included. Two reviewers independently read each preprint/publication and extracted relevant data from individual studies. Data were pooled using the random-effects model and expressed as risk ratio (RR) with 95% confidence interval (CI).

Results: A total of 13 206 patients from 12 randomised controlled trials were included. There was no significant difference in clinical improvement rate (RR = 1.00, 95% CI: 0.98–1.02, $p = 0.96$) or time to clinical improvement (median difference of 1.08 days with 95% CI ranging from −0.15 to +2.30 days) between convalescent plasma versus placebo/standard of care therapy. The use of convalescent plasma was not associated with significantly reduced risk of death (RR = 0.81, 95% CI: 0.65–1.02, $p = 0.08$). Reassuringly, overall incidence of infusion-related serious adverse events was low (3.25%) and not significantly different (RR = 1.14, 95% CI: 0.93–1.40, $p = 0.22$) for convalescent plasma transfusion compared to placebo/standard of care therapy.

Conclusions: There is low to moderate certainty evidence that the addition of convalescent plasma to current standard of care therapy is generally safe but, does not result in any significant clinical benefit or reduction of mortality in COVID-19.

KEYWORDS
antibody, convalescent, coronavirus, plasma, randomised, therapeutics

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has engulfed over 200 countries since being declared a pandemic[1] by the World Health Organisation (WHO) and continues to grow exponentially in several parts of the world with over 140 million confirmed cases and 3 million deaths globally[2] by the time of this report. Over 150 medical and pharmacological therapies are currently being investigated in >1000 randomised controlled trials (RCTs) across the world with an

aim to generate high-quality evidence to inform and guide clinical practise during the ongoing pandemic.[3] However, even a year and half after it was initially described, COVID-19 is still largely managed empirically worldwide with few effective or proven therapies. Dexamethasone was the first drug to demonstrate significant reduction in mortality in COVID-19 patients requiring ventilatory support or supplemental oxygen.[4] Recently, remdesivir become the first drug to receive United States (US) Food and Drug Administration (FDA) approval for the treatment of hospitalised COVID-19 patients based on significant reduction in the duration of hospitalisation[5] for COVID-19 patients of varying disease severity. Amongst various other promising therapies, convalescent plasma[6,7] enriched in human antibodies against COVID-19 from recovered patients and humanised monoclonal antibodies[8] have received emergency use authorization (EUA) from US FDA till date.

The use of convalescent blood products (whole blood, plasma, serum, and isolates such as immunoglobulins and antibodies) collected from recovered patients to confer passive immunity in the recipients is not entirely new and has strong scientific rationale and historical precedence.[9,10] Convalescent plasma therapy is a passive antibody therapy that involves the transfusion of plasma rich in antibodies against a given pathogen to a susceptible individual for the purpose of preventing or treating an infectious disease. Efficacy of such therapy largely correlates with titres of anti-SARS-CoV-2 specific neutralising antibodies present in convalescent plasma.[7,10,11] In addition to the neutralising antibodies, other components in donor plasma such as anti-inflammatory cytokines, clotting factors, natural antibodies, defensins, and pentraxins may also provide further benefit through their immunomodulatory effects and amelioration of systemic inflammatory response. [11] Convalescent plasma with neutralising antibodies has previously demonstrated clinical efficacy[9,10] against other virus-borne illnesses such as Ebola, human influenza A (H1N1), SARS, and Middle East respiratory syndrome (MERS). Over 50 RCTs are currently underway testing convalescent plasma against the present standard of care therapy in COVID-19 disease. However, many of these trials have limitation of numbers (small sample size) which would be inadequate to detect clinically meaningful and/or statistically significant differences, if any. Timely provision of COVID-19 convalescent plasma in resource-constrained settings poses significant logistic difficulties, challenges, and impediments in clinical trial accrual.[12,13] In addition, the unexpected presence of neutralising immunoglobulin G (IgG) antibodies against SARS-CoV-2 in recipients can even result in premature termination of the study, affecting statistical power and rigour. Given the context, a structured systematic review with appropriate statistical pooling of data in a direct comparison meta-analysis of all RCTs evaluating the safety and efficacy of convalescent plasma therapy in COVID-19 was necessary to create an evidence-base and facilitate rapid translation of research findings into clinical practise to inform and guide therapeutic decision-making globally.

2 | MATERIALS AND METHODS

This systematic review was carried out in accordance with Cochrane methodology for systematic reviews of interventional studies.[14] The analysis, interpretation, and reporting included a risk of bias assessment using the Cochrane Risk of Bias tool that assigns studies as having low, unclear, or high risk of bias. Quality of evidence and strength of recommendation was based on the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach[15] that involves consideration of methodological quality, directness of evidence, heterogeneity, precision of effect estimates, and publication bias.

Literature search strategy: For the purpose of this systematic review, priority sources for retrieval of relevant studies included PubMed (https://pubmed.ncbi.nlm.nih.gov) and its curated version LitCOVID; National Library of Medicine database of clinical studies (https://clinicaltrials.gov); WHO International Clinical Trials Registry Platform (https://www.who.int/ictrp/en/); medRxiv (https://www.medrxiv.org); Cochrane living registry of COVID-19 studies (https://covid-19.cochrane.org) and Living mapping and living systematic review of Covid-19 studies (https://covid-nma.com). A systematic search of the medical literature (Table S1) without any language restrictions was conducted on 25 September 2020 and later updated from December 2020 through March 2021 in accordance with international guidelines. A reference list of selected articles was also screened for identifying additional potentially eligible studies.

Study eligibility: Only prospective clinical trials randomly assigning patients with COVID-19 infection to convalescent plasma plus standard of care therapy (test arm) versus placebo/standard of care therapy (control arm) were included. Given the lack of globally accepted standard of care therapy, this could vary across trials, but needed to be similar in both the arms within individual studies. Multi-arm trials were eligible if they directly compared convalescent plasma versus standard of care therapy, with appropriate arms being included in the meta-analysis. Trials allowing co-enrolment of patients across multiple studies were also eligible provided the co-interventions (concurrent medical treatment) were delivered similarly in each of the randomised arms. Emulated RCTs, quasi-randomised trials, propensity matched analyses, nonrandomised comparative studies, or observational studies were not considered in this review. Trials testing complementary and alternative medicines, traditional Chinese medicine, nutraceuticals, phytoceuticals, and herbal formulations were also ineligible.

Outcome measures: The selection of outcome measures for this systematic review was based on the outcome sets developed by WHO for research in COVID-19 hospitalised patients identified through COMET initiative (http://www.comet-initiative.org/Studies/Details/1538). The primary outcomes of interest included clinical benefit as measured on WHO[16] or similar ordinal scale and all-cause mortality. Clinical improvement was defined as becoming asymptomatic and/or discharged (achieving a score of 1 or 2 on the ordinal scale). Relevant endpoints included clinical improvement rate (CIR) on specified days (defined as proportion of patients with clinical improvement by Day7, Day14, Day28 of randomization), time-to-clinical improvement (TTCI), and death due to any cause by Day28 of randomization. Secondary outcomes included viral negativity rate on specified days (defined as proportion of patients with viral negativity on Day3, Day7, Day14 of randomization) and time to viral clearance based on COVID-19

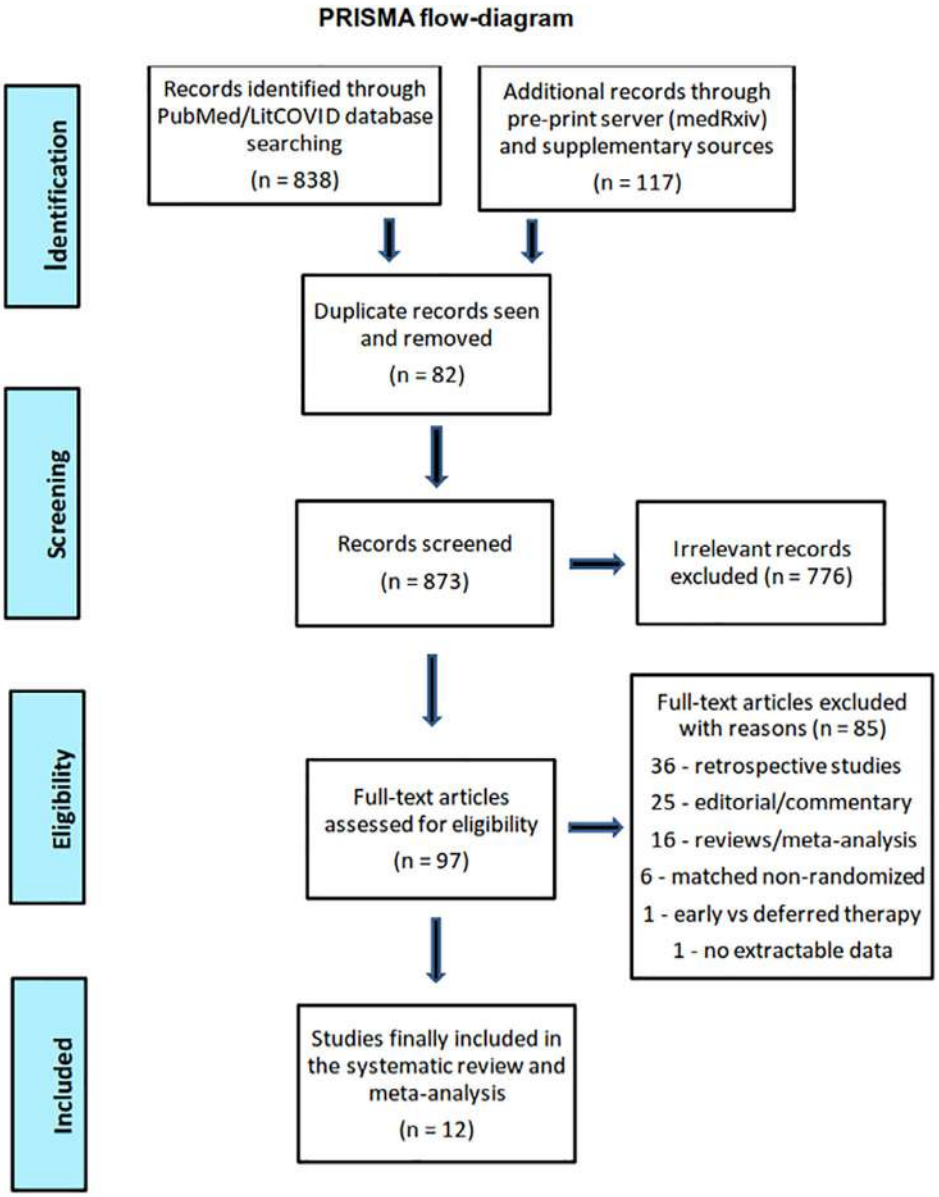

FIGURE 1 Flow-diagram of study selection and inclusion in the meta-analysis as per PRISMA guidelines [Color figure can be viewed at wileyonlinelibrary.com]

negativity as assessed by reverse transcriptase polymerase chain reaction (RT-PCR). In addition, safety outcomes included comparison of infusion-related serious adverse events between the two arms.

Data extraction and analyses: Two reviewers (BK and PT) independently read each preprint, publication, protocol, or any other available study report and extracted relevant data from individual primary studies. Discrepancy, if any, was resolved through consensus interpretation by a third reviewer (TG). In case of publication following a preprint report, data from the peer-reviewed article was used for statistical pooling. Extracted data included study characteristics (such as first author, publication year and journal), number of participants randomised, patient characteristics (severity of clinical presentation), intervention details (class and type of treatment), and outcome measures. For all dichotomous outcomes (CIR, viral negativity rate, adverse event rate, and mortality), the number of events of interest and the number of participants in each study arm were extracted per outcome. Data was pooled using the random-effects model and expressed as risk ratio (RR) with 95% confidence interval (CI). For continuous outcomes (TTCI and time to viral clearance), mean/median values with their dispersion as reported were extracted and expressed as difference in median time (in days) with 95% CI. Any p-value <0.05 was considered as statistically significant. Sensitivity analysis, subgroup analysis, and publication bias was also assessed as appropriate. All analyses were done using Review Manager (RevMan) version 5.3 & GRADE profiler (GRADEpro) version 3.6.1 (The Nordic Cochrane Centre, Cochrane Collaboration, 2008), Stata 14.0 (StataCorp LP, TX, USA) and R Studio. All data were reported in accordance with Preferred Reporting of Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[17] No source of funding was involved in study conduct, data extraction and analysis, or reporting of results. The protocol is registered with the International Platform of Registered Systematic Reviews and Meta-analysis Protocols (INPLASY202090092).

TABLE 1 Baseline patient and disease characteristics in randomised controlled trials of convalescent plasma therapy in COVID-19

Author [reference] (study name)	Treatment arms	Patient numbers (N)	Disease severity	Median/mean age (years)	Comorbidity[a] (%)	Male patients (%)	Baseline swab positivity (%)
Agarwal A [18] (PLACID)	Convalescent plasma	235	Moderate disease	52	71.1%	75%	100%
	Standard of care	229		52	64.2%	77%	100%
AlQahtani M [19]	Convalescent plasma	20	Severe disease	52.6	35%	85%	100%
	Standard of care	20		50.7	45%	75%	100%
Avendano-Sola C [20] (ConPlas)	Convalescent plasma	38	Mild to moderate	61.3	52.6%	52.6%	68.4%
	Standard of care	43		60.3	27.9%	55.8%	79.1%
Bajpai M [21]	Convalescent plasma	14	Severe disease	48.1	Not known	78.6%	100%
	Fresh Frozen pasma	15		48.3	Not known	73.3%	100%
Gharbharan A [22] (ConCOVID)	Convalescent plasma	43	Moderate to severe	61	30%	67.4%	100%
	Standard of care	43		63	26%	76.7%	100%
Horby P [23] (RECOVERY)	Convalescent plasma	5795	Moderate to severe	63.6	55%	63%	96%
	Standard of care	5763		63.4	56%	66%	96%
Li L [24]	Convalescent plasma	52	Severe disease	70	29%	51.9%	100%
	Standard of care	51		69	27%	64.7%	100%
Libster R [25]	Convalescent plasma	80	Mild disease	76.4	86.2%	32.5%	100%
	Placebo	80		77.9	77.5%	42.5%	100%
O'Donnell M [26]	Convalescent plasma	150	Severe disease	60	37%	64%	100%
	Normal plasma	73		63	38%	70%	100%
Rasheed M [27]	Convalescent plasma	21	Severe to critical	55.7	47.6%	Not known	100%
	Standard of care	28		47.8	39.3%	Not known	100%
Ray Y [28]	Convalescent plasma	40	Severe disease	59	Not known	75%	100%
	Standard of care	40		61	Not known	67.5%	100%
Simonovich V [29] (PlasmAr)	Convalescent plasma	228	Severe disease	62.5	64.9%	71.6%	100%
	Placebo	105		62	64.8%	61%	100%

[a]Percentages represent either any morbidity or highest proportion of one morbidity as reported in each arm of individual studies.

TABLE 2 Summary efficacy and safety outcomes in RCTs comparing convalescent plasma versus placebo/standard of care therapy in COVID-19 included in the meta-analysis

Author [reference] (study name)	Treatment Arms	Patient numbers (N)	Day7 CIR (%)	Day14 CIR (%)	Day28 CIR (%)	TTCI (in days)	Day28 Mortality (%)	Day3 VNR (%)	Day7 VNR (%)	Infusion-related severe toxicity (%)
Agarwal A [18] (PLACID)	Convalescent plasma	235	75.2%	Not known	Not known	14	14.5%	42.9%	67.6%	1.3%
	Standard of care	229	65.7%	Not known	Not known	13	13.5%	36.6%	55%	0%
AlQahtani M [19]	Convalescent plasma	20	Not known	Not known	Not known	Not known	5%	Not known	Not known	0%
	Standard of care	20	Not known	Not known	Not known	Not known	10%	Not known	Not known	0%
Avendano-Sola C [20] (ConPlas)	Convalescent plasma	38	42.1%	76.3%	89.5%	8.5	0%	34.6%	50%	5.3%
	Standard of care	43	39.6%	86%	90.7%	9	9.3%	11.8%	26.5%	0%
Bajpai M [21]	Convalescent plasma	14	Not known	Not known	Not known	12.1	21.4%	Not known	Not known	0%
	Fresh frozen plasma	15	Not known	Not known	Not known	16.1	6.7%	Not known	Not known	0%
Gharbharan A [22] (ConCOVID)	Convalescent plasma	43	37.2%	55.8%	76.7%	12.5	13.9%	Not known	Not known	0%
	Standard of care	43	32.6%	51.2%	72.1%	13.5	25.6%	Not known	Not known	0%
Horby P [23] (RECOVERY)	Convalescent plasma	5795	Not known	Not known	66.4%	11	24%	Not known	Not known	3.3%
	Standard of care	5763	Not known	Not known	66.7%	11	24%	Not known	Not known	3%
Li L [24]	Convalescent Plasma	52	9.6%	32.7%	51.9%	28	15.7%	87.2%	Not known	1.9%
	Standard of care	51	9.8%	14.6%	43.1%	30	24%	37.5%	Not known	0%
Libster R [25]	Convalescent plasma	80	Not known	Not known	Not known	Not known	2.5%	Not known	Not known	0%
	Placebo	80	Not known	Not known	Not known	Not known	5%	Not known	Not known	0%
O'Donnell M [26]	Convalescent plasma	150	Not known	Not known	72%	5	12.6%	Not known	Not known	2.7%
	Normal plasma	73	Not known	Noy known	65.8%	7	24.6%	Not known	Not known	4.2%
Rasheed M [27]	Convalescent plasma	21	Not known	Not known	Not known	19.3	4.8%	Not known	Not known	0%
	Standard of care	28	Not known	Not known	Not known	23.4	28.6%	Not known	Not known	0%
Ray Y [28]	Convalescent plasma	40	9.5%	51.3%	75.7%	13	25%	Not known	Not known	Not known
	Standard of care	40	2.8%	41%	61.8%	17	35%	Not known	Not known	Not known
Simonovich V [29] (PlasmAr)	Convalescent plasma	228	21.2%	56.3%	74%	12	10.9%	Not known	Not known	5.7%
	Placebo	105	29.4%	55.1%	76.2%	12	11.4%	Not known	Not known	1.9%

Abbreviations: CIR, clinical improvement rate; COVID-19, coronavirus disease 2019; RCT, randomised controlled trials; TTCI, time to clinical improvement; VNR, viral negativity rate.

3 | RESULTS

The flow-diagram of study selection and inclusion in the meta-analysis as per the PRISMA guidelines is depicted in Figure 1. Detailed PRISMA cheque-list is also provided as online a Table S2. Systematic search of PubMed/LitCOVID identified 838 records with an additional 117 records being retrieved through supplementary search of other sources. After removing duplicates (n = 82) and excluding irrelevant/inappropriate records (n = 776) through rigorous screening all titles/abstracts, a total of 97 full-text articles (including preprints) were assessed for eligibility, of which 12 RCTs[18–29] were finally included and pooled in this systematic review and meta-analysis.

Description of included studies: Patient characteristics, treatment details, and relevant outcomes of all 12 RCTs randomly assigning COVID-19 patients to convalescent plasma plus standard of care therapy versus placebo/standard of care therapy are briefly summarised in Tables 1 and 2 respectively. These trials were conducted between February 2020 to January 2021 in various parts of the world ensuring good geo-ethnic representation. Patients included in these RCTs were largely representative of the typical COVID-19 patient population seen in routine clinical practise. Trials enrolled patients with wide range of severity ranging from mild/moderate illness to severe/critical and life-threatening disease with varying primary endpoints and outcome measures. Convalescent plasma was administered only once

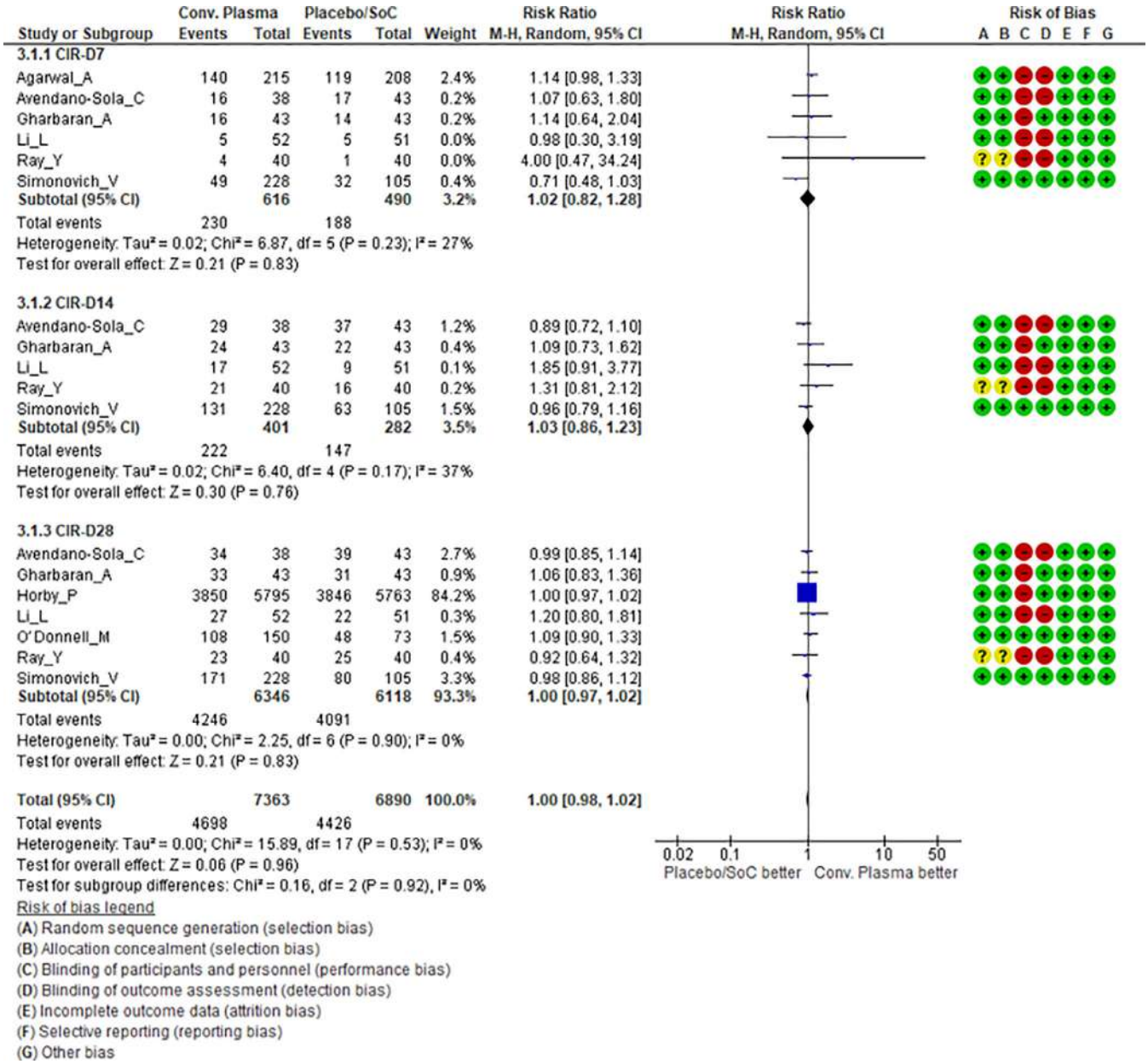

FIGURE 2 Forest plots including risk of bias in individual studies comparing convalescent plasma plus standard of care therapy versus placebo/standard of care therapy for clinical improvement rate (CIR) on specified days from randomization (Day7, Day14, Day28) and overall CIR in COVID-19 [Color figure can be viewed at wileyonlinelibrary.com]

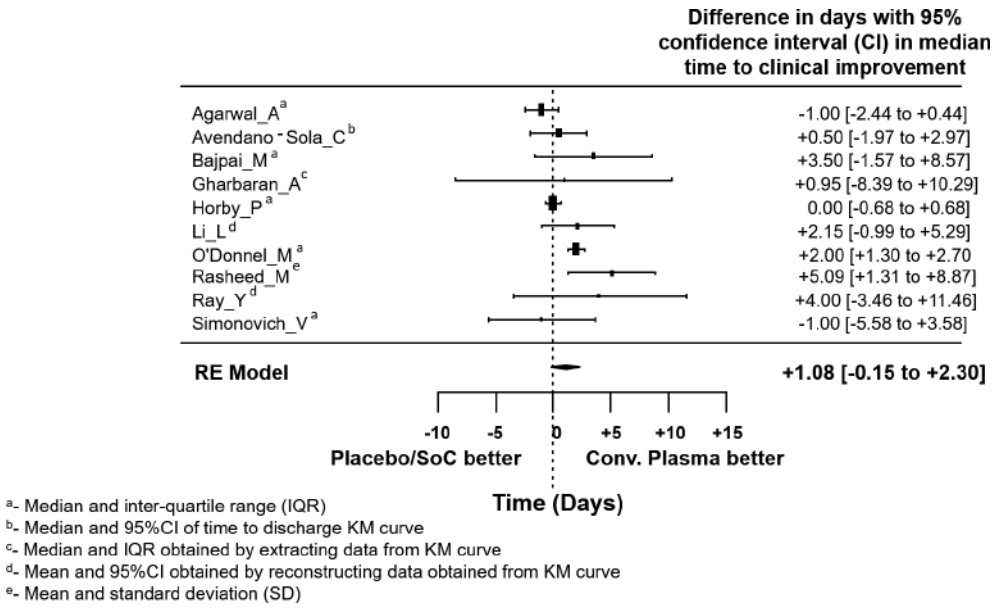

FIGURE 3 Median difference (in days) in time to clinical improvement (TTCI) between convalescent plasma plus standard of care therapy versus placebo/standard of care therapy in COVID-19

either using fixed dose of 250–500 ml[20,25–27,29] or 4–13 ml/kg body weight[24] or twice at a fixed dose of 200–275 ml given 12 to 24-h apart. [18,19,21,23,28] One trial[22] gave a single fixed dose of 300 ml convalescent plasma on day of inclusion but allowed a second such dose 5 days later in patients without clinical response and persistently positive RT-PCR. Only one trial[29] used convalescent plasma with very high neutralising antibody titres (minimum 1:800) while two other studies[23,26] used plasma with antibody titres >1:100 for transfusion. The standard of care though different in the included trials were in keeping with institutional protocols and national guidelines dictated by the best available evidence at the time and comprised of antimalarials (chloroquine, hydroxychloroquine), anti-virals (oseltamivir, lopinavir/ritonavir, remdesivir), broad-spectrum antibiotics (azithromycin), immunomodulators (steroids, tocilizumab, anakinra), traditional herbal medicines, and supportive care (oxygen inhalation and ventilatory support) as appropriate.

Evidence syntheses: There was no significant methodologic heterogeneity across the 12 included studies allowing statistical pooling of data from a total of 13 206 randomised patients in the meta-analysis. The addition of convalescent plasma to standard of care therapy was not associated with any significant or meaningful clinical benefit. There were no significant differences in rates of clinical improvement (Figure 2) between convalescent plasma plus standard of care therapy (test arm) versus placebo/standard of care therapy (control arm) either in terms of overall CIR (RR = 1.00, 95% CI: 0.98–1.02, $p = 0.96$) or CIR on Day7 (RR = 1.02, 95% CI: 0.82–1.28, $p = 0.83$); Day14 (RR = 1.03, 95% CI: 0.86–1.23, $p = 0.76$); and Day28 (RR = 1.00, 95% CI: 0.97–1.02, $p = 0.83$) respectively. Similarly, there was no significant difference in TTCI between the two arms (Figure 3) with a median difference of 1.08 days (95% CI: −0.15 to +2.30 days) favouring the convalescent plasma arm. The use of convalescent plasma was not associated with significantly reduced risk of death (Figure 4); RR of Day28 mortality was 0.81 (95% CI: 0.65–1.02, $p = 0.08$). Convalescent plasma however resulted in higher rates of viral clearance early after randomization, although based on a much smaller dataset comprising of just over 500 patients enrolled in three RCTs. Viral negativity rates both overall (RR = 1.55, 95% CI: 1.16–2.06, $p = 0.003$) and on Day3 (RR = 1.82, 95% CI: 1.02–3.23, $p = 0.04$) from randomization were higher in the convalescent plasma arm (Figure S3). Data regarding time to viral clearance was not reported consistently precluding statistically pooling of results. Reassuringly, the overall incidence of convalescent plasma transfusion-related serious adverse events was low with a weighted-mean pooled estimate of 3.25% (95% CI: 2.82–3.72%) confirming the safety of convalescent plasma transfusion. There was no significant difference (RR = 1.14, 95% CI: 0.93–1.22, $p = 0.22$) in treatment-related toxicity (Figure 5) between convalescent plasma plus standard of care therapy compared to placebo/standard of care therapy. Sensitivity analysis showed that no single trial was driving the results, inferences, and conclusions of the meta-analysis (Figure S4). Subgroup analysis stratified by disease severity (mild–moderate vs. severe-critical), timing of transfusion (early vs. later), sample size (small vs. large trials), and study design (open-label vs. placebo-controlled) suggested that the risk of dying was reduced with convalescent plasma transfusion in patients with more severe disease (RR = 0.62, 95% CI: 0.42–0.90, $p = 0.01$, 855 patients) and with early transfusion (RR = 0.51, 95% CI: 0.30–0.89, $p = 0.02$, 383 patients), based on much smaller patient numbers precluding definitive conclusions. A formal statistical analysis did not show any asymmetry in the funnel plot (Figure S5) indicating lack of significant publication bias.

Strength of recommendation: All RCTs[18-29] were of moderate to good quality with low risk of bias for most domains for the relevant outcomes of interest excepting high risk of performance and detection bias due to open-label nature of most included studies without placebo controls with lack of blinding of patients and/or physicians. Based on the above, there is low to moderate certainty evidence that the addition of convalescent plasma to standard of care therapy is not associated with significant clinical benefit or harm in patients with COVID-19 (Table 3).

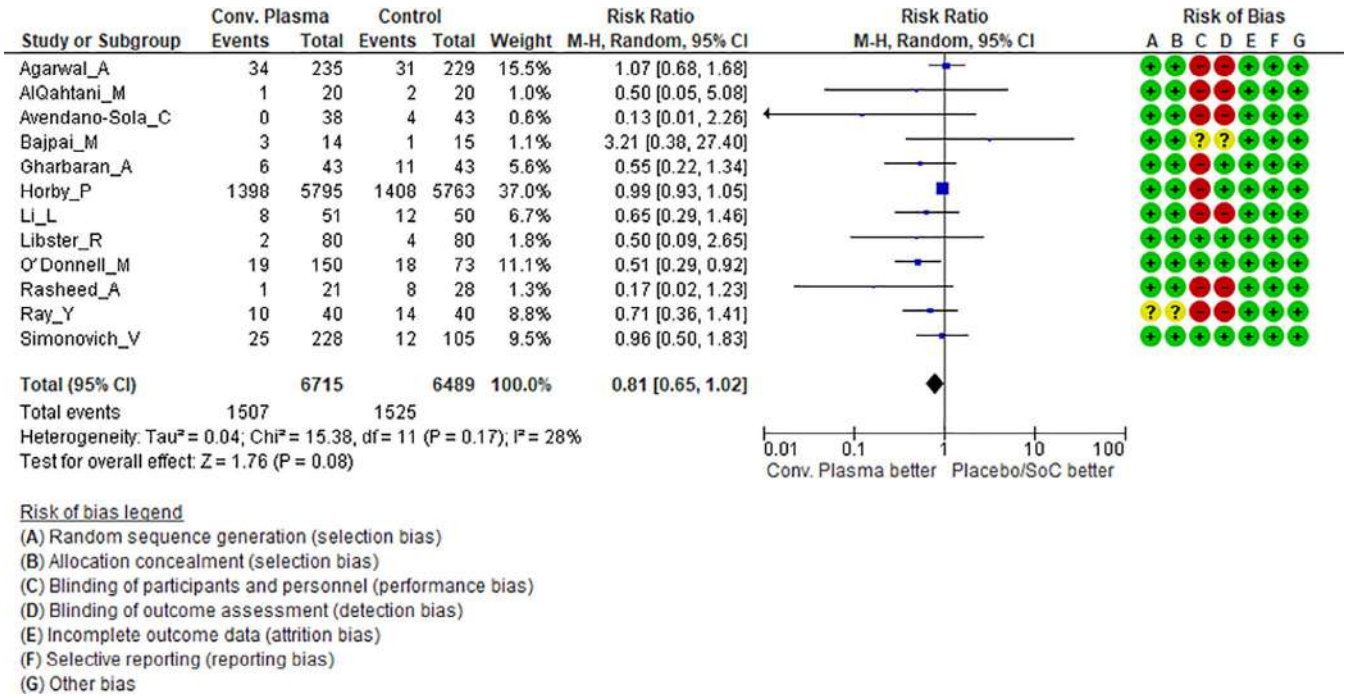

FIGURE 4 Forest plots including risk of bias in individual studies comparing convalescent plasma plus standard of care therapy versus placebo/standard of care therapy for all-cause mortality (by Day28 of randomization) in COVID-19 [Color figure can be viewed at wileyonlinelibrary.com]

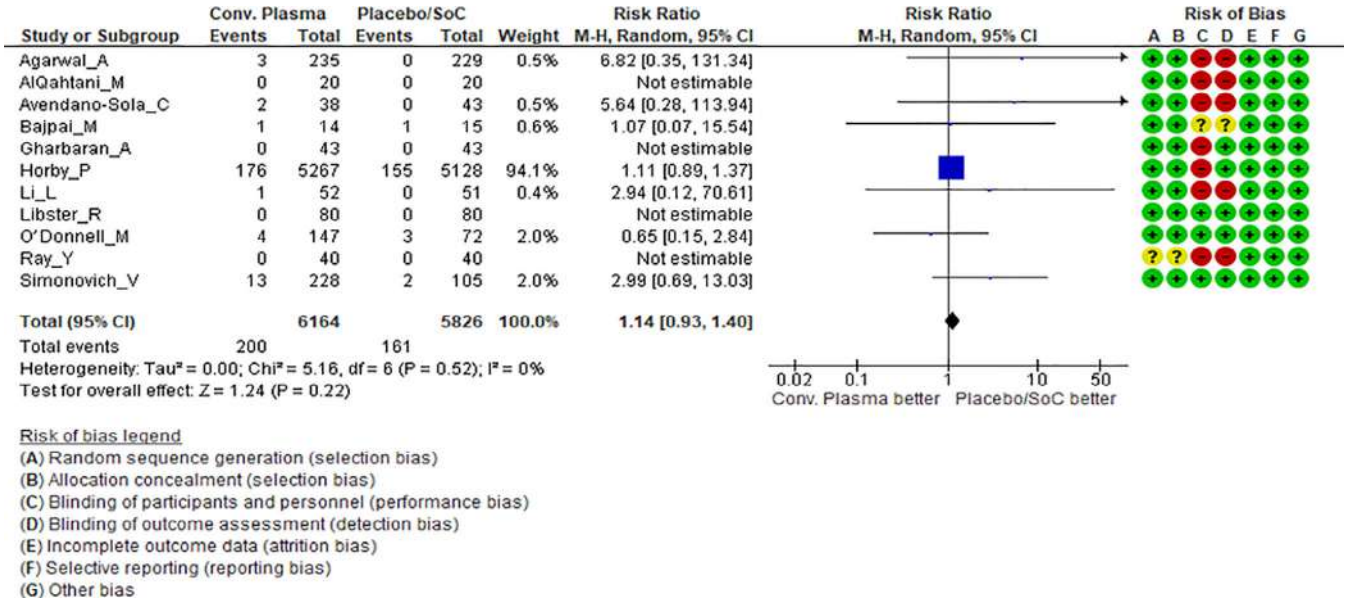

FIGURE 5 Forest plots including risk of bias in individual studies comparing convalescent plasma plus standard of care therapy versus placebo/standard of care therapy for infusion-related serious adverse events in patients with COVID-19 [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

The lack of an effective prophylactic and/or therapeutic agent against COVID-19 infection combined with strong scientific rationale and historical precedence demonstrating clinical benefit with convalescent plasma therapy in previous viral outbreaks[9,10] has prompted its widespread use hoping that this might be the magic potion for COVID-19 pandemic.[30]

Quite understandably, the use of convalescent plasma in COVID-19 infection has gained significant traction not only within the medical

TABLE 3 Summary of findings including relative effect and anticipated absolute effects with quality of evidence for benefits or harms of convalescent plasma therapy in COVID-19

Convalescent Plasma for COVID-19					
Outcomes	No of participants (studies) follow up	Quality of the evidence (GRADE)	Relative effect (95% CI)	Anticipated absolute effects	
				Risk with control	Risk difference with convalescent plasma (95% CI)
Clinical improvement rate (Clinical)	14 253(8 studies)	⨁⨁◯◯LOW[a,b]due to risk of bias, imprecision	RR 1.00 (0.98 to 1.02)	Study population	
				642 CIR per 1000	0 fewer per 1000(from 13 fewer to 13 more)
				Moderate	
				542 CIR per 1000	0 fewer per 1000(from 11 fewer to 11 more)
Day28 mortality (Clinical)	13 206(12 studies)	⨁⨁⨁◯MODERATE[b]due to imprecision	RR 0.81 (0.65 to 1.02)	Study population	
				235 per 1000	45 fewer per 1000(from 82 fewer to 5 more)
				Moderate	
				188 per 1000	36 fewer per 1000(from 66 fewer to 4 more)
Serious adverse events (Clinical)	11 990(11 studies)	⨁⨁◯◯LOW[a,b]due to risk of bias, imprecision	RR 1.14 (0.93 to 1.4)	Study population	
				28 per 1000	4 more per 1000(from 2 fewer to 11 more)
				Moderate	
				0 per 1000	-

Note: The basis for the assumed risk (e.g. the median control group risk across studies) is provided in footnotes. The corresponding risk (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). *GRADE Working Group grades of evidence*: High quality: Further research is very unlikely to change our confidence in the estimate of effect. Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate. Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate. Very low quality: We are very uncertain about the estimate.

Abbreviations: CI, confidence interval; RR: risk ratio.

[a]Most studies were open-label with no placebo-control resulting in potential performance bias.

[b]The 95% CI straddles the line of unity and increases/decreases the RR by more than 25% in several studies.

and scientific community across the globe but also within the lay public.[31] Despite lack of definitive evidence of efficacy, convalescent plasma was granted EUA by US FDA in late August 2020. Prior to this authorization, large scale clinical usage in the US was regulated through FDA's expanded access program,[32,33] that collected data on clinical outcomes and side effects in over 100 000 patients from 2700 hospitals across US in a span of 5 months (April to August 2020) and judged that convalescent plasma 'may be effective' and hence should be eligible for wider use under EUA. Safety data was derived from 20 000 patients initially and then over 35 000 hospitalised patients in the US which reported a very low incidence (<1%) of adverse events related to transfusion (circulatory overload, acute lung injury, severe allergic reactions), in the first few hours which was no different from standard blood/plasma transfusions.[32,33] Reassuringly, it largely eliminated concerns exacerbation of illness due to antibody-dependent enhancement. Further mining of this data suggests that patients who receive convalescent plasma early (within 3 days of their diagnosis) fared better than those who receive it later.[34,35] However, this observation has recently been challenged by a small RCT[33] that failed to report any significant benefit in the composite primary outcome of mechanical ventilation, hospitalisation for >14 days, or death in patients treated with upfront convalescent plasma at diagnosis compared to deferred therapy at further clinical deterioration for COVID-19 infection with an odds ratio (OR) of 0.95 (95% CI: 0.32–2.94, $p > 0.99$). There is some suggestion of a dose–response relationship, as those who receive plasma units with high titres of neutralising antibodies having lower mortality rate than patients receiving units with lower titres.[34,35] A minimum neutralising antibody titre in convalescent plasma needs to be determined to achieve desired efficacy yet maintain safe and sufficient supply[36] despite the negative impact of COVID-19 pandemic and resultant disruption of blood bank services.[37] The US FDA currently recommends anti-SARS-CoV-2 specific neutralising antibody titre >1:160 in donor plasma which corresponds to high efficacy based on the plaque reduction neutralisation test (PRNT) assay. It is now increasingly being recognised that evolutionary strain on the viral genome through the use of monoclonal

antibodies targeting the spike protein or convalescent plasma with low levels of neutralising antibodies for COVID-19 infection can potentiate immune escape allowing newer and novel mutations[38,39] with potential for increased infectivity, disease severity and even mortality. Consequent to the EUA, it has now become increasingly difficult to recruit patients on clinical trials evaluating convalescent plasma therapy clearly reflecting a missed opportunity to firmly establish its efficacy in COVID-19.[35]

An updated living Cochrane review[40] of convalescent plasma in COVID-19 involving 38 160 participants enrolled in 19 studies (two RCTs, eight nonrandomised controlled studies, and nine uncontrolled studies) reported an overall high risk of bias (due to study design, types of participants, and other previous or concurrent treatment) and concluded that the beneficial effects (improvement of clinical symptoms and reduction in mortality) or harms (severe/serious adverse events) of convalescent plasma therapy in patients with COVID-19 infection were very uncertain at the present time. More recently, Janiaud et al.[41] reported no significant clinical benefit (decrease in all-cause mortality, increase in rates of clinical improvement, or reduced length of hospitalisation) with convalescent plasma in COVID 19 infection compared to placebo/standard of care therapy in a pooled analysis of 1060 patients from four RCTs published in peer-reviewed journals, 316 patients from five RCTs posted on preprint servers and 10 406 patients from one RCT reported via press briefing. The summary risk ratio (RR) for all-cause mortality with convalescent plasma in the four peer-reviewed RCTs was 0.93 (95% CI: 0.63–1.38) with low certainty of the evidence due to imprecision. After adding results of six more RCTs (from preprints/press release), the summary RR was 1.02 (95% CI: 0.92–1.12) with moderate certainty of evidence. The authors further reported that limited data on clinical improvement, clinical deterioration, and serious adverse events showed no significant differences between the two treatments.

The current meta-analysis provides the most robust and best contemporary evidence regarding the safety and efficacy of convalescent plasma in the treatment of COVID-19 infection. The addition of convalescent plasma to the current standard of care therapy is not associated with statistically significant clinical improvement or reduction in mortality. Overall, the risk of infusion-related serious adverse events is quite low and not significantly different compared to placebo/standard of care therapy. The clinical significance of early viral negativity following convalescent plasma transfusion is unknown and its benefit when given early in the course of the disease and in patients with more severe disease should be considered exploratory findings from this meta-analysis based on much smaller cohort size for such analyses.

Strengths and limitations: Despite being the largest dataset (comprising over 13 000 patients) derived only from RCTs and pooled using modern meta-analytic methods, certain caveats and limitations remain. The efficacy of convalescent plasma largely correlates with high titres of neutralising antibodies in the donor plasma and lack/low-level of such antibodies in recipients. Only three RCTs transfused convalescent plasma with high titres of neutralising antibodies (measured quantitatively using the PRNT assay), while others did not mandate a quantitative estimation of such antibodies prior to transfusion. This was further confounded by the presence of anti-SARS-CoV-2 specific IgG antibodies in a significant proportion of convalescent plasma recipient patients even prior to transfusion in four studies. Detection of such neutralising IgG antibody was an exclusion criterion in only a single RCT, with other trials allowing such patients to be randomised. It is also hypothesized that early transfusion (within few days of symptom onset and/or disease of mild to moderate severity) of convalescent plasma is more effective than delayed/deferred transfusion (>7 days of symptom onset and/or severe to critical illness). However, most trials included patients somewhat late in the course of their illness with median time from symptom onset to transfusion being beyond 7 days in most studies. Four of the included RCTs were exploratory pilot studies with relatively small sample size and four others were terminated prematurely without achieving the specified target accrual further reducing statistical power and rigour. Only three of 12 included RCTs used placebo-controlled design, with remaining nine studies being open-label without blinding of patients/physicians with potential for performance and detection bias leading to downgrading of the quality of evidence. Finally, evidence synthesis and subgroup analyses were primarily based on data reported in preprints/publications without access to individual patient data which would be a more robust method to identify subgroups that might benefit with convalescent plasma transfusion.

Implications for research: Key considerations in clinical trials evaluating convalescent plasma for COVID-19 should include timing of administration relative to onset of disease, timing of donation relative to resolution of symptoms in the donor, severity of disease, pretransfusion serology, and antibody titres.[42,43] A scoping review[44] of registered clinical trials of convalescent plasma therapy for COVID-19 infection was conducted early in the course of the pandemic to provide a framework for accelerated synthesis of trial evidence. The review identified 48 such registered trials (29 controlled studies) projected to enrol over 5000 patients, combined analysis of which would be sufficient to determine meaningful improvements in mortality, intensive-care admission, or mechanical ventilation faster than any individual RCT determining effectiveness of convalescent plasma therapy. A more recent search of clinical trial registries identified 64 studies in 22 countries using convalescent plasma therapy for COVID-19 infection during an international survey.[45] Twenty of the 64 centres responded to the survey, of which only nine were RCTs, the remaining being single arm prospective case series. Only four RCTs planned to include over 400 patients (adequately powered) and only three RCTs were blinded (low risk of bias). The survey reported significant variability in donor antibody testing with no consensus towards an optimal cut-off of anti-SARS-CoV-2 IgG neutralising antibody titres in the donor plasma for transfusion.[45] Current trials of convalescent plasma therapy include patients with wide spectrum of COVID-19 illness (from mild to critical), have variable need for molecular evidence of viral infection, use nonstandardised intervention (differing antibody titres, dose, and timing), have no universally accepted standard of care (as comparator), are mostly open label without placebo control (such as normal plasma) with key differences in primary outcomes between trials.[46] It is conceivable that the treatment effect of convalescent plasma may differ by illness severity, by

dose in terms of volume, concentration of neutralisation antibody, and the risk of antibody dependent enhancement along with other adverse events during COVID-19 illness. The National Institutes of Health (NIH) COVID-19 treatment guidelines panel[47] recently stated that it cannot recommend convalescent plasma as a standard of care for treating COVID-19 at this time as currently the data are insufficient to recommend for or against its usage. Their report further states that prospective, well controlled, and adequately powered RCTs are needed to determine whether convalescent plasma and other passive immunotherapies are safe and effective in COVID-19.

Since the press release declaring closure of RECOVERY trial to recruitment on the convalescent plasma arm, three other RCTs, the REMAP-CAP (NCT02735707), CONCOR-1 (NCT04348656), and NIH-led C3PO study (NCT04355767) have issued public statements announcing cessation of recruitment based on reaching prespecified endpoints of statistical futility on interim analysis of available data. Many more RCTs of convalescent plasma including an ongoing large placebo-controlled trial of 1000 patients (PassITON)[48] are currently underway; an updated living pooled analysis[49] of yet unreported trials might further enhance the certainty of evidence and improve the strength of recommendation in the future.

The next generation of convalescent plasma trials should also determine desirable product attributes, optimal dose and timing of administration, as well as appropriate patient population for its usage.[46,50] All reported RCTs evaluating convalescent plasma in COVID-19 till date have included only hospitalised adults with mild/moderate to severe/critical disease, excepting one study conducted in the outpatient setting for elderly patients with milder disease to prevent symptomatic worsening. If the main mechanism of action of convalescent plasma is through virus neutralisation, it would possibly be most efficacious when used very early in the course of the disease and/or even for prophylaxis in high-risk individuals.[50] In addition, there may be specific groups who are more likely to benefit such as those with impaired immune responses secondary to an immunocompromised state (inherited or acquired immunodeficiency, cancer patients, transplant recipients on suppressive medication) leading to delayed viral clearance.[50] Continuous monitoring of pooled international trials of convalescent plasma for COVID-19 hospitalised patients (COMPILE) project is presently pooling individual patient data from RCTs of convalescent plasma in real-time[49] under a shared regulatory and statistical framework (http://nyulmc.org/compile). A similar initiative from the European Union COVID-19 convalescent plasma platform (https://www.euccp.dataplatform.tech.ec.europa.eu/) could be considered to further strengthen the evidence-base.

5 | CONCLUSIONS

There is low to moderate certainty evidence that the addition of convalescent plasma to current standard of care therapy is generally safe with low risk of transfusion-associated serious adverse events but does not result in significant clinical benefit or reduction of mortality in patients with COVID-19 infection. An updated meta-analysis including other ongoing large RCTs of convalescent plasma therapy may help improve this evidence-base in the future.

CONFLICT OF INTEREST

None of the authors have any conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Tejpal Gupta: Conceptualization, methodology, analysis, and writing–original draft. **Sadhana Kannan**: Methodology, literature search strategy, and analysis. **Babusha Kalra**: Data curation and writing–review & editing. **Prafulla Thakkar**: Data curation and writing–review & editing.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Volume of packed red blood cells and fresh frozen plasma is associated with intraoperative hypocalcaemia during large volume intraoperative transfusion

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Abstract

Background: Severe hypocalcaemia is associated with increased transfusion in the trauma population. Furthermore, trauma patients developing severe hypocalcaemia have higher mortality and coagulopathy. Electrolyte abnormalities associated with massive transfusion have been less studied in the surgical population. Here, we tested the primary hypothesis that volume of packed red blood cells and fresh frozen plasma transfused intraoperatively is associated with lower nadir ionised calcium in the surgical population receiving massive resuscitation.

Methods: We performed a retrospective observational study at an academic quaternary care centre to characterise hypocalcaemia following large volume (4 or more units packed red blood cells) intraoperative transfusion. We used multivariable linear regression to assess if volume of transfusion with packed red blood cells and fresh frozen plasma were independently associated with a lower ionised calcium. We then used multivariable logistic regressions to assess the association between ionised calcium and transfusion with: (i) mortality, (ii) acute kidney injury, and (iii) postoperative coagulopathy.

Results: Hypocalcaemia following large volume resuscitation in the operating room is a very frequent occurrence (70% of cases). After controlling for demographic variables and intraoperative variables, the volume transfused intraoperative was independently associated with hypocalcaemia on multivariable linear regression. Hypocalcaemia, intraoperative transfusion of packed red blood cells, and intraoperative transfusion of fresh frozen plasma were not shown to be associated with clinical outcomes.

Conclusions: Hypocalcaemia was associated with increased transfusion volume in this single-centre study. Unlike the trauma population, hypocalcaemia was not associated with increased mortality during surgical care. Our findings suggest that despite improved practice patterns of calcium supplementation, intraoperative hypocalcaemia occurs with relatively high frequency following large volume intraoperative transfusion.

KEYWORDS
calcium repletion, hypocalcaemia, intraoperative transfusion, massive transfusion, perioperative medicine

1 | INTRODUCTION

Massive transfusion is essential in the treatment of hypovolemic shock, but is associated with multiple infectious, immunologic, and physiologic complications.[1] Because blood products contain citrate, a calcium binder, to minimise coagulation during storage, massive transfusion can lead to systemic citrate toxicity with associated electrolyte abnormalities—hypocalcaemia and hypomagnesaemia. Calcium in the ionised form is required for coagulation of blood and muscular contraction. Citrate-associated hypocalcaemia can cause reduced vascular tone and myocardial contractility leading to hypotension and arrhythmias including prolongation of the QT interval and ventricular fibrillation.[1–3] Furthermore, severe hypocalcaemia has been linked with increased mortality in critically ill patients and an increased incidence of adverse cardiac events.[4,5]

The incidence and associated risk factors for hypocalcaemia following massive transfusion were recently evaluated in trauma patients.[6] In this population, severe hypocalcaemia was associated with increased transfusion of packed red blood cells and fresh frozen plasma. Additionally, patients developing severe hypocalcaemia had higher mortality and higher activated partial thromboplastin time (PTT) than those who did not experience hypocalcaemia. Electrolyte and metabolic abnormalities associated with massive transfusion have been less extensively studied in the surgical population, as compared to the trauma population. An earlier study of massive transfusion in elective surgical patients demonstrated that despite no calcium supplementation, patients developed only transient hypocalcaemia, without postoperative haemodynamic instability or metabolic acidosis.[7] Differences in clinical significance between the trauma and perioperative populations are hypothesised to result from alterations in citrate clearance secondary to hypotension, acidosis, and hypothermia in the trauma cohort.[6] Recent studies on intraoperative and perioperative massive resuscitation have been limited to specific surgeries, such as abdominal aortic aneurysm,[8] placenta accreta,[9] or liver transplantation,[10] which may not be widely generalizable. The largest study in non-cardiac surgery patients found that transfusion with 5 or more units of red blood cells was associated with increased 30-day mortality and greater rate of postoperative complications, however, this study did not specifically characterise the incidence and risk factors for abnormalities, like hypocalcaemia, in the massive transfusion population.[11]

Studies in the perioperative population are limited to non-generalizable surgical sub-populations[8–10] or are not reflective of current clinical practice.[7] Furthermore, trauma may precipitate altered citrate metabolism, which limits generalizability between trauma and surgical populations.[6,12] Therefore, a comprehensive characterisation of hypocalcaemia following massive transfusion in the perioperative period and the associated clinical consequences is needed. We thus tested the primary hypothesis that volume of packed red blood cells and volume of fresh frozen plasma transfused are associated with nadir ionised calcium in the surgical population receiving large volume (4 or more units of packed red blood cells) resuscitation. Secondarily, we tested whether nadir ionised calcium is associated with postoperative mortality, acute kidney injury (AKI), or coagulopathy.

2 | MATERIAL AND METHODS

2.1 | Study design

For this retrospective observational study performed at our academic quaternary care centre, we obtained Institutional Review Board (HUM00052066) approval. This article was prepared in accordance with the standards set forth by the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[13] Study methods including data collection, outcomes, and statistical analyses were established prospectively and presented at an institutional peer-review committee on 21 March 2018 prior to data access.[14]

2.2 | Data collection

Study data were collected via combined queries of the electronic perioperative anaesthesia database (Centricity; General Electric Healthcare, Waukesha, WI) and the hospital electronic health record (Epic, Verona, WI).[15,16] Methods for data input, validation, storage, and extraction within the MPOG consortium have been described elsewhere[17] and utilised in previous studies. Quality assurance was maintained through a standardised set of data diagnostics with limited manual review by clinicians to assess and attest to the accuracy of data extraction and source data.

2.3 | Study population

Inclusion criteria for the study were adult patients (≥18 years) who underwent a surgical procedure involving intraoperative transfusion with at least 4 units of packed red blood cells. We studied cases between 1 January 2008 and 1 August 2018. We excluded cardiac surgeries, liver transplantations, other cases requiring preoperative or intraoperative cardiovascular support (cardiopulmonary bypass, extracorporeal membrane oxygenation, ventricular assist devices, or intraaortic balloon pump), and *American Society of Anesthesiologists* (ASA) physical classification 6.

2.4 | Primary outcome

The primary outcome of this analysis was nadir (lowest value during the operation) ionised calcium (mmol/L) occurring *after* transfusion of the *first* unit of packed red blood cells and prior to completion of the procedure.

2.5 | Secondary outcomes

Secondary outcomes included: (i) 30-day post-operative all-cause mortality, (ii) post-operative AKI, and (iii) post-operative coagulopathy. AKI was defined according to the *Kidney Disease—Improving Global Outcomes* (KDIGO) definition[18] (specifically an increase in serum creatinine by ≥0.3 mg/dl within 48 h of anaesthesia end time, or a ≥50% increase within seven post-operative calendar days. Coagulopathy was defined by an abnormal PT/INR or PTT within 24 h of anaesthesia completion.

2.6 | Exposure variables

The exposure variables tested were volume of packed red blood cells and volume of fresh frozen plasma transfused. At our institution, packed red blood cells and fresh frozen plasma are typically documented in unit increments, which typically are 350 ml for packed red blood cells and 250 ml for fresh frozen plasma. In cases where the clinical provider documented transfusion in ml, instead of units, the transfusion was converted to units.

2.7 | Covariables

Covariables were divided into preoperative and intraoperative categories. Preoperative variables were those defined prior to induction of anaesthesia and remained unchanged throughout the course of the procedure. Categories of preoperative variables included: (i) demographic (age, sex, race, height, weight, admission type, ASA classification, and emergency surgery),[19] (ii) social history, (iii) comorbidities,[16] (iv) preoperative medications, and (v) baseline laboratory results. Dynamic intraoperative variables were also defined based upon the anaesthetic and surgical record and included: (i) procedural details (case duration, general anaesthetic), (ii) fluid resuscitation and transfusion, (iii) vasopressor/inotrope requirement, and (iv) calcium repletion. To ensure the predictive utility of our model, all variables were censored at the time point corresponding to our primary outcome: *nadir ionised calcium*. For example, *case duration* does not reflect overall case duration, but is the duration of time from anaesthesia start until time corresponding with nadir ionised calcium, nor does volume transfused reflect the whole case but only the amount transfused before nadir ionised calcium. The full list of preoperative and intraoperative variables collected can be seen in Table S1.

2.8 | Statistical analyses

Perioperative characteristics were summarised using means and SDs for normally distributed continuous covariates, medians, and interquartile range for non-normally distributed continuous variables, and counts and percentages for categorical covariates. Statistical analysis was performed in R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).[20] We used multivariable regression models to determine associations between our exposure variables (transfusion of packed red blood cells and transfusion of fresh frozen plasma) and our primary outcome, nadir ionised calcium. To analyse this outcome, we performed a multivariable linear regression with variable selection by least absolute shrinkage and selection operator (LASSO) to identify which preoperative and intraoperative factors were independently associated. As previously described, we used least absolute shrinkage and selection operator using the glmnet package (Palo Alto, CA; http://www.jstatsoft.org/v33/i01/) in R to select variables for inclusion in our final models.[16,21] Next, we assessed independent association between our exposure variables (transfusion of packed red blood cells and transfusion of fresh frozen plasma) and each of our secondary, clinical outcomes using multivariable logistic regressions with variable selection by LASSO. Our primary outcome was also included as a covariable in each of these logistic regressions.

2.9 | Power analysis

Preliminary power analysis was calculated based upon a mean of 10.07 units of PRBC in the severe hypocalcaemia group, a mean of 6.35 units of PRBC in the group without severe hypocalcaemia, and a common SD of 5.96. These numbers selected were based upon descriptive statistics obtained as part of an unpublished departmental quality improvement initiative. While the inclusion criteria for the study were transfusion with at least 4 units of packed red blood cells, we expected that most patients included would actually receive more than 4 units. In order to have 90% power to detect a difference between the two groups using a two-sided *t*-test at 0.05 significance level, 55 patients were needed per group. The power analysis was conducted using PASS version 20.0.2.

3 | RESULTS

A total of 1614 procedures met our inclusion criteria. The most common surgeries were as follows: abdominal ($n = 272$, 16.9%), vascular/plastics ($n = 229$, 14.2%), neurosurgery ($n = 227$, 14.1%), and hepatobiliary/transplant (kidney, pancreas) ($n = 198$, 12.3%). Patients had a mean age of 56 ± 17 years, and mean BMI of 28.3 ± 7.3 kg/m^2. Fifty-nine percent ($n = 959$) were male and the mean ASA Physical Status Classification system was 3 ± 1. Other notable *preoperative covariates* include: (i) 32.2% ($N = 519$) of patients had a history of coagulopathy, (ii) 35.2% ($n = 568$) cardiac arrhythmia, and (iii) 16.6% ($n = 268$) unintended weight loss. At the time nadir ionised calcium

TABLE 1 Characteristics of patients requiring intraoperative transfusion with at least 4 units of packed red blood cells with comparison by 30-day mortality

Characteristics of patients requiring ≥ 4 units packed red blood cells																								
		All data ($n = 1614$)							No in-hospital mortality ($n = 1408$)							In-hospital mortality ($n = 206$)							*p* value	
Variable	Level	*N*	%	Mean	SD	Median	IQR		*N*	%	Mean	SD	Median	IQR		*N*	%	Mean	SD	Median	IQR		χ^2	*t*-test
Age (years)		1614	100.0	56	17	59	45	68	1408	100.0	55.7	16.7	58.0	45.0	68.0	206	100.0	58.2	17.1	61.0	48.3	71.0		0.046
Emergent		569	35.3						422	30.0						147	71.4						<0.001	
ASA status	I	25	1.5						24	1.7						1	0.5						<0.001	
	II	267	16.5						260	18.5						7	3.4							
	III	721	44.7						681	48.4						40	19.4							
	IV	503	31.2						400	28.4						103	50.0							
	V	98	6.1						43	3.1						55	26.7							
BMI (kg/m^2)		1598	99.0	28.3	7.3	26.9	23.5	32.1	1401	99.5	28.2	7.2	26.8	23.4	31.9	197	95.6	29.2	8.1	27.7	23.7	34.9		0.097
Height (cm)		1599	99.1	170.7	10.8	171.5	162.6	177.8	1401	99.5	170.7	10.8	170.4	162.6	177.8	198	96.1	170.5	11.4	172.7	163.1	177.8		0.820
Weight (kg)		1613	99.9	83.8	23.4	80.7	67.4	96.3	1407	99.9	83.6	23.4	80.3	67.3	95.8	206	100.0	85.3	23.6	81.6	68.8	98.4		0.334
Gender	Female	655	40.6						580	41.2						75	36.4							0.219
	Male	959	59.4						828	58.8						131	63.6							
Race	White/Caucasian	1181	73.2						1050	74.6						131	63.6						<0.001	
	Other	163	10.1						155	11.0						8	3.9							
	Unknown	270	16.7						203	14.4						67	32.5							
Procedure	Abdominal	272	16.9						220	15.6						52	25.2						<0.001	
	Neurosurgery	227	14.1						211	15.0						16	7.8							
	Obstetrics/gynaecology/ urology	150	9.3						146	10.4						4	1.9							
	Oral/maxillofacial/ dentistry/ otolaryngology	123	7.6						122	8.7						1	0.5							
	Orthopaedics	159	9.9						155	11.0						4	1.9							
	Thoracic	39	2.4						32	2.3						7	3.4							
	Transplant/hepatobiliary	198	12.3						179	12.7						19	9.2							
	Trauma	68	4.2						41	2.9						27	13.1							
	Vascular/plastics	229	14.2						190	13.5						39	18.9							
	Other/unknown/ radiology	149	9.2						112	8.0						37	18.0							
Elixhauser comorbidities	Alcohol or drug abuse	185	11.5						152	10.8						33	16.0						0.007	
	Anaemia (iron deficiency)	190	11.8						176	12.5						14	6.8						0.066	
	Cardiac arrhythmias	568	35.2						483	34.3						85	41.3						0.003	
	Valvular diseases of the heart	108	6.7						88	6.3						20	9.7						0.031	
	COPD	316	19.6						270	19.2						46	22.3						0.087	
	Coagulopathy	519	32.2						414	29.4						105	51.0						<0.001	

TABLE 1 (Continued)

Characteristics of patients requiring ≥ 4 units packed red blood cells																							
		All data ($n = 1614$)							No in-hospital mortality ($n = 1408$)							In-hospital mortality ($n = 206$)						p value	
Variable	Level	N	%	Mean	SD	Median	IQR		N	%	Mean	SD	Median	IQR		N	%	Mean	SD	Median	IQR		χ^2	t-test
	Diabetes	347	21.5						305	21.7						42	20.4						0.745	
	Fluid and electrolyte disorders	725	44.9						597	42.4						128	62.1						<0.001	
	Hypertension	854	52.9						761	54.0						93	45.1						0.348	
	Hypothyroidism	197	12.2						179	12.7						18	8.7						0.292	
	Liver disease	338	20.9						263	18.7						75	36.4						<0.001	
	Metastatic cancer	295	18.3						277	19.7						18	8.7						0.001	
	Neurologic disorders	21	1.3						19	1.3						2	1.0						1.000	
	Peripheral vascular disorders	308	19.1						242	17.2						66	32.0						<0.001	
	Pulmonary circulation disorders	142	8.8						117	8.3						25	12.1						0.029	
	Renal failure	304	18.8						255	18.1						49	23.8						0.064	
	Unexpected or unanticipated weight loss	268	16.6						238	16.9						30	14.6						0.884	
Other comorbidities	Cerebrovascular disease	63	3.9						50	3.6						13	6.3						0.086	
Baseline labs	Serum creatinine (Cr)	1614	100.0	1.2	1.2	0.9	0.6	1.3	1408	100.0	1.2	1.2	0.9	0.6	1.3	206	100.0	1.4	1.2	1.1	0.8	1.7		0.002
	Blood urea nitrogen (BUN)	1614	100.0	21.0	17.5	17.0	11.0	26.0	1408	100.0	20.0	16.2	16.0	11.0	25.0	206	100.0	27.8	23.5	21.0	14.3	33.0		<0.001
	Haematocrit (Hct)	1579	97.8	30.9	7.4	30.6	24.9	36.4	1380	98.0	31.3	7.3	31.1	25.5	36.7	199	96.6	27.8	7.6	27.4	22.0	33.3		<0.001
	Total calcium	1425	88.3	8.6	1.6	8.7	7.9	9.4	1237	87.9	8.6	1.4	8.8	7.9	9.4	188	91.3	8.8	2.2	8.4	7.6	9.4		0.358
	Ionised calcium (iCal)	547	33.9	1.2	0.2	1.2	1.1	1.2	420	29.8	1.2	0.1	1.2	1.1	1.2	127	61.7	1.1	0.2	1.2	1.1	1.2		0.326
	Albumin	1264	78.3	3.5	0.9	3.6	2.7	4.2	1094	77.7	3.5	0.8	3.7	2.8	4.2	170	82.5	3.0	0.9	2.9	2.3	3.8		<0.001
	Partial thromboplastin time (PTT)	1419	87.9	1.7	3.4	1.1	1.0	1.5	1227	87.1	1.6	3.6	1.1	1.0	1.4	192	93.2	2.0	1.3	1.5	1.1	2.2		0.016
Intraoperative data (at nadir)	Estimated blood loss (L)	1614	100.0	1.5	1.9	1.0	0.0	2.3	1408	100.0	1.5	1.8	1.0	0.0	2.3	206	100.0	1.1	2.2	0.0	0.0	1.2		0.011
Fluid resuscitation	Urine output (ml)	1614	100.0	8.8	11.8	5.6	2.1	11.9	1408	100.0	9.3	11.7	6.2	2.7	12.7	206	100.0	5.5	12.2	1.0	0.0	5.6		<0.001
	Lactated ringer (LR) (L)	1614	100.0	1.7	1.7	1.3	0.4	2.6	1408	100.0	1.9	1.7	1.5	0.6	2.7	206	100.0	0.9	1.3	0.4	0.0	1.3		<0.001
	Crystalloid (L)	1614	100.0	2.8	2.0	2.4	1.4	3.8	1408	100.0	2.9	2.0	2.5	1.5	3.9	206	100.0	2.0	1.9	1.6	0.6	3.0		<0.001
	Colloid (L)	1614	100.0	0.5	0.7	0.3	0.0	1.0	1408	100.0	0.5	0.7	0.5	0.0	1.0	206	100.0	0.4	0.9	0.0	0.0	0.5		0.169
	Calcium repletion (mEq)	1614	100.0	52.8	1054.3	12.9	5.5	23.5	1408	100.0	42.1	977.4	12.5	5.7	22.6	206	100.0	126.1	1477.8	16.3	4.6	28.2		0.430
Case details	Duration (hour)	1614	100.0	4.5	3.1	3.9	2.1	6.2	1408	100.0	4.7	3.1	4.2	2.3	6.4	206	100.0	2.9	2.5	2.1	1.1	4.0		<0.001

(Continues)

TABLE 1 (Continued)

Characteristics of patients requiring ≥ 4 units packed red blood cells																						
		All data ($n = 1614$)							No in-hospital mortality ($n = 1408$)							In-hospital mortality ($n = 206$)							p value	
Variable	Level	*N*	%	Mean	SD	Median	IQR		*N*	%	Mean	SD	Median	IQR		*N*	%	Mean	SD	Median	IQR		χ^2	*t*-test
	Haematocrit (Hct)	1609	99.7	22.1	4.2	22.0	19.0	25.0	1403	99.6	22.2	4.1	22.0	19.6	25.0	206	100.0	21.9	5.2	21.0	18.0	24.0		
	Mean arterial pressure < 55 mmHg (min)	1614	100.0	11.9	21.0	4.0	1.0	13.0	1408	100.0	11.6	21.1	4.0	1.0	13.0	206	100.0	13.8	20.5	6.0	1.0	18.0		0.157
Medications	Norepinephrine administered (1 mcg)	226	14.0						1408	100.0	79.1	481.6	0.0	0.0	0.0	206	100.0	216.4	645.9	0.0	0.0	149.6		0.004
	Vasopressin administered (1 unit)	314	19.5						1408	100.0	1.0	3.4	0.0	0.0	0.0	206	100.0	3.3	6.3	0.0	0.0	4.0		<0.001
	Epinephrine administered	376	23.3						1408	100.0	0.1	0.7	0.0	0.0	0.0	206	100.0	0.9	2.5	0.0	0.0	0.2		<0.001
Transfusion	Packed red blood cells (pRBC) (units)	1614	100.0	4.2	3.4	4.0	2.0	5.0	1408	100.0	4.0	2.9	4.0	2.0	5.0	206	100.0	5.5	5.5	4.0	2.0	6.0		<0.001
	Fresh frozen plasma (FFP) (units)	1614	100.0	2.0	3.0	1.0	0.0	3.0	1408	100.0	1.8	2.7	1.0	0.0	3.0	206	100.0	3.2	4.3	2.0	0.9	4.0		<0.001
	Platelets (5-packs)	1614	100.0	0.1	0.5	0.0	0.0	0.0	1408	100.0	0.1	0.5	0.0	0.0	0.0	206	100.0	0.3	0.7	0.0	0.0	0.0		0.004
	Cryoprecipitate (5-packs)	1614	100.0	0.1	0.3	0.0	0.0	0.0	1408	100.0	0.0	0.2	0.0	0.0	0.0	206	100.0	0.2	0.6	0.0	0.0	0.0		0.007
	Cell salvage (ml)	1614	100.0	133.8	629.6	0.0	0.0	0.0	1408	100.0	121.5	466.8	0.0	0.0	0.0	206	100.0	217.9	1270.9	0.0	0.0	0.0		0.283
Primary outcome	Ionised calcium (iCal)	1614	100.0	0.92	0.18	0.93	0.82	1.03	1408	100.0	0.92	0.17	0.93	0.83	1.03	206	100.0	0.90	0.25	0.93	0.77	1.04		0.205

Abbreviations: ASA, American Society of Anesthesiologists; BMI, body mass index; cm, centimetre; kg, kilograms; L, litre; m, metre; mcg, micrograms; mEq, milliequivalents; min, minutes; mmol, millimoles.

TABLE 2 Multivariable linear regression for primary outcome: Nadir ionised calcium

Nadir ionised calcium			
	Estimate	**95% CI**	***p* Value**
Exposure variables			
Transfusion packed red blood cells (units)	−0.013	−0.022 to −0.005	0.002
Transfusion fresh frozen plasma (units)	−0.012	−0.020 to −0.003	0.009
Preoperative variables			
Weight (10 kg)	0.005	0.002 to 0.0116	0.170
Male gender	−0.019	−0.015 to 0.053	0.266
Preoperative ionised calcium (mmol/L)	0.1531	0.044 to 0.263	0.006
History of cardiac arrhythmia	0.020	−0.011 to 0.050	0.212
History of coagulopathy	0.037	0.005 to 0.070	0.026
History of weight loss	0.058	0.022 to 0.095	0.002
Vascular/plastic surgery procedure	0.052	0.009 to 0.095	0.019
Intraoperative variables			
Estimated blood loss (1 L)	0.015	0.003 to 0.027	0.015
Calcium repletion (10 mEq)	0.015	0.001 to 0.028	0.037
Crystalloid resuscitation (1 L)	−0.020	−0.029 to −0.010	<0.001
Case duration (hours)	0.007	−0.001 to 0.0143	0.091
Epinephrine administered (100 mcg)	−1.291	−2.601 to 0.019	0.054
Vasopressin administered (4 units)	0.024	0.007 to 0.041	0.005
Norepinephrine administered (80 mcg)	0.001	−0.001 to 0.004	0.290

Abbreviations: kg, kilograms; L, litre; mcg, micrograms; mEq, milliequivalents; mmol, millimoles.

occurred, a median of 4 (interquartile range = 2–5) units of packed red blood cells and median 1 (0–3) fresh frozen plasma units had been transfused. *Intraoperatively*, nadir calcium occurred 4.5 ± 3.1 h into the case. At the time of nadir calcium, patients had been replete with 12.9 mEq (5.5, 23.5) of calcium. Twenty-three percent of patients received epinephrine, 20% received vasopressin, and 14% received norepinephrine. Patients spent 12 ± 21 min with a mean arterial pressure (MAP) less than 55 mmHg. A full description of our cohort can be found in Table 1.

3.1 | Primary outcome: Nadir ionised calcium

The mean nadir ionised calcium was 0.92 ± 0.18 mmol/L. Most patients (n = 1099, 70%) developed intraoperative hypocalcaemia (ionised calcium ≤1.0 mmol/L). Twenty-two percent (n = 378) demonstrated severe hypocalcaemia (ionised calcium ≤0.80 mmol/L). The distribution of severity of hypocalcaemia can be visualised in Figure S1. Using multivariable linear regression to adjust for other factors that may be associated with calcium levels (e.g., patient age, baseline laboratory values, medical comorbidities, and intraoperative details), we found that transfusion of each additional unit of packed red blood cells was independently associated with only a slight decrease (−0.013 mmol/L, 95% CI, −0.0218 to −0.0048; p = 0.002) in nadir calcium and each additional unit of fresh frozen plasma was similarly associated with a lower ionised calcium (−0.012 mmol/L; 95% CI, −0.0202 to −0.0029; p = 0.009). History of coagulopathy and unintended weight loss were also associated with higher ionised calcium. Cases involving larger resuscitation with crystalloid, more calcium repletion, and larger vasopressin receipt were associated with higher ionised calcium. Full details of the multivariable linear regression can be found in Table 2.

3.2 | Secondary outcome: 30-day mortality

Patients receiving at least 4 units of packed red blood cells intraoperatively had a 30-day mortality of 13% (n = 206). The mean ionised calcium in the group with no in-hospital mortality was 0.93 ± 0.17 and was 0.90 ± 0.25 in the mortality group (p = 0.205). Nadir ionised calcium was not associated with 30-day mortality (adjusted odds ratio [aOR] = 0.787; 95% CI, 0.258–2.398; p = 0.674). Emergent surgery (aOR = 1.946; 95% CI, 1.196–3.166; p = 0.007), history of peripheral vascular disorders (aOR = 2.137; 95% CI, 1.360–3.357; p = 0.001), history of coagulopathy (aOR = 1.652; 95% CI, 1.050–2.599; p = 0.030), and transfusion of platelets (aOR = 1.189; 95% CI, 1.063–1.330; p = 0.002) were all associated with *higher* 30-day mortality on logistic regression, while amount of RBC or FFP units transfused had no association with mortality. Full details of the multivariable logistic regression can be found in Table 3.

3.3 | Secondary outcome: Post-operative AKI

AKI occurred following 24% (n = 382) procedures involving large volume resuscitation. The mean ionised calcium in the group that did not

TABLE 3 Multivariable logistic regressions for secondary outcomes

A. 30-day mortality (c-statistic = 0.845)				
Variable		**aOR**	**95% CI**	***p* Value**
Nadir ionised calcium		0.787	0.258–2.398	0.674
Emergent surgery		1.946	1.196–3.166	0.007
Race	Unknown	3.480	2.126–5.696	<0.001
Procedural category	Trauma	4.272	1.861–9.805	0.001
	Other/radiologic	2.168	1.158–4.060	0.016
History of peripheral vascular disorders		2.137	1.360–3.357	0.001
History of liver disease		1.400	0.865–2.266	0.171
History of coagulopathy		1.652	1.050–2.599	0.030
History of fluid or electrolyte disorder		1.560	0.949–2.511	0.067
Case duration (min)		0.998	0.997–0.999	0.005
Vasopressin administered (4U)		1.101	0.912–1.329	0.317
Norepinephrine administered (8mcg)		1.002	1.000–1.004	0.033
Platelet transfusion (5-packs)		1.189	1.063–1.330	0.002
B. Postoperative acute kidney injury (c-statistic = 0.806)				
Variable		**aOR**	**95% CI**	***p* Value**
Nadir ionised calcium		0.733	0.286–1.877	0.518
Age (years)		1.012	1.001–1.023	0.028
Weight (kg)		1.011	1.004–1.018	0.003
Procedural category				
	Neurosurgery	0.201	0.097–0.415	<0.001
	Obstetrics/gynaecology/urology	1.722	1.0195–2.908	0.042
	Oral surgery/ENT/dentistry	0.509	0.234–1.108	0.089
	Orthopaedic surgery	0.207	0.097–0.442	<0.001
	Transplant	2.171	1.349–3.454	0.001
	Vascular surgery/plastics	1.685	1.077–2.634	0.022
History of coagulopathy		1.149	0.820–1.610	0.420
History of fluid or electrolyte disorder		1.836	1.324–2.545	<0.001
Preoperative creatinine (mg/dl)		0.569	0.440–0.735	<0.001
EBL at nadir (L)		1.105	0.905–1.221	0.389
Transfusion FFP at nadir (units)		1.029	0.941–1.125	0.532
Urine output at Nadir (500 ml)		0.000	0.000–0.399	0.033
Norepinephrine administered (8 mcg)		1.004	1.000–1.007	0.027
Phenylephrine administered (250 mcg)		1.009	1.011–1.017	0.018
EBL at case completion (L)		1.000	0.905–1.105	0.776
Transfusion FFP at case completion (units)		0.995	0.926–1.070	0.898
Transfusion pRBC at case completion (units)		1.018	0.958–1.081	0.565
Platelet transfusion at case completion (5-packs)		1.082	0.961–1.212	0.191
Cryoprecipitate transfusion at case completion (5-packs)		1.014	0.789–1.302	0.917
C. Postoperative coagulopathy (c-statistic = 0.784)				
Variable		**aOR**	**95% CI**	***p* Value**
Nadir ionised calcium		0.507	0.218–1.180	0.115
Weight (kg)		0.986	0.979–0.992	<0.001
Emergent surgery		1.317	0.941–1.844	0.108
Race	Unknown	1.625	1.125–2.347	0.010

TABLE 3 (Continued)

C. Postoperative coagulopathy (c-statistic = 0.784)				
Variable		**aOR**	**95% CI**	***p* Value**
Procedural category				
	Neurosurgery	0.401	0.257–0.623	<0.001
	Obstetrics/gynaecology/urology	0.620	0.374–1.026	0.063
	Orthopaedic surgery	0.620	0.387–0.992	0.046
	Transplant	2.305	1.342–3.959	0.003
	Vascular surgery/plastics	1.132	0.757–1.691	0.547
History of coagulopathy		1.568	1.153–2.133	0.004
History of fluid or electrolyte disorder		1.093	0.819–1.457	0.546
History of renal failure		1.542	1.082–2.199	0.017
History of liver disease		1.692	1.145–2.500	0.008
History of chronic pulmonary disease		1.416	1.023–1.961	0.036
Preoperative serum albumin (g/dl)		0.672	0.560–0.809	<0.001
Colloid resuscitation at Nadir (L)		1.856	1.471–2.340	<0.001
Phenylephrine administered at nadir (250 mcg)		1.009	1.002–1.016	0.011
Transfusion pRBC at case completion (units)		1.016	0.983–1.050	0.338
Final haematocrit (%)		0.989	0.952–1.026	0.551
Norepinephrine administered (8 mcg)		1.001	0.994–1.003	0.178
Time with MAP <55 mmHg (minutes)		1.008	1.002–1.014	0.007

Abbreviations: dl, decilitre; ENT, ear, nose, and throat (otolaryngology); FFP, fresh frozen plasma; L, litre; m, metre; MAP, mean arterial pressure; mcg, micrograms; mEq, milliequivalents; mmol, millimoles; pRBC, packed red blood cells.

develop postoperative AKI was 0.92 ± 0.17 compared with 0.93 ± 0.19 in the group that did develop an AKI. Nadir ionised calcium was not associated with post-operative AKI (aOR = 0.733; 95% CI, 0.286–1.877; $p = 0.518$), so could not serve as an intermediate variable for mediation analysis. Furthermore, none of our transfusion exposure variables were associated with post-operative AKI. Age (aOR = 1.012; 95% CI, 1.001–1.023; $p = 0.028$), weight (aOR = 1.011; 95% CI, 1.004–1.018; $p = 0.003$), history of fluid/electrolyte disorders (aOR = 1.836; 95% CI, 1.324–2.545; $p < 0.001$) were associated with higher incidence of post-operative AKI. Administration of norepinephrine (aOR = 1.004; 95% CI, 1.000–1.007; $p = 0.027$) and phenylephrine (aOR = 1.009; 95% CI, 1.011–1.017–1.018; $p = 0.018$) were also associated with higher rates of post-operative AKI. Full details of the multivariable logistic regression can be found in Table 3.

3.4 | Secondary outcome: Post-operative coagulopathy

Post-operative coagulopathy occurred following 32% ($n = 519$) procedures involving large volume resuscitation. The mean ionised calcium in the group that did not develop post-operative coagulopathy was 0.93 ± 0.18 and 0.91 ± 0.18 in the group that did develop coagulopathy. Nadir ionised calcium was not associated with post-operative coagulopathy (aOR = 0.507; 95% CI, 0.218–0.180; $p = 0.115$), so could not serve as an intermediate variable for mediation analysis. Furthermore, none of our transfusion exposure variables were associated with post-operative coagulopathy. Increasing weight (aOR = 0.986; 95% CI, 0.979–0.992; $p < 0.001$), increasing preoperative serum albumin (aOR = 0.672; 95% CI, 0.560–0.809; $p < 0.001$), neurosurgical (aOR = 0.401; 95% CI, 0.257–0.623; $p < 0.001$) and orthopaedic (aOR = 0.620; 95% CI, 0.387–0.992; $p = 0.046$) procedures were associated with lower rates of coagulopathy. Transplant surgeries (aOR = 2.305; 95% CI, 1.342–3.959; $p = 0.003$), history of renal failure (aOR = 1.542; 95% CI, 1.082–2.199; $p = 0.017$), history of liver disease (aOR = 1.692; 95% CI, 1.145–2.500; $p = 0.008$), and phenylephrine administration before nadir (250 mcg doses) (aOR = 1.009; 95% CI, 1.002–1.016; $p = 0.011$) were associated with *higher* rates of post-operative coagulopathy. Colloid resuscitation (Litres) (aOR = 1.856; 95% CI, 1.471–2.340; $p < 0.001$) and minutes with mean arterial pressure (MAP) < 55 mmHg (aOR = 1.008; 95% CI, 1.002–1.014; $p = 0.007$) were also associated with increased rates of coagulopathy. Full details of the multivariable logistic regression can be found in Table 3.

3.5 | Calcium repletion

We also determined the amount of elemental calcium (in mEq) per unit of packed red blood cells or fresh frozen plasma transfused in the cohort never developing hypocalcaemia compared with the cohort

developing severe hypocalcaemia (defined as nadir ionised calcium ≤0.80 mmol/L). We found 4.01 ± 2.76 mEq of calcium were administered per unit of citrate containing blood products in the group not developing hypocalcaemia compared with 2.90 ± 2.32 mEq per unit of citrate containing blood products in the group developing severe hypocalcaemia. We then assessed repletion strategy. The majority of providers repleted entirely with calcium gluconate ($n = 945$, 59%). Fourteen percent ($n = 222$) repleted exclusively with calcium chloride, 23% ($n = 378$) adopted a mixed repletion, and 4% ($n = 69$) had no intraoperative calcium repletion. Patients repleted with calcium chloride had higher nadir ionised calcium than those replete entirely with calcium gluconate (0.94 ± 0.22 compared with 0.92 + 0.16, $p < 0.001$), on univariate analysis; however, repletion strategy was not selected in the LASSO multivariate models. Ionised calcium had normalised (defined as ≥1.0 mmol/L) at case completion in 73% of cases and the mean calcium at case completion was 0.95 ± 0.18 mmol/L.

4 | DISCUSSION

We found the volume of packed red cells and volume of fresh frozen plasma are independently associated with intraoperative hypocalcaemia during large volume transfusion. We did not detect an association between intraoperative hypocalcaemia or intraoperative transfusion and post-operative clinical outcomes of 30-day mortality, AKI, or coagulopathy.

4.1 | Concordance with previous studies

Our primary findings that volume of blood products are associated with hypocalcaemia agree with a smaller, retrospective study of massive resuscitation in the trauma population.[6] Unlike the trauma population, we could not demonstrate any association between hypocalcaemia and mortality or coagulopathy. This difference could be caused by multiple mechanisms, including differences in baseline health between populations, a more controlled environment in the operating room, and improved calcium repletion processes. While differing from the trauma population, the lack of association between hypocalcaemia and clinical outcomes agrees with previous reports from the perioperative, non-trauma population.[7] Additionally, a patient's hepatic and renal function may decrease the metabolism of citrate, putting these patients at higher risk of hypocalcaemia following massive transfusion. Pre-existing liver disease and renal failure based upon prior *International Classification of Diseases* (ICD) diagnoses,[22] as well as, preoperative serum creatinine were included in our model (Table S1), but were ultimately not selected for inclusion within the final regression based upon the LASSO selection.

Our research suggests that despite improvements in the administration of blood products, specifically when compared with a prior study of intraoperative transfusion where calcium repletion was not performed as standard practice,[7] hypocalcaemia still occurs with high frequency following large volume transfusion in the operating room. Specifically, we noted severe hypocalcaemia (defined as nadir ionised calcium ≤0.80 mmol/L) occurred in 22% of cases and mild hypocalcaemia (defined as nadir ionised calcium ≤1.00 mmol/L) occurred in 70% of cases. Our inability to demonstrate an association between intraoperative hypocalcaemia and meaningful postoperative outcomes is hypothesis generating. Potential reasons may be (i) more frequent monitoring and aggressive resuscitation in the operating room, compared to the emergency department or the intensive care unit (ii) differences in aetiology of bleeding between surgery versus trauma, and (iii) more rapid, transient control of surgical bleeding. In fact, ionised calcium had normalised by case completion in 73% of cases and the mean calcium at case completion was 0.95 + 0.18.

Recommendations on the rate of calcium repletion in massive transfusion vary greatly and range from 2.28 to 4.56 mEq of calcium gluconate or 1.36–3.4 mEq of calcium chloride per unit of packed red blood cells.[23,24] Our results showed 4.01 ± 2.76 mEq of calcium were administered per unit of citrate containing blood products in the group not developing hypocalcaemia compared with 2.90 ± 2.32 mEq per unit of citrate containing blood products in the group developing hypocalcaemia. This suggests that perhaps clinicians should replete towards the upper limit of recommended, as the patients in the severe hypocalcaemia group received a mean dose of calcium that was still within the recommended range. As calcium chloride contains more elemental calcium and has greater bioavailability than calcium gluconate (13.6 mEq per 1000 mg of chloride compared to 4.56 mEq of gluconate), calcium chloride provides more rapid correction of hypocalcaemia; however, the greater toxicity to blood vessels makes it less desirable for prolonged administration.[5,25] Patients repleted with calcium chloride had higher nadir ionised calcium than those replete entirely with calcium gluconate (0.94 ± 0.22 compared with 0.92 + 0.16, $p < 0.001$) on univariate analysis; however, since this was not demonstrated on multivariable modelling, additional research is necessary on optimal repletion strategy in different surgical populations.

4.2 | Cohort definition

The classic definition for *massive transfusion*, ≥10 units packed red blood cells in a 24-h period, approximates total blood for an average adult patient.[26,27] Because of the potential for drastic changes in blood volume over a much shorter duration, this classic definition is not always generalizable to the surgical and trauma populations.[27] Newer metrics that account for both rate and timing have, therefore, been proposed.[26] Our inclusion criteria: transfusion with ≥4 units of packed red blood cells intraoperatively was selected to capture the largest cohort for analysis. Because this is notably different from the definition used in the trauma population: ≥3 units of packed red blood cells over a single hour,[12] we distinguish our population as a *large volume* intraoperative transfusion (instead of *massive* transfusion).

4.3 | Strengths and limitations of study methodology

Our study has multiple limitations. As a single-centre effort, our results may not be generalizable to other institutions or populations. Because the

study was done retrospectively, significant covariates may be associated, but we cannot speculate a causal relationship with our outcomes—limiting the influence on clinical practice. A notable strength of our study is that we account for the confounding effect of calcium administration through the intraoperative period (showing that every 10 mEq of calcium repletion increases nadir ionised calcium by 0.015 mmol/L (95% CI, 0.001–0.028; $p = 0.037$). Future studies will attempt to further understand changes in supplementation strategy and characterise successful versus inadequate repletion strategies.

5 | CONCLUSION

In patients requiring intraoperative transfusion with at least 4 units of packed red blood cells, we retrospectively observed that volume of packed red blood cells and volume of fresh frozen plasma are both associated with lower nadir of intraoperative ionised calcium. We failed to demonstrate that intraoperative hypocalcaemia or transfusion is associated with meaningful post-operative clinical outcomes including mortality, AKI, or coagulopathy. Our findings suggest that despite improved practice patterns of calcium supplementation,[7,28] intraoperative hypocalcaemia occurs with relatively high frequency following large volume transfusion. Our regression models also provide insight into populations with higher or lower risk for hypocalcaemia and optimal repletion strategies.

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CONFLICT OF INTEREST

Nicholas Douville, MD, PhD reports grant from Foundation for Anesthesia Education and Research (FAER) during the conduct of the study. Ryan Davis, MD, declares no conflicts of interest. Elizabeth Jewell, MS, declares no conflicts of interest. Douglas A. Colquhoun, MBChB, MSc, MPH, declares research funding paid to his Department from Merck Inc. Satya Krishna Ramachandran, MD, FRCA, is a scientific advisor to Fresenius Kabi USA. Milo C. Engoren, MD, declares no conflicts of interest. Paul Picton, MBChB, declares no conflicts of interest.

AUTHOR CONTRIBUTIONS

Nicholas J. Douville: Responsible for the conception and design of the work; the interpretation of data for the work; developing first and final drafts of the work; and the assimilation of intellectual content from all co-authors. **Ryan Davis:** Responsible for the acquisition and analysis of data for the work; interpretation of data for the work, and critically revising the work for important intellectual content. **Elizabeth Jewell:** Responsible for the acquisition and analysis of data for the work; interpretation of data for the work, and critically revising the work for important intellectual content. **Douglas A. Colquhoun:** Responsible for the interpretation of data for the work, and critically revising the work for important intellectual content. **Satya Krishna Ramachandran:** Responsible for the conception and design of the work; interpretation of data for the work, and critically revising the work for important intellectual content. **Milo C. Engoren:** Responsible for the conception and design of the work; the interpretation of data for the work; developing first and final drafts of the work; and the assimilation of intellectual content from all co-authors. **Paul Picton:** Responsible for the conception and design of the work; interpretation of data for the work, and critically revising the work for important intellectual content.

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SUPPORTING INFORMATION

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

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ORIGINAL ARTICLE

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Assessment the knowledge of blood transfusion in Iranian nurses of Tehran's hospitals

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Abstract

Objective: To investigate the knowledge of nurses about pre and post blood transfusion processes.

Background: To ensure a safe blood transfusion, apart from the role of blood banking to perform safe donation, attention must be paid to equally important but rather neglected factors including nursing practice and knowledge of blood transfusion.

Method and materials: Data was collected using a valid blood transfusion questionnaire consisting of 43 questions. We analysed data using SPSS 22. Percentages and analytical statistics such as Mann–Whitney, Kruskal-Wallis were used to report the results. The significant level of *p*-value was assumed to be <0.05.

Results: In this study, 325 nurses participated and their knowledge scores ranged from 24% to 85% (mean 56.16, standard deviation: 5.92) and the majority of nurses lacked knowledge in pre-transfusion activities. The analysis also revealed there was a significant correlation between the knowledge score and academic degree. Out of all nurses, 48% ($N = 156$) declared that they need further training in haemovigilance. As the minimum and maximum scored questions, it was revealed that only 39 nurses (12%) have enough knowledge to act properly in case of ambiguous orders; on the other hand, 94% ($N = 304$) have sufficient knowledge of the agents administered with transfusion. A large proportion of the involved nurses are unaware of the risk of improper identification.

Conclusion: All the efforts taken to prepare a safe and matched blood unit would be futile by inattentive administration of blood. That is why mandatory ongoing blood transfusion training for nurses is required urgently.

KEYWORDS
blood transfusion, knowledge, nursing assessment, questionnaire and surveys

1 | INTRODUCTION

Blood transfusion is one of the commonest treatments worldwide from the early 19th century to date, and each year millions of patients have been candidates to receive blood components. These could range from thalassemic patients to those who need massive blood in surgeries. With regard to World Health Organization report, in 90 various countries more than 9 million patients received blood components annually.[1,2] Despite remarkable improvements in blood transfusion procedure, there are considerable side effects and potent risk factors that could threaten patient health. Unfortunately, there is not any useful policy to evaluate the knowledge of involved staff to assure performing correct transfusion. According to the Serious Hazards of Transfusion (SHOT), inappropriate transfusion accounts for almost 70% of adverse outcomes.[2] Nurses play a crucial role in blood transfusion chain and thus having adequate knowledge and skill

is indispensable considering their direct involvement with patients. Therefore, measuring this knowledge has become a current trend between researchers. Misidentification accounts for the common morbidity rate during the transfusion procedure. Consequently, in order to perform a safe transfusion, evaluation of nurse's knowledge could be a useful approach to reduce the risk and decrease blood components wastage. A survey undertaken in the United Kingdom in 1993 and a similar one in the United States revealed that wrong identification and transfusion to the wrong patients account for the majority of fatality rate.[3] Qualified clinical staff include midwives and particularly nurses whose role is important in right and safe transfusion. Therefore, the expertise (or the lack thereof) of this staff could be the Achilles' heel in blood transfusion. Blood transfusion administration has five interwoven stages four of which directly related to nursing role including preparation before collecting blood units from the storage site, blood bag collection, pre-transfusion activities, post transfusion activities and monitoring patients.[4] To date, there have been only limited studies about the knowledge and expertise of the nurses performing blood transfusion. The majority of articles published in the Middle East point to the fact that the nurses suffer from insufficient knowledge in this regard.[2,4–7] Since there was no study preformed in this area in Tehran city, capital of Iran country it was deemed that a study must be undertaken to evaluate the knowledge of nursing staffs about blood transfusion so we can make sure the least people will be affected by low-level nurses' knowledge. The present research has been carried out on university based hospitals affiliated to Shahid Beheshti University of Medical Sciences.

2 | METHODS

2.1 | Design

This was a descriptive study carried out by field-related interviewers (the first and second authors) using the valid questionnaire. Our target participants are nurses who worked and are involved in the administration of blood transfusion in eight wards including intensive care unit, emergency, oncology, orthopaedics, surgeries, internal medicine, urology and paediatrics in 13 university affiliated training public hospitals.

The study subjects included about 2000 registered nurses with experience at in-patient areas and with at least 5 months of work experience. The required sample size is 325 nurses (Setting $\alpha = 0.05$, confidence level = 95%, and bound on error = 5%) which was performed using simple random sampling by SPSS software version 19 (United States) (www.raosoft.com/samplesize.html).

2.2 | Measurement tool

We used a pre-made and used questionnaire by Mr Hijji who had modified and developed this Routine Blood Transfusion Knowledge Questionnaire (RBTKQ) earlier (for which we have his kind permission).[6,7] The questionnaire was then supervised and confirmed by the elite scientists in this field based on Iranian Blood Transfusion Organization Standards. The questionnaire includes seven sections with 43 questions related to blood transfusion knowledge. All nurses accepted to complete the questionnaire. There are three yes/no questions concerning hospital policy and the importance of regulating transfusion rate, whilst the rest of the questions are designed as multiple choices. Each correct answer is given one score and there were no negative scores for questions left unanswered. The total score was determined to be 55 for those who had experience in both adults and infant's units and the figure was 54 for the nurses with the working experience just in one. Furthermore, there are two correct answers to the question 9 in section E which concerns the agents administered with transfusion (b. normal saline 0.9% and d. morphine 1 mg/ml in NS). However, since in accordance with Iranian Blood Transfusion Organization policy doctors should avoid direct morphine compound injections with transfusion, in this questionnaire we limited the correct answer to only one choice (normal saline 0.9%) rather than two.

The current version of RBTKQ included 32 knowledge multiple-choice questions (2 true-false; 20 multiple-choice; 10 multiple-response). Demographics and training information are asked in section A and other sections (B–G) are listed as follows respectively: knowledge aspects of blood bag collection from blood bank and patient preparation prior to it, pre-transfusion initiation nursing responsibilities, post transfusion initiation nursing responsibilities, complications related to blood transfusion (33 items) and the issues related to hospitals' blood transfusion policies and procedures.[7]

2.3 | Validation and pilot study

Readability, validity and content clarity of the questionnaire were evaluated as a pilot study by 50 readers consisting of qualified registered nurses, post-graduate students, and the relevant specialists. They all indicated that content is understandable and readable, and that it had a content validity index of 90% which is well within the acceptable range. All these 50 questionnaires filled out as the pilot were not counted in the main final results. In order to check the internal consistency, we obtained an excellent Cronbach's alpha which was 0.91. We also checked its readability using the Flesch Reading Ease Index (yielding an index of ~68). All modifications were applied after the pilot study.

2.4 | Data collection

After obtaining permission from Director of Nurses in each hospital, two qualified haematology and blood banking students collected the questionnaire data. Besides, the questionnaire was completed by volunteer nurses during 30–60 min in presence of the research assistant. In order to keep anonymity, the involved nurses' names were not asked and the data were collected in 2017–2018.

2.5 | Data analysis

The total score in this questionnaire was 55 points and each correct response gains one point. Since the population did not have normal distribution, non-parametric analyses were carried out. By the time the questionnaires were completed, the data were entered onto SPSS software (United States) and then the descriptive analyses were carried out. For each section, the mean knowledge score was calculated and the overall knowledge score was reported. The chi-squared test was used in order to check the relation between nurses' characteristics (such as age, number of transfusions in year, experience, education) and the knowledge mean score. Statistical significance was set at $p < 0.05$.

2.6 | Ethical considerations

An official permission from the responsible authorities in Shahid Beheshti University of Medical Science by the Ethics Approval Code no: IR.SBMU.RETECH.REC.1395.1034 was obtained to legally start the study. Each nurse participated voluntarily in the study. Anonymity and confidentiality were all guaranteed.

3 | FINDINGS AND DISCUSSION

3.1 | Participants' characteristics and trainings

The number of nurses who answered the questionnaire was 325, out of which 251 (77%) were female. Most of the nurses were in the age group of 20–29 ($n = 190$, 58%). Out of the total number of the participants, 58% (188 nurses) had clinical experience between one to five years; besides, there were 89 nurses (27%) with more than five years of experience. Of the studied nurses, nearly 50% ($n = 153$) acknowledged that they had in-service training on blood transfusion, whereas the rest of them ($n = 170$) had never have such trainings and 156 and 148 (48% and 46%) of them expressed a strong need for training in haemovigilance and adverse reactions, respectively.

3.2 | Overall knowledge

The obtained scores on nurses' knowledge were scaled to 100%; the results showed that the scores of nurses ranged from 24% to 85% (mean 56.16, standard deviation: 5.92) indicating that no one had correctly answered all the questions. Based on previous studies we categorised knowledge scores in three groups where lower than 30% (<30%) indicates poor ($N = 10$, 3%), from thirty to sixty-five (30%–65%) indicates moderate ($N = 262$, 81%) and more than 65% (>65%) indicates good knowledge ($N = 53$, 16%).[4] According to this category the majority of nurses (81%) have been located in the average group.

In comparison with similar conducted studies in the Middle East, all the nurses have an average and insufficient knowledge score. Our results were congruent with other studies including one on 117 nurses in medical training hospitals of Shahrekord University of Medical Sciences in 2004[5] which reported only 51.6% of nurses had good knowledge score.[5] The other conducted by Mr Hijii in Abu Dhabi Emirates (2011) and Jordan (2009) also reported the mean knowledge scores being 40.8 and 51.8, respectively[6,7] and finally, one study performed in Morocco in 2014 on 42 nurses showed the same insufficiency in the overall knowledge of nurses.[8] In addition, in Egypt a survey on 286 nurses showed 61.2 as the mean knowledge score which is higher than other Middle East countries.[2] Furthermore, results obtained from another survey conducted in turkey on 100 health care staffs which 71% of them were nurses, revealed that the average knowledge is adequate. Also they reported that the knowledge of health care professionals was higher than the mean.[9] In another hand, there were reports from Qazvin University of Medicals Science which showed that the average knowledge of 124 nurses calculated to be as medium level.[10]

3.3 | Correlations

3.3.1 | Work experience

In order to measure the strength and correlation that exist between variables, non-parametric tests were performed. The spearman correlation analysis revealed that there is a significant and positive correlation between nurse's work experience and the obtained scores ($p = 0.000$, rs $= 0.229$). Also, Kruskal-Wallis H test confirmed that there was a statistically significant difference in work experience between the different knowledge groups, χ^2 (2) $= 14.458$, $p = 0.001$, with a mean rank work experience of 129.41 for poor, 149.07 for moderate and 197.83 for good. Based on the results and the personal communication of nurses, the more the work experience in blood transfusion, the more the knowledge in that field that is most likely through learning from experienced reactions and interactions between colleagues. But the point is 'what's more important: qualifications or experiences?'.

Generally, learning through academic courses brings about deeper and better understanding whilst the experience only teaches you what happens in practice. Consequently, due to the vital role of blood transfusion even making a simple mistake is inexcusable. SHOT program also provided a checklist which should be completed before transfusion is indicated. However, the errors are inevitable even with years of experience and seniority.[3]

3.3.2 | Degree and transfusion attempts

Mann–Whitney test also was performed to find the possible correlation between the academic degree with the knowledge score. Results illustrated that nurses who have master degree ($N = 59$, mean rank $= 195.46$) tend to have more knowledge score in comparison with the nurses with bachelor degree ($N = 255$, mean rank $= 148.72$)

($p = 0.000$, Mann–Whitney $U = 5283.000$). Moreover, as shown in Table 1 and estimated by Kruskal-Wallis test, the majority of nurses who had administered more than 12 times ($N = 111$, Mean rank = 161.45) of transfusion attempts within the last 6 months showed no higher mean rank than the other nurses with lower experience. The finding illustrated that there was no significant correlation between the number of experiences in administering transfusion and the obtained knowledge score ($p = 0.203$, Kruskal-Wallis = 5.952). The results stressed that academic knowledge is really more important than experience.

3.4 | Issues relating to patient preparation

This section stresses the proper timing for blood collection, availability of intravenous access line and, the appropriate times for vital sign recording. Table 2 shows that nurses lacked the awareness about incomplete medical orders with only 12% ($N = 39$) of nurses refusing to collect and authorise the blood; most likely because of the low knowledge level of nurses and their busy workload. Consequently, this could result in the increasing fatality rate of blood transfusion. On the contrary, about the aspects of information given to patients and baseline vital signs recording, the nurses had enough knowledge. About checking patency of IV after blood bag collection, the results showed a really low knowledge that might be because of hospital crowding and lack of time for nurses which could lead to increase in the holding time of blood units in ward and may raise the bacterial infection potential.

Sufficient knowledge in patient preparation field can prevent the occurrence of complications and blood transfusion reactions. The mean score for this section was 55.78% which is insufficient and as we know, this score illustrates the importance of patient preparation to be neglected most likely due to shortage of personnel in the departments and the hospital crowding. Similar studies including ones conducted by Hamed Abd Elhy et al.[2] and Tetteh[11] showed that nurses' knowledge was fair enough in patient preparation.[11]

3.5 | Blood bag collection

In this section which is about transporting blood units, we have got the highest mean score (83.48%) of all the other sections of the questionnaire.

Table 3 shows that almost all nurses (92%, $n = 299$) would transport blood bags with validated special boxes. About information to ensure collecting the right blood from the blood bank, 84% of nurses ($n = 274$) would check the identification details which are identical on the blood bag and blood request form. In the case of receiving A− blood bag from blood bank for A+ patient, 241 nurses (74%) would check with the physician and obey their orders. Since the earliest blood transfusions, it has always been a concern to transfuse compatible blood type to the recipient. With the advancement in blood typing, it is expected to have only few fatalities caused by incompatible transfusion. Yet, according to global reports human error is still a considerable factor in incorrect transfusion. This is why it is crucial for all hospitals to have very strict policies. Although the nurses in this study have shown a sufficient level of ABO-terminology knowledge, there needs to be more assessment.

TABLE 1 Correlation of categorised knowledge scores with demographic data

	Total knowledge			
Variable	**Poor ($N = 17$)**	**Moderate ($N = 254$)**	**Good ($N = 54$)**	**Test result**
Sex				0.071
Female	17 (6.8%)	139 (76.9%)	41 (16.3%)	
Male	0 (0%)	61 (82.4%)	13 (17.6%)	
				0.246
Age, median, range	28 (26–41)	28 (22–45)	29 (23–41)	
Score, median, range	15 (13–19)	15 (13–19)	30 (21–35)	0.000
Work experience, median, range	2.35 (1–6)	3 (0.1–24)	4 (0.9–12)	0.001
Degree				$X^2(2) = 5.878$ $p = 0.05$
B.S.	13 (5.1%)	204 (80%)	38 (14.9%)	
MSC	1 (1.7%)	42 (71.2%)	16 (27.1%)	
Transfusion				$X^2(8) = 16.889$ $p = 0.031$
0	2 (11.8%)	20 (8.7%)	0 (0%)	
1 to 4	9 (52.9%)	54 (25.6%)	10 (31.5%)	
5 to 8	0 (0%)	43 (16.5%)	4 (24.1%)	
9 to 12	0 (0%)	25 (14.2%)	6 (14.8%)	
More than 12	6 (35.3%)	64 (35%)	15 (29.6%)	

TABLE 2 Issues relating to patient preparation

Section B	Question	Correct answer	Number of correct answers	Percentage of correct answers
1	Checking patency of IV after blood bag collection	F	96	30%
2	Collecting blood bag from blood bank should take place before the administration of any prescribed pre-medication	F	182	56%
3	Decisions to be taken by the nurse with incomplete order	Refuse to collect and administer blood	39	12%
4	Three aspects of information given to patient	Reasons for blood	272	84%
		Transfusion risk of blood transfusion	149	46%
		Reaction symptoms	246	76%
5	Baseline vital signs recording	Within ½ h before transfusion	285	88%

TABLE 3 Blood bag collection

Section C	Question	Correct answer	Number of correct answers	Percentage of correct answers
1	Information to ensure collecting the right blood from blood bank	Patient's identification details are identical on the blood bag and blood request form	274	84%
2	Blood bag transport method	Validated special box	299	92%
3	Receiving A− blood bag from blood bank for A+ patient	Check with the physician and obey their orders	241	74%

TABLE 4 Pre-transfusion initiation nursing activities

Section D	Question	Correct answer	Number of correct answers	Percentage of correct answers
1	Most important nursing action before starting the transfusion	Patient identification	**177**	**54%**
2	Clinical indications for blood warming	Exchange transfusion for infant	**213**	**66%**
		Rapid transfusion	**159**	**49%**
		Patient with cold agglutinins	**106**	**33%**
3	Best time to start the transfusion if delivered to the ward at 4 PM	4:10 PM	**109**	**34%**
4	Blood handling after delivery to ward	Start immediately	**68**	**21%**
5	Steps for patient identification	Ask patient to state name and date of birth	**274**	**85%**
		Patient's identification details are identical on ID band	**207**	**65%**
		Blood request form	**182**	**56%**
6	Suitable filter size of transfusion set	170–200 micron	**96**	**30%**
7	Omitting the final bedside identity check	Never acceptable	252	78%

Note: Bold values indicate the nurses' activities before starting the blood components transfusion.

The promising results in this section may indicate the existence of strict rules and standards in hospital blood banks.

Based on similar studies conducted in Menoufia University Hospital, they also reported a good situation on blood bag collection and it is not in the same line with Hijji which reported that the majority of their targeted nurses lacked knowledge with basic ABO terminology.[2,7]

3.6 | Pre-transfusion initiation nursing activities

This part is about proper patient identification, documentation, use of warm blood, and determination of the right time to start the transfusion.

As shown in Table 4, only 68 nurses (21%) would start transfusion immediately after blood is delivered to the ward. In the clinical

TABLE 5 Post transfusion initiation nursing activities and issues

Section E	Question	Correct answer	Number of correct answers	Percentage of correct answers
1	Three activities for nurses to perform routinely after starting the blood transfusion	Setting up the flow rate	218	67%
		Documentation of relevant information	261	80%
		Observation for transfusion reaction	199	61%
2	The rate to initiate a transfusion on an adult patient	Not more than 120 ml/h	78	27%
3	Regulation of blood transfusion is important	Yes	320	98%
	Regulation of transfusion flow rate	Manual	226	69%
		Via electronic pump	94	28%
4	Maximum duration of using a blood administration set for continuous multiple transfusions	4 h	243	75%
5	The rate to initiate a transfusion at on an infant	Not more than 0.5 ml/kg/h	52	28%
6	Maximum duration for completing a unit of blood.	4 h	249	77%
7	Indications for slow blood transfusion	Patients with heart disease	279	86%
		severe anaemia	94	29%
8	Agents compatible with blood	Normal saline 0.9%	304	94%
9	Vital signs recording after starting a transfusion at 2:00 PM	2:05 and 2:15	280	86%
		3:15	167	51%
		4:15	107	33%
		5:00	72	22%
10	Timing and duration when it is essential to physically observe a patient for possible transfusion reaction	First 10–15 min	151	46%

Note: Bold values indicate clinical complications/complains after starting blood components transfusion.

indications for blood warming only 106 nurses (33%) are aware of patients with cold agglutinins. A high percentage of nurses ask patients to state their date of birth ($n = 274$, 85%) and then also check for identical details on ID bands for proper identification.

Section D has got 51.58% as the mean knowledge score and this almost moderate percentage can lead to many transfusion complications such as acute haemolytic transfusion reactions and microbial infections. There is a crisis about 'blood handling after delivery to ward' because most of the nurses think they should wait for half an hour and then start the transfusion but in fact, this is a common mistake because in principle there is a maximum of half an hour to onset the transfusion. Another remarkable issue is about suitable filter size of transfusion set about which most of nurses do not exactly know and can definitely be due to lack of knowledge in this area.

We had the lowest score for this section of questionnaire and in comparison with other undertaken studies namely Hamed Abd Elhy et al.[2] The majority of participants would act inappropriately regarding pre transfusion responsibilities and as we said earlier this irresponsibility accounts for the high fatality rate. Moreover, Hijji also reported a skimped knowledge base in Jordanian nurses about pre-transfusion initiation nursing activities.[7]

3.7 | Post transfusion initiation nursing activities and issues

Section E is about setting a convenient flow rate, proper duration of transfusion, simultaneous use of drug/solutions with blood and surveillance over the patient for plausible transfusion reactions.

Almost all the nurses are aware of the importance of the regulation of the flow rate of blood transfusion but only 27 percent ($N = 78$) and 28 percent ($N = 52$) of nurses are aware of the suitable rate to initiate a transfusion respectively on adult and infant patients. Seventy-five percent ($N = 243$) acknowledged that maximum duration for completing a unit of blood is 4 h. Concerning the importance of using warm blood to avoid serious side effects such as ischaemia 86% and 29% ($N = 279$, $N = 94$) of nurses respectively know that setting the slow transfusion rate is necessary for patients with heart disease and severe anaemia.

Section E has got 59.11% as the mean knowledge score.it is almost fair or moderate level of knowledge but a poor awareness about blood administration rate can definitely be due to lack of awareness and can leads to blood transfusion complications for example in patient with transfusion-associated circulatory overload which setting a slower administration rate can be helpful.[12]

TABLE 6 Complications related to blood transfusion

Section F	Question	Correct answer	Number of correct answers	Percentage of correct answers
1	Nursing interventions that could minimise the risk of developing transfusion reaction	Administering compatible blood	**266**	**82%**
		Starting transfusion within 20 min	**102**	**31%**
		Total duration of administration 4 h	**67**	**21%**
		Avoid incompatible drugs/solutions	**179**	**55%**
2	Signs and symptoms of acute haemolytic reaction	Tachycardia	**272**	**84%**
		Chest pain	**234**	**72%**
		Hypotension	**122**	**38%**
		Nausea/vomiting	**197**	**61%**
3	Nursing management of AHTR	Stop blood transfusion	**314**	**97%**
		KVO with N/S	**107**	**33%**
		Check V/S	**274**	**84%**
		Notify the doctor and begin emergency treatment	**253**	**78%**
4	A unit of blood was kept in nurses' station for 90 min without starting the transfusion, what should the nurse do?	Not to start the transfusion, notify the blood bank and return the blood	185	57%
5	The usual presenting complaint of a mild allergic transfusion reaction	Urticarial rash	239	74%
6	The first action the nurse should take with mild allergic transfusion reaction	Slow the transfusion rate and notify the doctor	72	22%
7	The commonest cause of fatal transfusion reaction	Identification error of patient	89	27%
8	Complication of rapid transfusion of cold blood	Cardiac arrhythmia	**166**	**51%**

Abbreviations: AHTR, acute hemolytic transfusion reaction; KVO, keep the vein open.

TABLE 7 Issues related to blood transfusion policies and procedures

Section G	Question	Correct answer	Number of correct answers	Percent of correct answers
1	Availability of a written policy for the administration of blood.	Yes	267	82%
		No	30	9%
		I do not know	22	7%
2	If yes, have you read the policy?	Yes	255	95%
		No	52	16%

Our findings were aligned with Hamed Abd Elhy, Hijji and Khalil[2,7,13] which all revealed theirs finding scores were generally inadequate (Table 5).

3.8 | Complications related to blood transfusion

Section F is about sign and symptoms and actions to be taken when acute hemolytic transfusion reaction (AHTR) and allergic transfusion reactions happen.

Administering compatible blood, starting transfusion within 20 min, administering blood during 4 h and avoiding incompatible drugs/solutions are four things that are less likely to lead to a transfusion reaction with timely intervention by nurses with the scores for these four being 82%, 31%, 21% and 55% ($N = 266$, $N = 102$, $N = 67$ and $N = 179$), respectively. About the sign and symptoms of a haemolytic reaction, 84% ($N = 272$) knows that tachycardia is one of the signs, that chest pain, hypotension and nausea/vomiting are the other important symptoms about which the awareness of nurses was 72% ($N = 234$), 38% ($N = 122$) and 61% ($N = 197$), respectively. Only 27% ($N = 89$) believe that the error of patient identification is the commonest cause of fatal transfusion reaction.

Here we got 56.81% as the mean score for this important section. Lack of knowledge in this area can be very disastrous, so continuous

education about the effects of blood transfusion should be given to nurses (Table 6).

3.9 | Issues related to blood transfusion policies and procedures

As we all know presence of a written policy is necessary in the wards of hospitals (http://www.southend.nhs.uk/media/64178/administration_of_blood_and_blood_components.pdf). In this questionnaire the availability of a written policy for the administration of blood was asked and 82% ($N = 267$) said yes out of whom 95% declared that they have read the policy. Nine percent ($N = 30$) of nurses said they have not seen any (Table 7).

4 | CONCLUSION

Annually extensive efforts have been taken in order to collect and produce blood components, encourage people to donate their blood and of course to screen them to obtain a safe donation. Consequently, the role of nurses in this chain is really crucial to reach the optimum efficiency with regard to patient treatment and to reduce component wastage. All the conducted surveys illustrated that the majority of nurses have suffered from insufficient knowledge concerning blood transfusion in all aspects. The main reason for this knowledge deficit is the lack of such factors as supervision policy for nurses and course units on blood transfusion in their curricula. There has also been a lack of a system for regular observations of the nurses to keep them abreast with the latest developments. In comparison with other similar studies on nurses' knowledge level, our target population knowledge was considered to be between the poor and average score border. However, with respect to the vital role of safe and proper transfusion in patient treatment, this knowledge score is inadequate and a post-qualification training is highly recommended to improve nurses' knowledge and skill.

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AUTHOR CONTRIBUTIONS

Amir Yami and Arezoo Darbandi: analysis. **Esmaeil Saber:** collecting data. **Mehdi Tabrizi:** some proofing and addressing a part of questionnaire. **Ahmad Gharehbaghian:** main analytical and discussion issues.

CONFLICT OF INTEREST

The authors have no competing interests.

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ORIGINAL ARTICLE

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Alloimmunisation rate of patients on Daratumumab: A retrospective cohort study of patients in England

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Abstract

Objective: Whilst small-scale studies on rates of alloimmunisation of patients on Daratumumab have been undertaken, no large-scale study has been performed to date on this cohort of patients.

Background: Patients with multiple myeloma (MM) who are relapsed or refractory to standard treatment are treated with the anti-CD38 therapeutic monoclonal antibody, Daratumumab. Due to the complexity of pre-transfusion compatibility testing, many MM patients in England are referred to Red Cell Immunohaematology (RCI) laboratories for investigation and provision of Red Blood Cell (RBC) components.

Methods: Over a 4-month period, patients due to commence, or currently on anti-CD38 therapy were identified and flagged on the RCI Laboratory Information Management System (LIMS). Data was identified and extracted for further analysis. Interrogation of data was performed independently by two subject matter experts, with discrepancies resolved through further enquiry.

Results: Of 734 English MM patients, we report an alloimmunisation rate of 0.4% whilst on an anti-CD38 TMAb. This is in line with other smaller cohort studies.

Conclusion: Given the low rate of RBC alloimmunisation, consideration should be given to revising the pre-transfusion testing regimen in this cohort. This may improve testing costs, turn-around times and evidence-based patient care.

KEYWORDS
alloimmunisation, CD38, compatibility testing, daratumumab, immunohaematology, multiple myeloma, therapeutic monoclonal antibody, transfusion

1 | INTRODUCTION

Patients in England with multiple myeloma (MM) who are relapsed or refractory to standard treatment are treated with the anti-CD38 therapeutic monoclonal IgG antibody, Daratumumab (also known as DARA or Darzalex®). Daratumumab is used either as a monotherapy, or more commonly in combination with other drugs such as bortezomib (Velcade) and dexamethasone (DVD combination therapy) through the cancer drugs fund.[1,2,3] It is thought that DVD combination therapy could benefit up to 2900 myeloma patients in England who fail to respond to first line treatments. Daratumumab is a Therapeutic Monoclonal Antibody (TMAb), which is specific for the CD38 antigen. CD38 is found in increased quantities on the surface of plasma cells (the primary subset of cells responsible for MM).[4] Once bound to Daratumumab, the plasma cells are selectively removed by the patient's immune system, leading to tumour cell death via immune-mediated apoptosis pathways.

Daratumumab also binds to weakly expressed RBC-bound CD38 antigen, and as a consequence causes pan-reactivity in pre-transfusion compatibility testing, affecting antibody identification (ABID), phenotyping, Direct Antiglobulin Testing (DAT) and cross-matching (XM). All of which can prolong serological investigations and delay provision of blood.[5]

A 2017 addendum to the British Society of Haematology (BSH) Pre-Transfusion Compatibility Procedures in Blood Transfusion Laboratories describes a model laboratory investigation protocol for patients who are being treated with targeted therapeutic monoclonal antibodies (TMAb)[6]. The protocol requires patients who are due to undertake a course of anti-CD38 TMAb treatment to be tested as follows:

- ABO and D group
- Antibody screen/identification
- DAT
- Extended phenotype or genotype (C, c, E, e, K, (k if K+), MNSs, Jk^a, Jk^b, Fy^a and Fy^b)

Once the patient has commenced anti-CD38 therapy, the protocol then makes the following recommendations regarding pre-transfusion compatibility testing and provision of blood components, if required:

- ABO and D typing as per normal method.
- Antibody screening, and antibody identification if required, using a strategy to avoid the effect of anti-CD38, for example, reagent cells treated with 0.2 M Dithiothreitol (DTT). Other strategies and techniques for overcoming the effect of anti-CD38 on red cells may become available in the future.
- Red cells should be matched for Rh and K as well as for any alloantibodies.

The overall aim of the protocol is to aid in the timely investigation and provision of blood components, and to mitigate against alloimmunisation in these patients. In addition, before beginning treatment with a drug which is known to interfere with pre-transfusion testing, it is helpful to establish phenotype and antibody status because it will be difficult to do so once treatment has begun. Performing this testing provides information and assurance when selecting RBCs for transfusion. The outcomes of this pre-therapy compatibility testing protocol should be documented in the transfusion laboratory, in the patient's notes and ideally on a patient-held shared-care record. In practice, however, this does not always occur, with lapses and failures in communication a common theme in transfusion-related incidents.[7]

Alternative mitigation strategies to remove pan-reactivity in pre-transfusion compatibility testing include the use of the reducing agent 0.2 M Dithiolthreitol (DTT)[8] which denatures the CD38 antigen, removing pan-reactivity and allowing the identification of underlying alloantibodies. However, treatment of reagent Red Blood Cells (RBCs) with 0.2 M DTT also removes antigens in the Kell, Lutheran, YT, JMH, LW, Cromer, Indian, Dombrock and Knops blood group systems.[9] This prevents recognition by corresponding alloantibodies, where present. Other mitigation strategies include the use of trypsin treated RBCs,[10] chloroquine diphosphate, soluble recombinant proteins and FAB fragments.[11] In addition, the use of cells that express reduced levels of CD38 antigen has been described; including cord cells or Lu(a−b−) cells of the dominant In(LU) phenotype.[12] However, most of these investigative strategies are undertaken in reference laboratories due to the expense and availability of many of the reagents involved. Due to the complexity of pre-transfusion compatibility testing, many of the MM patients in England who are on DARA are referred to the specialist NHS Blood & Transplant (NHSBT) Red Cell Immunohaematology (RCI) laboratories for investigation and provision of Red Blood Cell (RBC) components for transfusion.

Studies in the available literature report favourable outcomes to transfusion in this cohort of patients where ABO/RH/K matched or extended phenotype matched units have been given, with very few transfusion reactions reported and instances of alloimmunisation reported to be low, once daratumumab therapy has commenced. Published rates of alloimmunisation are between 0% and 3% in this cohort of patients[13–20] (see Table I) and studies report a variety of transfusion approaches, including providing RBCs matched for ABO/Rh/K antigens, which is congruent with current BSH guidance, or routine provision of extended matched RBCs to this cohort of patients.

However, a limitation of published studies to date has been the size of the cohorts studied, and data obtained may not reflect the true rate of alloimmunisation. Additionally, the use of extended phenotype matched blood in these studies is not reflective of current BSH guidance,[6] and therefore, does not reflect alloimmunisation rates under current practice. Routine extended matching for all antigens is not achievable with most hospital blood bank stock and may deplete blood service extended phenotype matched stock for other transfusion dependent patients who are at a greater risk of alloimmunisation (e.g., those with sickle cell anaemia).[21]

NHSBT RCI laboratories routinely perform investigations on patients undergoing anti-CD38 TMAb treatment. Therefore, NHSBT is in a unique position to analyse this cohort's data. RCI laboratories have seen an increase in requests for the investigation of patients undergoing anti-CD38 TMAb therapy. However, it is not yet known exactly how many of these patients RCI laboratories are investigating and how many units of blood are being issued, nor the frequency of transfusion and importantly, the frequency of alloimmunisation in this cohort. In order to answer these questions, the authors undertook a retrospective cohort study over a 4-month period in 2019.

2 | METHODOLOGY

Over a 4-month period in 2019 (June–Sept), patients who were referred to RCI laboratories in England were identified either on the RCI test request form by the referring hospital laboratory as due to commence or currently on anti-CD38 therapy, alternatively, they were identified by RCI through the result of serological investigation and further enquiry. Once identified, the patients were flagged on the RCI Laboratory Information Management System (LIMS) as being on an anti-CD38 TMAb. At the end of the 4-month data collection period, the cohort of patients was identified using a Business Objects (BObs—business intelligence software) search to enable the extraction of the discrete data set, using the CD38 LIMS flag.

TABLE I Published rates of alloimmunisation in cohorts of MM patients receiving treatment with anti-CD38 therapeutic monoclonal antibodies

Study	Number of patients transfused	Alloantibodies detected pre-therapy	Underlying rate of alloimmunisation before anti-CD38 therapy commenced (%)	New alloantibodies detected post-therapy	Rate of alloimmunisation once anti-CD38 therapy commenced (%)	Extended phenotype matched blood Y/N
Chari et al.[14]	14	anti-D, anti-E, anti-K, anti-Jk^b, anti-Fy^a, anti-Fy^b, anti-S and anti-Knops	14% ($n = 2$)	None	0%	Y
Deneys et al.[15]	11	None	0%	None	0%	Y
Bub et al.[16]	5	None	0%	None	0%	Y
Ye et al.[17]	45	None	0%	None	0%	Y[a]
Solves et al.[18]	44	None	0%	None	0%	N—ABO/Rh/K matched only
Anani et al.[19]	62	Anti-Jk^a	1% ($n = 1$)	None	0%	
Cushing et al.[20]	91	Alloantibodies/ Autoantibodies—Specificities not given	26.4% ($n = 24$) Alloantibodies 6 (6.6%) Warm autoantibodies 12 (13.2%) Cold autoantibodies 9 (9.9%) Nonspecific reactivity 5 (5.5%)	Anti-C anti-S and anti-Co^b	3% ($n = 3$)	N—ABO/K matched only
Carreño-Tarragona et al.[21]	33	anti-D, anti-C, anti-E and anti-c	9% ($n = 3$)	None	0%	Y

[a]ABO-Rh compatible and ABO compatible plus phenotypically matched RBcs were given. Ratios of each not stated.

Data extracted was collated onto an Excel spreadsheet for further analysis. Interrogation of data was performed independently by two subject matter experts, with discrepancies solved through further discussion and enquiry. Crossmatch requests received by RCI over the 4-month period were analysed to establish the average number of units requested and subsequently provided by RCI to capture patients who had received repeated transfusions over an extended period, for example, at least 3 months. Patient samples received during the audit period who were identified as being alloimmunised once anti-CD38 therapy had begun had their transfusion history examined and followed up with the referring hospital to establish potential sources of alloimmunisation. Other patient data, such as samples received, and number of crossmatch requests was analysed from the 4-month study period only.

3 | RESULTS

Over the 4-month course of the study period, samples were received by RCI labs in England from a total of 734 patients who were flagged on the LIMS as being on Daratumumab. The number of Males was 418 (57%); Females was 296 (41%); sex unknown, 20 (2%). The average age of patients seen was 68 years (68y Males; 69y Females). The range of ages seen was 9–91 years (9–88 years males; 12–91 years females).

The total number of samples received for these patients over the 4-month study period was 1629. The average number of samples received per patient was 2.2 (range 1–13), with some regional variation in sample referral frequency observed.

A total of 46% (341/734) of patient referrals were accompanied by a request for crossmatched units. Over the 4-month study period, the average of units requested and subsequently provided by RCI per patient was 2.8 (range 1–13) although it is not known if all units issued were subsequently transfused.

The majority of patients had an extended phenotype or genotype performed. This comprised of the following; an extended phenotype (58%, $n = 426$); extended genotype (32%, $n = 234$) or neither (10%, $n = 74$). Patients who had no extended typing by RCI labs may indicate testing is being performed by some hospital transfusion laboratories, or alternative local transfusion strategies (limiting to ABO, Rh/K matching) are being employed.

Antibodies to red cell antigens were detected in a total of 4% (30/734) patients seen over the course of the study period. Antibodies to both self antigen (autoantibodies) and non-self antigen

(alloantibodies) were detected. Of the 30 patients in whom RBC antibodies were detected, antibodies where a specificity could not be determined (SNDT) were excluded ($n = 4$), leaving a total of 3% (26/734) of patients on anti-CD38 TMAb therapy who had an identifiable specific underlying allo or autoantibody(ies). Patients were shown to have developed single and multiple specificities of RBC antibodies (see Figure 1).

The detected antibodies can be divided into two categories; pre-existing (before commencement of Dara) or newly formed (detected after the initiation of Dara treatment). In 80% (21/26) of patients with an underlying antibody, the antibody detected was pre-existing in samples received prior to the start of anti-CD38 treatment. The five remaining patients developed newly-formed antibodies whilst on anti-CD38 therapy. The specificities of these identifiable antibodies were as follows:

- 1 x allo anti-D
- 1 x allo anti-E
- 1 x allo anti-Fya
- 1 x auto anti-C (detected in eluate only)
- 1 x auto anti-M (detected in eluate only)

Autoantibodies were then excluded from the dataset, as in the absence of haemolysis, RBC are not selected to take into account autoantibody specificity, as prevention of the development of alloantibodies is usually of more importance.[6]

Therefore, the rate of RBC alloimmunisation for patients whilst on anti-CD38 therapy is 0.4% (3/734 patients included in the study).

4 | CONCLUSION/DISCUSSION

The findings of this study, the largest of its kind to date, found a rate of RBC antigen alloimmunisation in patients whilst on daratumumab of 0.4% (3/734). This is concordant with other, much smaller cohort group studies.[13–20] The overall rate of alloimmunisation in the cohort, defined as the presence of an antibody to a RBC antigen, either existing or newly-formed once daratumumab therapy had begun, was 4% (30/734).

This alloimmunisation rate is lower than in other transfusion dependent cohorts, where alloimmunisation rates as high as 60% of regularly transfused patients are reported.[21] The decreased rate of RBC alloimmunisation in the study cohort may be due to a combination of the disease pathology and immunosuppressive therapeutic regimen. Reduced alloimmunisation has been reported in patients with immunosuppression.[22–23]

Alloimmunisation to RBC antigens occurred despite matching RBC in accordance with BSH guidelines.[6.] In particular, 2 out of the 3 alloimmunised patient were alloimmunised to Rh blood group system antibodies despite receiving RBC fully matched for Rh antigens which would suggest prior alloimmunisation and senescence or sensitisation through an alternative immunological stimulus. In examining the instances of alloimmunisation whilst on Dara more closely, all alloimmunised patients were female and their past medical and serological history was as follows.

The patient who developed alloanti-D started Daratumumab in March 2019. No alloantibodies were detected at the end of April 2019 in the 1st sample received by RCI. Allo anti-D was first detected by RCI in July 2019 and once again in August 2019. The hospital

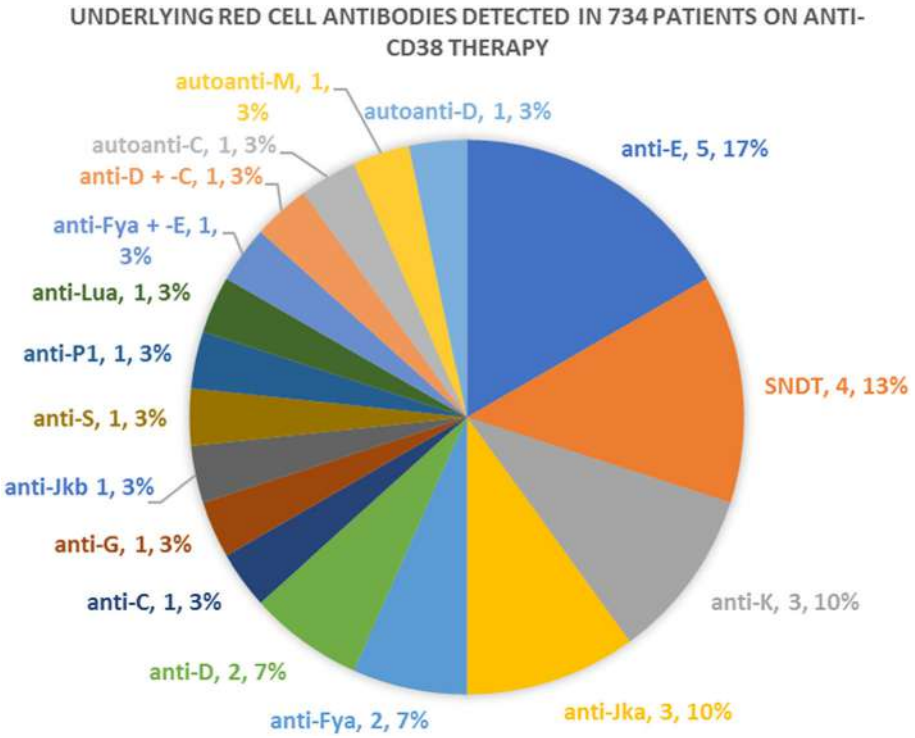

FIGURE 1 Pie chart showing underlying red cell antibodies detected in 734 anti-CD38 TMAb patient referrals to RCI, June 2019-Sept 2019. The most frequently detected, identifiable alloantibodies were Anti-E, Anti-K, anti-Fy^a and Anti-Jk^a. Alloantibodies were detected both prior to commencing Daratumumab therapy (pre-existing, $n = 21$), and once Daratumumab therapy had commenced (newly-formed, $n = 3$). The specificities of the newly-formed alloantibodies were as follows; 1 x alloanti-D, 1 x alloanti-E and 1 x alloanti-Fy^a

confirmed the patient only received D negative RBCs during the course of their treatment, but they did receive one unit of group A, D positive pooled platelets at the beginning of April 2019. Therefore, the formation of alloanti-D was either due to RBC contamination of the pooled platelets,[24] or possibly following a previous pregnancy/sensitising event the anti-D became senescent, and an anamnestic response was subsequently stimulated by administration of D positive pooled platelet components. Modern blood component manufacturing and processing methods now result in very low residual RBC contamination in pooled and apheresis platelet components, however, the risk of antibody alloimmunisation to residual RBCs in these components is not totally obviated. The age/sex of the patients in the study may have meant that a dose of RAADP (250 IU to cover 5 ATD of platelets in a 6-week period) was not issued following the D incompatible platelet transfusion described above, but this should be considered if a patient is D negative, of childbearing potential and D negative platelets cannot be supplied.

The patient who developed anti-E received an autologous Haematopoietic Stem Cell Transplant (HSCT) in early 2019. The patient started Daratumumab in October 2019. RCI crossmatched units for the patient twice in September 2019, and once in October and November 2019 before detection of allo anti-E in November 2019. On all occasions, RBCs provided to the patient from RCI were fully matched for Rh and K antigens, in line with BSH guidance.[6] There was no historical record at the referring hospital of the patient having an allo anti-E prior to commencing Daratumumab. The patient received transfusions of pooled platelets in September (×3), October (×2) and November (×1) 2019. Therefore, formation of alloanti-E may have been due to either of the following; the formation of a naturally occurring alloanti-E, RBC contamination of pooled platelets, or possibly following a previous pregnancy/sensitising event.

The last alloimmunised patient who developed alloanti-Fy^a received an autologous HSCT in 2012, and again in 2016. The patient subsequently started Daratumumab in May 2019. No alloantibodies were detected in May 2019 in the 1st sample received by RCI. RCI first detected alloanti-Fy^a in July 2019. There was no historical record of alloanti-Fy^a at the referring hospital before Daratumumab treatment commenced. The patient received three units of RBC in May and two units of RBC in June. One of the RBC units issued in May was Fy(a+b+), and is, therefore, a possible source of alloimmunisation in this instance.

As discussed, development of autoantibodies did not change the provision of blood components as in the absence of haemolysis, RBC are not selected to take into account autoantibody specificity, as prevention of the development of alloantibodies is usually of more importance.[6] Therefore, they are not considered to be significant in the context of clinical management and are excluded from the scope of these findings. However, it is important to note that despite immunosupression, the patients above were still able to mount an immunological response to RBC derived antigens, producing antibodies to both self and non-self antigenic structures.

These findings come with the caveat that any antibodies detected may have been senescent but present in the patients identified, and that for the purposes of the study alloimmunisation was defined as a newly detected/identified antibody which had not been reported/identified prior to commencement of anti-CD38 therapy. Additionally, as DTT was used for ABID, there may have been an underestimation of the alloimmunisation rate in the patient population, especially for Kell system antibodies and additional specificities not detected by using DTT treated cells, due to antigen removal following DTT treatment. There is also a risk that despite having audited a large number of patients, the time frames studied may not be representative of the exposure and risk, and hence underestimate alloimmunisation. Our data may also underrepresent the number of patients/transfusions, as some labs order extended matched (ABO, Rh, K, MNS, FY, JK) blood directly from NHSBT through the Online Blood Ordering System (OBOS) once a genotype or phenotype has been performed and do not refer samples to RCI for repeat antibody investigation and crossmatching of units.

On the basis of the findings of this study, current protocols to phenotype or genotype all patients prior to commencement of anti-CD38 therapy may be considered excessive given the low rate of alloimmunisation. Particularly when comparing this pre-transfusion testing strategy to antigen matching strategies in other cohorts of patients, for example, in sickle cell anaemia where rates of alloimmunisation are much higher. Consideration should be given to removing the requirement for extended phenotyping or genotyping in all cases prior to the commencement of anti-CD38 therapy, limiting this to those with pre-existing or newly formed alloantibodies. An alternative suggested pre-therapeutic approach to pre-compatibility testing may include the approach shown in Figure 2.

If the initial antibody screen is negative, it is unlikely that alloantibody formation will occur, and therefore, the standard approach of transfusing ABO compatible, Rh/K matched units, issued as suitable would be appropriate for the majority of patients in this cohort.

If the initial antibody screen is positive at commencement of therapy, or becomes positive as a result of antibodies other than the TMAb whilst on treatment, then it would be prudent to phenotype or genotype the patient, as they have previous/current history of antibody formation; and therefore, may make further antibodies if transfused. This would then guide RBC component selection.

Anecdotal reports suggest that some hospital transfusion laboratories already order extended matched RBC routinely, to avoid alloimmunisation and referral of samples to a reference laboratory for investigation and RBC provision. This places additional demand on the stock of extended phenotype units which are needed for other cohorts of transfusion dependent patients who have a higher risk of alloimmunisation, or whom may already have multiple antibodies. This study data should provide confidence that ordering extended phenotype matched units beyond ABO/Rh and K as routine is unnecessary. It also should allow some confidence when assessing the possibility of extending sample validity periods to 7 days from 3 days due to the low risk of alloimmunisation between transfusions. This should enable reduced hospital visits, which with the increased risk of Coronavirus (COVID-19) in these immunocompromised patients is a sensible precautionary measure.

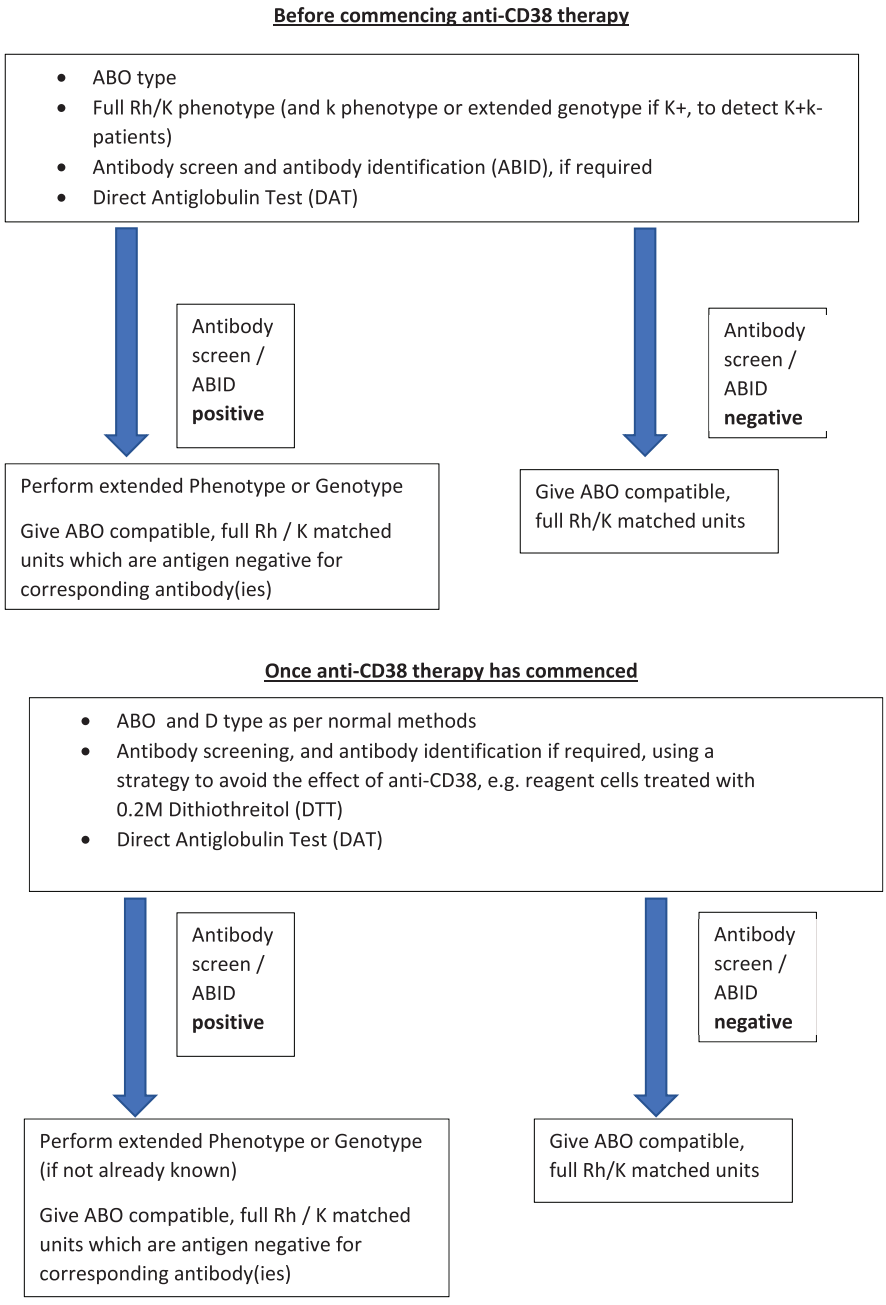

FIGURE 2 Proposed workflow for pre-transfusion compatibility testing for patients on anti-CD38 TMAbs. For each transfusion episode patients should have an ABO Rh/K (and k type, if K+)

The risks of not performing extended typing on MM patients on anti-CD38 therapies who are not alloimmunised may include a delay in blood provision in those few instances when a new antibody is detected, to allow for a phenotype or genotype to be performed. Additionally, it is known that the incidence of MM amongst White populations is significantly lower than Black populations[25] and a genotype may be useful in detecting antigenic variants and guiding RBC selection. A percentage of MM patients also go on to have an allogeneic Stem Cell Transplant and in the long term, a genotype or phenotype would not be beneficial to their treatment due to the different antigenic profile between the recipient and donor. There is also the initiative for personalised medicine which includes genotype-matched blood provision. The current large-scale feasibility of this approach means that it may be limited to transfusion dependent cohorts whom are at greater risk of alloimmunisation. The data from this study indicates that for this cohort, matching routinely beyond ABO/Rh/K may be unnecessary for most MM patients.

Pre-transfusion compatibility testing is necessary to prevent haemolytic transfusion reactions, however, as more TMAbs are developed to treat varying disease states, those working in blood transfusion will need to be aware of any subsequent challenges in blood compatibility testing and supply. Determining the risk of RBC alloimmunisation in patients treated with these novel therapies may help to reduce testing costs and turn-around times and enable evidence-based patient care.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Tom Bullock wrote the paper, analysed and reported the data. Amie Foster contributed to the paper, supplied and analysed the data. Bryony Clinkard contributed to the data analysis.

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ORIGINAL ARTICLE

Improvement of the understanding of blood donors with human T-cell leukaemia virus type 1 using a new information booklet

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Abstract

Background: Human T-cell leukaemia virus type 1 (HTLV-1) tests have been mandated in Japan since 1986, and notification of HTLV-1-seropositive donors started in 1999. However, donor knowledge and response to notification has not been assessed.

Study design and Methods: A questionnaire survey was conducted among blood donors notified of HTLV-1 seropositivity regarding their knowledge of HTLV-1 and unmet information needs. To reduce anxiety among notified individuals and raise awareness of their infection status, we created a booklet containing information that would be useful for these individuals without causing unnecessary anxiety while also requesting that they refrain from donating blood in the future.

Results: A questionnaire survey conducted before the distribution of a new booklet revealed that 15.0% of respondents donated blood again despite receiving an HTLV-1-seropositive notification at the previous donation. While 62.2% of respondents reacted to the notification favourably, 40.2% expressed anxiety and 32.5% requested information on related diseases and medical institutions for consultation. In the secondary survey after distribution of the new booklet, 87.9% of respondents reported that the information was comprehensible, and an increase in consultations of medical institutions by notification recipients was observed. Furthermore, no re-visiting donors were observed among the HTLV-1-seropositive recipients who were notified using the new information booklet.

Conclusion: The new information booklet provided enlightenment on HTLV-1 infection and facilitated the consultation of medical institutions by seropositive donors, leading to an improvement in the health-related quality of life of seropositive blood donors and the safety of blood products.

KEYWORDS
blood donors, blood safety, education, HTLV-1, information booklet, notification

1 | INTRODUCTION

Human T-cell leukaemia virus type 1 (HTLV-1) is a causative agent of human T-cell malignancy, adult T-cell leukaemia/lymphoma (ATL) and HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[1–3] The number of HTLV-1-infected individuals is estimated to be 10–20 million worldwide[4] and over 1 million in Japan,[5] and >95% of infected patients will remain asymptomatic throughout their lifetime. Therefore, asymptomatic HTLV-1 carriers could be at risk of becoming blood donors in Japan. Regarding the transfusion-transmission of HTLV-1, a serological test for all blood donors was mandated by the Japanese Red Cross Blood Centre (JRC) in 1986. At the same time, the JRC has permanently declined blood donation from HTLV-1-seropositive donors, and subsequently, in 1999, a notification programme for HTLV-1-seropositive blood donors was started in order to ensure the safety of blood products for transfusion, according to the recommendation from the government committee on notification of HTLV-1 infection. Although a unified document for HTLV-1-seropositive blood donors was prepared in the JRC headquarters, the format and information content were not put practical use among the regions at this point. The reason why uniform materials were not used nationwide is that the required information varies according to the prevalence in the region.

The Kyushu region, located in the south-western part of Japan, is well-known to have the highest prevalence of HTLV-1 among developed countries. In the Kyushu region, we annually detect >300 HTLV-1-seropositive blood donations, and approximately 2% of seropositive donors visit for repeated blood donation. As the only facility to collect and supply blood products in Japan, the JRC has a responsibility to maintain the safety of blood products by instructing HTLV-1-seropositive blood donors to refrain from blood donation. However, whether or not the notified donors correctly understand the results and what information they need has not been investigated. In order to promote the awareness of HTLV-1-seropositive blood donors, it is important to provide accurate and up-to-date information that addresses the unmet needs of notification recipients.

In this study, we conducted a questionnaire survey to define the unmet needs and knowledge on HTLV-1 infection among donors who were notified of HTLV-1-seropositivity. Based on the responses, we created a new information booklet that contains updated information on HTLV-1 and HTLV-1-specialised medical institutions, with a comment instructing the individual to refrain from blood donation in the future. To assess the impact of the new information booklet on the comprehension of notified donors and their consultation of designated medical institutes, a follow-up survey was conducted. And the number of repeating HTLV-1-seropositive blood donors was compared before and after the distribution of the new information booklet.

2 | MATERIALS AND METHODS

2.1 | Study design

From December 2018 to March 2020, 388 donors (male, $n = 222$; female, n = 166) were notified of their seropositivity on a confirmatory test of HTLV-1. We mailed the notification along with an explanation of the purpose of this study, a consent form, a questionnaire survey form (Appendix S1) and a postage-paid envelope to the notified donors.

In the first survey, the donors who received the notification were asked about their knowledge of HTLV-1, their feelings on receiving the notification, their unmet information needs, the tools they used to obtain on-demand information, whether or not they wished to visit a medical institution and any problems they encountered when receiving the notification. When introducing medical institutions for HTLV-1 carrier consultation in the booklet, we referred to accredited institutions registered in the Japanese Society of HTLV-1 and Associated Diseases (JSHAD). Consent to include the name and reception hours of each medical institution designated for consultation in the attachment of the new information booklet was obtained from all nine certified HTLV-1-specialised medical institutions in the Kyushu region.

The new information booklet was created through consideration of the responses to the first questionnaire survey, and distribution with notification of HTLV-1-seropositive test results started in June 2019. We assessed the recipients' impressions and comprehension of the information in the new booklet, as a second survey targeting newly notified seropositive individuals. Next, we investigated the change in the number of the newly notified blood donors who visited the medical institutions listed in the attachment. In addition, the number of repeating HTLV-1-seropositive blood donors was compared before and after receipt of

TABLE 1 Characteristics of the respondents to the questionnaire survey

	Total (*n*)	Age (years) 16–19	20–29	30–39	40–49	50–59	60–69	Median (range)
Male								
Notified donors	222	15	18	36	41	88	24	50.0 (17–65)
Respondents	46	0	2	4	3	31	6	56.0 (20–64)
Response rate (%)	20.7	0.0	11.1	11.1	7.3	35.2	25.0	
Female								
Notified donors	166	8	10	16	28	75	29	52.0 (17–67)
Respondents	57	2	2	8	6	31	8	53.0 (18–66)
Response rate (%)	34.3	25.0	20.0	50.0	21.4	41.3	27.6	

the above booklet as an evaluation study of new information booklet with attachment from January 2017 to March 2021.

2.2 | Ethical approval

Ethical approval for this study was obtained through the JRC Ethics board (Infection-112, 2018-037-1).

3 | RESULTS

3.1 | Questionnaire survey for notified HTLV-1-seropositive blood donors and the preparation of the new information booklet

Of the 388 notified HTLV-1-seropositive donors, 103 donors (male, $n = 46$; female, $n = 57$) gave their consent to participate in this

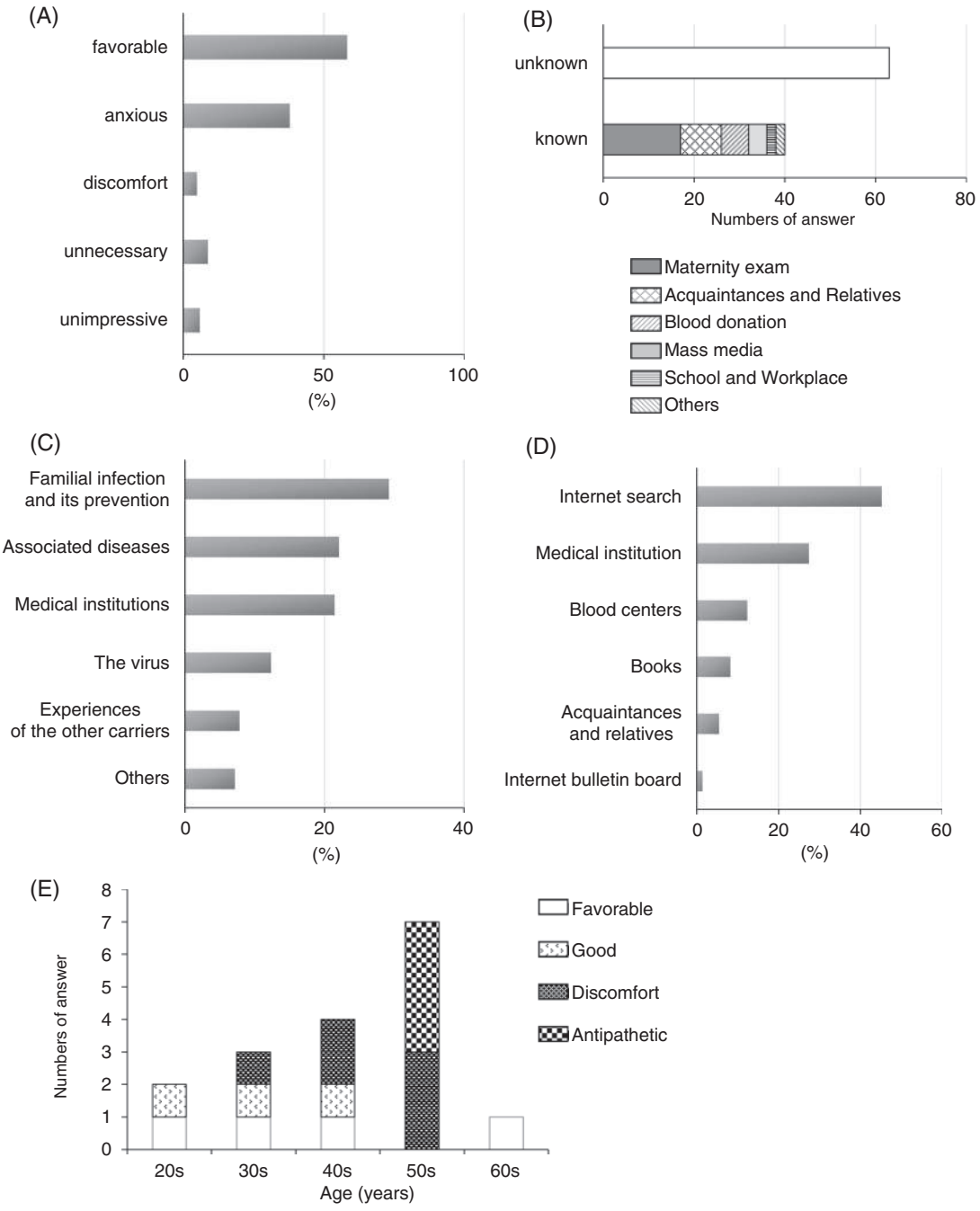

FIGURE 1 Answers to the first questionnaire survey. The questionnaire was sent along with a notification concerning seropositive test results for HTLV-1, and answers were obtained from respondents who consented to participate. (A) Donors' feelings at the time of receipt of the HTLV-1-seropositive notification (numbers of answers: 119), (B) Awareness of HTLV-1 when receiving the notification (numbers of answers: 103; white, did not know about HTLV-1 prior to the receipt of seropositive notification; shaded, knew about HTLV-1 prior to the receipt of seropositive notification). The horizontal axis shows the numbers of respondents. (C) Type of information requested (numbers of answers: 154), (D) Tools for obtaining on-demand information (numbers of answers: 73), (E) Impression about the MHLW Manga material

study and completed the questionnaire. The median age of the male and female respondents was 56.0 years (range 20–64 years) and 53.0 years (18–66 years), respectively (Table 1). Sixty (58.3%) of the 103 respondents accepted the notification of HTLV-1 infection calmly and viewed the contents of the booklet favourably. Thirty-nine (37.9%) experienced anxiety and 5 (4.9%) experienced discomfort after being notified of their HTLV-1 infection status (Figure 1A). Forty donors answered that they had been aware of HTLV-1 before receiving the notification, and 17 (42.5%) of them had learned of HTLV-1 through maternity examinations and prenatal (pre-mom) classes. Six (15.0%) had received the same notification at their previous blood donations. Two of the four responders who answered 'Other' revealed how they had learned about HTLV-1 (at school, $n = 1$; at their workplace, $n = 1$). Nine (22.5%) had received information on HTLV-1 from acquaintances and relatives, possibly reflecting the fact that this study was conducted in a highly endemic area (Figure 1B).

We obtained 154 answers from 80 donors about the information they needed. Forty-five (29.2%) requested knowledge about the transmission of the virus among family members and its prevention. Following that, 34 (22.1%) sought information about HTLV-1-associated diseases, 33 (21.4%) sought information about available medical institutions and 19 (12.3%) and 12 (7.8%) sought information about the virus itself and experiences of other HTLV-1 carriers, respectively (Figure 1C). The most commonly used tools to obtain on-demand information were an Internet search engine ($n = 33$, 45.2%), followed by consulting an HTLV-1-specialised doctor at a medical institution (n = 20, 27.4%; Figure 1D).

In addition, we received 35 telephone inquiries, saying that the word 'HTLV-1' was unfamiliar and difficult to remember and pronounce for ordinary people or even the notification recipients. Therefore, when creating a booklet, we chose 'HAD', as the easy-to-remember and easy-to-pronounce word; this was taken from JSHAD. Namely, 'HAD' is the abbreviation of 'HTLV-1 and associated diseases'.

We collected the latest information for the contents of the new information booklet to address the unmet needs of notification recipients as follows: the virological and epidemiological aspects of HTLV-1 virus, the routes of infection, associated diseases, transmission and prevention of transmission in normal life among the family and in the workplace, and medical institutions to consult, along with comments from and experiences of other HTLV-1 carriers. A question-and-answer format that used easy-to-understand expressions was adopted, with technical terms eliminated when possible. The illustrations, which were drawn by an illustrator, an HTLV-1 carrier who had also learned about the infection after donating blood, were appropriately placed in order to promote understanding.

The new information booklet was reviewed by virologists, haematologists, neurologists, an ophthalmologist and a transfusionist, who were all authorities and experts in the field of HTLV-1. Considering the high rate of respondents who retrieved information using Internet search engines, we introduced the Ministry of Health, Labour and Welfare (MHLW) website, as well as a search map for medical institutions and attached a guide to consulting the HTLV-1-specialising medical institutions available in each prefecture in the Kyushu region.

As the most important issue for the improvement of the safety of blood products, we explicitly stated in the new information booklet that future blood donations from the notified recipients would be declined.

3.2 | Follow-up survey to assess comprehension after distribution of the new information booklet

The reviewed and revised information booklet (available at: https://www.bs.jrc.or.jp/bc9/bbc/special/m6_05_04_index.html) has been distributed to the HTLV-1-seropositive donors since June 2019. A follow-up survey was conducted to assess the comprehension of the notification recipients and their status of HTLV-1 infection.

For the follow-up survey, we distributed a questionnaire about the notification to 233 HTLV-1-seropositive blood donors, and 58 donors (male, $n = 30$; female, $n = 28$; 24.9%) replied. The median age of the male and female respondents was 56.0 years (range, 20–64 years) and 52.5 years (range, 24–64 years), respectively; and 19 (63.3%) of the male respondents and 16 (57.1%) of the female respondents were in their 50s (Table 2). Fifty-eight respondents reported 66 impressions of the new information booklet; 33 (50.0%) found it 'easy to understand', 11 (16.7%) found it

TABLE 2 Characteristics of the respondents to the follow-up questionnaire survey

	Total (*n*)	Age (years) 16–19	20–29	30–39	40–49	50–59	60–69	Median (range)
Male								
Notified donors	147	12	10	20	29	58	18	50.0 (17–65)
Respondents	30	0	2	2	2	19	5	56.0 (20–64)
Response rate (%)	20.4	0.0	20.0	10.0	6.9	32.8	27.8	
Female								
Notified donors	86	0	5	5	16	44	16	53.0 (20–66)
Respondents	28	0	2	2	4	16	4	52.5 (24–64)
Response rate (%)	32.6	0.0	40.0	40.0	25.0	36.4	25.0	

'useful' and 14 (21.2%) found it 'difficult to understand but still comprehensive', meaning that 87.9% of the respondents were able to gather the necessary information from the contents of the new information booklet (Figure 2). By attachment of the consultation guide for available medical institutions specialising in HTLV-1 consultation, seven of the nine introduced hospitals confirmed that they had outpatient visits from blood donors with an HTLV-1-seropositive notification.

3.3 | Deterrent effect of the new information booklet on repeated donation by HTLV-1-seropositive notification recipients

The first questionnaire survey revealed that 38.8% of respondents had been notified of their HTLV-1-seropositive status before their latest blood donation. After the distribution of the new information booklet, we investigated the change in the rate of repeating donors who had already received the notification of their HTLV-1-seropositive status at their previous donation.

To evaluate the utility of the new information booklet, we assessed the re-visiting rate of notified HTLV-1-seropositive donors from January 2017 to March 2021. Among 1383 HTLV-1-seropositive donors, 853 were identified before the distribution of the new information booklet. Among these 853 donors, 19 donations were made by 17 donors (1.99%) who had been notified of their HTLV-1-seropositive status at their previous donation. Five recipients (0.59%) had re-visited for blood donation within 1 year after HTLV-1-seropositive notification. A total of 530 of 1383 received our new information booklet after the initiation of delivery in July 2019. Among these recipients, 310 were observed for more than 1 year, and none had re-visited for blood donation (Table 3).

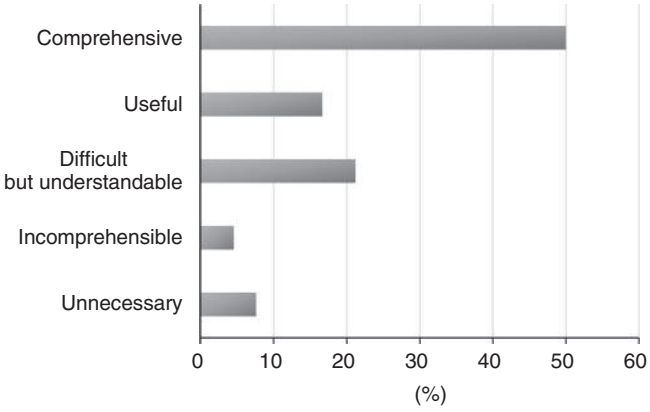

FIGURE 2 Impressions of the new information booklet. After the distribution of the new information booklet with the seropositive notification, a follow-up survey was conducted. Fifty-eight respondents gave 66 answers about their impressions of the new information booklet

4 | DISCUSSION

Japan is the only developed country where HTLV-1 is endemic.[4] In the Kyushu region, in particular, it was estimated that there were approximately 450 000 HTLV-1 carriers.[5]

The WHO reported that 37 countries conduct mandatory testing of all blood donors for HTLV-1 and HTLV-2 and that seven countries conduct selective testing of new donors or donors who have not been previously tested.[6] It is a worldwide consensus in blood programmes that the notification and counselling of blood donors who show seropositive test results are important to blood safety; however, there are no fixed standards for either the regulatory requirements (legally prescribed criteria for notification) or the guidelines for notifying blood donors.[7,8] Notification of HTLV-positive blood donors was reported in Canada,[9] Australia[10] and the United States[11] in the 1990s. For example, in the UK,[12] notification recipients are asked to contact the blood service to arrange a discussion about their test results and onward clinical care. In Japan, this notification program started in 1999. The notification of healthy blood donors about seropositive test results can cause confusion, anxiety, and lack of understanding. In the recent report on health-related quality of life among blood donors who were notified viral infection, cases shown anxiety and depression had been 2.67-fold in HTLV carriers comparing to the control uninfected donors.[13] However, we have not adequately followed up the outcomes of notification.

In the present study, we defined the knowledge of HTLV-1 among notified blood donors and the unmet information needs according to the findings of a questionnaire. Taking the respondents' voice into consideration, we then created a new information booklet to provide the most necessary and up-to-date information in an easy-to-understand format. In the new information booklet, with the aim of improving health-related quality of life of the notification recipients, we included phrases to mitigate their anxiety, recommended early consultation to those with any symptoms, and listed the HTLV-1-specialised medical

TABLE 3 A comparison of the numbers of re-visiting recipients before and after the distribution of the new information booklet

	All notified donors in this study		Recipients tracked over 1 year	
	n	Re-visiting recipients (%)	*n*	Re-visiting recipients within 1 year (%)
Before distribution	853	17 (1.99)	853	5 (0.59)
After distribution	530	0 (0.00)	310	0 (0.00)

institutions for the consultation. In addition, we conducted a questionnaire survey to investigate the comprehension of recipients. In this survey, 90% of the respondents answered that the new information booklet was understandable, indicating that their knowledge had dramatically improved thanks to the contents, which coincided with the unmet needs of the notification recipients.

No HTLV-2-seropositive individuals have been confirmed among Japanese blood donors since the start of the notification program for HTLV-1-seropositive blood donors; thus, we did not mention HTLV-2 in the latest new information booklet. However, we might need to prepare an additional description about HTLV-2 in the future, as the first case of an HTLV-2-infected Japanese pregnant woman was recently reported.[14]

HTLV-1 antibody testing became mandatory in antenatal pregnancy screening throughout the nation in 2010. Simultaneously, the recommendation for mothers with positive results to refrain from breastfeeding was implemented for the prevention of mother-to-child transmission via breast milk. Following that, the MHLW of Japan collaborated in the production of the Japanese animation series, *Cells at Work!*, to conduct a public awareness campaign about HTLV-1 in 2018.[15] Enlightenment posters using popular comic book character have been distributed to health centres throughout Japan.

In our study, regarding the knowledge of HTLV-1, 17 recipients answered that they had learned about HTLV-1 in maternity examinations and prenatal (pre-mom) classes, suggesting that the education system for pregnant women had helped to spread knowledge about HTLV-1 in Japan; however, the efforts to disseminate knowledge regarding the ways to prevent horizontal transmission via transfusion remain insufficient.

Surprisingly, despite the receipt of a HTLV-1-seropositive notification following prior donations, 15% of respondents donated blood again. Five recipients had re-visited for blood donation within 1 year after seropositive notification, suggesting that we had not provided sufficiently useful information before the distribution of the new information booklet. Continuous blood donation by notified HTLV-1-seropositive donors poses a risk to both the donor and patients, namely; a risk of an adverse effect of unnecessary blood collection for the donor and a risk of transfusion-transmission of the virus for patients. To reduce these risks, we clearly stated in the new information booklet that blood donation by those individuals would be refused. As a result, no repeated blood donations by recipients of the new information booklet were observed, indicating that appropriate presentation of information that addressed with the unmet needs of notified donors corrected their understanding of their HTLV-1 infection status and that blood donation would be declined.

In a study conducted among blood donors in India, donors were notified of their seropositive status in order to prevent transfusion-transmission of blood-borne infectious agents (TTIs).[16] A study in Thailand[17] showed that the behaviour of blood donors could be affected by providing a deeper knowledge about their HIV status, indicating that proper notification is necessary in order to prevent repeated blood donation. These investigations demonstrated that donor notification is an efficient method of curtailing TTIs, which is consistent with the results of our study.

Several limitations associated with the present study should be mentioned. First, the comprehension of recipients was evaluated by self-stated answers for the questionnaire, suggesting that the understanding might not have been sufficient. Second, recipients of the new information booklet could not be tracked for a long enough period to obtain an accurate evaluation of the re-visiting rate compared with before distribution. Third, there may have been some bias, as only 26.5% of recipients participated in this survey. Thus, recipients who did not send their answer sheet might have understood less than the participants. However, since no re-visiting donors were observed after the distribution of the new information booklet, the new information booklet might have improved their understanding of HTLV-1 infection.

We recently received an e-mail from a foreign student living in Kyushu, writing that his Japanese girlfriend had recently been notified that she was HTLV-1-seropositive and that he was strongly concerned about transmission through sexual intercourse. He was anxious to learn about infection routes and the frequency of HTLV-1 transmission, and he would like to visit a medical institution for consultation to HTLV-1-specialised doctors. A basic strategy for preventing TTIs is to notify and counsel infected blood donors. Although counselling of individuals infected with HTLV-1/2 has been recommended,[18] a nationwide consultation system has not yet been fully developed in Japan. The aforementioned international student wrote in his e-mail, 'Unfortunately I live in an HTLV-1 endemic area'. There is thus an urgent need to formulate nationally acceptable guidelines for the notification and follow-up of HTLV-1-seropositive individuals in health checks and to prevent the spread of HTLV-1, both domestically and abroad.

In this study, HTLV-1-seropositive blood donors expressed a strong wish for information about medical institutions capable of counselling HTLV-1 carriers. In response to our request, all nine certified medical institutions in the Kyushu region accepted that the notification of HTLV-1 test results from the JRC would be regarded as a patient referral document and that recipients who visited the designated medical institutions would be exempted from the additional fee for a first-time patient who presented no referral. Owing to the reduction in the additional fee for consultation, the number of consultations for recipients of the new information booklet increased, and visits from those recipients were observed in seven of the nine designated medical institutions. In fact, visits from HTLV-1-seropositive donors increased 1.44-fold at the introduced medical institutions following the distribution of the new information booklets. The result indicated that the disclosure of available medical institutions and the reduction of medical expenses are effective measures for notified donors who are anxious about their status and who desire to visit appropriate medical institutions for consultation. The new information booklet was fruitful in two aspects: one was the facilitation of consultations of HTLV-1-seropositive notification recipients; the other was the deterrent effect in relation to repeated donation by the recipients, leading

to improvement of both the health-related quality of life of seropositive blood donors and the safety of blood products.

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CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Hitomi Nakamura and Yasuko Sagara designed this study, analysed data, edited the information booklet and wrote this manuscript. Midori Yamamoto collected data. Atae Utsunomiya and Toshiki Watanabe reviewed the information booklet and supervised this manuscript. Masahiro Satake also reviewed the information booklet, supervised this study and supervised this manuscript. Kazuo Irita supervised this study.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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TRANSFUSION MEDICINE WILEY

LETTER TO THE EDITOR

Use of recombinant Von Willebrand factor during transcatheter aortic valve replacement in a patient with acquired von Willebrand syndrome

To the Editor,

Acquired von Willebrand syndrome (aVWS) is a clinical and biological entity similar to congenital von Willebrand disease (VWD). Although aVWS is rare, its true incidence is most likely underestimated due to the diagnostic complexity. Many underlying diseases are associated with aVWS, such as autoimmune, cardiovascular, lymphoproliferative, myeloproliferative or malignant disorders. Cardiovascular disorders especially include congenital heart defects, aortic stenosis, and the use of left ventricular assist devices.[1,2] The therapeutic strategy consists in identifying the underlying pathology in order to treat the underlying condition. In cases where such remedies are not possible, only symptomatic treatment is available. The management of surgeries in patients with aVWS can be complicated in particular, due to the often reduced half-life of von Willebrand factor (VWF). We report on the use of vonicog alfa, the first recombinant VWF, during transcatheter aortic valve replacement (TAVR) in a patient with aVWS.

This case concerns a 72-year-old male patient with hypertension who presented with VWD diagnosed 10 years ago, with lower gastrointestinal (GI) bleeding. Factor VIII (FVIII), Willebrand antigen (VWF:Ag) and ristocetin cofactor activity (VWF:Rco) levels were respectively at 40% (50%–150%), 20% (50%–150%) and <10% (50%–150%), associated with loss of high molecular weight multimers (HMWM). An acquired aetiology was evoked, as there was no personal or family history of bleeding. The ratio between VWF propeptide and antigen was found to be 3.95 (<2.4), thus further supporting this hypothesis. A complete clinical and biological assessment led to the diagnosis of GI angiodysplasia, calcified aortic stenosis (AS), and monoclonal gammopathy of undetermined significance (MGUS) with IgM kappa. The latter seemed to be the leading aetiology of aVWS.

Recently, the patient's coronary syndrome and calcified AS worsened, and it was decided to perform a TAVR. First, on 19 August 2020, a coronary angioplasty with placement of two active stents on the proximal circumflex, was performed under a single injection of 40 IU/kg of vonicog alfa and 40 IU/kg of octocog alfa. The recovery of vonicog alfa was calculated during the coronary angioplasty at 0.75 IU/dl per IU/kg with a half-life of 12 h (Table 1). The TAVR procedure was performed via the left common carotid 1 month later, with implantation of one valve. The TAVR was preceded by a bolus of 91 IU/kg of vonicog alfa and 40 IU/kg of octocog alfa. The recovery during the procedure was 1 IU/dl per IU/kg. The operation lasted 3 h and no abnormal bleeding was observed. Moreover, given the existence of conduction disorders before TAVR and an episode of sinus node dysfunction post-procedure, placement of a pacemaker was indicated. A cardiac pacemaker was implanted 48 h after the TAVR, under a bolus of 91 IU/kg of vonicog alfa.

Following this double procedure, 64 IU/kg of vonicog alfa were administered daily for 4 days, and this brought the residual VWF:Rco level to between 40% and 50% (Figure 1). The haemoglobin level remained stable throughout the patient's stay (between 11 and 12 g/dl), with nevertheless some non-serious haematomas at the incisions. The patient left the cardiology department after 10 days, including seven in intensive care. Upon discussion with colleagues, Clopidogrel 75 was introduced for 1 month. The antiplatelet therapy was nevertheless stopped on the seventh day after the patient's discharge due to an episode of epistaxis which required nasal packing and the injection of 65 IU/kg of vonicog alfa. The patient was seen again 1 month after the procedure. His clinical condition was satisfactory from a cardiological point of view, but he reports experiencing epistaxis on a daily basis.

The management of surgery in patients with aVWS is often complicated. From a therapeutic point of view, there are several options: desmopressin when there is no contraindication, intravenous immunoglobulins (IVIG), plasma VWF concentrates with or without FVIII, FVIII alone or, if these fail, recombinant activated factor VII. IVIG are especially useful in cases associated with MGUS, but are usually ineffective in IgM-MGUS.[2] Because the half-life of endogenous or exogenous VWF can be very short, a pharmacokinetic (PK) study before any major surgery is justified. The VWF dose needed depends on the clinical situation and can range from 30 to 100 IU/kg.[3] There are no consistent guidelines for the treatment of aVWS, likely due to its rarity.

In this context, we report here the case of a polypathological patient with aVWS in connection with a MGUS who underwent a TAVR under vonicog alfa. Vonicog alfa is the first recombinant VWF product and contains no FVIII. It is manufactured in the absence of animal or other human plasma proteins, which eliminates all risks of pathogenic transmissions.[4] PK assessments performed as part of prospective phase studies in patients with type 3 or severe type 1 VWD have demonstrated that the terminal half-life of vonicog alfa is longer than that of plasma-derived VWF (pd-VWF). The peak plasma vonicog alfa levels are observed less than 60 min after injection and its half-life is 21.9 h.[5] The efficacy of vonicog alfa in surgery was studied in a phase 3 study that included 15 surgeries performed in patients with congenital VWD (10 major and 5 minor).[6]

TABLE 1 Comparative pharmacokinetics between pd-VWF (Wilfactin®) and vonicog alpha

	VIII:C (%)		VWF:Ag (%)		VWF:Rco (%)	
	40 IU/kg octocog alpha 40 IU/kg Wilfactin®	40 IU/kg octocog alpha 40 IU/kg vonicog alpha	40 IU/kg octocog alpha 40 IU/kg Wilfactin®	40 IU/kg octocog alpha 40 IU/kg vonicog alpha	40 IU/kg octocog alpha 40 IU/kg Wilfactin®	40 IU/kg octocog alpha 40 IU/kg vonicog alpha
T0	29	31	21	41	<10	<10
T1h	93	85	103	89	30	36
T4h	80	98	84	87	<10	33
T8h	73	91	67	84	<10	25
T12h	60	89	62	74	<10	21

Abbreviations: Rco, ristocetin cofactor activity; VWF, Willebrand factor.

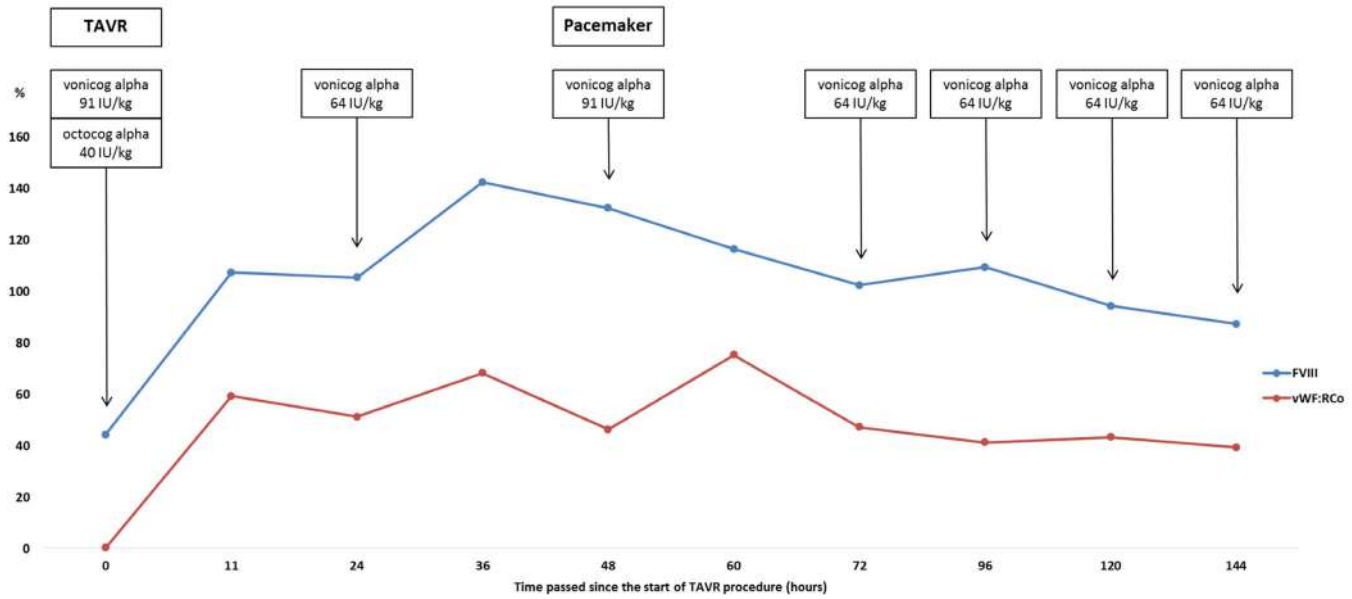

FIGURE 1 Evolution of Willebrand factor (VWF):ristocetin cofactor activity (Rco) and Factor VIII (FVIII) levels during and after the procedures [Color figure can be viewed at wileyonlinelibrary.com]

Regarding our patient, a PK analysis was conducted both with pd-VWF (Wilfactin®) and with vonicog alfa. Blood samples were collected at baseline (T0) and respectively 1 h (T1h), 4 h (T4h), 8 h (T8h) and 12 h (T12h) after infusion (Table 1). PK parameters with vonicog alfa (half-life of 12 h and recovery of 0.75 IU/dl per IU/kg) seemed to be better than those obtained 3 weeks ago with pd-VWF, which prompted us to prescribe vonicog alfa for the TAVR. Though TAVR is not a major surgery, it is still a complex procedure associated with a real risk of perioperative bleeding.[7]

In the case reported here, the efficacy of vonicog alfa was deemed to be excellent. However, the total quantity of vonicog alfa administered (502 IU/kg over 6 days) was greater than the overall median surgical dose of vonicog alfa (220.4 IU/kg, range 63.8–648.4 IU/kg) used in the phase 3 study.[6] Aside from the preoperative bolus of FVIII, no further injection of FVIII was necessary, which attests to the endogenous stabilisation of FVIII by vonicog alfa.

Vonicog alfa could represent an interesting alternative for surgery in patients with aVWS, who often have greatly reduced HMWM. During the production process, vonicog alfa has no exposure to ADAMTS 13 and therefore contains intact HMWM with significant haemostatic power and probably a better capacity to stabilise FVIII and the clot. Indeed HMWM play a crucial role in primary haemostasis because of their binding capacity for collagen and platelet receptors, thus facilitating platelet aggregation under shear stress.[8]

To date, there have been few reports in the literature of experience with vonicog alfa in the perioperative management of patients with aVWS. Weyand et al reported the use of vonicog alfa in a 2-year-old child with moderate aVWS during a valve replacement.[9] Furthermore, Tran et al reported in their review a clinical case similar to ours, of a patient with aVWS associated with myeloma who underwent a valve replacement, in whom vonicog alfa was ultimately not used in due to the high concomitant need for FVIII.[10]

While global conclusions cannot be drawn from the case we present here, the role of vonicog alfa in the management of surgery in patients with aVWS should be studied in greater detail, particularly in the current context of IGIV shortage.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTIONS

Dominique Desprez: Designed the study. **Laurent Sattler** and **Dominique Desprez:** Analysed the data and wrote the paper. **Laurent Sattler**, **Olivier Feugeas**, **Ulun Crimizade**, **Sébastien Hess**, **Lélia Grunebaum** and **Dominique Desprez:** Critically reviewed the manuscript. All authors approved the final version.

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