How do I implement a whole blood program for massively bleeding patients?

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Building on the successful military experience, interest has been rekindled in transfusing whole blood (WB) early in the resuscitation of traumatically injured civilians, often before their ABO group is known. WB efficiently provides treatment for shock and coagulopathy, as well as platelet hemostatic function, to patients losing large volumes of blood. Unlike group O uncrossmatched red blood cells (RBCs), group O WB contains a substantial amount of plasma, which is incompatible with the RBCs of all non–group O recipients. Thus, when implementing a WB program, it is important to decide how to mitigate the risk of immune-mediated hemolysis. Other questions that a hospital needs to answer before implementing a WB program include determining which patients will be eligible for this product, how many units eligible patients can receive, for how long it should be stored and under what conditions, and how to monitor for adverse events. The donor center needs to consider if the WB should be leukoreduced, how to comply with the AABB's transfusion-related acute lung injury risk mitigation standard, and into which storage solution it should be collected. This report describes the multidisciplinary approach taken to implementing a civilian WB program at a multihospital health care system in the United States.

"For the good times past, that are come again
I am your man."

First tempter of Thomas à Beckett in *Murder in the Cathedral* by T.S. Eliot.

WHAT'S OLD IS NEW AGAIN

Using whole blood (WB) for trauma resuscitation is not a new idea, but rather the reincarnation of the product used to resuscitate casualties on the battlefield. From the First World War through today's conflicts,[1,2] WB has been an important staple of wartime medicine. WB was also the mainstay of hospital-based transfusion therapy until the advent of component therapy.[1] As the importance of early plasma and platelet (PLT) transfusion to resuscitate traumatically injured patients has become better appreciated,[3] so too has the interest in using cold-stored WB for civilian trauma patients.[2] Reducing death from hemorrhage is essential since the mortality for patients with traumatic hemorrhagic shock is high (at least 20%) and there are approximately 30,000 preventable civilian deaths due to traumatic hemorrhage per year in the United States alone.[4] The early, prehospital administration of blood has recently been demonstrated to significantly improve the

ABBREVIATIONS: CTS = Centralized Transfusion Service; LR = leukoreduced; WB = whole blood.

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survival of military casualties compared to those who received delayed transfusions or those who did not receive transfusions.[5] WB offers several benefits over component therapy including providing simultaneous treatment for both oxygen debt and the coagulopathy of trauma;[6,7] it is a more concentrated product compared to reconstituting WB using component therapy; and cold-stored WB contains PLTs that appear to have equivalent or better hemostatic effect in both in vitro tests[8-11] and in clinical trials,[12,13] compared to PLTs that have been stored under conventional room temperature conditions. Another benefit of WB that is perhaps harder to quantify is the simplification of the resuscitation effort with its use, especially in the prehospital environment. In such settings, where the clinical staff are task saturated, patient intravenous access is limited, and storage space in helicopters and ambulances is very limited, having the ability to provide a balanced resuscitation fluid in one bag instead of up to four bags is valuable. This is important because any delay in the provision of blood products in hemorrhagic shock can be lethal; mortality is increased by 5% for each minute there is a delay in the delivery of blood products.[14] In addition, WB has a similar volume and identical storage and transportation temperature requirements as a red blood cell (RBC) unit, so it provides all the necessary resuscitation fluids without having to reengineer the transportation and storage of blood products outside of the hospital. Other benefits of transfusing WB include a potentially reduced risk of bacterial contamination compared to a room temperature–stored PLT product as the WB is stored at refrigerator temperature and fewer donor exposures for the recipient.

The use of WB for civilian trauma patients is growing in the United States. Currently there are at least 10 hospitals and air and ground ambulance systems that use WB as the initial resuscitation fluid, and others are planning on establishing a WB program in the near future (P. Spinella, personal communication, October 2017). This "How do I . . ." article will describe some of the questions that arise when implementing a WB program and address some of the solutions.

REGULATORY CONSIDERATIONS DIRECTING THE USE OF WB

Standard 5.15.1 in the 30th edition of the AABB Standards for Blood Banks and Transfusion Services required WB to be administered in a manner that is ABO identical with the recipient. The wording of this standard made it impossible to use WB before a recipient's ABO group was known, thereby making it nearly impossible to administer it in a patient's prehospital or early in-hospital course when they would benefit from balanced resuscitation. However, in the 31st edition of the Standards that will be published online in early January 2018 and become effective April 1, 2018, this prohibition has been removed. Instead, the transfusion of WB will be now permitted in a manner such that the RBC component of the WB must be compatible (but not necessarily identical) with the RBCs of the recipient. Effectively this means that low-titer group O WB will be allowed to be administered in the same way that group O uncrossmatched RBCs are administered. The new standard will read as follows: "Recipients shall receive ABO group-compatible Red Blood Cell components, ABO group-specific WB, or low titer group O WB (for non-group O or for recipients whose ABO group is unknown)." The standards go on to require transfusion services that offer WB to develop local policies for the definition of low titer, how many units of WB each patient can receive, which patients are eligible for WB, and procedures for adverse event monitoring (Standard 5.27.1). Thus, effective in 2018, hospitals that are accredited by the AABB can decide on their own policy for using WB, as the regulatory barrier to using it in an uncrossmatched manner for recipients of unknown ABO group will be eliminated. Nevertheless, as mandated by the new standards, the transfusion service will have to develop policies that guide the practice of transfusing WB, and this includes deciding on which titer threshold to use even if their blood supplier is performing the titers.

IMMUNOHEMATOLOGIC CONSIDERATIONS FOR TRANSFUSING GROUP O WB

A significant minority of patients in most populations around the world will be group O and thus are not at risk of a hemolytic reaction from the anti-A and –B that is found in the plasma component of group O WB. However, for non–group O recipients, an incompatibility between the plasma in the group O WB and the antigens on their RBCs exists. The new standards permit the use of low-titer group O WB if the recipient's ABO group is not known at the time of transfusion, and this leads to several questions that each hospital must answer when implementing a WB transfusion program for traumatically injured or massively bleeding patients.

What method should be used to titer the anti-A and -B in group O WB and what is a safe titer threshold?

A full discussion of these issues is beyond the scope of this review, and has been reviewed elsewhere,[15] but several key points should be mentioned. At the moment, a gold standard method for performing antibody titers does not exist and it is known that the method of performing the titer affects the results. Belin and colleagues[16] reviewed six different studies that evaluated different methods of titering anti-A and/or -B and found that, despite the heterogeneous methods used in these studies,

gel-based methods were more reproducible and generally produced titers that were one to two dilutions higher than saline tube–based methods. While these differences in titers could potentially complicate studies that compare titer results between centers, within a center the laboratorians and clinicians will become accustomed to what the reported titer means at their institution and can proceed accordingly as long as the same method is consistently employed. Thus, each center needs to determine which titer method produces the optimal balance of cost, ease of use, precision, and accuracy (again, a gold standard method for determining the quantity of antibody in a sample is not yet available). At the Centralized Transfusion Service (CTS) in Pittsburgh, which supplies low-titer group O WB to a total of five Level 1 or 2 trauma centers, the immediate-spin saline tube method without enhancement additives or extended incubation time is the titer method of choice.

In terms of selecting a titer threshold, a universally recognized titer level of anti-A and -B below which a hemolytic reaction is absolutely guaranteed not to occur if the WB is transfused in an incompatible manner does not exist. Using the reports of hemolytic reactions after the transfusion of incompatible PLTs as a guide, most of these reactions tend to occur with units that have an obviously high titer, although there are some exceptions.[17,18] Thus, it is likely that an absolute "safe" or "low-enough" titer below which hemolysis will not occur does not exist; instead, titering the antibodies in group O WB should be considered a hemolysis risk mitigation strategy, not a risk elimination step. To implement a WB program where group O WB is administered to recipients of unknown ABO group, a hospital must be comfortable with the small risk of hemolysis in a low-titer unit balanced against the benefits that WB affords the patient and the trauma or emergency department teams. Among the civilian hospitals that utilize group O WB, there is a range of critical antibody titer thresholds; at the CTS in Pittsburgh, all group O WB units must have anti-A and -B titers of less than 50 (immediate spin), while at the Mayo Clinic the threshold is less than 200 (immediate spin). The US Army has set its titer threshold for WB at less than 256, although individual sites can impose a more stringent threshold.[19] Given the titers at which hemolytic reactions to PLT occurred, and the civilian[20] and military[1] experience with transfusing group O WB, as well as supportive anecdotal unpublished evidence from civilian transfusion services that use WB, a "low-titer" threshold of up to 256 seems reasonable. Interestingly, in the Safety of the Use of Group A Plasma Units in Trauma (STAT) study, 354 group B and AB trauma patients received a mean of 4 units of group A plasma and had mortality and hospital length of stays that were not significantly different from those of the 809 group A trauma patients who also received group A plasma. In this study, 76% of the participating centers did not titer the anti-B in the group A plasma units, perhaps suggesting that an even higher titer threshold might be suitable for this product.[21] Although hemolysis was not an outcome measure in the STAT study, others have investigated the incidence of hemolysis after receipt of incompatible plasma in WB transfusions (see below).

While patient safety should be the main factor in deciding what constitutes a low titer, some consideration for the selected titer's impact on the number of donated units that will be deferred should be given. At the CTS in Pittsburgh, the titer threshold of less than 50 by the immediate-spin technique results in a deferral of approximately 20% of the donated WB units. These high-titer units are immediately converted into group O RBCs and stored for up to 21 days in CPD (see below). On a related note, the rate of group A plasma unit deferral due to a high-titer anti-B is approximately 14% at the CTS in Pittsburgh, where the same titer threshold and method as for WB is used.

How often should group O donors be titered?

There exists no standard for answering this question, but there is some experience to guide decision making. In a study of 56 healthy adult volunteers in southern Denmark whose anti-A and/or -B was measured every 3 months for a period of 1 year using an automated solid-phase instrument, the overall pooled standard deviation (SD) between these serial titer measurements ranged from 0.30 to 0.47 $\log_2$ titer steps.[22] For reference, a titer of 8 would be equivalent to a $\log_2$ titer step of 3 and a titer of 16 would be equivalent to a $\log_2$ titer step of 4, so the pooled SDs of these 56 volunteers reflects a variation of less than half of a titer dilution. These volunteers did not have any restrictions on their eating habits, ability to procure vaccines, becoming pregnant, or anything else that could potentially have changed their antibody titers. Thus, at least in this population, the antibody titers are very stable, which can inform the decision about how often their isohemagglutinins should be titered if potentially incompatible blood products are to be employed.

In addition, the rate of high-titer (>50), group O male WB donors at the CTS in Pittsburgh did not show any seasonal variation over an approximately 2-year period (Fig. 1). Nevertheless, the antibody titers are determined on each donated WB unit—even from repeat donors—at this center to prevent the transfusion of a high-titer unit.

Should the group O WB units be D+ or D–?

Centers that are contemplating the use of WB for patients whose ABO group has not yet been determined have likely encountered this question when determining the appropriate D type for their uncrossmatched RBCs. Briefly, the reason to consider the D type for the WB and/or RBCs is the potential for D– females of childbearing age to

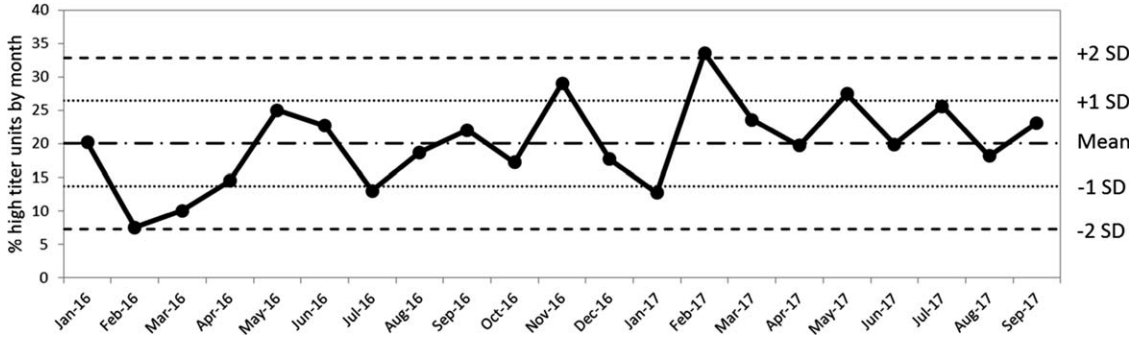

Fig. 1. Levey-Jennings chart of high-titer (>50), group O WB units at the CTS in Pittsburgh by month. Note that this does not reflect all male, group O donors, only those who were selected to donate WB.

become alloimmunized to D if they are exposed to D+ RBCs, which could adversely affect future pregnancies if the fetus is D+. A variety of primarily retrospective studies have demonstrated that the rate of D alloimmunization among hospitalized patients who received at least one D+ RBC unit is approximately 20% to 25%,[23-29] which is much lower than the often cited alloimmunization rate of approximately 80% among healthy D– volunteers who receive D+ transfusions.[30-32] Thus, hospitals should evaluate the number of times that females of childbearing age who would be eligible for WB therapy are treated at their center; if a significant number of females of childbearing age are treated then stocking D– WB could be considered. At all four of the adult Level 1 or 2 hospitals in Pittsburgh, only D+ WB is stocked as the majority of WB-eligible trauma patients are males in whom D alloimmunization is of minor, non–life-threatening consequence; females under the age of 50 are not eligible to receive WB. At the Level 1 pediatric hospital in Pittsburgh, D– WB is stocked so that both traumatically injured boys and girls can be treated with this product. The question of the D status of the unit is also relevant if WB is to be utilized in the prehospital period, such as by being transported to the scene of the accident by ambulance or helicopter, where the sex and/or age of the potential recipient might not be known in advance.

NONIMMUNOHEMATOLOGIC CONSIDERATIONS FOR TRANSFUSING WB

The new AABB standards for WB transfusion defer much of the decision making about the policies and procedures that guide the WB program to the hospital and the transfusion service. This offers significant flexibility to accommodate local practices and patient populations.

For example, the new standards do not specify the nature of the patient(s) that can receive WB. At the adult and pediatric hospitals in Pittsburgh where WB is available, only patients with hypotension from traumatic bleeding are eligible for WB therapy. Thus, patients who are having massive bleeding in the operating room, gastrointestinal bleeding, and so forth are not currently eligible for WB. This restrictive policy is likely to be amended in the near future. In the meantime, traumatically injured adults can receive up to 4 units of WB and then they are transitioned to conventional component therapy based on point-of-care or near-patient test results and the impressions of the attending surgeons and physicians. Initially, the maximum dose at these hospitals was 2 units of WB per patient; this was a conservative approach and was based on the local experience of transfusing ABO-mismatched PLT units without observing hemolysis. Once the safety of transfusing up to 2 WB units per adult patient was demonstrated,[20] the quantity was increased to 4 units and surveillance for hemolysis is ongoing. Through the middle of October 2017 there have been approximately 15 adult non–group O recipients of 3 and 4 WB units, and compared to the approximately 10 adult group O recipients of 3 and 4 WB units, no laboratory or clinical evidence of hemolysis among the non–group O recipients has been detected (unpublished observations, see below). These 4 units are kept in the emergency department's monitored blood refrigerator for up to 14 days, and additional WB units are kept in the blood bank to replenish the supply in the emergency department. Having WB units readily available in the emergency department is essential to promote their utilization early in the resuscitation, rather than administering conventional components first while waiting for the WB to be sent to the emergency department from the blood bank after the patient arrives. A cooler is kept in the emergency department such that if all 4 units are not administered while the patient is there, the remainder can be packed in the cooler, the chemical coolant can be activated, and the WB can accompany the patient to the operating room or to the radiology department. Again, this is done to promote the use of WB as the first products to be administered to a hemorrhaging trauma patient while the point-of-care or near-patient testing is being conducted.

At the San Antonio Military Medical Center in Texas, more than 40 units of low-titer WB (≤210 using PK7300 instrument, Beckman-Coulter) have been transfused to eight adult patients without clear evidence of hemolysis or other adverse events. The blood bank maintains an inventory of both group O+ and O– WB units, which are collected from male donors or females without a pregnancy history, and these units are kept exclusively in the blood bank. They are issued when ordered to male patients of any age and females who are over age 50 with severe bleeding from traumatic injury. For female patients under age 50 or patients whose D type is unknown, O– WB units are provided if available. If O– WB units are not available, O– RBC units are issued. WB units are kept as WB for up to 21 days and as such cannot be manufactured into RBC units if they are unused. The local policy requires the blood bank physician to discuss with the attending anesthesiologist how to proceed with the resuscitation after 8 units of WB have been issued to the patient, that is, whether to continue using WB or whether to switch to component therapy, as there is no policy-specified upper limit on the number of WB units that can be issued to a patient (A.P. Cap, personal communication, November 2017).

At the pediatric Level 1 hospital in Pittsburgh, only traumatically injured patients who are both at least 3 years old and weigh at least 15 kg can receive WB. These guard bands, although arbitrary, ensure that a non–group O recipient should have A and/or B antigen(s) expressed on their tissues and in their secretions that can adsorb the corresponding antibodies from the WB thereby preventing hemolysis. Pediatric recipients at this hospital can receive up to 30 mL/kg WB before being transitioned to component therapy. The WB units stocked at the pediatric hospital also have isohemagglutinin titers of less than 50, and they otherwise have the same attributes as those stocked at the adult hospitals with the exception that they are D–.

CLINICAL CONSIDERATIONS WHEN IMPLEMENTING A WB TRANSFUSION PROGRAM

While the transfusion service is responsible for providing, storing, and monitoring the use of the WB, the clinical trauma team is responsible for monitoring the recipients for clinical efficacy and adverse events associated with the transfusion, especially hemolysis. The transfusion service in Pittsburgh worked closely with the trauma teams at the adult and pediatric hospitals to develop the protocols for WB administration. As stated, in addition to close clinical observation for signs and symptoms of immune-mediated hemolysis, the algorithm for hemolysis monitoring at the adult and pediatric hospitals in Pittsburgh involves measuring the lactate dehydrogenase, total bilirubin, and haptoglobin on the day of WB receipt and for the next 2 days. Ideally the first measurement of these variables should be before the patient receives the WB but this is often logistically impossible, so the clinicians are encouraged to order these laboratory tests as close to the time of WB administration as is feasible in the context of the resuscitation effort. A special electronic order set is being created that will automatically order these laboratory markers at the prescribed times so that the clinicians do not have to remember to order them each day, although they are required to manually initiate the order set on the day of WB transfusion. The same protocol for hemolysis monitoring is also employed at San Antonio Military Medical Center.

In Pittsburgh, the clinicians are also responsible for ensuring that the correct patients receive the WB. Because the WB units maintained at the adult hospitals are D+, males of any age and female trauma patients over the age of 50 years can receive WB. Furthermore, until the WB eligibility criteria at this center are expanded to include other massively bleeding patients, the clinical trauma team is responsible for ensuring that only traumatically injured patients receive WB and for educating their colleagues in the emergency department, intensive care unit, and the operating room about which patients qualify for WB administration. They also investigate and produce a corrective action plan should WB be administered to ineligible recipients.

DONOR CENTER CONSIDERATIONS FOR SUPPORTING A WB TRANSFUSION PROGRAM

Whole blood units contain a substantial quantity of plasma, and therefore the transfusion-related acute lung injury (TRALI) mitigation standards that apply to regular plasma units must also be applied to WB. Rather than screening WB units for HLA antibodies if donated by females with a pregnancy history, the blood bank that supports the CTS in Pittsburgh only collects WB units from male donors to comply with the TRALI risk mitigation standard.

Another issue for the donor center to consider is whether the WB should be leukoreduced (LR). Most of the studies that compared outcomes such as mortality, microchimerism, lung injury, and so forth between trauma patients who received non-LR or LR RBCs did not find clinically or statistically significant differences.[33-36] Thus, it is not clear if LR WB should be provided to trauma patients. In addition, one of the potential benefits of WB in trauma patients is the provision of cold-stored PLTs. Many WB LR filters also remove the PLTs during filtration. There is only one Food and Drug Administration–approved, PLT-sparing WB LR filter that is available in the United States, the Terumo Imuflex WB-SP filter (Lakewood, CO). Thus, the decision to provide a LR WB product

should take into account any potential adverse effects that the filter might have on the PLT count or function in relation to the benefits that LR might provide to a severely bleeding patient. A recent study of LR WB collected using the Imuflex WB-SP filter that was agitated under several different conditions found the mean PLT concentration on Storage Day 3, the earliest time point after LR where the concentration was measured, to be more than 100×10^9/L for the unrocked and rocked WB units, roughly half the number of PLTs in a single WB PLT unit.[37] The Imuflex WB-SP filter is attached to a storage bag containing CPD that gives the WB a 21-day shelf life. Whether the WB should be maintained as such for 21 days, or for a shorter period, depends on how one interprets the literature on cold-stored PLT function cited. In Pittsburgh, the decision was made to keep WB for up to 14 days in the refrigerator as it was clear from the literature that cold-stored PLT function was well maintained for at least that length of time. Continuous agitation of WB is not recommended as it does not enhance PLT quality and contributes to increased hemolysis during storage.[37] On Day 15 the unused units of WB are returned to the CTS laboratory where the WB is concentrated into an RBC unit by removing the PLT-rich plasma, and the resulting RBC unit can be stored for an additional 6 days. As these units are group O they are readily issued to recipients who require RBC transfusion and very few are wasted. A different reading of the literature might lead one to maintain the WB as such for the maximum 21 days if stored in CPD. In fact, WB collected into CPDA-1 could be maintained as WB for up to 35 days at refrigerator temperatures, but the literature on cold-stored PLT function for that length of time is not well developed.

It will be necessary for the donor center to create new policies and procedures to handle the selection of suitable WB donors, having special LR collection kits with filters available at the sites where WB is to be collected if LR is to be performed, performing HLA antibody screening if necessary, and inventory management issues such as keeping track of units with a shorter shelf life than most RBC-containing products. There will likely be other one-time efforts required to implement WB such as validating the collection kits and modifying the information technology system to handle the new product. Issues such as permitting the return of unused WB units that could be processed into RBC units from hospitals and pricing of the WB would need to be decided on a case-by-case basis. For example, one pricing model for WB might be to charge the sum of the costs of the individual components with perhaps a surcharge for leukoreduction, and in Pittsburgh a new product code was created in the transfusion service's computer system to accommodate the WB.

Implementing a WB program requires a multidisciplinary approach to answer the logistic and practical questions that transfusing this product poses. In the absence of well-defined answers to questions like what is a safe titer threshold or the ideal titer method to use, one must rely on the literature and one's personal experience to guide the program's policies and procedures and realize that as the local experience with WB transfusion accumulates, aspects of the program can change to accommodate new ideas or efficiencies that will improve the program or make it safer for future recipients.

CONFLICT OF INTEREST

MHY received honoraria from Terumo BCT. The other authors have disclosed no conflicts of interest.

REFERENCES

1. Murdock AD, Berséus O, Hervig T, et al. Whole blood: the future of traumatic hemorrhagic shock resuscitation. Shock 2014;41 Suppl 1:62-9.
2. Spinella PC, Cap AP. Whole blood: back to the future. Curr Opin Hematol 2016;23:536-42.
3. Zielinski MD, Stubbs JR, Berns KS, et al. Prehospital blood transfusion programs: capabilities and lessons learned. J Trauma Acute Care Surg 2017;82:S70-8.
4. Spinella PC, Cap AP. Prehospital hemostatic resuscitation to achieve zero preventable deaths after traumatic injury. Curr Opin Hematol 2017;24:529-35.
5. Shackelford SA, Del Junco DJ, Powell-Dunford N, et al. Association of prehospital blood product transfusion during medical evacuation of combat casualties in Afghanistan with acute and 30-day survival. JAMA 2017;318:1581-91.
6. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. JAMA 2015;313:471-82.
7. Davenport RA, Brohi K. Cause of trauma-induced coagulopathy. Curr Opin Anaesthesiol 2016;29:212-9.
8. Pidcoke HF, McFaul SJ, Ramasubramanian AK, et al. Primary hemostatic capacity of whole blood: a comprehensive analysis of pathogen reduction and refrigeration effects over time. Transfusion 2013;53 Suppl 1:137S-49S.
9. Reddoch KM, Pidcoke HF, Montgomery RK, et al. Hemostatic function of apheresis platelets stored at 4°C and 22°C. Shock 2014;41 Suppl 1:54-61.
10. Nair PM, Pandya SG, Dallo SF, et al. Platelets stored at 4°C contribute to superior clot properties compared to current standard-of-care through fibrin-crosslinking. Br J Haematol 2017;178:119-29.
11. Nair PM, Pidcoke HF, Cap AP, et al. Effect of cold storage on shear-induced platelet aggregation and clot strength. J Trauma Acute Care Surg 2014;77:S88-93.
12. Manno CS, Hedberg KW, Kim HC, et al. Comparison of the hemostatic effects of fresh whole blood, stored whole blood, and components after open heart surgery in children. Blood 1991;77:930-6.

13. Becker GA, Tuccelli M, Kunicki T, et al. Studies of platelet concentrates stored at 22 C and 4 C. Transfusion 1973;13:61-8.
14. Meyer DE, Vincent LE, Fox EE, et al. Every minute counts: time to delivery of initial massive transfusion cooler and its impact on mortality. J Trauma Acute Care Surg 2017;83:19-24.
15. Yazer MH, Seheult JN, Kleinman S, et al. Who's afraid of incompatible plasma? A balanced approach to the safe transfusion of blood products containing ABO-incompatible plasma. Transfusion 2018;58:532-38.
16. Belin TR, Yazer MH, Meledeo MA, et al. An evaluation of methods for producing low-titer group O whole blood to support military trauma resuscitation. J Trauma Acute Care Surg 2017;82:S79-s86.
17. Josephson CD, Castillejo MI, Grima K, et al. ABO-mismatched platelet transfusions: strategies to mitigate patient exposure to naturally occurring hemolytic antibodies. Transfus Apher Sci 2010;42:83-8.
18. Lozano M, Cid J. The clinical implications of platelet transfusions associated with ABO or Rh(D) incompatibility. Transfus Med Rev 2003;17:57-68.
19. Bahr MP, Yazer MH, Triulzi DJ, et al. Whole blood for the acutely haemorrhaging civilian trauma patient: a novel idea or rediscovery? Transfus Med 2016;26:406-14.
20. Seheult JN, Triulzi DJ, Alarcon LH, et al. Measurement of haemolysis markers following transfusion of uncrossmatched, low-titre, group O+ whole blood in civilian trauma patients: initial experience at a level 1 trauma centre. Transfus Med 2017;27:30-5.
21. Dunbar NM, Yazer MH; Biomedical Excellence for Safer Transfusion (BEST) Collaborative and the STAT Study Investigators. Safety of the use of group A plasma in trauma: the STAT study. Transfusion 2017;57:1879-84.
22. Sprogoe U, Yazer MH, Rasmussen MH, et al. Minimal variation in anti-A and -B titers among healthy volunteers over time: implications for the use of out-of-group blood components. J Trauma Acute Care Surg 2017;82:S87-90.
23. Yazer MH, Triulzi DJ. Detection of anti-D in D- recipients transfused with D+ red blood cells. Transfusion 2007;47:2197-201.
24. Frohn C, Dümbgen L, Brand JM, et al. Probability of anti-D development in D-patients receiving D+ RBCs. Transfusion 2003;43:893-8.
25. Gonzalez-Porras JR, Graciani IF, Perez-Simon JA, et al. Prospective evaluation of a transfusion policy of D+ red blood cells into D- patients. Transfusion 2008;48:1318-24.
26. Selleng K, Jenichen G, Denker K, et al. Emergency transfusion of patients with unknown blood type with blood group O Rhesus D positive red blood cell concentrates: a prospective, single-centre, observational study. Lancet Haematol 2017;4:e218-24.
27. Meyer E, Uhl L. A case for stocking O D+ red blood cells in emergency room trauma bays. Transfusion 2015;55:791-5.
28. Miraflor E, Yeung L, Strumwasser A, et al. Emergency uncrossmatched transfusion effect on blood type alloantibodies. J Trauma Acute Care Surg 2012;72:48-52; discussion 52-3.
29. Dutton RP, Shih D, Edelman BB, et al. Safety of uncrossmatched type-O red cells for resuscitation from hemorrhagic shock. J Trauma 2005;59:1445-9.
30. Gunson HH, Stratton F, Cooper DG, et al. Primary immunization of Rh-negative volunteers. Br Med J 1970;1:593-5.
31. Urbaniak SJ, Robertson AE. A successful program of immunizing Rh-negative male volunteers for anti-D production using frozen/thawed blood. Transfusion 1981;21:64-9.
32. Pollack W, Ascari WQ, Crispen JF, et al. Studies on Rh prophylaxis. II. Rh immune prophylaxis after transfusion with Rh-positive blood. Transfusion 1971;11:340-4.
33. Nathens AB, Nester TA, Rubenfeld GD, et al. The effects of leukoreduced blood transfusion on infection risk following injury: a randomized controlled trial. Shock 2006;26:342-7.
34. Utter GH, Nathens AB, Lee TH, et al. Leukoreduction of blood transfusions does not diminish transfusion-associated microchimerism in trauma patients. Transfusion 2006;46:1863-9.
35. Watkins TR, Rubenfeld GD, Martin TR, et al. Effects of leukoreduced blood on acute lung injury after trauma: a randomized controlled trial. Crit Care Med 2008;36:1493-9.
36. Phelan HA, Sperry JL, Friese RS. Leukoreduction before red blood cell transfusion has no impact on mortality in trauma patients. J Surg Res 2007;138:32-6.
37. Yazer MH, Glackin EM, Triulzi DJ, et al. The effect of stationary versus rocked storage of whole blood on red blood cell damage and platelet function. Transfusion 2016;56:596-604.