Whole blood for hemostatic resuscitation of major bleeding

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Recent combat experience reignited interest in transfusing whole blood (WB) for patients with life-threatening bleeding. US Army data indicate that WB transfusion is associated with improved or comparable survival compared to resuscitation with blood components. These data complement randomized controlled trials that indicate that platelet (PLT)-containing blood products stored at 4°C have superior hemostatic function, based on reduced bleeding and improved functional measures of hemostasis, compared to PLT-containing blood products at 22°C.

WB is rarely available in civilian hospitals and as a result is rarely transfused for patients with hemorrhagic shock. Recent developments suggest that impediments to WB availability can be overcome, specifically the misconceptions that WB must be ABO specific, that WB cannot be leukoreduced and maintain PLTs, and finally that cold storage causes loss of PLT function. Data indicate that the use of low anti-A and anti-B titer group O WB is safe as a universal donor, WB can be leukoreduced with PLT-sparing filters, and WB stored at 4°C retains PLT function during 15 days of storage. The understanding that these perceived barriers are not insurmountable will improve the availability of WB and facilitate its use. In addition, there are logistic and economic advantages of WB-based resuscitation compared to component therapy for hemorrhagic shock. The use of low-titer group O WB stored for up to 15 days at 4°C merits further study to compare its efficacy and safety with current resuscitation approaches for all patients with life-threatening bleeding.

Damage control resuscitation (DCR) and hemostatic resuscitation are concepts recently developed to optimize resuscitative and transfusion approaches to patients with traumatic

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hemorrhagic shock and immediately life-threatening injuries. DCR has many components all of which are aimed at preventing or treating shock and coagulopathy and thereby reducing morbidity and mortality from severe traumatic injuries causing massive hemorrhage.[1] Hemostatic resuscitation is the central tenet of DCR. This concept developed with the recognition that a blood-based transfusion strategy would be optimal for severe bleeding and that crystalloid or colloid-based resuscitation cause hemodilution, acidosis, and a steady decline in oxygen delivery, which aggravate the underlying coagulation and metabolic disorders that evolve after injury and blood loss.[2] Although a natural and obvious hemostatic resuscitation product would be whole blood (WB), it is not commonly available in the developed world, so many substitute components transfused at high ratios of plasma and platelets (PLTs) to red blood cells (RBCs) that range between 1:1:2 and 1:1:1 units, respectively. Goal-directed hemostatic resuscitation is also being explored as a method to alter empiric ratios of blood products and provide specific therapies based on the rapid results from point-of-care coagulation and shock monitoring. Recognizing the lack of robust clinical trial data available to support the development of optimal resuscitative strategies in patients with traumatic hemorrhagic shock, the following will review the history of trauma resuscitation and the evidence regarding the use of WB in all patient populations in which it has been analyzed. We suggest that cold WB (CWB) is an underutilized potential source of hemostatically functional PLTs, plasma, and RBCs and that it needs to be explored in large clinical trials for all patients with hemorrhagic shock, to include, for example, traumatic, operative, obstetric, and gastrointestinal bleeding.

HISTORICAL TO CURRENT TRANSFUSION PRACTICE FOR SEVERE BLEEDING

The use of WB has a long history in military medicine, which began almost 100 years ago in World War (WW)I. Briefly, in WWI, the Canadian surgeon Major Bruce L. Robertson demonstrated that direct transfusion of uncrossmatched WB was a lifesaving procedure in combat casualties with severe blood loss.[3] Later, when the United States entered the war in 1917, US Army Captain Oswald Hope Robertson documented that citrated and cold-stored universal, group O WB could be given safely in forward casualty clearing stations.[4] This was one of the most important medical advances of the war and made British, Canadian, and American forces able to supply the front-line hospitals with group O WB as the universal donor product. In WWII the lessons from WWI were initially discounted and US Forces planned for a plasma-based resuscitation strategy with freeze-dried plasma and albumin. This approach, although practical, was found to be inadequate to the task of preventing or reversing shock physiology.[5] The British Forces primarily transfused WB during WWII based on the concept that both shock and coagulopathy needed to be equally addressed for optimal outcomes. After the US leadership recognized the successes the British Forces were having with this approach they also shifted back to a WB-based resuscitation strategy.[6,7]

US Forces in the WWII established what were termed "field blood banks," where fresh WB was collected from immediately available donors and either used on site immediately or packaged and delivered as far forward as possible for resuscitation near point of wounding. Units actively engaged in combat used freeze-dried plasma and any available WB until casualties could be evacuated to surgical facilities. This approach was replicated successfully in Korea where group O WB was used exclusively and group O low titer (<1:200 by saline dilution) was reserved for all non-group O recipients.[8,9] This experience informed resuscitation planning in the Vietnam conflict. In early 1965, it was decided that only universal-donor low-titer group O would be shipped to Vietnam. However, as the requirements for blood increased it became necessary to utilize fully the donor population and the first shipment of non-group O WB appeared in December 1965. Eventually group-specific WB became available, but universal-donor low-titer group O WB was the only blood product used in forward, prehospital settings.[6,10]

During and after the Vietnam War Era, crystalloids and colloids replaced blood to become the primary initial resuscitative solution for hemorrhagic shock. This was in part due to the risks of infectious disease transmission with blood products, but also due to research performed by Carrico and colleagues[11] indicating that the interstitial compartment or "third space" needed to be resuscitated with 1 to 2 L of crystalloids to perfuse the tissues, after which the transfusion of WB would be indicated only if hemodynamic instability persisted. Their philosophy for resuscitation in patients with traumatic bleeding was misapplied and led to the overuse of crystalloids, to the detriment of patients with severe bleeding who commonly received 5 to 10 L of crystalloids before any blood product administration. The result was dilutional coagulopathy and severe interstitial edema. Indeed, the term "Da Nang lung" was coined to describe the effects of massive crystalloid resuscitation in causing pulmonary edema. In civilian practice blood banks gradually transitioned to component inventories and WB became unavailable. RBC units that lacked plasma and PLTs took the place of WB but clinicians did not recognize that RBC units compared to WB units were not as efficacious at treating both shock and coagulopathy (hemorrhagic shock). The misapplication of the data of Carrico and colleagues and the disappearance of WB from the clinical armamentarium ushered in an era of acute respiratory distress syndrome, abdominal compartment syndrome, multiorgan failure, and anasarca in intensive care units (ICUs).[12,13] These outcomes were actually predicted by Shoemaker in

1976,[14] when he challenged the notion that the interstitial compartment required resuscitation and instead emphasized the need for WB to treat significant bleeding when the hematocrit fell below 30%. The overuse of crystalloids occurred despite a call for moderation by Moore and Shires as early as 1967. In their editorial, Moore and Shires state, "Blood should still be replaced during major operative surgery as it is lost. The use of balanced salt solutions appears to be a physiological adjunct to surgical trauma, not a substitute for blood." Subsequent research has demonstrated that a crystalloid-based resuscitation strategy leads to increased inflammation and vascular permeability compared to WB.[15,16]

The pendulum has now swung back to focusing on a blood-based strategy for patients with life-threatening traumatic bleeding. Recent conflicts in Iraq and Afghanistan reignited interest in the concept of transfusing WB or "reconstituted WB" with components in a 1:1:2 to 1:1:1 unit ratio as a result of many factors. The large volume of patients presenting in hemorrhagic shock with high mortality forced physicians to think of alternative methods to improve outcomes. Before 2005, PLT units were not available in Iraq or Afghanistan and even after that they were in limited supply. There was increased awareness that shock can directly lead to coagulopathy and that a balanced resuscitation that corrected both conditions might reduce death from hemorrhage.[17] Physicians with prior combat experience maintained institutional knowledge of the successful use of WB in previous conflicts and the ease with which a WB donor program could be implemented and maintained.[18,19] As a result, the use of WB at large combat support hospitals increased in 2003 to 2004 from a "rescue" therapy in the initial phases to a more widely adopted strategy that was employed earlier in the resuscitation of patients with life-threatening hemorrhagic shock. It was during the period of 2004 to 2006 that the concepts of DCR were solidifying into one bundle of care, with hemostatic resuscitation as the centerpiece of clinical practice guidelines for the treatment of traumatic hemorrhagic shock. The DCR concept and specific use of WB were codified into on-line CPGs and routinely reevaluated and updated by the US Department of Defense Joint Trauma System.[20] In view of the accumulated experience and data, in 2014, the Tactical Combat Casualty Care Committee recommended that WB should be the preferred resuscitative product for patients with traumatic hemorrhagic shock over all other blood product combinations and fluids.[21]

The concept of hemostatic resuscitation as a component of DCR continues to be an area of active research, with unprecedented attention given to optimizing resuscitation strategies. The use of WB for hemostatic resuscitation is attracting interest in the civilian trauma community as evidenced by its inclusion as a research priority in the NHLBI Transfusion Medicine State of the Science Symposium Summary Statement. In addition, highly detailed clinical protocols on how to collect and transfuse low-titer group O blood safely in a combat environment have also been recently published and implemented in Special Operations Forces units in multiple countries.[22,23] Surprisingly, the use of WB on vacation cruise liners at sea has also become common in which ABO-specific warm fresh WB (WFWB) is used routinely and very successfully for patients with life-threatening (mainly gastrointestinal) hemorrhage.[24]

WB DEFINITIONS

Approximately 450 to 500 mL of WB can be collected into a variety of non–adenine-containing anticoagulant solutions (CPD, CP2D, etc.) and stored for up to 21 days between 1 and 6°C. WB can be stored for up to 35 days if collected in CPDA-1. All required transmissible disease tests are performed on this product before it is issued for transfusion. WB retains all the components of the donation without additional processing. The Food and Drug Administration (FDA) Circular of Information for Use of Human Blood and Blood Components states that WB is indicated for symptomatic anemia with large-volume deficits.[25] The Circular of Information also states that WB must be of the same ABO group as the recipient, although as described below, some are challenging this requirement. To differentiate this product from WB used in military settings, it is called CWB. When CWB is used within 48 hours of collection it is cold fresh WB (CFWB).

In military or austere settings, where formal testing for transfusion transmitted diseases (TTDs) is not possible, WB is collected from pretested donors (before deployment or every 90 days during deployment) and stored warm at 22°C for up to 8 hours and then for a maximum of an additional 24 hours at 4°C. However, it is typically transfused immediately after collection. This product is termed WFWB. Since WFWB is transfused before results of TTD testing are available (testing of each unit is retrospective since samples are sent from combat treatment facilities to US laboratories) it is not approved for use by the FDA. WFWB presents a higher risk of disease transmission and is reserved for situations in which tested blood products are unavailable or ineffective. It is important to understand the differences between cold and warm storage of WB since there are reports of both in the literature.

The use of the term "fresh" when the WB unit has been stored for less than 48 hours is arbitrary and is largely related to the observation that PLTs stored at 1 to 6°C have reduced circulation time after transfusion compared to fresh or room temperature–stored PLTs. Historically, cold-stored PLTs were not considered to be "viable" or therefore functional. How circulation time came to be considered a measure of PLT hemostatic function is not

clear. Acute hemostatic capacity, which is better preserved with cold storage, is a more relevant measure of function in patients with severe bleeding and at high risk of death within hours than the ability of the PLTs to circulate for days. A reappraisal of the concept of WB "freshness" or efficacy is indicated.

CWB

The two largest randomized controlled trials (RCTs) that have exclusively compared WB to blood components were performed in pediatric cardiac surgery patients. These two trials with significantly different methods reported contradictory results. In a double-blinded study, Manno and coworkers[26] evaluated postoperative blood loss in 161 children undergoing open heart surgery with cardiopulmonary bypass whose immediate postoperative transfusion requirements were met with either WB or reconstituted WB (RBCs, fresh-frozen plasma [FFP], and PLTs given together in a 1:1:1 unit ratio in an attempt to re-create WB). The transfusion of WB was associated with significantly less postoperative blood loss than the transfusion of RBCs, FFP, and PLTs in children less than 2 years of age who underwent complex cardiac surgery (mean 24-hr postoperative blood loss 52.3 ± 10.8 mL/kg vs. 96.2 ± 10.7 mL/kg, p = 0.001). In that study, WB was associated with improved PLT function according to aggregometry results. Mou and colleagues[27] compared the use of fresh WB with the use of a combination of RBCs and FFP (reconstituted blood) for priming of the cardiopulmonary bypass circuit in children less than 1 year of age undergoing cardiac surgery. Children were randomly assigned to receive either fresh WB (96 subjects) or reconstituted blood (104 subjects) for bypass-circuit priming. This study did not continue randomization of the intervention in the ICU, where outcomes were measured. The use of fresh WB for cardiopulmonary bypass priming presented no advantage over the use of a combination of RBCs and FFP during surgery for congenital heart disease. Moreover, circuit priming with fresh WB was associated with an increased length of stay in the ICU and increased perioperative fluid overload. Unfortunately, this secondary outcome was not adjusted for confounding variables.

The only available prospective evidence in trauma patients that utilized WB came from a single RCT of 107 patients requiring massive transfusion.[28] One arm received leukoreduced modified WB (with PLTs removed during filtration) supplemented with room temperature–stored PLTs, and the other was treated exclusively with components (RBCs, plasma, PLT units). While the primary outcome of 24-hour transfusion volume was equivalent between groups, a secondary analysis excluding patients with severe traumatic brain injury demonstrated significantly reduced 24-hour RBC and plasma use for the modified WB group with a trend toward lower PLT use as well.

It is difficult to know whether the addition of PLTs to WB sufficiently changed the results to limit generalizability, and thus the study was not designed to definitively establish the efficacy of WB.

A small RCT published in 1990 that compared WFWB to CFWB enrolled 36 adult patients requiring cardiac surgery and cardiopulmonary bypass. The hemostatic effect of fresh nonleukoreduced WFWB was compared to CFWB stored between 1 and 6°C for both 5 and 24 hours, and investigators found that blood loss increased postoperatively in those transfused with WB stored between 1 and 6°C, suggesting that WFWB may be superior to CFWB.[29] The small sample size in this one report limits its generalizability, but it may reflect that WFWB retains hemostatic function better than stored CWB when storage time is less than 24 hours. Since WFWB is not clinically available, except for in austere or military environments, these results may be relevant only for this population of patients.

In vitro data indicate that the hemostatic capacity of WB is higher than that of blood reconstituted from individual components in a 1:1:1 ratio.[29] In one analysis in which PLTs were stored at 22°C for up to 5 days, and RBCs were stored between 1 and 6°C for up to 35 days, investigators compared reconstituted WB to WFWB and found that the hemostatic capacity of PLTs was reduced as storage duration increased.[30] Conversely, as RBC and PLT storage age increased, more thrombin–antithrombin complexes were detected, indicating increased thrombin generation; this was confirmed in a thrombin generation assay. Similarly, WFWB PLT aggregation response to collagen was consistently better than that of reconstituted WB. A similar in vitro study compared warm and CWB with and without additional PLTs to reconstituted WB with components in a 1:1:1 unit ratio. No differences in PLT function or viscoelastic monitoring results were noted between warm and CWB and reconstituted WB when RBCs and PLTs were at their maximum storage duration.[31] Differences in study methods reported for these two studies may account for the conflicting results.

In vitro studies also indicate that considerable CWB hemostatic capacity is maintained for at least 14 days. One analysis of nonleukoreduced WB stored between 1 and 6°C reported normal PLT function as reflected by thromboelastogram maximum amplitude values for all 21 samples out to 14 days of storage, with the median thromboelastogram maximum amplitude values within normal range out to 21 days of storage.[32] In addition, light transmission aggregometry analysis of PLT function exhibited no change from Day 1 to Day 21 with adenosine diphosphate and epinephrine stimulation, but did report a decline in response to collagen (Day 7) and ristocetin (Day 17). These results were reported without adjusting for a dramatic reduction in PLT count over time, which

dropped to a median value of less than 100×10^9/L by Day 7 of storage.

In vitro measures of hemostasis studied in WB units stored at 4°C versus 22°C over 21 days reported superior prothrombin time and partial thromboplastin time, impedance PLT aggregation, and TEG results in cold-stored blood.[33] In this study nonleukoreduced WB was stored for up to 21 days. WB stored at 4°C was superior to those units stored at 22°C based on prothrombin time and partial thromboplastin time, impedance PLT aggregation, and TEG variables. Refrigeration of WB units also increased shear-induced PLT aggregation and ristocetin-induced PLT agglutination as well as the proportion of GPIb-expressing PLTs. Furthermore, PLT function in stored WB measured by shear- and von Willebrand factor–dependent methodology is retained at 4°C for up to 7 days.[34]

These findings are similar to those regarding PLT units stored at 4°C. Refrigerated PLTs perform better than room temperature–stored PLTs in aggregation studies as has been reported by multiple investigative teams.[35-39] This includes an RCT demonstrating that PLTs stored at 4°C were superior in reducing bleeding time in both patients on aspirin and in patients with thrombocytopenia when compared to PLTs stored at 22°C.[40]

It is unknown what the effect of storing WB at 1 to 6°C for up to 10 to 15 days has on the endothelial effects of plasma that appear to be protective in freshly thawed or reconstituted plasma products.[41] An analysis of liquid plasma stored for 14 days at 1 to 6°C indicated similar endothelial protective properties to that of immediately thawed plasma.[42] This suggests that perhaps the plasma in WB stored at 1 to 6°C may also retain endothelial protective properties. The examination of the effect of all available blood products on endothelial function is of great interest since the endothelium is critically affected by shock physiology and tissue trauma.

WARM WB

Retrospective data in adults further support the use of WB compared to exclusive use of components, including an analysis of US casualties with 100% follow-up, which reported an independent association between survival and the addition of WB as an adjunct to resuscitation.[43] Additionally, a study of US and non-US casualties reported no difference in outcomes for those transfused WB as part of their resuscitation compared to those who only received blood components; however, results should be viewed with caution as approximately 33% of patients were lost to follow-up.[44] These two studies of combat casualties are unique in that the WB transfused in combat was given immediately after collection or was stored very briefly (<24 hr). They also differ from civilian data in that WB accounted for only a portion of the total blood used for resuscitation during the initial 24 hours of admission. In the study that found an association with survival, 30% of the 24-hour blood volume transfused was WB, whereas in the study that demonstrated equivalence, only 20% of the 24-hour blood volume transfused was WB.

In a retrospective civilian study, the use of WB as part of a resuscitation was associated with similar outcomes compared to the exclusive use of blood components. In patients with multiple etiologies of hemorrhagic shock requiring massive transfusion, there were no differences in blood utilization rates and clinical outcomes.[45]

Two small RCTs have compared WFWB to PLT concentrates stored between 20 and 24°C. One study, published in 1989, compared WFWB to PLT concentrates stored at 20 to 24°C in 24 patients requiring cardiopulmonary bypass.[46] A scanning electron microscope was used to assess PLT aggregation. Their results indicated that 1 unit of WFWB had a hemostatic effect comparable to 8 to 10 WB-derived PLT units. In another small randomized study of 27 patients requiring cardiopulmonary bypass, published in 1988, WFWB was also found to be associated with improved PLT function compared to PLT units stored at 20 to 24°C as assessed with aggregation studies, the gold standard for PLT function analysis.[47]

WB AVAILABILITY

WB is widely available in much of the world, particularly in developing nations such as those of sub-Saharan Africa. For example, in Kenya at the Kijabe Hospital, group O, nonleukoreduced CWB, stored at 4°C for up to 35 days, is commonly provided to patients (J. Kibuchi and S. Letchford, oral communication, August 15, 2015). The use of WB in developing nations has historically been limited primarily due to the lack of systems or capacity to produce components; however, some areas have resisted the adoption of component therapy for multiple other reasons.[48]

In the developed world, the clinical availability of WB from the 1970s to 1980s has been dramatically reduced. The transition from WB to the use of individual components during this time was influenced by the economics of blood banking and the ability to provide the specific blood component needed for isolated deficits (RBCs for anemia, PLTs for thrombocytopenia, plasma for coagulopathy). The change in transfusion practice was also driven by increased need for plasma for fractionation to provide sufficient amount of coagulation factor concentrates. Furthermore, the majority of oncology patients required only RBCs or PLT concentrates to treat cytopenias associated with cancer and chemotherapy. WFWB essentially disappeared from US hospitals during the early days of the HIV epidemic as a result of infectious concerns. The combination of these trends accelerated the disappearance of

CWB, even though it is fully tested for TTDs. Four other factors virtually guaranteed its disappearance.

The first is that regulatory bodies have mandated that WB be ABO group identical with the recipient. While it is clear that the RBCs in the whole unit, like any RBCs, must be compatible with the recipient, this mandate was put in place because of the fear of a hemolytic reaction caused by a minor ABO incompatibility, that is, anti-A or anti-B in the WB unit causing hemolysis in an A or B recipient. Providing ABO-specific WB introduces a significant logistic concern since maintaining sufficient inventory to ensure a ready supply of every ABO group would result in significant cost and waste as well as limit the use of WB in an urgent bleeding situation where the recipient's ABO group has not yet been confirmed.

The second perceived barrier is the lack of a PLT-sparing leukoreduction (LR) filter. Since prestorage LR has become either mandatory in most countries or widely used in others, the fact that WB could not be leukoreduced while maintaining PLTs further limited the willingness to supply WB.

The third barrier is due to the concern that the PLTs in CWB stored between 1 and 6°C might not be functional or viable. In short, there has existed a perception that CWB is not a truly functional PLT-containing product. Due to this concern, when WB is used in most developed countries it is used within 2 to 7 days of storage, even though it is licensed for storage between 21 and 35 days. This is in contrast to hospitals in the developing world that transfuse WB until the date of expiration if necessary.

A fourth belief that limited WB availability is the notion that blood components are equally efficacious compared to WB for patients with shock and coagulopathy. This concept has not been studied well, except in one pediatric cardiac surgery trial, where the use of WB did reduce blood loss likely due to improved PLT function compared to blood components.[26] As mentioned, in all other trials, either PLTs were added to WB when compared to blood components or WB was only given during a portion of the study period, potentially limiting generalizability.[27,28]

These four issues have caused concern regarding the efficacy, safety, and logistic feasibility of providing WB for patients with life-threatening bleeding and have led to the dramatic reduction of its use in much of the developed world, including Australia, the United Kingdom, and Canada (D. Irving, H. Doughty, D. Devine, oral communication, August 15, 2015). While the concepts above have been challenged recently in an effort to provide patients with a better, more efficacious resuscitation product, acceptance and implementation have been slow, and thus WB availability remains limited. Some developed countries do still use WB for pediatric transfusion, including France (A. Sailliol, oral communication, August 15, 2015), Norway (G. Strandenes, oral communication, August 15, 2015), and 15% of children's hospitals in the United

States.[49] In both the United States and France, despite licensing for up to 35 days, CWB storage duration is limited to 2 to 7 days in clinical settings (D. Jobes and A. Sailliol, oral communication, August 15, 2015).

Group-specific versus O– low-titer CWB

Transfusion of group O low titer CWB is an alternative solution to group-specific CWB, but despite being the standard of care for treating hemorrhagic shock up to and during the Vietnam War, it is currently not standard practice according to the AABB. Warm or cold group O low-titer WB is a potentially accepted alternative during combat operations in a few countries, such as the United States, the United Kingdom, France, Australia, and Norway.[50] The reluctance to adopt group O low-titer WB is difficult to understand. Hundreds of thousands of units of this product were used during both World Wars, the Korean War, and the war in Vietnam.[51-53] The threshold for low titer set by the US military during the Korean War was less than 256.[9] A report by Nessen and coworkers[54] indicated that, at US military forward surgical bases, in a subset of patients, the use of nontitered group O WB was associated with improved outcomes when compared to RBCs and plasma alone.

In addition to decades of use with few reports of adverse complications,[55] there are multiple reasons to consider that group O low-titer CWB is safer than group-specific CWB. First, the risk of hemolysis caused by the transfusion of group O CWB to a non-group O recipient is likely to be low as many adult patients, especially oncology patients, routinely receive ABO minor-mismatched PLT transfusions without experiencing acute hemolysis, albeit usually in lower doses and over longer periods of time. The risk of ABO plasma incompatibility with group O CWB or with the use of ABO-mismatched PLTs is typically a mild to moderate hemolytic reaction, with an incidence of only 1:120,000 transfusions of minor-incompatible PLT units according to the UK SHOT hemovigilance database.[46] The risk of hemolysis from the plasma of a group O CWB unit is likely to be even lower than the risk cited above, as group O CWB donors can be selected to have low titers of anti-A and anti-B, a selection not routinely performed on most other donors. Also, massively bleeding patients are likely to receive numerous group O units of RBCs, thereby further reducing the likelihood of hemolysis from the plasma in the group O CWB. It is important to recognize that the amount of plasma in an apheresis unit of PLTs, or a typical adult-sized pool of WB-derived PLTs, is similar to the amount of plasma in a unit of WB. In contrast to the typically nonfatal risk posed by incompatible plasma, ABO group–specific transfusion of RBCs or WB carries a higher risk (1:80,000) of often-fatal ABO-incompatible hemolytic reactions, largely due to human error in matching donor and recipient

appropriately.[46] Thus, the safety gain of using group O CWB is directly proportionate to the risk of an ABO-incompatible RBC transfusion, especially seeing as the risk of a human error leading to an ABO mismatch might be higher in the chaotic circumstances surrounding massive transfusion protocol activation in a severely bleeding patient.

In summary, our current standard practice of requiring WB to be ABO identical potentially puts patients at higher risk of severe transfusion reactions compared to the use of low-titer group O CWB. Furthermore, in a recent review of the plasma transfusion practices at mostly Level I trauma centers in the United States, 69% reported using group A plasma in trauma resuscitation when the recipient's ABO group is unknown, and the majority neither titered the anti-B in the plasma nor limited the amount that could be infused.[56] The concerns over hemolysis due to minor ABO plasma incompatibilities have been judged to be of limited relevance at civilian trauma hospitals.

Given the prevalence of group O donors, group O low-titer CWB is a viable alternative to group-specific WB, but to date it continues to be largely unavailable. Implementation of group O LTWB would require evaluation of current methods for determining titers, since multiple assays are available, and evidence-based standards are not available. In addition, a commonly accepted, data-driven threshold that defines "low-titer" anti-A and anti-B IgM and IgG is needed to replace the relatively arbitrary values used in previous military conflicts. It is also not known if low titers persist in donors, permitting classification as low-titer donors without requiring testing before each blood donation. In the report by Nessen and colleagues described, the authors also found that use of untitered group O WB was not associated with increased adverse events. However, with the exception of emergent cases or very austere environments, few would advocate this strategy given the small sample size and the relatively low cost of establishing titers.

PLT-sparing LR filters

Classically, LR filters also removed PLTs. As a result, WB in the past has not been leukoreduced since part of the benefit of WB was its PLT content. The FDA recently approved a WB LR filter that is PLT-sparing, paving the way for greater availability of leukoreduced WB as a PLT-containing product. The Terumo BCT IMUFLEX WB-SP set is an FDA-approved and CE-marked (for use within the European Union) blood bag system containing a PLT-sparing, WB LR filter. It was approved by the FDA in 2006 and is currently in use in the United States by the American Red Cross for WB supplied to centers in the Philadelphia region. It has also been available since 2000 in other countries including Germany, Greece, Italy, Norway, Spain and Sweden. Furthermore, it is the only WB LR filter licensed that is PLT sparing.[57] The IMUFLEX WB-SP set is currently configured for the LR of WB that is then to be separated into blood components. The US Air Force has funded an ongoing project to reconfigure the IMUFLEX WB-SP set so that the leukoreduced product is WB and not components derived from WB. This will improve its functionality and ease of use for WB that is either stored or to be used immediately in the field.

Hemostatic function of PLTs in WB stored between 2 and 6°C

Another concern is that the PLTs in WB stored between 2 and 6°C are not functional or viable, thus calling into question whether WB is truly a PLT-containing product. This misconception stems from the assumption that since cold PLTs have undergone shape change to become spherical and are more rapidly cleared from circulation than room temperature–stored PLTs, they must be nonviable and nonfunctional. The opposite is true. Cold-stored PLTs aggregate better than those stored between 20 and 24°C and produce stronger clots.[32-40] Most trials comparing a cold PLT-containing blood product to warm-stored products have indicated improved PLT aggregation, improved clotting times, and less blood loss.[26,40] The FDA in 2015 announced the approval of alternative storage for apheresis PLTs under 21 CFR 640.120 Alternatives and Exceptions, commonly known as a variance. It states: "Alternative procedure approved per 21 CFR 640.120 5. 21 CFR 606.65(e) & 610.53(c) To store apheresis PLTs at refrigerator temperature (1-6 C) without agitation for up to 3 days. The cold stored PLTs will only be used in the resuscitation of actively bleeding patients. The new storage conditions will be reflected in Circular of Information."

While the standard approach for WB has been to limit its storage to 48 to 72 hours due to concerns regarding PLT function, a few centers in the United States have increased storage duration past this point. The Children's Hospital of Philadelphia transfuses leukoreduced, ABO-identical WB stored for up to 7 days to children with significant bleeding secondary to craniofacial surgery. The University of Pittsburgh Medical Center Trauma Program is using uncrossmatched, leukoreduced, low-titer (<100) group O WB stored at 4°C for up to 10 days for severely bleeding patients upon admission. The Mayo Clinic Trauma Program is planning to use nonleukoreduced, low-titer group O WB stored between 1 and 6°C for up to 14 days for severely bleeding patients in the hospital setting and may use it in the prehospital phase of care in the future. Data from these trauma centers will supplement in vitro studies completed by the US Army and Norwegian Navy that demonstrated adequate preservation of hemostatic function over 2 weeks of storage.[33,58] Additional safety data in massively transfused patients are needed to

determine if the use of cold-stored low-titer group O WB affects outcomes.

Efficacy and safety of WB compared to components

The human and in vitro data evaluating the efficacy of WB has been summarized in the sections on CWB and warm whole blood (WWB) data. The belief that there is no benefit to WB compared to components is not based on data, but rather rooted in decades-old theory that patients with severe bleeding would not benefit from early administration of PLTs or PLT-containing products, such as WB. This, in combination with the idea that CWB does not contain functional PLTs, strengthened the misperception that there is no potential increased efficacy with CWB. Current in vitro data in combination with the limited in vivo data on CWB have led to a reconsideration of whether it would be more efficacious than the use of blood components for patients with life-threatening bleeding. Furthermore, the convenience of administering only one product, particularly in the prehospital setting, has made CWB very attractive to military and civilian trauma system directors.

Biologic risks of leukoreduced CWB theoretically include thrombosis due to PLT activation and immunologic consequences secondary to white blood cells (WBCs). Experiments have previously concluded that PLTs stored at 4°C became activated based on irreversible shape change. As a result, there was concern that transfusion of WB stored at 4°C would increase the risk of thrombosis. In trials of either WB or PLT units stored at 4°C, there has not been an increased risk of thrombotic events. Two trials that compared component blood products to WB in children requiring cardiac surgery did not reveal any increased risk of adverse events.[27] In a trial of adults randomized to receive PLT units at 4 or 22°C, there was also no increased risk of adverse events.[40] No increase in thrombosis has been observed with the clinical use of CWB at children's hospitals where it is frequently transfused in children who require small, 3.5-mm-diameter gortex aortopulmonary shunts that already present a high risk of thrombosis.[59]

Recent in vitro studies suggest that cold storage of PLT-containing blood products primes PLTs for activation, but that these PLTs still respond to inhibitory signals produced by the endothelium. Under static and flow conditions, PLTs stored at 4°C compared to the control group of freshly drawn unstored PLTs displayed similar ability to respond to control signals like nitric oxide and prostacyclin as measured by PLT aggregometry, adhesion to collagen in microfluidic flow cells, and viscoelastic measures.[60]

WB is generally stored without agitation, but it is unknown if agitation is required to reduce PLT clumping and adherence to bag surfaces. In addition, if agitation is required it is not known which method of agitation (manual, end-over-end, or horizontal agitation) is optimal. Investigations are ongoing to determine optimal WB storage and handling protocols.

Finally, studies are also needed to determine if LR is necessary to improve outcomes in this patient population. While it is standard of practice in most developed countries, the evidence that it is associated with improved outcomes is controversial. One RCT in 268 trauma patients reported that LR of RBCs was not associated with a reduction in acute lung injury.[61]

RATIONALE FOR WB

There are sound biologic, logistic, and economic rationales support the use of WB rather than components for patients with severe life-threatening hemorrhagic shock. Biologically, WB provides a balanced amount of RBCs, plasma, and PLTs, as well as an increased concentration of cellular components and improved function compared with stored components in a 1:1:1 unit ratio.[43,62] Each of the separate component units, RBCs, plasma, and PLTs, contains a considerably increased amount of anticoagulants and additives that will contribute to dilutional coagulopathy compared with 1 unit of WFWB or CWB.[43] These characteristics of WB are important since patients with severe life-threatening bleeding are at high risk of shock and coagulopathy. Shock and coagulopathy potentiate each other leading to organ failure and death if not rapidly reversed. Therefore, a resuscitative product that provides a balanced approach for preventing or treating both shock and coagulopathy is essential. Mitigation of oxygen debt accumulation not only prevents adverse effects of hypoxia, but also prevents the exacerbation of coagulopathy.[2] WB meets this need since it has a 30% higher oxygen-carrying capacity than components in 1:1:1 ratio.[63] The quality of the RBCs, plasma, and PLTs provided for patients in hemorrhagic shock is also important. This highly vulnerable patient population requires a resuscitative approach that is highly efficacious at improving oxygen delivery to treat shock as well as hemostatic to prevent or treat coagulopathy-related bleeding. The safety of the resuscitative solution is also important. The adverse effects of resuscitation-induced dysregulation of immune, coagulation, and endothelial function can obviate potential benefits.

Other potential advantages of WB compared to the use of components are that if the WB is limited to 10 to 15 days of storage between 1 and 6°C, the RBC storage lesion will be less developed, and PLT hemostatic function may actually be improved compared to PLT units stored at 20 to 24°C. It is apparent that RBC quality is not directly related to storage duration based on the results of the ARIPI, RECESS, and ABLE studies that did not reveal improved outcomes for critically ill premature neonates or adults who received fresh RBCs compared to older RBC

units.[64-66] These trials did not typically include patients who required large amounts of blood products and did not include a significant number of patients with traumatic injury, who may be most vulnerable to storage lesion effects.[67] In addition, due to donor variability in RBC quality over storage time, RBC age may not be the best surrogate for RBC quality. Studies are needed to determine which RBC quality measures are accurate surrogates for efficacy regarding oxygen delivery. In addition, the above trials did not specifically examine the effects of RBC storage age at the end of expiration (28-42 days of storage). Patients who require massive transfusion protocol activation due to life-threatening bleeding are often exposed to large volumes of RBCs at the end of expiration and as a result are exposed to RBCs with reduced efficacy and safety. These patients are also transfused PLTs stored at 22°C, which might have less hemostatic capacity than the PLTs in WB stored at 4°C.[68]

The use of WB exposes the recipient to one donor compared to three for reconstituted WB with RBC, FFP, and PLT units, while providing a more concentrated and perhaps functional and safer product. Reduction of exposure to additional donors is important to the massively transfused patient. The reduction of donor exposures with the use of WB compared to components has been documented by the cardiac program at the Children's Hospital of Philadelphia in a study of over 4000 patients during a 15-year period.[59]

As suggested in studies of blood component products, residual WBCs in WB may cause some degree of immunosuppression in the transfusion recipient, although this may also be mediated by cellular microparticles generated over time with storage. Direct clinical effect of residual WBCs or cellular microparticles on immune function has not been well studied. One trial examined inflammatory effects of WB compared to the use of RBCs and plasma units to prime cardiopulmonary bypass circuits in children. It reported that measures of inflammation were similar between the two study groups except for an increase in lipopolysaccharide-binding protein in the CWB group at 48 and 72 hours.[27] Microparticle content is similar in WB stored at 4 versus 22°C. Therefore, if microparticles have any clinical effect, the risks are potentially similar between CWB and PLT units stored at 22°C. The clinical relevance of the effects of microparticles from all cell lines in blood products requires further study.

Viable WBC in blood products can cause transfusion-associated graft-versus-host disease (TA-GVHD). TA-GVHD is a very rare and almost always fatal condition that occurs due to engraftment of donor WBCs into the recipient. The primary risk factor for TA-GVHD in noniatrogenically immunosuppressed recipients is transfusion of viable donor lymphocytes that share common antigens with host cells at either HLA Class I or II loci. Most cases of TA-GVHD have occurred when blood products were

transfused within 10 days of collection. LR may reduce, but not completely eliminate the risk of TA-GVHD; only gamma irradiation is licensed for inactivation of donor lymphocytes and TA-GVHD prevention.[69] Microchimerism is low-level (<5%) engraftment of donor lymphocytes or peripheral blood progenitor cells into the recipient. The clinical consequences of microchimerism are unknown, but the condition may persist for decades. The risk of TA-GVHD and microchimerism is related to the presence of viable WBCs in vulnerable recipients, particularly those suffering from traumatic injury. The risk of microchimerism developing with WB is similar to those with LR-RBCs stored for less than 10 to 15 days.

Risks with WWB are increased risk of infection and an immunogenic response. Increased risk of TTDs occurs in austere settings when there is not adequate time or infrastructure to perform the standard testing for infectious agents. One study calculated the risk of TTD transmission in a military setting where US personnel were donors and were not screened with rapid tests for TTDs. In this setting there was a 1/800 rate of HCV transmission in recipients.[70] With 10,238 units of WWB transfused to patients treated at US Military hospitals since 2001, there has been one case of HTLV and one case of HCV transmission (LTC Audra Taylor, Director, US Army Blood Program, personal communication, December 1, 2015). To mitigate these risks, rapid screening tests for HCV, HBV, and HIV can be used to screen potential donors. Donors are also vaccinated against HBV, which would prevent HBV infection in the donor and reduce risks for future WB recipients. US Military donors are screened for HIV every 2 years and immediately before deployment. Registered donors in military walking blood banks are currently fully prescreened according to standard donor protocols and undergo repeat testing every 90 days during deployment. In the French Army, to mitigate these risks, before deployment overseas, all military personnel are vaccinated against HBV and all volunteer WB donors are selected with clinical and biologic tests. In the field, rapid screening tests for HCV, HBV, and HIV are used to screen WB collected, and samples are send to the French Military Blood Transfusion Center to perform all the regular serologic and nucleic acid testing required.

Currently, PLT-sparing LR filters have not been implemented in military settings for WWB transfusion. Since WWB transfusion is generally an emergency procedure undertaken in the face of exsanguinating hemorrhage, the modest benefits of LR have been deemed insufficient to warrant the time delays in WB unit preparation, logistic requirements, and cost when collection is required immediately before transfusion. Whether LR can or should be accomplished in the military context for WB that is collected and then stored is currently controversial. It is unknown if the potential risks of residual WBCs present in nonleukoreduced WB are different for WWB that is

transfused immediately compared to CWB that is stored. The clinical relevance of LR is controversial due to conflicting reports regarding its effect on outcomes in critically ill populations. Aside from reducing febrile reactions, CMV transmission, and HLA sensitization, LR has not consistently been associated with improved morbidity or mortality.[71] Furthermore, in these studies the blood products that were not leukoreduced were stored for a period of time. There may be a different biologic effect of transfusing a blood product with normal amounts of WBCs that has been stored for weeks compared to one that is transfused immediately. The fresh WBCs in WFWB may or may not be harmful. They may even be helpful in patients who are in an immune-deficient state immediately after traumatic injury or due to other critical illnesses. Additional research is needed in this area to thoroughly understand the benefits of LR in WB that is transfused immediately compared to WB that is stored.

The risk of both TTD transmission and immunologic challenges posed by viable donor WBCs (such as TA-GVHD) may be substantially mitigated by development of the Mirasol Pathogen Reduction Technology for WB (Terumo BCT, Lakewood, CO), which is based on the photochemical degradation of nucleic acids by riboflavin and UV light. Several pathogen reduction technologies prevent WBC replication and thus inactivate WBCs, although the Mirasol technology is the only one demonstrated to be effective in WB.[72] The importance of WBC inactivation may be different for WWB compared to stored CWB and requires further study.[73]

Logistic benefits

According to a recent survey of 132 trauma centers, blood products are used for prehospital resuscitation by only 34% of first responders.[74] A smaller minority is carrying both RBCs and plasma, and very few, if any, ever carry PLTs, although multiple studies demonstrate that they are important for hemostasis. The use of blood products to resuscitate patients in the prehospital phase has the potential to reduce the large risk of death from hemorrhagic shock before hospital admission. Military data indicate that 90% of potentially survivable deaths occur from hemorrhagic shock.[75] Improved hemorrhage control and early blood product use are required to reduce this toll. The logistic constraints of transporting RBCs, plasma, and PLTs in the prehospital phase are considerable. In addition to the extra weight and complexity of transfusing product from multiple bags, intravenous or intraosseous catheters have a limited number of access ports. The use of WB stored at 4°C for less than 10 to 14 days would dramatically reduce the logistic burden compared to the current approach.

Costs and benefits

WB is very likely to be cost-effective from a patient and hospital perspective compared to the use of blood components for life-threatening bleeding. A formal cost-effectiveness analysis has not been performed, but when the following are considered it very well may be associated with reduced costs while at the very least maintaining equal efficacy if not improving it. A transition to the use of WB would include reduced costs associated with the fractionation of WB, including both equipment-related and staffing costs. If the shelf life of CWB was limited to 15 days this would virtually triple the inventory of PLT-containing products for patients who require them for severe bleeding. Contrary to the widely made assumption that the vast majority of PLT units, perhaps 80%, are transfused prophylactically to cancer patients with thrombocytopenia, the most recent survey of blood use in the United States, conducted in 2011 by the Department of Health and Human Services, demonstrated that 25% of PLT units are transfused for surgical or trauma patients, with an additional 12% consumed in the ICU setting. This clearly indicates that a substantial proportion of hospitalized patients require PLTs for acute bleeding and are therefore likely to require not only PLTs but also RBCs and plasma—all of which could be conveniently supplied by WB. The improved hemostatic function of PLTs in CWB may also reduce costs compared to the standard PLTs stored at 22°C. Reduced bleeding and donor exposure will improve safety and costs. The use of CWB instead of PLTs at 22°C would also eliminate the cost of bacterial testing required for products stored at room temperature. The additional costs associated with the use of CWB are the costs of performing anti-A/B titers and the cost of lost revenue if the WB unit expires. While RBCs might be recoverable from a WB unit stored between 1 to 6°C, plasma and PLTs from that unit are not suitable for further separation and storage. The quality of recovered RBCs should be evaluated thoroughly in clinical studies.

Presuming that at the very least WB is just as efficacious as blood components, the presumed net reduced costs associated with its use in all patients with hemorrhagic shock are substantial and may in and of itself justify the adoption of this product for this patient population. Formal cost-effectiveness studies are warranted to quantify the economic impact of transitioning to a CWB transfusion strategy for all patients with hemorrhagic shock.

SUMMARY

In summary, for all patients with hemorrhagic shock, current blood component products may not be optimal. CWB is an underutilized potential source of hemostatically functional PLTs, plasma, and RBCs that may be more

efficacious, safe, and logistically feasible and may have the potential to positively affect the economics of critical care medicine.

CONFLICT OF INTEREST

MY, PS, HP, PN, and TH, TerumoBCT. All other authors have disclosed no conflicts of interest.

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