Society of Cardiovascular Anesthesiologists

Cardiovascular Anesthesiology Section Editor: **Charles W. Hogue, Jr.**
Perioperative Echocardiography and Cardiovascular Education Section Editor: **Martin J. London**
Hemostasis and Transfusion Medicine Section Editor: **Jerrold H. Levy**

SPECIAL ARTICLE

CME

Fresh Whole Blood Use for Hemorrhagic Shock: Preserving Benefit While Avoiding Complications

Philip C. Spinella, MD,* Heather L. Reddy, PhD,† Jennifer S. Jaffe, MPH,* Andrew P. Cap, MD, PhD, FACP,‡ and Raymond P. Goodrich, PhD†

Transfusion support of patients with hemorrhagic shock has changed over time with the development of storage and processing methods. Transfusion medicine developed during World War I with the use of whole blood, and now in the developed world, component therapy predominates. In contrast, there is still clinical use of fresh whole blood (FWB) in the developing world, in a minority of children's hospitals, and in combat settings. Although there is a rationale for the use of FWB in massively bleeding patients compared with the use of individual components, it has rarely been analyzed in prospective randomized clinical trials. Recent retrospective studies in adult trauma and mixed critically ill patients have revived this decades-old controversial question of the value of FWB for patients with severe shock and coagulopathy or those at risk. The risks of FWB use have also been highlighted recently, which has caused some to focus on reducing these risks with alternative processing and storage methods. It is important to recognize that current processing and storage methods for components have also not been adequately explored to determine whether they affect clinical outcomes. In this article, we review potential benefits and risks of FWB use for patients with hemorrhagic shock from any cause, and how current and future processing and storage methods may affect efficacy and safety of FWB in this population. We intend this review to stimulate hypothesis generation and clinical investigation in determining when FWB may be indicated and how to optimally process and store FWB to maximize its risk-benefit ratio. (Anesth Analg 2012;115:751–8)

Component therapy has replaced whole blood (WB) as the standard in most environments because of well-demonstrated availability of individual products for patients with specific deficits (anemia, thrombocytopenia, coagulation factor deficiency), and improved blood bank economics.[1–3] However, component usage in austere settings (developing world and combat) is often limited by storage requirements for blood products (refrigeration for red blood cells [RBCs], room temperature with agitation for platelets [PLTs], and frozen for plasma products).[4–6] When these storage technologies are unavailable, fresh whole blood (FWB) becomes the default option. WB also continues to be used in the developed world in certain pediatric populations because of the belief that it is superior to components for hemorrhagic shock.[7,8] The definition of FWB has varied in the literature and depends mainly on the storage duration and temperature. The United States (US) military defines WB as fresh when it is used within 24 hours of collection.[9] Current US military clinical practice guidelines state that "FWB will have a shelf-life of 24-hours and should be stored at 1–6° within 8-hours after collection, unless otherwise directed by medical staff due to insufficient or no red blood cell (RBC) or plasma product inventory" (Ref. 1 in Table 1). Most civilian institutions define it as fresh if it is stored for <48 hours at 2°C to 6°C.[10] We will refer to each as either warm or cold FWB, respectively, in this review for clarity. There are additional differences between warm and cold stored FWB other than the duration and temperature of storage. The effect of storage temperature on PLT activation and survival and recovery was first documented by Murphy and Gardner.[11] Room temperature storage of PLTs was associated with improved recovery and survival compared with cold storage, whereas cold stored PLTs were noted to have increased markers of activation.[11] Another major difference between warm and cold FWB is that when it is transfused warm for emergency use in combat scenarios, complete transfusion-transmitted disease screening is not possible, whereas when stored cold, it can be fully tested before use and it is collected in a manner that meets Food and Drug Administration (FDA) standards.

From the *Department of Pediatrics, Washington University in St. Louis, St. Louis, Missouri; †Terumo BCT Biotechnologies, LLC, formerly known as Caridian BCT Biotechnologies, LLC, Lakewood, Colorado; and ‡U. S. Army Institute of Surgical Research, Fort Sam Houston, Texas.

Accepted for publication May 11, 2012.

Conflicts of Interest: See Disclosures at the end of the article.

Reprints will not be available from the authors.

Address correspondence to Philip C. Spinella, MD, Department of Pediatrics, Washington University in St. Louis, St. Louis Children's Hospital, Campus Box 8116, One Children's Place/NWT 10th fl., St. Louis, MO 63110. Address e-mail to spinella_p@kids.wustl.edu.

DOI: 10.1213/ANE.0b013e318261f40e

Table 1. Web Site References

Reference	URL
1	Joint Theater Trauma System Clinical Practice Guideline: Fresh Whole Blood Transfusion, 30 MAR 2011. Available at: http://www.usaisr.amedd.army.mil/assets/cpgs/Fresh_Whole_Blood_Transfusion_30_Mar_2011.pdf. Accessed February 23, 2012.
2	Brinsfield K. Reserve Donor Strategies. 2007. Available at: http://www.hhs.gov/ash/bloodsafety/presentations/brinsfield0807.pdf. Accessed August 20, 2011.
3	Early Whole Blood in Patients Requiring Transfusion After Major Trauma. 2011. Available at: http://clinicaltrials.gov/ct2/show/NCT01227005. Accessed May 11, 2011.
4	Cryer H. Transfusion of Stored Fresh Whole Blood in a Civilian Trauma Center: A Prospective Evaluation of Feasibility and Outcomes. 2011. Available at: http://www.nationaltraumainstitute.org/research/2011studies/cryer.html. Accessed February 23, 2012.

The transition over time in the developed world from WB to components occurred with very few clinical trials to determine which populations would or would not benefit from this change.[3] In fact, there have been many processing methods, to include not only component preparation from WB, but also irradiation, and washing, for example, that have also emerged over time without clinical trial evidence to allow for risk-benefit analyses within different patient populations.

The importance of determining risk-benefit ratios in different populations is highlighted in the "two-hit" hypothesis. Based on this concept, the risk-benefit ratio for blood product administration is dependent on the baseline physiologic state. For example, patients with critical illness are at increased risk of adverse effects from transfusion of blood products because they are already in a dysfunctional physiologic state and have minimal if any reserve to absorb any additional pathologic perturbations.[3] Unfortunately, because there is no standard approach to quantify severity of illness, or thorough understanding of which of the many aspects of critical illness (immune, endothelial, coagulation, vasoregulation dysfunction, etc.) affects this relationship, it is very difficult to systematically analyze the risk-benefit ratio of blood products in these patients. Recognizing that there are genomic influences that affect this relationship also further confounds the determination of the risk-benefit ratio of blood products, especially in patients with hemorrhagic shock. One approach to differentiating risk-benefit ratios among distinct cohorts is to perform clinical trials within each of them. This would be difficult with the standard approach to clinical trial design but would be more feasible with a comparative effectiveness design.[12]

Compared with elective surgery patients requiring transfusion or those requiring chronic transfusions, critically ill patients who are in a state of shock and coagulopathy (e.g., hemorrhagic shock) are in a precarious state regarding the effects of transfusion.[3,13] These patients require rapid resolution of shock and coagulopathy to prevent morbidity and mortality, and they are also at highest risk for complications of transfusion.[3] With increased storage duration, oxygen delivery capability and PLT function decrease and the generation of immunomodulating microparticles increases in both RBC and PLT concentrates. Classically, it has been presumed that these storage lesion effects were not clinically relevant and would not affect clinical outcomes. It is currently unknown whether storage duration of RBCs and PLTs affects outcomes. The overwhelming weight of biologic evidence suggests that RBC and PLT storage age affect immune, vasoregulatory, and coagulation systems. An intense scientific effort is underway and seeks to determine the magnitude and clinical significance of these deleterious changes.[3,14,15]

Ironically, because of allocation strategies and the need to minimize blood product waste, critically ill patients are at increased risk of being exposed to blood products of increased storage age. This occurs because of the common practice of transferring older RBCs (approaching the expiration date) from community hospitals to medical centers.[16] This allows blood near expiration to be used and not wasted. In addition, this standard policy of allocating the oldest RBC unit in inventory increases the risk of exposure to RBCs of increased storage duration to the sickest patients because they require the most blood.[17] This standard blood use practice in effect shuttles older blood products with known reduced efficacy and safety to the patients who presumably require the most efficient and safest product. The availability of FWB could potentially improve efficacy in critically ill patients. The safety of FWB compared with stored components is much less clear. Whereas FWB seems to be safer in some respects, it may not be in others.

It is essential that future research focus on developing optimal blood products with the highest benefit-to-risk ratio for patients with hemorrhagic shock from any cause (traumatic, gastrointestinal, obstetric, postoperative, etc.). Our review focuses on the potential risks and benefits of FWB for hemorrhagic shock and then explores processing methods that may reduce these risks. In addition, we discuss the value of developing trials comparing FWB with components in populations at high risk for hemorrhagic shock.

BIOLOGIC RATIONALE FOR FWB WITH MASSIVE BLEEDING

There are many biologic and theoretical reasons that support the use of warm FWB rather than components for patients with severe life-threatening hemorrhagic shock.[1,18–21] FWB 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.[21,22] Each of the separate component units, RBCs, plasma, and PLTs, contains a considerable increased amount of anticoagulants and additives that will contribute to a dilutional coagulopathy compared with 1 U of FWB.[21] Other potential advantages of FWB are that it provides fresh (not stored) fully functional hemoglobin, coagulation factors, and PLTs for patients at high risk of

mortality from hemorrhagic shock. The RBC, PLT, and plasma quality of FWB is higher than stored components.[18] For example, microcirculatory flow and the ability to increase oxygen use decreases with RBC storage,[23–27] and stored PLTs demonstrate decreased hemostatic function because of a decrease in expression of high-affinity thrombin receptors during storage.[18,28] The use of FWB decreases the use of stored components of increased storage duration, which have been associated with increased risk of organ failure and death in critically ill patients.[29–33] Additional potential advantages of warm FWB for massively bleeding patients are reduced risk of hypothermia and hyperkalemia, limited impact of processing on function, and reduced donor number exposure.[3,9,18] Clinically, FWB has been demonstrated to reverse dilution coagulopathy, and there is evidence that one single warm FWB unit has hemostatic effect similar to 10 U of PLT concentrates.[18,34]

CURRENT CLINICAL DATA AND USE OF FWB

Clinical data comparing FWB with components have been published in both military and nonmilitary settings. FWB has been transfused by the US Military in every conflict since World War I and its use continues to be taught at predeployment training sites. The current US Emergency War Manual's chapter on the use of FWB is largely based on the experience of its use in Somalia in 1993.[35,36] Hemorrhage resulting from combat-related injuries is the most common cause of potentially preventable death in military operations.[37,38] Early shock and coagulopathy have been strongly associated with mortality in this population and are interrelated.[1,39,40] In an effort to rapidly reverse shock and coagulopathy and death from hemorrhage, FWB is often used in combat settings by the US military. In fact, the development of a 1:1:1 unit strategy of providing components to massively bleeding combat trauma patients early in the war in Iraq was based on attempting to approximate the use of FWB.[2] Many retrospective studies have since indicated that increased (>1:2) plasma-to-RBC and PLT-to-RBC ratios are independently associated with improved outcomes.[41] Current US military doctrine allows for FWB use in emergency, life-threatening scenarios in combat zones when tested, stored blood components are unavailable or the patient is not responding to stored components.[42] More than 8000 U of FWB have been transfused in the injured in both Iraq and Afghanistan since 2001.[42] Recent retrospective analyses of partial FWB use compared with stored components indicate that it is independently associated with improved survival when analyzed in US casualties from Iraq and Afghanistan.[21] One analysis that included US casualties only reported improved adjusted 30-day survival for patients with severe traumatic injuries transfused partially with FWB compared with patients transfused with blood components with similar severity of injury.[21] However, it is limited by its retrospective nature, inability to adjust for all potential confounders, and survivorship bias. Another recent report[5] comparing partial FWB use with components reported improved adjusted 24-hour survival using FWB that approached significance but did not indicate any difference in outcomes at 30 days. Because this study included non-US personnel, it was limited by a significant loss (approximately 33%) of patients before 30 days. A very recent study from Australia by Ho and Leonard[43] indicated that the use of FWB in a mixed population of cardiac surgery, trauma, and others was not associated with improved long-term outcomes. It is essential to note that in all of these retrospective FWB studies, none of the patients received only FWB and the proportions of FWB to all blood given varied. In addition, a survival advantage was associated with FWB when 30% of all blood was in the form of FWB,[21] whereas the use of FWB in negative reports ranged between 10% and 20% of all blood products transfused.[5,43]

The strongest evidence analyzing the clinical effects of FWB (warm and cold) to stored components is a randomized controlled trial (RCT) in children requiring cardiac surgery by Manno et al.[10] This study indicated that FWB (warm or cold) exhibited both improved PLT function and decreased bleeding compared with components.[10] The RCT by Manno et al.[10] was not completely randomized, because randomization to the FWB group was dependent on the availability of an FWB donor. Nevertheless, Manno et al.[10] concluded in an adjusted analysis that the transfusion of FWB significantly decreased postoperative blood loss compared with the transfusion of packed RBCs, fresh frozen plasma, and PLTs in children younger than 2 years old who underwent cardiac surgery. Contradictory evidence was published from another RCT, in children requiring bypass for cardiac surgery, which showed no differences in their composite primary outcome, but did report increased intensive care unit (ICU) length of stay (LOS) for children transfused with FWB.[44] In this study, ICU LOS was a secondary unadjusted outcome and FWB was only given intraoperatively and not postoperatively in the ICU, limiting ICU LOS validity as an outcome.

Warm and cold FWB are also used frequently for transfusion in austere settings and in the nondeveloped world.[42] At a recent symposium focused on FWB, Dr. J. P. Allain explained that the use of FWB in sub-Saharan Africa is not due to lack of technical means or higher costs but due to the concept that FWB is the product of choice for massive bleeding and acute primary malaria.[8] It is clear that there will always be a need for FWB in austere combat situations and in the developing world. Questions remain regarding the need for FWB in the developed world for nonmilitary use to include large civilian disasters where stored component therapy has been exhausted[19] (Ref. 2 in Table 1). Prospective RCTs are still needed to determine whether FWB improves outcomes for trauma or other patients with severe life-threatening hemorrhagic shock and additional large multicenter studies are needed in pediatric cardiac surgery patients that are powered to analyze morbidity and mortality. Whereas it is possible to design trials with stored FWB, it will be extremely difficult to perform RCTs of warm FWB in military and developed world locations. Warm FWB trials may be possible in the developing world but their external validity to the developed world will be limited. We are aware of 2 ongoing trials of WB. One is a clinical trial that has started in Houston, TX to determine whether the combination of stored WB and PLTs compared with component therapy can reduce the volume of blood transfused for patients with severe traumatic injury (Ref. 3

in Table 1). The other is a feasibility and outcomes study in Los Angeles, CA that will compare stored WB to components in severe trauma patients (Ref. 4 in Table 1).

FWB transfusions are also used regularly in countries such as Japan and Israel.[45] In addition, a recent survey of transfusion practices in the US and Canada revealed that 15% of children's hospitals use cold FWB for patients with massive blood requirements such as those who require cardiac, liver transplant, or spinal fusion surgery.[7] Although it is not frequently available in the US, WB can be provided by blood collection centers because it is an FDA-approved product. It is not typically made available because there is a general view that WB is not necessary and that for it to be beneficial it must not be stored for >48 hours because PLTs stored at 2°C to 6°C will not remain in circulation.[2] Unfortunately, there are no high-quality clinical data supporting this belief. It is possible that WB could be stored at 4°C for up to 14 days and still have adequate hemostatic function in bleeding patients.[46–50] If clinical studies indicate improved outcomes with the use of FWB for patients with severe hemorrhagic shock, logistic systems will need to improve to allow for increased FWB availability.

PROCESSING AND STORAGE EFFECTS

The effect of FWB storage temperature and duration on efficacy in vitro is contradictory. Nilsson et al.[51] showed good stability of coagulation proteins in WB during 4°C storage. Fibrinogen and plasminogen activities did not change during 4°C storage, and FII, FVII, FIX, FX, FXII, and FXIII remained above the lower normal limit even after 35 days at 4°C. The most labile factors, FV and FVIII, remained above 30% after prolonged storage.[47,51] Interestingly, it is unknown if this frequently used threshold of 30% coagulation factor function is appropriate to apply to patients with traumatic hemorrhage because it was established in patients with congenital coagulation factor deficiencies.[52–54] In a recent study of properties of refrigerated WB stored for 31 days, Jobes et al.[47] demonstrated preservation of normal integrated coagulation function to a minimum of 11 days and up to 14 days with FWB collections with TEG® and light transmission PLT aggregometry. However, according to the other reports, the most labile factors V and VIII have reduced activity within 12 to 18 hours and PLT function decreases within 5 hours of storage at 4°C compared with warm FWB.[55] Hughes et al.[56] evaluated protein stability of FWB at room temperature for 72 hours and showed significant reduction in pH and 2.3 diphosphoglycerate, but modest reduction in PLT function and plasma coagulation factor activity and no bacterial growth. Preliminary in vitro data by Pidcoke and Cap at the US Army Institute of Surgical Research indicate that PLT function measured by TEG® and multiplate impedence WB aggregometry is retained in WB stored at 4°C despite a reduction in coagulation factors for up to 14 days.[46–50] These in vitro findings open the possibility for an extended availability of WB. Although WB can be stored for up to 21 days at 4°C, the classic belief that PLT function is reduced after 48 hours has precluded any use of WB past 48 hours in the developed world. The potential benefit of increased availability of WB is its ability to restore oxygen delivery and hemostasis in patients with hemorrhagic shock without exacerbating hemodilution and exposing these vulnerable patients to potential adverse consequences of the storage lesion of RBCs stored for more than 14 to 21 days and PLTs stored at room temperature.[3]

The standard practice of storing PLTs at room temperature for transfusion to all patients including those with massive bleeding has also recently come into question. The decision to store PLTs at room temperature in the 1970s was based on improved survival and recovery compared with cold storage at lower temperatures, but cold stored PLTs were noted to have improved function according to PLT activation variables.[11] It is unknown whether a massively bleeding patient would benefit from an activated PLT that is more rapidly removed from the circulation than one that is less responsive but remains in the circulation. In fact, data from an RCT in postoperative cardiac surgery in children indicate that both warm and cold FWB reduced postoperative bleeding because of improved PLT function compared with PLTs stored for 72 hours or longer at 20°C to 24°C.[10] These clinical data contradict some of the above-referenced in vitro data indicating superior function with warm compared with cold FWB and PLT concentrates.

Typically, FWB is not leukoreduced because it is being given in part to support hemostasis, and until recently, all leukoreduction filters also removed PLTs. There are now a few PLT-sparing leukoreduction filters that are available for clinical use that have been approved based on survival and recovery data.[57] The in vitro or in vivo function of PLTs filtered by these leukoreduction devices has not been examined. This is another area of research that needs attention.

There are also different processing methods for RBCs (WB derived, apheresis, buffy coat) and plasma (from WB held up to 8 or to 24 hours, and apheresis) and there are no in vivo data that compare efficacy or noninfectious safety between these processing methods. There needs to be a thorough investigation using in vivo experiments that evaluate the optimal storage temperature, duration, storage solutions, and even plasticizers generated from the different types of storage bags used for FWB and blood components if we are going to analyze the safety and efficacy of all blood products. RBC efficacy should be determined by directly measuring oxygen consumption in the setting of shock, and with functional measures of hemostasis for PLTs. Because FDA guidelines do not require this level of data for blood products and do not have clearly defined criteria for efficacy and safety to be determined in vivo, it is difficult for investigators to design appropriate experiments. One novel approach that is being explored to determine in vivo efficacy of a blood product uses a model in which immune-deficient mice are transfused either human RBCs or PLTs.[26,27,58] This method and others in animals and humans need to be explored to establish a well-accepted approach to determining efficacy and safety of blood products.

RISKS ASSOCIATED WITH TRANSFUSION OF WARM FWB

The risk of severe hemolytic reactions from the transfusion of nonidentical ABO FWB is a concern, because in austere

settings there is often no time for standard ABO testing. In large combat hospital facilities, ABO typing is performed but this is not always possible in remote far-forward settings. All attempts in austere settings should be made to transfuse ABO-identical FWB if possible. The use of non-compatible ABO FWB can be used however in dire circumstances and is required often as was reported during the Vietnam War.[59] Risk analysis of this practice has been recently described.[7] In far-forward military settings, transfusion of ABO-compatible blood to combat wounded is facilitated by identification on the combatants, but this identification may be in error because it is self-reported and not based on actual ABO testing.[60] A rapid test of ABO type can help mitigate the risks in all austere settings, where standard blood bank ABO testing is unavailable. A recently developed rapid test, developed with funding from the US Army, is now available. The new Micronics ABO/Rh Card is a disposable, credit card–sized device that can accurately determine the ABO blood type and Rh factor from a single drop of blood in <30 seconds. It is the first device that does not require refrigeration or supporting equipment and that works in a closed system to protect the blood sample and reagents from environmental contamination.[61] The capability to perform rapid and accurate ABO testing will facilitate safer FWB transfusion in the developing world in addition to its potential use in civilian disasters.

In comparison to components available from standard blood bank collections, warm FWB collections have greater risk associated with the potential for pathogen transmission (range of 1/500 to 1/800 for hepatitis C virus)[20,62] and for white blood cell (WBC)-related complications. Because warm FWB is currently always given in remote settings, it is not possible for formal transfusion-transmitted disease testing to be performed before transfusion and as a result the transfusion of WB contaminated with pathogens can occur.[63] Whereas the risk of infectious disease transmission with FWB can be significantly reduced for the pathogens human immunodeficiency virus, hepatitis B virus, and hepatitis C virus with the use of rapid (20- to 30-minute) screening tests,[20] infectious pathogens for which no screening tests are available are also of concern. Donor screening questionnaires help to reduce the risk of exposure to other types of infectious pathogens, but the use of questionnaires can be impractical under the conditions in which warm FWB is usually needed. The risk of transmitting parasitic diseases because of local exposure in austere settings is also a concern with the use of FWB that currently cannot be mitigated.

Transfusion of high numbers of leukocytes has been shown to lead to a number of donor antirecipient responses, such as transfusion-associated graft versus host disease (TA-GvHD), transfusion-associated microchimerism, febrile nonhemolytic transfusion reactions, and induction of alloantibodies. Transfusion of nonleukoreduced FWB may be responsible for the association of FWB with renal failure and acute respiratory distress syndrome in recent retrospective reports.[8,23,26] TA-GvHD can be mitigated by γ-irradiation (current protocol is exposure to 25 Gy), but this is logistically impractical to perform in austere settings. Prestorage leukoreduction can be an effective tool for mitigation of some but not all leukocyte-associated risks.

The risks associated with pathogens could be mitigated by a pathogen reduction device. Currently, no such devices are approved by the FDA. Methods for the pathogen reduction of blood components (treated after separation from WB) are in use or in development. Pathogen reduction technologies for the treatment of plasma are approved and used in other parts of the world.[64] These technologies use different mechanisms for inactivation of pathogens (such as solvent detergent, methylene blue, amotosalen with UV light, and riboflavin with UV light). Treatment of cellular components is more complicated because of the potential for altering the function of the cells. Two of the devices used to treat plasma are also used to treat PLTs[64]: the Intercept Blood System (amotosalen and UV light, Cerus Corp., Concord, CA) and the Mirasol® Pathogen Reduction Technology (PRT) System (riboflavin and UV light, Terumo BCT, Inc., Lakewood, CO). These technologies also inactivate leukocytes[65–68] and mitigate the risk of TA-GvHD.[69,70]

For the treatment of erythrocytes, various technologies have been tested, with 2 technologies reaching phase III clinical studies. In both cases, the studies were halted because of observations of recipient antibodies against the treated RBCs. One of those technologies (Inactine) is no longer in development. The other technology (S-303) has been modified to increase the concentration of quencher and thereby reduce the membrane modifications due to S-303. The S-303 technology has been evaluated with recovery and survival studies in healthy volunteers.[71]

Treatment of WB with a pathogen reduction and leukocyte inactivation step would be the simplest method to use, both when considering the transfusion of WB and the preparation of components. The S-303 technology has been tested in vitro to assess its effectiveness in the treatment of WB.[72] Data for a limited number of replicates and a few viruses and bacteria are presented by Mufti et al.[72] Safety of the S-303 compound has been evaluated in studies of toxicity[73] with relevance to the treatment of RBCs (which are centrifuged after treatment to remove the S-303 solution and replace with fresh additive solution). Neoantigenicity of the S-303 is of concern, even at the low levels present after removal by washing in the red cell treatment system. After observations of immunogenicity in clinical trials in patients, the process for treating RBCs was modified to decrease the immunogenicity with an increase in the amount of glutathione and tested in RBC recovery and survival trials.[71] Removal of the S-303 from WB after treatment would need to be accomplished by use of special filtration or adsorption devices, because centrifugation and removal of the plasma from WB would change the nature of the blood product. Such methods have not yet been described and may still be in development.

Leukocyte inactivation and pathogen reduction have also been tested with the Mirasol System for Whole Blood. This system was adapted from the Mirasol PRT System, and uses the same illuminator, disposable kit, and solution. Similar to the Mirasol PRT System for Platelets and Plasma, the Mirasol System for Whole Blood uses riboflavin and UV light to reduce pathogens and inactivate leukocytes. This technology has been evaluated in vitro for blood quality

and WBC inactivation, as well as for virus, bacteria, and parasite reduction,[66,74,75] and in an early-phase in vivo study.[76]

The Mirasol System for Whole Blood has been tested for efficacy with respect to leukocyte inactivation and pathogen reduction. The WBC inactivation studies in previous publications provided in vitro data[77] that indicated that the device would be effective at preventing GvHD. Recent in vitro work[66] provides a comparison of γ-irradiation with Mirasol treatment. Treatment with the Mirasol System and treatment by γ-irradiation were similarly effective in reducing viable T cells in treated WB, and Mirasol treatment was more effective in suppressing antigen presentation, cellular activation, and cytokine secretion.

Pathogen reduction efficacy has been tested with an in vitro assay for *Trypanosoma cruzi*,[78] in which the level of reduction observed was ≥3.5 log and <4.5 log. Reduction of another parasite, *Babesia microti*, was 5.0 log when tested with an in vivo model.[74] Tests of virus reduction have been reported for model viruses and for bacteria that have been associated with transfusion-transmitted infections.[77] The reduction of nonenveloped and enveloped viruses ranged from 1 to 5 log and the reduction of low-titer spikes of bacteria (consistent with levels in donated blood) eliminated the growth of *Yersinia enterocolitica* and *Serratia liquefaciens*. The pathogen reduction levels observed for this device are expected to provide increased safety relative to disease transmission by reducing levels of infectious agents present in donated units.

Riboflavin is a vitamin, and is generally regarded as safe. It is not removed from WB or blood components after treatment. Safety of the WB treated with the Mirasol System has been assessed in preclinical studies (including tests of neoantigenicity), and is supported by the toxicology work done[79] to support PLT and plasma components treated with the Mirasol PRT System for Platelets and Plasma. Mirasol PRT-treated PLTs and plasma are in routine use in countries outside of the US.[64] Future preclinical testing of treated WB, beyond the FDA standards of survival and recovery, will evaluate the efficacy of treated FWB to increase oxygen use and clot formation in vivo. Future clinical studies of treated WB will involve tests of the efficacy of treated components derived from the treated WB, as well as safety and efficacy of transfused, treated WB itself. Clinical studies of treated WB will provide additional evidence regarding the safety and efficacy of untreated WB, which would be the appropriate control blood product for such studies.

CONCLUSION

FWB continues to be used in a variety of clinical settings. Except for pediatric cardiac surgery, the decision to use FWB in these situations is based on perceived risk-benefit ratios that are not supported with adequate data. The limited data on both FWB and component efficacy and noninfectious safety need to be addressed in a multidisciplinary manner. Continuing to transfuse patients with blood products in which the ability to improve oxygen use or support hemostasis has not been established is concerning, especially in the critically ill. The multiple processing and storage methods used for WB and components also require thorough investigation. For WB to be a feasible option in nonaustere settings, the ability to have it fully tested for pathogens and stored for at least 7 to 14 days is optimal. Data on prolonged WB storage at 2°C to 6°C are encouraging but in vivo studies are needed. The risks of pathogen transmission and of WBC-related complications in warm FWB may be mitigated with technologies in development. The optimal resuscitation strategy for patients with severe hemorrhagic shock is undetermined. Exploring whether FWB is an appropriate option in certain circumstances is warranted. Efforts to preserve FWB benefit while avoiding complications are also essential.

DISCLOSURES

Name: Philip C. Spinella, MD.
Contribution: This author helped write the manuscript.
Attestation: Philip C. Spinella approved the final manuscript.
Conflicts of Interest: Philip C. Spinella consults for Terumo BCT Biotechnologies.
Name: Heather L. Reddy, PhD.
Contribution: This author helped write the manuscript.
Attestation: Heather L. Reddy approved the final manuscript.
Conflicts of Interest: Heather L. Reddy works for Terumo BCT Biotechnologies. Dr. Reddy is a program manager at Terumo BCT Biotechnologies.
Name: Jennifer S. Jaffe, MPH.
Contribution: This author helped write the manuscript.
Attestation: Jennifer Jaffe approved the final manuscript.
Conflicts of Interest: The author has no conflicts of interest to declare.
Name: Andrew P. Cap, MD, PhD, FACP.
Contribution: This author helped write the manuscript.
Attestation: Andrew Cap approved the final manuscript.
Conflicts of Interest: Andrew Cap received research funding from Caridian BCT Biotechnologies.
Name: Raymond P. Goodrich, PhD, Biotechnologies, LLC, Lakewood, CO.
Contribution: This author helped write the manuscript.
Attestation: Raymond P. Goodrich approved the final manuscript.
Conflicts of Interest: Raymond P. Goodrich works for Terumo BCT Biotechnologies. Dr. Goodrich is the Vice President of Scientific and Clinical Affairs for Terumo BCT Biotechnologies.
This manuscript was handled by: Jerrold H. Levy, MD, FAHA.

REFERENCES

1. Borgman MA, Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, Sebesta J, Jenkins D, Wade CE, Holcomb JB. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. J Trauma 2007;63:805–13
2. Repine TB, Perkins JG, Kauvar DS, Blackborne L. The use of fresh whole blood in massive transfusion. J Trauma 2006;60: S59–69
3. Spinella PC, Doctor A, Blumberg N, Holcomb JB. Does the storage duration of blood products affect outcomes in critically ill patients? Transfusion 2011;51:1644–50
4. Bolan CE, Webster MS. Military Transfusion Practice: Anesthesia and Perioperative Care of the Combat Casualty. Washington, DC: Headquarters, Department of the Army, 1994:341–73
5. Perkins JG, Cap AP, Spinella PC, Shorr AF, Beekley AC, Grathwohl KW, Rentas FJ, Wade CE, Holcomb JB. Comparison of platelet transfusion as fresh whole blood versus apheresis platelets for massively transfused combat trauma patients (CME). Transfusion 2011;51:242–52

6. Perkins JG, Cap AP, Weiss BM, Reid TJ, Bolan CD. Massive transfusion and nonsurgical hemostatic agents. Crit Care Med 2008;36:S325–39
7. Pidcoke HF, Delgado KK, Mora AG, Reddy HL, Goodrich R, Cap AP. Pathogen reduction in whole blood with riboflavin and ultraviolet light does not affect platelet count or function. Transfusion 2011;51(s3):64A–65A
8. Spinella PC, Strandenes G, Rein EB, Seghatchian J, Hervig T. Symposium on fresh whole blood for severe hemorrhagic shock: From in-hospital to far forward resuscitations. Transfus Apher Sci 2012;46:113–7
9. Spinella PC. Warm fresh whole blood transfusion for severe hemorrhage: U.S. military and potential civilian applications. Crit Care Med 2008;36:S340–5
10. Manno CS, Hedberg KW, Kim HC, Bunin GR, Nicolson S, Jobes D, Schwartz E, Norwood WI. 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
11. Murphy S, Gardner FH. Platelet storage at 22 degrees C: metabolic, morphologic, and functional studies. J Clin Invest 1971;50:370–7
12. Luce BR, Kramer JM, Goodman SN, Connor JT, Tunis S, Whicher D, Schwartz JS. Rethinking randomized clinical trials for comparative effectiveness research: the need for transformational change. Ann Intern Med 2009;151:206–9
13. Tinmouth A, Fergusson D, Yee IC, Hebert PC. Clinical consequences of red cell storage in the critically ill. Transfusion 2006;46:2014–27
14. Glynn SA. The red blood cell storage lesion: a method to the madness. Transfusion 2010;50:1164–9
15. Spinella PC, Sparrow RL, Hess JR, Norris PJ. Properties of stored red blood cells: understanding immune and vascular reactivity. Transfusion 2011;51:894–900
16. Sayers M, Centilli J. What if shelf life becomes a consideration in ordering red blood cells? Transfusion 2012;52:201–6
17. van de Watering L. Pitfalls in the current published observational literature on the effects of red blood cell storage. Transfusion 2011;51:1847–54
18. Kauvar DS, Holcomb JB, Norris GC, Hess JR. Fresh whole blood transfusion: a controversial military practice. J Trauma 2006;61:181–4
19. Spinella PC, Borgman MA, Azarow KS. Pediatric trauma in an austere combat environment. Crit Care Med 2008;36:S293–6
20. Spinella PC, Perkins JG, Grathwohl KW. Risks associated with warm whole blood transfusions compared to PRBC transfusions during combat. Crit Care Med 2005;33:A44
21. Spinella PC, Perkins JG, Grathwohl KW, Beekley AC, Holcomb JB. Warm fresh whole blood is independently associated with improved survival for patients with combat-related traumatic injuries. J Trauma 2009;66:S69–76
22. Armand R, Hess JR. Treating coagulopathy in trauma patients. Transfus Med Rev 2003;17:223–31
23. Kiraly LN, Underwood S, Differding JA, Schreiber MA. Transfusion of aged packed red blood cells results in decreased tissue oxygenation in critically injured trauma patients. J Trauma 2009;67:29–32
24. Arslan E, Sierko E, Waters JH, Siemionow M. Microcirculatory hemodynamics after acute blood loss followed by fresh and banked blood transfusion. Am J Surg 2005;190:456–62
25. Berezina TL, Zaets SB, Morgan C, Spillert CR, Kamiyama M, Spolarics Z, Deitch EA, Machiedo GW. Influence of storage on red blood cell rheological properties. J Surg Res 2002;102:6–12
26. Fitzgerald RD, Martin CM, Dietz GE, Doig GS, Potter RF, Sibbald WJ. Transfusing red blood cells stored in citrate phosphate dextrose adenine-1 for 28 days fails to improve tissue oxygenation in rats. Crit Care Med 1997;25:726–32
27. Raat NJ, Verhoeven AJ, Mik EG, Gouwerok CW, Verhaar R, Goedhart PT, de Korte D, Ince C. The effect of storage time of human red cells on intestinal microcirculatory oxygenation in a rat isovolemic exchange model. Crit Care Med 2005;33:39–45
28. Lozano ML, Rivera J, Gonzalez-Conejero R, Moraleda JM, Vicente V. Loss of high-affinity thrombin receptors during platelet concentrate storage impairs the reactivity of platelets to thrombin. Transfusion 1997;37:368–75
29. Eikelboom JW, Cook RJ, Liu Y, Heddle NM. Duration of red cell storage before transfusion and in-hospital mortality. Am Heart J 2010;159:737.e1–43.e1
30. Inaba K, Lustenberger T, Rhee P, Holcomb JB, Blackbourne LH, Shulman I, Nelson J, Talving P, Demetriades D. The impact of platelet transfusion in massively transfused trauma patients. J Am Coll Surg 2010;211:573–9
31. Koch CG, Li L, Duncan AI, Mihaljevic T, Cosgrove DM, Loop FD, Starr NJ, Blackstone EH. Morbidity and mortality risk associated with red blood cell and blood-component transfusion in isolated coronary artery bypass grafting. Crit Care Med 2006;34:1608–16
32. Spinella PC, Carroll CL, Staff I, Gross R, McQuay J, Keibel L, Wade CE, Holcomb JB. Duration of red blood cell storage is associated with increased incidence of deep vein thrombosis and in-hospital mortality in patients with traumatic injuries. Crit Care 2009;13:R151
33. Weinberg JA, McGwin G Jr, Vandromme MJ, Marques MB, Melton SM, Reiff DA, Kerby JD, Rue LW III. Duration of red cell storage influences mortality after trauma. J Trauma 2010;69:1427–31
34. Mohr R, Martinowitz U, Lavee J, Amroch D, Ramot B, Goor DA. The hemostatic effect of transfusing fresh whole blood versus platelet concentrates after cardiac operations. J Thorac Cardiovasc Surg 1988;96:530–4
35. Mabry RL, Holcomb JB, Baker AM, Cloonan CC, Uhorchak JM, Perkins DE, Canfield AJ, Hagmann JH. United States Army Rangers in Somalia: an analysis of combat casualties on an urban battlefield. J Trauma 2000;49:515–28
36. Borden Institute (U.S.). Emergency War Surgery. 3rd U.S. revision. Washington DC: Office of the Surgeon General, U.S. Army, Borden Institute, Walter Reed Army Medical Center, 2004
37. Bellamy RF. The causes of death in conventional land warfare: implications for combat casualty care research. Mil Med 1984;149:55–62
38. Holcomb JB, McMullin NR, Pearse L, Caruso J, Wade CE, Oetjen-Gerdes L, Champion HR, Lawnick M, Farr W, Rodriguez S, Butler FK. Causes of death in U.S. Special Operations Forces in the global war on terrorism: 2001–2004. Ann Surg 2007;245:986–91
39. Brohi K, Singh J, Heron M, Coats T. Acute traumatic coagulopathy. J Trauma 2003;54:1127–30
40. Niles SE, McLaughlin DF, Perkins JG, Wade CE, Li Y, Spinella PC, Holcomb JB. Increased mortality associated with the early coagulopathy of trauma in combat casualties. J Trauma 2008;64:1459–63
41. Spinella PC, Holcomb JB. Resuscitation and transfusion principles for traumatic hemorrhagic shock. Blood Rev 2009;23:231–40
42. Spinella PC, Dunne J, Beilman G, O'Connell R, Borgman MA, Cap AP, Rentas FJ. Constant challenges and evolution of US military transfusion medicine and blood operations in combat. Transfusion 2012;52:1146–53
43. Ho KM, Leonard AD. Lack of effect of unrefrigerated young whole blood transfusion on patient outcomes after massive transfusion in a civilian setting. Transfusion 2011;51:1669–75
44. Mou SS, Giroir BP, Molitor-Kirsch EA, Leonard SR, Nikaidoh H, Nizzi F, Town DA, Roy LC, Scott W, Stromberg D. Fresh whole blood versus reconstituted blood for pump priming in heart surgery in infants. N Engl J Med 2004;351:1635–44
45. Thaler M, Shamiss A, Orgad S, Huszar M, Nussinovitch N, Meisel S, Gazit E, Lavee J, Smolinsky A. The role of blood from HLA-homozygous donors in fatal transfusion-associated graft-versus-host disease after open-heart surgery. N Engl J Med 1989;321:25–8
46. Delgado KK, Pidcoke HF, Mora AG, Cap AP. Platelet function in stored whole blood measured by shear- and Von Willebrand factor-dependent methodology is retained during storage at 4 degrees C for up to 7 days. Transfusion 2011;51:65A

47. Jobes D, Wolfe Y, O'Neill D, Calder J, Jones L, Sesok-Pizzini D, Zheng XL. Toward a definition of "fresh" whole blood: an in vitro characterization of coagulation properties in refrigerated whole blood for transfusion. Transfusion 2011;51:43–51
48. Mora AG, Pidcoke HF, Valdez-Delgado KK, Fedyk CG, Reddy HL, Goodrich R, Cap AP. Pathogen reduction treatment of whole blood inhibits clot lysis and thrombin generation is preserved to 14 days by storage at 4°C. ASH Annual Meeting Abstracts 2011;118:2272
49. Pidcoke HF, Delgado KK, Mora AG, Cap AP. Whole blood stored at four degrees retains platelet function, normal prothrombin time and overall hemostatic function as measured by thromboelastography. Transfusion 2011;51:44A
50. Pidcoke HF, Delgado KK, Mora AG, Reddy HL, Goodrich R, Cap AP. Pathogen reduction in whole blood with riboflavin and ultraviolet light does not affect platelet count or function. Transfusion 2011;51:64A–5A
51. Nilsson L, Hedner U, Nilsson IM, Robertson B. Shelf-life of bank blood and stored plasma with special reference to coagulation factors. Transfusion 1983;23:377–81
52. Zhang B, Ginsburg D. Familial multiple coagulation factor deficiencies: new biologic insight from rare genetic bleeding disorders. J Thromb Haemost 2004;2:1564–72
53. McMillan CW, Roberts HR. Congenital combined deficiency of coagulation factors II, VII, IX and X: report of a case. N Engl J Med 1966;274:1313–5
54. Oeri J, Matter M, Isenschmid H, Hauser F, Koller F. Congenital factor V deficiency (parahemophilia) with true hemophilia in two brothers [in German]. Bibl Paediatr 1954;58:575–88
55. Golan M, Modan M, Lavee J, Martinowitz U, Savion N, Goor DA, Mohr R. Transfusion of fresh whole blood stored (4 degrees C) for short period fails to improve platelet aggregation on extracellular matrix and clinical hemostasis after cardiopulmonary bypass. J Thorac Cardiovasc Surg 1990;99:354–60
56. Hughes JD, Macdonald VW, Hess JR. Warm storage of whole blood for 72 hours. Transfusion 2007;47:2050–6
57. Snyder EL, Whitley P, Kingsbury T, Miripol J, Tormey CA. In vitro and in vivo evaluation of a whole blood platelet-sparing leukoreduction filtration system. Transfusion 2010;50:2145–51
58. Vostal JG, Reid TJ, Mondoro TH. Summary of a workshop on in vivo efficacy of transfused platelet components and platelet substitutes. Transfusion 2000;40:742–50
59. Neel SH. Medical Support of the U.S. Army in Vietnam, 1965–1970. Vietnam Studies. Washington, DC: Department of the Army, 1973:xv, 196 p
60. Rentas FJ, Clark PA. Blood type discrepancies on military identification cards and tags: a readiness concern in the U.S. Army. Mil Med 1999;164:785–7
61. Holloway T. TATRC Blood Testing Technology. Army Medicine News Releases 2009; http://www.armymedicine.army.mil/news/releases/20090805tatrc.cfm
62. Hakre S, Peel SA, O'Connell RJ, Sanders-Buell EE, Jagodzinski LL, Eggleston JC, Myles O, Waterman PE, McBride RH, Eader SA, Davis KW, Rentas FJ, Sateren WB, Naito NA, Tobler SK, Tovanabutra S, Petruccelli BP, McCutchan FE, Michael NL, Cersovsky SB, Scott PT. Transfusion-transmissible viral infections among US military recipients of whole blood and platelets during Operation Enduring Freedom and Operation Iraqi Freedom. Transfusion 2011;51:473–85
63. Spinella PC, Perkins JG, Grathwohl KW, Repine T, Beekley AC, Sebesta J, Jenkins D, Azarow K, Holcomb JB. Risks associated with fresh whole blood and red blood cell transfusions in a combat support hospital. Crit Care Med 2007;35:2576–81
64. Marschner S, Goodrich R. Pathogen reduction technology treatment of platelets, plasma and whole blood using riboflavin and UV light. Transfus Med Hemother 2011;38:8–18
65. Fast LD, Dileone G, Li J, Goodrich R. Functional inactivation of white blood cells by Mirasol treatment. Transfusion 2006;46:642–8
66. Fast LD, Marschner S, Nevola M, Reddy HL, Goodrich RP. Inactivation of leukocytes in whole blood treated with the Mirasol® System in comparison to gamma-irradiation. Transfusion 2011;51:41A
67. Grass JA, Hei DJ, Metchette K, Cimino GD, Wiesehahn GP, Corash L, Lin L. Inactivation of leukocytes in platelet concentrates by photochemical treatment with psoralen plus UVA. Blood 1998;91:2180–8
68. Hei DJ, Grass J, Lin L, Corash L, Cimino G. Elimination of cytokine production in stored platelet concentrate aliquots by photochemical treatment with psoralen plus ultraviolet A light. Transfusion 1999;39:239–48
69. Marschner S, Fast LD, Baldwin WM III, Slichter SJ, Goodrich RP. White blood cell inactivation after treatment with riboflavin and ultraviolet light. Transfusion 2010;50:2489–98
70. Grass JA, Wafa T, Reames A, Wages D, Corash L, Ferrara JL, Lin L. Prevention of transfusion-associated graft-versus-host disease by photochemical treatment. Blood 1999;93:3140–7
71. Cancelas JA, Dumont LJ, Rugg N, Szczepiorkowski ZM, Herschel L, Siegel A, Pratt PG, Worsham DN, Erickson A, Propst M, North A, Sherman CD, Mufti NA, Reed WF, Corash L. Stored red blood cell viability is maintained after treatment with a second-generation S-303 pathogen inactivation process. Transfusion 2011;51:2367–76
72. Mufti NA, Erickson AC, North AK, Hanson D, Sawyer L, Corash LM, Lin L. Treatment of whole blood (WB) and red blood cells (RBC) with S-303 inactivates pathogens and retains in vitro quality of stored RBC. Biologicals 2010;38:14–9
73. North A, Ciaravino V, Mufti N, Corash L. Preclinical pharmacokinetic and toxicology assessment of red blood cells prepared with S-303 pathogen inactivation treatment. Transfusion 2011;51:2208–18
74. Tonnetti L, Thorp AM, Reddy HL, Keil SD, Goodrich RP, Leiby DA. Evaluation of Babesia microti reduction with the Mirasol® System for Whole Blood. Transfusion 2011;51:27A–8A
75. Reddy HL, Doane S, Marschner S, Wilkinson S, Miklauz M, Rouse L, Hovenga N, Hansen E, Ettinger A, Goodrich R. Riboflavin-and-UV-light-treated whole blood stored at room temperature for twenty-four hours compared to paired controls. Transfusion 2011;51:72A
76. Cancelas JA, Rugg N, Fletcher D, Pratt PG, Worsham DN, Dunn SK, Marschner S, Reddy HL, Goodrich RP. In vivo viability of stored red blood cells derived from riboflavin plus ultraviolet light-treated whole blood. Transfusion 2011;51:1460–8
77. Goodrich RP, Doane S, Reddy HL. Design and development of a method for the reduction of infectious pathogen load and inactivation of white blood cells in whole blood products. Biologicals 2010;38:20–30
78. Tonnetti L, Thorp AM, Reddy HL, Keil SD, Goodrich RP, Leiby DA. Evaluating pathogen reduction of Trypanosoma cruzi with riboflavin and ultraviolet light for whole blood. Transfusion 2012;52:409–16
79. Reddy HL, Dayan AD, Cavagnaro J, Gad S, Li J, Goodrich RP. Toxicity testing of a novel riboflavin-based technology for pathogen reduction and white blood cell inactivation. Transfus Med Rev 2008;22:133–53