Use of Cold-Stored Whole Blood is Associated With Improved Mortality in Hemostatic Resuscitation of Major Bleeding

A Multicenter Study

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Objective: The aim of this study was to identify a mortality benefit with the use of whole blood (WB) as part of the resuscitation of bleeding trauma patients.

Background: Blood component therapy (BCT) is the current standard for resuscitating trauma patients, with WB emerging as the blood product of choice. We hypothesized that the use of WB versus BCT alone would result in decreased mortality.

Methods: We performed a 14-center, prospective observational study of trauma patients who received WB versus BCT during their resuscitation. We applied a generalized linear mixed-effects model with a random effect and controlled for age, sex, mechanism of injury (MOI), and injury severity score. All patients who received blood as part of their initial resuscitation were included. Primary outcome was mortality and secondary outcomes included acute kidney injury, deep vein thrombosis/pulmonary embolism, pulmonary complications, and bleeding complications.

Results: A total of 1623 [WB: 1180 (74%), BCT: 443(27%)] patients who sustained penetrating (53%) or blunt (47%) injury were included. Patients who received WB had a higher shock index (0.98 vs 0.83), more comorbidities, and more blunt MOI (all $P < 0.05$). After controlling for center, age, sex, MOI, and injury severity score, we found no differences in the rates of acute kidney injury, deep vein thrombosis/pulmonary embolism or pulmonary complications. WB patients were 9% less likely to experience bleeding complications and were 48% less likely to die than BCT patients ($P < 0.0001$).

Conclusions: Compared with BCT, the use of WB was associated with a 48% reduction in mortality in trauma patients. Our study supports the use of WB use in the resuscitation of trauma patients.

Keywords: blood component therapy, hemorrhagic shock, trauma resuscitation, whole blood transfusion

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Despite developments in resuscitation and transfusion practices, hemorrhage remains a leading cause of potentially preventable death in trauma patients.[1,2] The practice of balanced blood product resuscitation with an equal (1:1:1) ratio of plasma, packed red blood cells (PRBC), and platelets is the standard of care for hemorrhagic shock.[3] This balanced "damage control resuscitation" provides fixed ratios of blood components thereby replacing blood loss with an approximation of reconstituted whole blood (WB).[4] More recently, fresh WB transfusion was standard military practice for battlefield resuscitation, in addition to blood component therapy (BCT) and reports from the conflicts in Iraq and Afghanistan demonstrated successful outcomes with this method of resuscitation.[5–8]

Over the last decade, the expanding trauma literature has demonstrated a renewed interest in the use of WB, specifically

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cold-stored WB, in civilian trauma resuscitation. One limitation to the widespread adoption of WB for emergency transfusion has been the lack of any true "universal donor" WB product. However, numerous reports from both the military and civilian literature suggest that the use of low-titer, cold-stored type-O WB is a safe practice without an increased risk of transfusion reactions compared with standard BCT transfusion.[6,9–12] Furthermore, the use of WB is shown to reduce the need for ongoing transfusions over 24 hours,[13] and other authors have suggested that the use of WB simplifies the resuscitation process by eliminating the need to track product ratios during massive transfusion.[14,15]

Although the military has published multiple large series on their experience with warm fresh WB, there is a lack of corresponding large or prospective series examining the use of cold-stored type O WB. While the military data is promising, a direct comparison cannot be easily made with warm fresh WB and the cold-stored WB available in the civilian trauma center. With a series of smaller studies and one large single-center study demonstrating improved survival with the use of cold-stored WB transfusion in hemorrhagic shock,[6,9,16–18] the need for a large comparative multicenter trial still exists. The purpose of our study was to assess the outcomes of patients who received cold-stored WB versus BCT during their initial trauma resuscitation using a large, multicenter trial. We hypothesized that the use of WB is independently associated with improved survival compared with patients receiving BCT alone.

METHODS

Study Design

We conducted a prospective, multicenter study consisting of 14 trauma centers in the United States. The patient cohort study has been conducted and reported in accordance with the STROBE guidelines for observational studies.[19] The study was an Eastern Association for the Surgery of Trauma (EAST) sponsored multicenter study. Ethical approval was obtained from each site's institutional review board.

Participants

All trauma patients who presented to participating trauma centers and received blood products as part of their initial trauma resuscitation, defined as the immediate time period from arrival to the hospital until they were discharged from the trauma bay/emergency department, were recruited for the study. Patients included those who received WB+BCT, and those who receive BCT only. The WB product used as all centers was leukoreduced, low-titer cold-stored type O WB, but the indications and utilization of WB was at the discretion of each center's local trauma resuscitation protocol. Each trauma center participating in the study had an existing protocol for the use of WB in trauma resuscitation, but no standardized protocol was used for the purpose of this study. Similarly, the exact cutoff for determining "low-titer" status was not standardized and was based on the local center and blood bank protocols. Data were collected prospectively starting on January 1, 2016 and completed on September 1, 2021.

Data Collection

Local institutional review board approval was required prior to participation and data use agreements were obtained and signed by all participating institutions. Data was collected via Redcap and imported into SAS 9.4 (SAS Institute Inc., Cary, NC) and R-studio (RStudio, Inc., Boston, MA). The secure data environment has been validated and approved for the use of large-scale data. Data captured included patient demographics, presence of preexisting comorbid conditions (hypertension, coronary artery disease, heart failure, diabetes, chronic obstructive pulmonary disease), preexisting use of antiplatelet or anticoagulant medication (Aspirin, Plavix, Coumadin, Xarelto, Eliquis, Pradaxa, Arixtra, Lovenox, Heparin), injury severity score (ISS), abbreviated injury score, mechanism of injury (MOI), blood products transfused including those given in the prehospital setting and at time 4 and 24 hours from arrival, arrival Glasgow coma scale, arrival systolic blood pressure, systolic blood pressure nadir while in the trauma bay, arrival heart rate, arrival shock index, use of hemostatic medications at arrival (tranexamic acid, Factor 7, 3-factor prothrombin complex concentrate (PCC), 4-factor PCC, Andexxa, Praxibind), initial labs (hemoglobin, hematocrit, platelet count, international normalized ratio (INR), prothrombin time (PT), partial thromboplastin time (PTT), and lactate), labs at both 4 and 24 hours from arrival, disposition from the trauma bay, need for operative intervention or angioembolization, and outcomes. Patients were characterized as undergoing a massive transfusion protocol (MTP), defined as 10 or more units of blood in the initial 24 hours, or ultramassive transfusion protocol (UMTP), defined as 20 or more units of blood in the initial 24 hours.

Outcomes

The primary outcome was in-hospital mortality. Secondary outcomes included acute kidney injury, deep vein thrombosis, pulmonary embolism, pulmonary complications (acute respiratory distress syndrome, transfusion associated circulatory overload), transfusion associated lung injury, and bleeding complications (uncontrollable hemorrhage/exsanguination, disseminated intravascular coagulation, gastrointestinal bleeding, unexpected need to return to the operating room, and unexpected need for interventional angioembolization).

Statistical Analysis

We performed all statistical analyses in SAS statistical software version 9.4 (SAS Institute) or R software, V.4.0.3 (R Core Team, Vienna, Austria) using 2-tailed 0.05 as the significance level tests. Univariate analyses included Student t tests for continuous variables and χ^2 tests for categorical variables.

We applied a generalized linear mixed-effects model with a random effect for the center (to control for center) to calculate the odds ratio and 95% confidence interval for each group of the cohort.[20] In the multivariable models, we adjusted for age, sex, prehospital blood product administration, MOI and ISS as confounders. Considering center factors such as comorbid diseases and conditions frequently associated with patients either receiving WB or BCT, we performed a sensitivity analysis to minimize potential confounding bias. We performed the analysis with the inverse probability of weight using the propensity score, which we calculated via a logistic regression analysis with the aforementioned covariates in the final model, to account for imbalances in the baseline data between patients who received WB and BCT.[21] Lastly, we constructed Kaplan-Meier curves were generated, and log-rank tests to test the null hypothesis of no difference in survival between patients who received WB and BCT.

RESULTS

A total of 1623 trauma patients from 14 centers were enrolled during the study period (January 1, 2016 through September 1, 2021). Of the total cohort, 83% were male, and median age was 35 years (interquartile range: 24–53). A total of 1180 (73%) received at least 1 unit of WB while 443 (27%) received only BCT during their initial resuscitation. Both penetrating MOI (53%) and blunt MOI (47%) were included.

Patients who received WB were older ($P = 0.0038$), more likely to be male ($P < 0.0001$), and were more likely to have preexisting medical comorbid conditions ($P = 0.0479$). WB recipients were also more likely to have a blunt MOI ($P = 0.0007$), have a higher Glasgow coma scale ($P = 0.004$), and a higher shock index on arrival ($P = 0.0001$) (Table 1). There was no difference between groups in preexisting use of antiplatelet or anticoagulant medications ($P = 0.1343$). There was no difference in the number of patients receiving tranexamic acid between groups [BTC = 133 (37.5%) vs WB = 388 (36.8%), $P = 0.8073$]. Low sample sizes of patients receiving other hemostatic medications rendered these values statistically insignificant.

Groups differed in laboratory values with the WB group having a higher initial hemoglobin ($P = 0.0184$) and higher 24-hour hemoglobin ($P = 0.0162$). Platelet count in the WB was found to be lower at the 4-hour time ($P = 0.0062$) (Fig. 1). The number of patients characterized as MTP and UMTP did not differ between groups. A total of 139 (31.7%) BCT patients and 378 (32.4%) WB patients where characterized as MTP recipients ($P = 0.7889$) and a total of 32 (9.6%) BCT patients and 103 (8.8%) WB patients were characterized as UMTP recipients ($P = 0.6659$).

Total number of blood products transfused, per unit, differed between groups with the BCT group having higher numbers of PRBCs transfused within the first 4 hours ($P = 0.003$) and within the first 24 hours ($P = 0.0041$). Expectedly, in the WB group there was more WB transfused across all time periods (all $P < 0.0001$). No difference was observed in total units of plasma, platelets, or cryoprecipitate between groups. Furthermore, there was no difference in total amount of product transfused between groups ($P = 0.1588$) (Table 2).

Patients who received WB were 9% less likely to experience a bleeding complication ($P < 0.0001$). There were no differences in the rates of acute kidney injury, deep vein thrombosis/pulmonary embolism, pulmonary complications, or LOS between the 2 groups (Table 3). There were no differences between WB and BCT in predicting hospital length of stay, intensive care unit length of stay or mechanical ventilations days in univariate analysis. Only variables that were significantly different on univariate analysis were used in the multivariable model.

The full results of the multivariable regression models for the main study outcomes are shown in Table 3. When controlling for age, sex, prehospital blood product administration, MOI, and ISS, receiving WB was associated with a 48% decreased in mortality compared with patients who received BCT alone ($P < 0.001$). Most of the deaths occurred during the initial resuscitation phase and within the first 24 hours from arrival. In the BCT group, 4 hours cause of death was more commonly due to hemorrhage (71%) followed by traumatic brain injury (TBI) (21%), with the WB group having similar causes with hemorrhage (54%) and TBI (27%). In the BCT group, 24 hours cause of death was hemorrhage (69%) and TBI (22%), and in the WB group hemorrhage (44%) and TBI (23%). Within the first 4 hours, 24 of 436 (5.5%) BCT patients died while 41 of 1171 (3.5%) WB patients died ($P = 0.0864$). Within the first 24 hours, 138 of 436 (32%) of BCT patients died while 167 of 1171 (14%) of WB patients died (Fig. 2, $P < 0.0001$). Furthermore, there was a sustained association with survival beyond the initial 24 hours ($P < 0.0001$, Fig. 3). The most common cause of death amongst all study patients was hemorrhage, followed by TBI. Due to multicollinearity within the models, we could not include shock index and WB in the model.

DISCUSSION

Our study is the first prospective, multicenter observational trial to demonstrate and association with WB transfusion and decreased mortality for resuscitation in trauma. It is also the largest and only civilian multicenter study examining the outcomes associated with the specific product of cold-stored low-titer type O WB as a universal donor resuscitation product. After controlling for age, sex, prehospital blood product transfusion, MOI, and ISS, patients resuscitated with WB were 48% less

TABLE 1. Baseline Demographic and Clinical Comparison of the 1623 Patients

	Component Therapy (n = 443)	Whole Blood (n = 1180)	*P*
Age	32 (23, 51)	36 (25, 54)	0.0038
Sex, male (%)	71	87	< 0.0001
Penetrating MOI (%)	60	51	0.0007
Comorbid conditions (%)	29	33	0.0479
Arrival HR	87 (33, 111)	101 (77, 121)	0.4438
Arrival SBP, mm Hg	97 (50, 127)	93 (76, 116)	0.8853
SBP Nadir, mm Hg	76 (0, 96)	75 (60, 89)	0.5019
Shock index	0.83 (0.63, 1.16)	0.98 (0.75, 1.33)	0.0001
Arrival GCS	10 (3, 15)	14 (3, 15)	0.0004
ISS	21 (10, 30)	22 (12, 30)	0.3265
AIS head	0 (0, 3)	0 (0, 3)	0.5470
AIS face	0 (0, 1)	0 (0, 1)	0.5546
AIS chest	3 (0, 4)	2 (0, 3)	0.3184
AIS abdomen	2 (0, 3)	2 (0, 3)	0.3166
AIS extremity	0 (0, 3)	2 (0, 3)	0.3152
AIS external	1 (0, 1)	2 (0, 3)	0.3178

Data presented as median with interquartile range (IQR), unless otherwise noted.
Comorbid conditions include, hypertension, coronary artery disease, heart failure, diabetes, chronic obstructive pulmonary disease.
GCS indicates Glasgow coma scale.

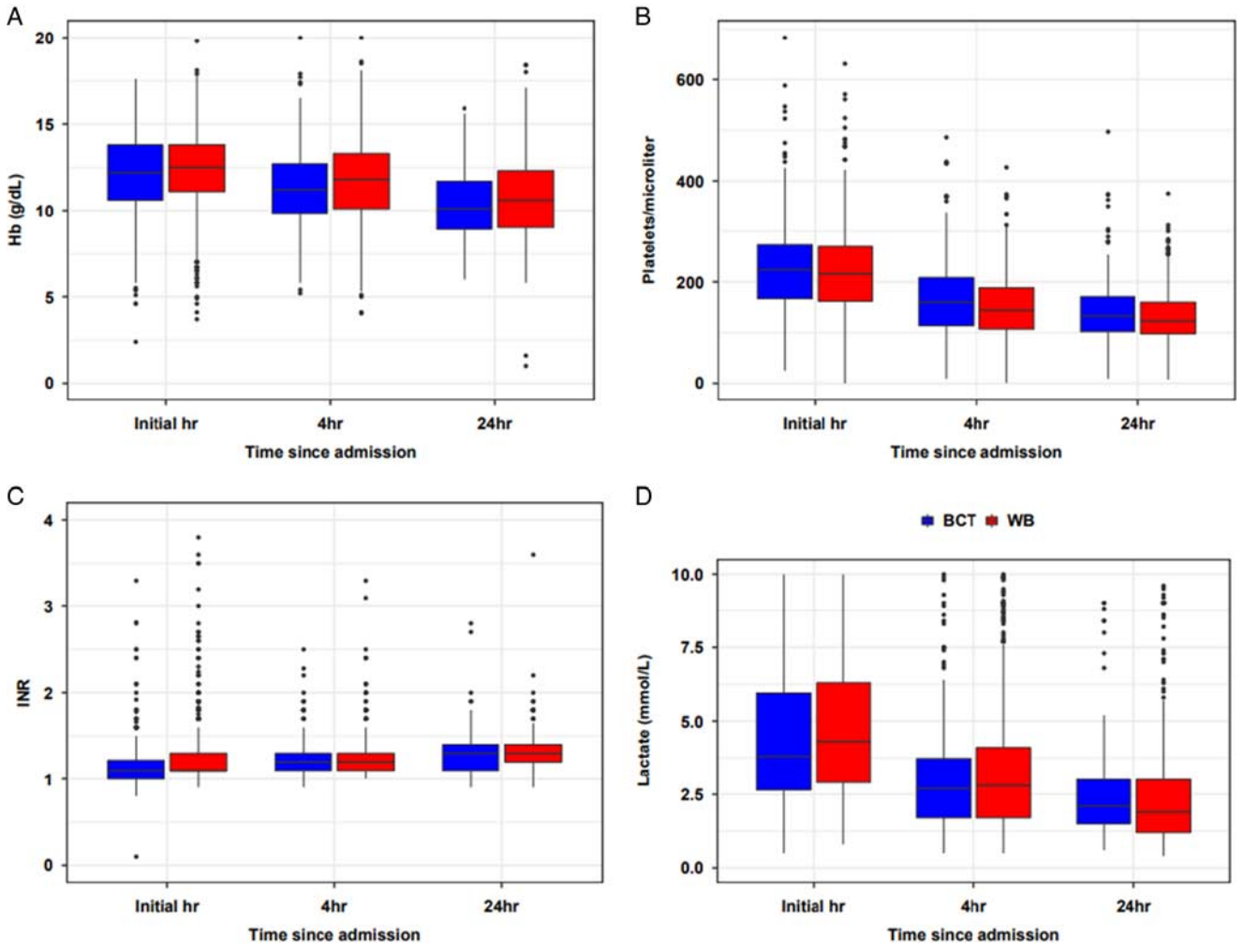

FIGURE 1. Laboratory values at time of arrival, 4 hours and 24 hours. Hb indicates hemoglobin.

TABLE 2. Transfusion of Blood Products per Group, Displayed as Units

	Component Therapy (n = 433)			Whole Blood (n = 1165)			
	Minimum	Median (IQR)	Maximum	Minimum	Median (IQR)	Maximum	*P*
Arrival to 4 h blood products administered							
WB	0	0	0	0	2 (1, 2)	15	< 0.0001
PRBC	0	3 (2, 6)	123	0	1 (0, 5)	90	0.003
Plasma	0	2 (0, 5)	122	0	0 (0, 4)	116	0.3265
Platelets	0	0 (0, 1)	18	0	0 (0, 1)	12	0.2031
Cryoprecipitate	0	0	4	0	0	5	0.1598
4–24 h blood products administered							
WB	0	0	0	0	0	8	< 0.0001
PRBC	0	0	52	0	0	69	0.3354
Plasma	0	0	52	0	0	63	0.653
Platelets	0	0	8	0	0	8	0.5557
Cryoprecipitate	0	0	4	0	0	5	0.9708
Arrival to 24 h cumulative blood products administered							
WB	0	0	0	0	2 (1, 3)	15	< 0.0001
PRBC	1	3 (2, 8)	123	0	2 (0, 6)	113	0.0041
Plasma	0	2 (0, 6)	122	0	1 (0, 5)	116	0.3135
Platelets	0	0 (0, 1)	18	0	0 (0, 1)	15	0.2106
Cryoprecipitate	0	0	4	0	0	5	0.0895
Total product (sum)	1	6 (2, 16)	263	1	4 (1, 12)	237	0.1588

Data presented as median with interquartile range (IQR), unless otherwise noted.

TABLE 3. Morbidity and Mortality Outcomes

Outcome	Adjusted OR (95% CI)	*P*
Mortality	0.52 (0.39–0.70)	< 0.0001
Bleeding complications	0.91 (0.91–0.91)	< 0.001
Acute kidney injury	1.51 (0.92–2.48)	0.10
Deep vein thrombosis/pulmonary embolism	1.33 (0.76–2.31)	0.38
Pulmonary complications	0.86 (0.36–2.05)	0.73

Bleeding complications defined as: uncontrollable hemorrhage/exsanguination, disseminated intravascular coagulation, gastrointestinal bleeding, unexpected need to return to the operating room, and unexpected need for angioembolization.

Acute kidney injury: defined as per each institutions definition of "AKI."

Pulmonary complications defined as: acute respiratory distress syndrome, transfusion associated circulatory overload, and transfusion associated lung injury.

CI indicates confidence interval; OR, odds ratio.

likely to die during their hospital admission than those who did not receive WB. Our results are consistent with previous studies demonstrating an associated decrease in mortality in patients receiving WB, although the majority of these previous studies examined warm fresh WB and in military environments.

Brill et al[18] showed in their single-center observational study a 4-fold reduction in mortality with the use of WB for resuscitation in trauma patients 16 years of age and older. Their analysis also demonstrated a 60% reduction in the total blood volume transfused in patients that received WB. Furthermore, in their subgroup analysis, patients with and without TBI both sustained a significant survival benefit with the use of WB. Additionally, a nationwide analysis of WB use by Hanna et al[22] using the 2015 to 2016 Trauma Quality Improvement Program database demonstrated that in adult patients presenting in hemorrhagic shock, the use of WB in addition to component therapy was independently associated with reduced 24-hour mortality. Our study is consistent with these previous reports in demonstrating a mortality benefit to using WB. Furthermore, this mortality benefit was sustained throughout the hospital admission.

In the United States, trauma centers utilizing WB transfusion will do so in conjunction with BCT. WB is provided as cold stored, low-titer O positive or O negative units. The standard for BCT is to transfuse PRBCs, FFP, and platelets in a 1:1:1 ratio which provides a unit of "reconstituted" WB equivalent. The mechanism for the mortality benefit observed in our study with WB still needs to be elucidated. Potential mechanisms for improved outcomes with WB include provision of higher concentrations of clotting factors, an improved hemostatic profile of WB, lower overall use of blood volume and blood preservatives, and reversal of the endotheliopathy of trauma.

A unit of WB contains higher concentrations of red blood cells, plasma proteins, fibrinogen, and platelets compared with an equivalent unit of reconstituted blood. For comparison, a unit of WB has a hematocrit of 38% to 50%, platelets of 150,000 to 400,000, 1 g of fibrinogen, and coagulation factor activity of 100% whereas a unit of reconstituted WB contains a hematocrit of 29%, platelets of 88,000, 150 mg of fibrinogen, and only 65% coagulation factor activity.[15] Furthermore, a unit of WB is 500 ml on average, versus 675 ml for a unit of reconstituted WB using BCT. This allows for a lower overall volume of fluid as well as a lower volume of preservatives that are contained in each unit of blood components.[15]

The provision of higher concentrations of red blood cells and clotting factors in addition to lower overall blood volumes

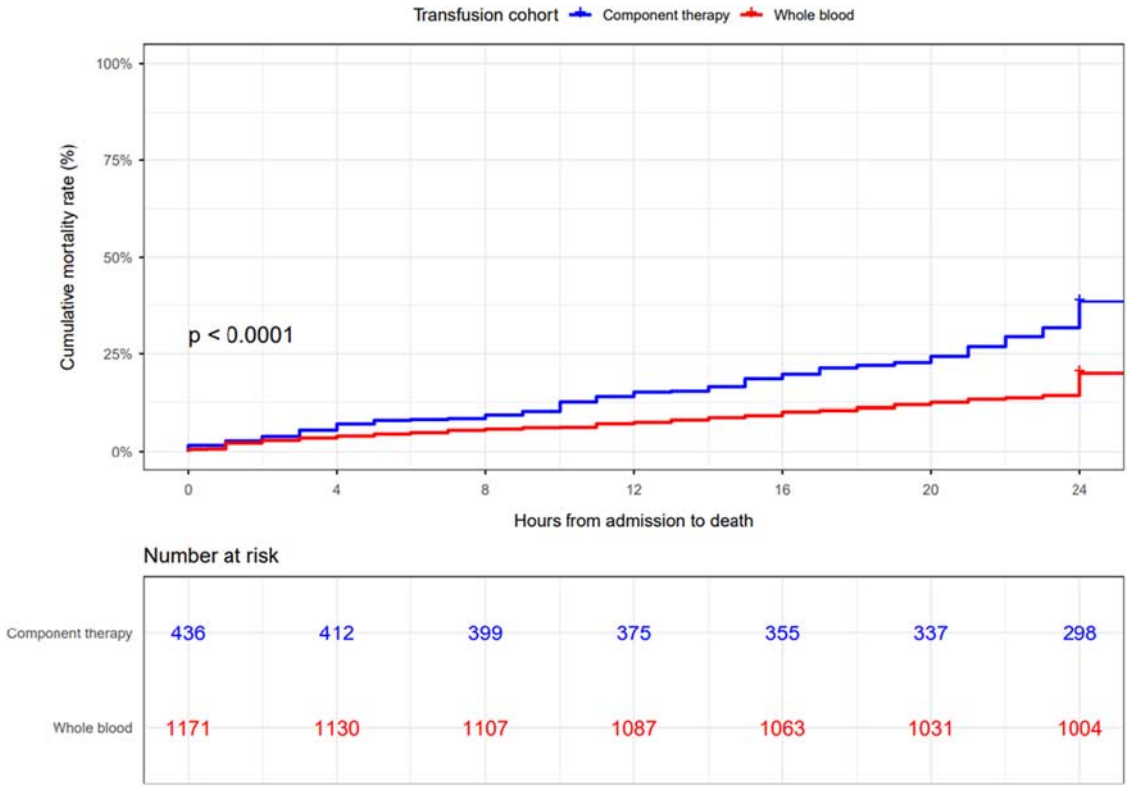

FIGURE 2. Kaplan-Meier curve demonstrating 24 hours mortality.

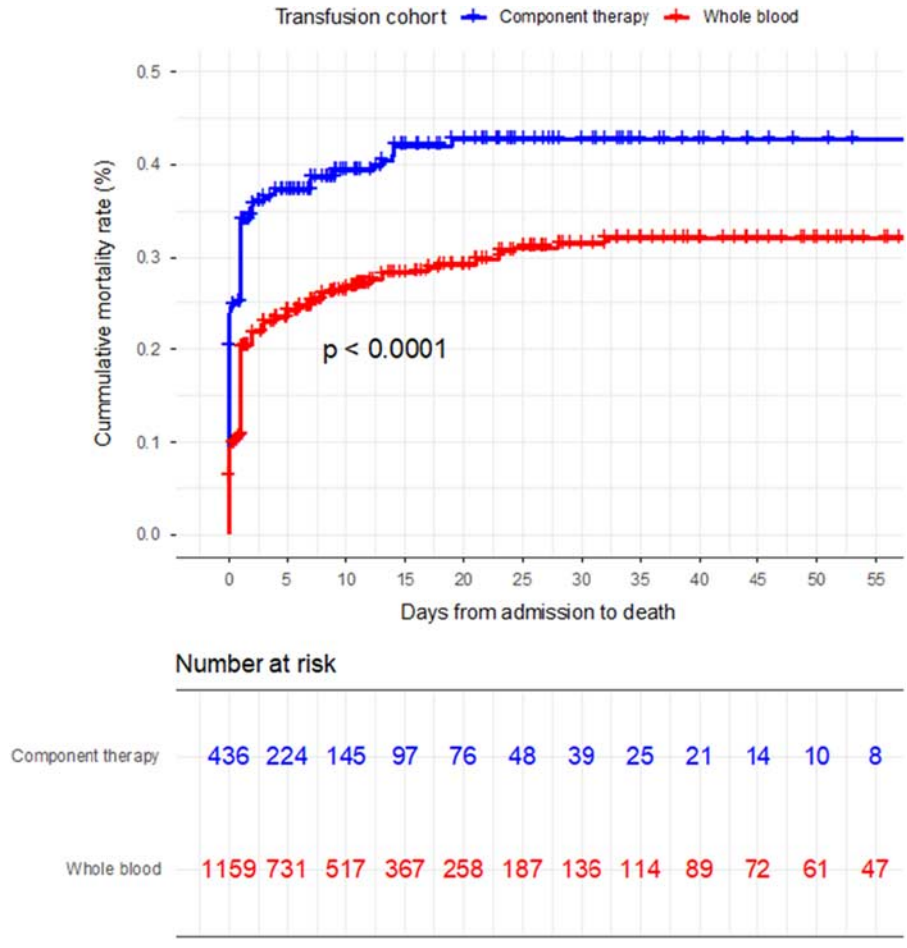

FIGURE 3. Kaplan-Meier curve demonstrating 60 days mortality.

has obvious theoretical benefits compared with BCT in terms of intravascular volume resuscitation and the correction of coagulopathy. An in vitro study performed by Kornblith et al[23] demonstrated that a unit of cold-stored WB in conjunction with a unit of plasma had a better hemostatic profile compared with a unit of reconstituted WB alone. Their study specifically demonstrated that WB in conjunction with platelets had superior clot strength compared with blood components in a 1:1:1 ratio. Interestingly, their study also demonstrated that in a unit of WB alone without an additional unit of platelets, there were no differences in clot strength compared with reconstituted WB. This suggests that the benefit of WB may not only depend on the reversal of the coagulopathy of trauma but other physiologic mechanisms. These mechanisms may also include the reversal of the endotheliopathy of trauma and hemorrhagic shock.[24] In shock, endotheliopathy occurs due to degradation of the glycocalyx as well as endothelial cell damage.[25] Multiple studies in animal models of hemorrhagic shock as well as in vivo cell cultures showed that blood components partially reverse this pathology.[26–28] Transfused donor platelets, plasma, and cryoprecipitate were individually studied and each demonstrated a reduction in vascular leakage and stabilization of the vascular endothelium. Furthermore, cold-stored platelets at 4°C showed superiority over room temperature platelets at 22°C in vascular permeability factor induced vascular leak.[29] Therefore, a unit of WB provides all of the factors required to modulate shock associated endotheliopathy in a single unit, and provides platelets in their most active form (4°C). Theoretically, providing a unit of WB early can modulate vascular leakage and possibly attenuate the subsequent organ failure and late mortality that is associated with it by providing the right components at the very beginning of resuscitation.

This study was limited due to its observational nature. Patients were not randomized to treatment groups of BCT versus WB resuscitation. Therefore, we would not be able to account for all potential confounding variables. However, patients that received WB had a higher shock index and were more likely to have a blunt trauma mechanism, therefore would be predicted to have a higher mortality. We also could not assess whether there was a dose related response associated with WB, largely due to the fact that most patients in the WB only received 1 or 2 units of WB. This is also a limitation when discussing the safety of cold-stored WB, as most civilian data does not report on high-volume cold-stored WB transfusions. There is currently no universally accepted antigen titer for WB, therefore WB may contain more antigenic proteins than type specific blood products. Therefore, there may be a dose-dependent effect where higher numbers of WB transfusion result in an increase in transfusion reactions, though recent military data studying the effects of warm fresh WB are promising.[30] This result could not be elucidated from our study due to the low number of WB units transfused per patient.

In conclusion, cold-stored WB used in the resuscitation of trauma patients was associated with decreased mortality, without an increase in complications compared with BCT alone. The mechanisms for improved outcomes will need to be further studied to include effects on coagulation parameters, inflammatory profiles, organ perfusion, and dose-dependent responses. Cold-stored WB may become the preferred primary resuscitation product for trauma patient with active hemorrhage or traumatic shock in the civilian setting.

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DISCUSSANT

Dr. Jeff Kerby (Birmingham, AL)

He is in the Ukraine providing support for our surgical colleagues there, so I'm happy to stand in for Dr. Holcomb and provide his discussion. I would like to thank the Association for the honor of discussing the paper, Use of Whole Blood Improves Mortality for Hemostatic Resuscitation of Major Bleeding, a multicenter study, Dr. Hazelton and colleagues for sending the paper well in advance, and Dr. Kerby for standing in for me.

Dr. Hazelton and colleagues have performed a great service. They present today a study that attempts to undo 40 years of transfusion dogma by addressing one of the really hot topics in trauma care. They performed a prospective observational study of 1623 patients who received blood products at 14 centers over six years. 1180, or 73%, received at least one unit of whole blood while the remaining 27% received only components. These studies require enormous effort, and I truly congratulate all the authors for their sustained efforts over a long period of time.

Let me be clear. I fully support their conclusions, but I do have a brief comment about the different types of whole blood and then suggestions that may help clarify some issues. Led by the US military, whole blood is now the preferred resuscitation fluid on the battlefield, and its use is spreading rapidly in civilian trauma centers, both in the US and abroad. The authors review many of the issues pertaining to the resurgence of whole blood in resuscitation of trauma patients. However, they should make clear the difference between fresh whole blood and the FDA-cleared low-titer type O whole blood used in their study. Given the differences in preparation and storage time, these products are not the same.

Additionally, were all of the whole blood units transfused in your study leukocyte reduced? There are data suggesting that non-leukocyte-reduced whole blood may be superior. The leukocyte reduction filter probably impairs platelet function, suggesting that not all whole blood transfused in the US is the same product.

Throughout the paper, including the title, you state that the use of whole blood improves mortality. Since this was not a randomized study, I would suggest you temper your conclusions and title to more accurately say there was an association with whole blood and improved survival. I appreciate you supplying units transfused at four and 24 hours, but you don't state what the four-hour and 24-hour and overall mortality was. Please add the median time of death and the cause of death into one of your tables.

In the results, you stated a definition of massive transfusion as 10 units of blood in 24 hours and that 32% of both groups reached this threshold. This definition ignores the issue of survival bias as many patients die before reaching a 10-unit threshold. Using a more modern definition of three units in four hours or something similar might enable you to avoid the bias issue and uncover some potentially interesting differences.

While I am a strong proponent of these type of studies, there are many issues with multicenter observational studies. It was unclear how many units of whole blood were available for transfusion. Were they always available? What were the transfusion triggers? Were they given first before components or somewhere in the middle of the early transfusion? Were women of childbearing age given low-titer O-positive whole blood or only low-titer O-negative whole blood? Was there an arbitrary limit to the number of whole blood units that could be transfused to an individual patient?

It is reassuring that safety issues didn't arise in the large number of patients who were transfused whole blood, although the median whole blood units transfused was only two and the median units within 24 hours was only six versus four.

Finally, the overall Kaplan-Meier curve documents that as expected, most of the mortality and separation between groups occurs very early. Please add a new Kaplan-Meier figure that shows the separation between groups just during the first 24 hours. The mechanism of why these differences are seen will not be sorted out until randomized studies with patients receiving only whole blood versus components are done. Hopefully, this type of trial will start soon.

In closing, these data are extremely important to our patients and to the trauma community as we move from small, single-center studies through large, multicenter observation studies to larger, definitive randomized trials. I really appreciated the opportunity to comment on this important paper.

Response from Joshua Hazelton

Thank you Dr. Kerby. We certainly wish for Dr. Holcomb's safe return. I would like to address several things that you mentioned. First, cold-stored whole blood is not the same blood that is used in the military. Warm, fresh whole blood is a different product, to use your own words, so comparing cold-stored whole blood to warm, fresh whole blood is very challenging. Certainly, the initiation of this paper was based on military data that has studied fresh whole blood.

In my personal clinical experience, the whole blood was leukoreduced with a platelet-sparing filter, but we could not control for this as there were 14 different centers and multiple different blood banks involved. My own center uses up to four different blood banks, so to control for the exact type of whole blood can be challenging, and we certainly recognize this as a limitation.

Another limitation would be the difference in what is determined as "low-titer" blood across different institutions and different blood banks. Again, something we could not dictate as each center used different blood suppliers.

We certainly recognize the survival bias in the MTP groups and we are planning to discuss that further in the paper.

In regards to special patient populations, we had very few patients in the whole blood group who were women of childbearing age, so I can't comment on outcomes of those patients specifically.

To further discuss the limitations, we really feel the biggest limitation is that transfusion triggers for whole blood are dependent upon each trauma center. This was a prospective observational study and we did not dictate which patients may or may not receive whole blood. The amount of whole blood given to each patient was determined by the guideline of that particular center or the clinician who ordered the transfusion and was running the resuscitation. The vast majority of patients in the whole blood group received only one or two units. While most of participating centers had guidelines that limited the amount of whole blood to four units per patient, we did see several patients who received more than 4 units of whole blood.

We recognize that the safety of whole blood has been determined, at least in the civilian studies and in our study, based on data in patients who were transfused with one to two units of whole blood, so larger studies looking at larger volumes of whole blood transfused may have different results as far as safety.

Finally, commenting on the hourly mortality. We plan to publish a Kaplan-Meier curve of the first 24 hours. Expectedly, that's when most of the deaths do occur in studies like this and, along with the 60-day mortality curve, we think will provide a more clear picture of the mortality differences.

I hope that answered all your questions, doctor, thank you.

Dr. Bryan Cotton (Houston, TX)

Bryan Cotton, UT Houston. Dr. Hazelton, fantastic presentation. One question, the RePHILL trial just came out in Lancet and showed no difference in prehospital blood product resuscitation versus saline, but PAMPer from Sperry and colleagues showed an improvement with plasma in the prehospital setting, and plasma and red cells especially. Our study just came out in JACS this month with whole blood showing a benefit to prehospital but a bigger benefit on arrival to the hospital. Did you tease out where they got their whole blood and whether there was an impact in benefit from prehospital? Thank you.

Response from Joshua Hazelton

Thank you Dr. Cotton. The results of the British trial were interesting. We had very few patients in our group who had prehospital blood, so I don't have any data to support or refute that study specifically. Many of the centers that were in our study do not use prehospital blood.

You mentioned in the PAMPer trial, plasma being of such great benefit in the prehospital world. I think that that's a good way of looking at whole blood, at least for me. What is the greatest benefit of whole blood? I do not believe that it is the oxygen-carrying capacity of the red cells. I believe that it is the ability to reverse coagulopathy as part of the initial resuscitation, so I think that is one area of research which could demonstrate the greatest impact whole blood has on resuscitation. Thank you again for your questions.

Dr. Gill Cryer (Los Angeles, CA)

Gill Cryer from Los Angeles. I enjoyed this paper. Dr. Holcomb asked most of my questions, but I have one. The mortality all happened very early on, and the only way that I can

see that that would have any effect based on whether they got whole blood or not would be the availability of the blood. Do you have a time to the first unit in the groups or was there a difference between the availability of a whole blood unit and a packed cell and one-to-one-to-one ratio type of thing? Thanks.

Response from Joshua Hazelton

Great question Dr. Cryer, thank you very much. The majority of trauma centers in this study have blood banks or blood refrigerators in the trauma bay or in close proximity. I don't have an exact time as far as the initial time of the first unit of blood for all patients, but the majority of these centers did have whole blood immediately available as opposed to a massive transfusion scenario, where a cooler may not arrive for several minutes. All of these centers had roughly the same availability of whole blood and packed red blood cells, so if a patient arrived to the trauma bay and needed a transfusion of blood, the immediate availability of whole blood or packed red blood cells as a choice was the same timeframe from arrival to potential transfusion time.

Dr. Mitch Cohen (Aurora, CO)

Hi, Mitch Cohen, Aurora. A really nice and very important study that fits my bias, although I would share Drs. Holcomb's and Kerby's suggestion that maybe we all temper our enthusiasm until we have better data. I have a little bit of a worry that what you've discovered is some propensity and some understanding by the clinicians of who should get whole blood versus who should get component therapy early that's not captured in your data or propensity matching. We all know that ISS is an imperfect score. You suggest appropriately that blood transfusion, however we give it, is supposed to be fixing coagulopathy and endotheliopathy, but I don't see any difference in your data. It doesn't look like the patients were more coagulopathic in one group versus the other, and it doesn't look like whole blood fixed coagulopathy, and as a surrogate for endotheliopathy, organ failure doesn't seem different in either of the two groups, so I ask you what's different between these two patient cohorts? What was in the mind of the clinician that gave one group component therapy and one group whole blood because I think there may be more signal there than there was in what component they got. There was something about, "Yeah, this patient, we'll give him a chance. We'll give whole blood. This patient we won't. This patient's too sick." Is there something different? Did they die from different reasons or is the timing different? I think there's just so much unmeasured confounding, and I applaud your ability to propensity match, and we all do the best we can with these sorts of data, but I worry that what we're really seeing here is something different about the two groups that's not captured in the usual matching of ISS and shock, et cetera, so help me understand that if you would.

Response from Joshua Hazelton

Thank you, Dr Cohen. I agree that injury severity score is not a great measure of the true extent of injury in our patients at the time of arrival. We found a collinearity between shock index and ISS, so that's why we used ISS in our model, and the whole blood group did have a higher shock index on arrival. One notable difference is the difference in the Glasgow Coma Score. Many of the guidelines don't necessarily restrict whole blood to patients with severe TBI, but patients with severe TBI as an isolated injury and as the cause of their hypotension or physiologic compromise, many of those patients were not given whole blood.

Although the number of patients in the groups, penetrating versus blunt, were the same, we did find a significantly better mortality benefit with patients suffering penetrating trauma versus blunt trauma. Whether that outcomes is the result of a selection bias at time of arrival, or that highlights the nature of the patient's injury and nature of their disease, it's hard to know exactly, but those are certainly things that we are still looking at.

Dr. Ernest Moore (Denver, CO)

Congratulations for completing a very difficult study, but one of the concerns I would add is the failure to correct for trauma center effect. You used mixed linear model that corrects for risk factors, and a randomization effect, which corrects within institutions, but comparison between trauma centers is apparently lacking. For example, have you analyzed your study population to determine high, medium, versus low performance or trauma volume or time to transport to the hospital to see if the differential between whole blood and component is preserved through these stratifications?

Response from Joshua Hazelton

I very much appreciate that question Dr. Moore, thank you. In sensitivity analysis we corrected for trauma centers in two ways. First, we explored the possible interaction between trauma centers and blood transfusion type. The interaction term was not significant. Next, trauma center was interested in the model as a fixed term based on number of patients contributed and no association between trauma centers and mortality was observed.

Dr. Rachael Callcut (Sacramento, CA)

Rachel Callcut from Sacramento. Thanks so much for this interesting study. It is really important for us to have prospective data on this topic. My question for you gets a little bit more to the heart of why people die and whether or not their death is modifiable or not modifiable. Without understanding the cause of death and exactly why people died, I think it's difficult to interpret the result seven using propensity matching. Have you've done any subset analysis such as separating out the traumatic brain injury patients as an example versus the people who received a lot of blood versus the people who received a little bit of blood? The presumption here is that the outcome difference, which is fairly stark, is being driven by that massive transfusion group. Yet. Massive transfusion occurred in only just under one-third in each group. The median amount of units used across each group was actually quite low at three versus two units of blood. So, help us understand a little bit more if you're able to, what happens in that group that doesn't receive a significant amount of blood? Is this helpful, harmful, or makes no difference? Thanks.

Response from Joshua Hazelton

Thank you Dr. Callcut. In terms of TBI patients, we looked at the AIS of head and found that there was no difference in patients who had an AIS greater than or equal to 2 in either group. We also looked at Injury Severity Score greater

than 15 and found that there was a reduction in the mortality association in the higher ISS but still significant in favor of the whole blood group. Finally, we looked at the patients who had undergone a massive transfusion and found that the association with whole blood and decreased mortality was sustained, although to a lesser degree. The question as to whether only one or two units of whole blood could have such a significant impact on survival has been raised before. While the number of units may not be impressive, the comparison of a unit of whole blood in terms of larger volume of products with less preservative, and the fact that whole blood recipients are receiving the plasma and platelets up front as opposed to PRBC recipients who are only receiving red cells up front is, I believe, the key to the difference in outcomes and one that should be further explored.

Dr. Sam Arbabi (Seattle, WA)

Sam Arbabi, Seattle. Great presentation and study. When you have a 48% improvement in mortality, people start thinking, "are there other issues associated with this finding." As Dr. Moore mentioned, the quality of the medical center may play a role. You performed a random effect modification to adjust for the "center" variable, which may not adjust for factors that are not random. High versatility to use whole blood may be a marker for a well-organized medical center. Did all medical centers in your study have equal capability to administer whole blood?

Response from Joshua Hazelton

Great question Dr. Arbabi. Every center in our study had the ability to transfuse whole blood. Part of our recruitment from the very beginning was just that. It needed to be a trauma center that used whole blood. This entire project now is turning into a registry. My goal would be to have a large nationwide registry of centers that use whole blood and centers which do not currently use whole blood so that we can compare data from a wider range of trauma centers.

One thing that was different is the experience level in these different trauma centers with whole blood. Some of the hospitals came online and began collecting data immediately after their whole blood program was implemented whereas other centers had had a whole blood program for several years prior to joining the study.