Age of Transfused Blood: An Independent Predictor of Mortality Despite Universal Leukoreduction

Jordan A. Weinberg, MD, Gerald McGwin, Jr., MS, PhD, Russell L. Griffin, MPH, Vu Q. Huynh, BSc, Samuel A. Cherry, III, MD, Marisa B. Marques, MD, Donald A. Reiff, MD, Jeffrey D. Kerby, MD, PhD, and Loring W. Rue, III, MD

Background: **The transfusion of relatively older stored blood has been associated with an increased risk of multiple organ failure, infection, and death. It remains unknown whether this phenomenon is mitigated by transfusion of leukoreduced red cell units. The purpose of this study was to evaluate the influence of stored blood age on mortality in injured patients who universally received leukoreduced blood.**

Methods: **Trauma patients who received ≥1 unit of blood during the first 24 hours after hospital arrival were selected for inclusion. Patients were stratified both according to total units and "old" units (≥14 days) versus "young" units (<14 days) received in the initial 24 hours. Odds ratios and 95% confidence intervals (CIs) were calculated for the association between mortality and the age and amount of blood transfused, adjusted for age, sex, injury severity, injury mechanism, number of units transfused, and length of stay.**

Results: **Over 7.5 years, 1,813 patients met study criteria. Among patients who received a total of 1 to 2 or 3 to 5 units in the first 24 hours, there was no association between the amount and age of transfused blood and mortality. For patients who received a total of ≥6 units, the presence of ≥3 units of young blood was associated with a 3.8-fold increased odds of death (CI: 1.1–12.7), compared with a 7.8-fold (CI: 2.3–26.3) increased odds of death associated with the presence of ≥3 units of old blood ($p = 0.0024$).**

Conclusion: **Although larger volumes of blood, irrespective of age, are associated with increased odds of mortality, the transfusion of blood stored beyond 2 weeks appears to potentiate this association despite a practice of universal leukoreduction. For patients who receive relatively smaller transfusion volumes, blood age appears to have no effect on mortality.**

Key Words: **Blood transfusion, Red blood cells, Blood storage, leukoreduction.**

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Allogeneic blood transfusion has consistently been demonstrated to be independently associated with postinjury mortality, multiorgan failure, infection, and pulmonary morbidity.[1–7] Moreover, the transfusion of red cell units with relatively longer storage age has been associated with both morbidity and mortality in critically ill patients.[8–11] It is generally accepted that passenger leukocytes accompanying the red cells in storage are implicated in deleterious proinflammatory phenomena that may account for the association between the transfusion of older red cell units and adverse outcome.[12] Although current blood storage techniques have extended the shelf life of packed red cell units to 42 days, reports suggest that the deleterious effect of stored blood transfusion becomes evident earlier, at a storage age beyond 2 weeks.[8,11,12]

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From the Center for Injury Sciences, Section of Trauma, Burns, and Surgical Critical Care, Department of Surgery (J.A.W., G.M., R.L.G., D.A.R., J.D.K., L.W.R.), Department of Epidemiology, School of Public Health (G.M., R.L.G.), Department of Pathology (M.B.M.), Department of Anesthesiology (S.A.C.), and School of Medicine (V.Q.H.), University of Alabama at Birmingham, Birmingham, Alabama.

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Address for reprints: Jordan A. Weinberg, MD, Department of Surgery, University of Alabama at Birmingham, 1530 3rd Avenue LHRB 112, Birmingham, AL 35294-0007; email: jweinberg@uab.edu.

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Leukoreduction, the process of removing a large portion of the offending leukocytes from the red cell unit, has been proposed as a potential mitigator of the deleterious effect of older blood.[11] Although universal leukoreduction of the red cell stock has been incorporated into the practice of many U.S. medical centers over the past decade, the clinical reports associating blood storage age with adverse outcomes originated from centers that did not practice universal leukoreduction at the time. Thus, it remains unknown as to whether or not these associations would persist in a setting of universal leukoreduction. In this study, we sought to evaluate the association between blood storage age and mortality in a patient population that exclusively received leukoreduced red cell units.

PATIENTS AND METHODS

A retrospective cohort study was conducted to evaluate the association between the age of transfused blood and mortality among trauma patients. The study base consisted of those patients admitted to the Trauma Center at the University of Alabama at Birmingham (UAB) between January 2000 and June 2007. From the study base, patients who received at least one allogeneic red blood cell transfusion during the first 24 hours after arrival to hospital were selected for inclusion. Patients who died or were discharged within 24 hours of admission were excluded. This study was approved by the Institutional Review Board of the UAB.

In addition to standard demographic characteristics (i.e., age, gender), information pertaining to injury severity (i.e.,

Table 1 Demographic, Injury, and Outcome Characteristics Among Patients Receiving Old and Young Blood Transfusions Overall and According to Total Units Transfused

No. Units <14 d Old	A 0	B 0	C 1–2	D 1–2	E 1–2	F ≥3	G ≥3	H ≥3	*p*
No. units ≥14 d old	1–2	≥3	0	1–2	≥3	0	1–2	≥3	
No. patients	398	208	350	190	138	213	140	176	
Demographic									
Age, mean (SD)	41 (19)	41 (19)	42 (18)	41 (18)	38 (17)	43 (21)	42 (18)	38 (17)	0.1035
Men, n (%)	255 (64)	142 (68)	220 (63)	116 (61)	88 (64)	148 (69)	97 (69)	119 (68)	0.4619
Injury									
Injury Severity Score, mean (SD)	23 (12)	28 (15)	23 (14)	25 (14)	29 (15)	27 (15)	28 (14)	32 (15)	<0.0001
Blunt injury, n (%)	313 (79)	160 (77)	268 (77)	144 (76)	97 (70)	158 (74)	109 (78)	120 (68)	0.1710
Units transfused/24 h, mean (SD)	1.7 (0.5)	5.5 (3.7)	1.7 (0.4)	2.8 (0.8)	6.8 (4.3)	5.5 (3.0)	7.6 (4.6)	16.0 (9.9)	<0.0001
Outcome									
Mortality, n (%)	23 (5.8)	25 (12.0)	24 (6.9)	17 (8.9)	21 (15.2)	17 (8.0)	17 (12.1)	37 (21.0)	<0.0001

Injury Severity Scale score, ISS), mechanism of injury (i.e., blunt vs. penetrating), and discharge disposition was obtained for each patient. Additionally, each patient's transfusion history was obtained from blood bank records and the storage age (days) of each unit of allogeneic red cells transfused during the first 24 hours of hospitalization was analyzed. During the study period, all red cell units transfused had undergone prestorage leukoreduction, a practice implemented in our center in 1999. Red cells were leukoreduced within 24 hours of collection by high-efficiency filters. As previous reports suggest that the deleterious effect of stored blood transfusion becomes evident at a storage age beyond 2 weeks, each unit of blood transfused was categorized as being less than 14 days old (i.e., "young" blood) or 14 or more days old (i.e., "old" blood) for the purposes of this study.[8,11,12] Patients were further categorized according to the amount of each type of blood they received: 0 units, 1–2 units, or ≥3 units.

Demographic and injury characteristics were compared between patients in the old and young blood groups using *t* and χ^2 tests for continuous and categorical variables, respectively. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between mortality and blood age (old vs. young) with adjustment for age, gender, ISS, mechanism of injury, total number of units transfused in the first 24 hours of hospitalization, and length of hospital stay. To evaluate the role of total blood volume on any observed associations, stratification according to total number of units transfused during the first 24 hours was performed. Interaction terms were added to the model to evaluate whether the observed associations for the old and young blood groups were significantly different. Additionally, subgroup analyses were conducted focusing on those patients who received exclusively old versus exclusively young red cell units. *p* values ≤0.05 (two-sided) were considered statistically significant.

RESULTS

During the 7.5-year period, 20,338 patients were admitted to the trauma service of UAB Hospital. Of these, 2,062 were transfused at least 1 unit of allogeneic red cells within the first 24 hours after hospital arrival. A total of 249 early (<24 hour) deaths or discharges were excluded, leaving 1,813 patients for analysis. The mean age was 41 years and 65% percent were male. The mean ISS was 26 and 76% had a blunt mechanism of injury. Total transfusions of RBC units during the first 24 hours ranged from 1 to 74, with a mean transfusion requirement of 4.95 units. Overall mortality across the cohort was 10%. As expected, nonsurvivors had received a significantly greater number of RBC transfusions during the first 24 hours versus survivors (7.9 units vs. 4.6 units, respectively; $p < 0.0001$). Mechanism of injury (blunt vs. penetrating), however, concerning nonsurvivors versus survivors was similar (74.8% vs. 81.2%, respectively; $p = 0.06$).

Table 1 presents demographic, injury and outcome characteristics according to age and amount of blood units transfused during the first 24 hours. As expected, higher age, ISS, and mortality were observed in those patients who received relatively larger total volumes of blood. It is also notable that patients who received similar total volumes of blood, regardless of blood age, had similar demographics and injury characteristics (for example, column "A" versus column "C").

Overall, patients who received 1–2 or 3 or more units of young blood had a 65% and 70%, respectively, increased odds of death compared with those who received no young blood (Table 2). A similar pattern of results was observed for old blood, however, for those receiving 3 or more units the association was of significantly greater magnitude when compared with those receiving a similar volume of young blood (OR 2.78 vs. OR 1.70, $p = 0.0013$). When stratified according to total transfusion volume in the first 24 hours, no association between blood age and mortality was observed for those receiving small (1–2 units) or moderate (3–5 units) transfusion volumes. For patients who received ≥6 units during the first 24 hours, the receipt of 1–2 units of young blood was not associated with a significantly increased odds of death; 1–2 units of old blood, however, was associated with a 5.2-fold increased odds of death. Further, though

Table 2 Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for the Association Between Mortality and the Age of Transfused Blood Overall and According to Total Units Transfused

	Total No. Units Transfused in First 24 h			Overall OR* (95% CI)
	1–2 Units OR* (95% CI)	3–5 Units OR* (95% CI)	≥6 Units OR* (95% CI)	
<14 d old				
0 Units	Reference	Reference	Reference	Reference
1–2 Units	1.38 (0.53–3.58)	1.09 (0.40–3.02)	1.90 (0.49–7.35)	1.65 (1.01–2.70)
≥3 Units	NA	0.42 (0.10–2.83)	3.79 (1.14–12.67)	1.70 (0.96–2.99)
≥14 d old				
0 Units	Reference	Reference	Reference	Reference
1–2 Units	1.34 (0.51–3.51)	0.73 (0.14–3.81)	5.21 (1.41–19.25)	1.78 (1.06–2.98)
≥3 Units	NA	0.89 (0.21–6.46)	7.78 (2.30–26.31)	2.78 (1.58–4.88)

* Adjusted for age, gender, Injury Severity Score, mechanism of injury, total number of units transfused in the first 24 h of hospitalization, and length of hospital stay.

receipt of 3 or more units of young blood was associated with a 3.8-fold increased odds of death, a significantly greater (7.8-fold) increased odds of death was observed when 3 or more units of old blood was transfused ($p = 0.0024$).

A subgroup analysis was performed among those patients who received exclusively old or exclusively young blood. Overall, the transfusion of old blood was not associated with increased odds of death (OR 1.27, CI 0.78–2.10). When stratified according to transfusion volume in the first 24 hours, a similar null association was observed for those who received 1–2 total units of blood (OR 1.00, CI 0.53–1.92). However, among those receiving a total of 3 or more units, receipt of old blood was associated with an over 2-fold increased odds of death (OR 2.18, CI 1.00–4.97).

DISCUSSION

Multiple studies have described an association between the transfusion of older blood and adverse outcome.[8–11,13] Although all of these reports contained some element of control or accounting for the total volume of blood transfused, it is nonetheless difficult to dissociate the effect of blood age from transfusion volume, as the two variables are inherently interdependent. It is intuitive that increased transfusion volume would be associated with adverse outcomes, and it is plausible that the observed associations between older blood and morbidity or mortality may actually be more reflective of the residual confounding of transfusion volume rather than a true association with storage age. To complete the picture, a comparative analysis concerning the association of younger blood with outcome is required. If the observed association between older blood and mortality is simply a reflection of transfusion volume rather than storage age, the association between younger blood and mortality would be expected to be similar in magnitude. Our results demonstrate that while larger volumes of blood, irrespective of storage age, are associated with increased odds of mortality, the transfusion of older blood appears to significantly potentiate this association.

Given the complexity of evaluating the independent role of blood age on mortality in a patient population that received a heterogeneous distribution of old and young blood (as defined for the purposes of the study), we performed a subgroup analysis limited to those patients that received exclusively old versus exclusively young blood. Under the assumption that blood is not distributed systematically with respect to age, focusing on the groups of patients who received exclusively old versus young blood mimics a randomized, albeit not prospective study. The subgroup analysis further demonstrates that older blood potentiates the odds of death; among those patients who received 3 or more units of blood, those who died were twice as likely to have received exclusively old versus exclusively young blood.

It is notable that the association between the receipt of older blood and mortality in the current study appears limited to those receiving relatively larger volumes of blood (≥6 units in the primary analysis and ≥3 units in the subgroup analysis). The results suggest that a certain transfusion threshold is necessary before the potentiating effect of older blood becomes apparent. We can only speculate as to the underlying cause of this phenomenon. Prior work has characterized the "two-hit phenomenon," whereby an initial insult (i.e., injury) primes the host such that a subsequent activating event (i.e., allogeneic red cell transfusion) provokes an exaggerated maladaptive systemic response.[14] Specifically, Biffl et al.[12,15] have demonstrated both the potential of red cell transfusion to delay neutrophil apoptosis postinjury, and the potentiation of this phenomenon by older cells. The present results may represent the clinical correlate of this phenomenon. The patients who received relatively smaller volumes of blood were less severely injured; perhaps the severity of injury in these patients did not meet the threshold for a significant "first hit," precluding an observable aggravating effect of the "second hit," i.e., transfusion.

All study patients were transfused with blood that had undergone prestorage leukoreduction. Although leukoreduction has well-documented efficacy related to specific clinical

circumstances, a generalized benefit remains unproven.[16] Nathens et al.[17] randomized 1,864 injured patients to receive prestorage leukoreduced versus standard nonleukoreduced transfusions during the course of hospitalization, and found no difference in mortality or infectious morbidity in the 268 patients eligible for analysis. They did not, however, account for the age of transfused blood in their study design. As the passenger leukocytes contained in a typical red cell unit have been implicated in the deleterious effect of older blood, it follows that leukoreduction might mitigate this effect. Biffl et al.[12] addressed this hypothesis in the laboratory, and observed that although the plasma from nonleukoreduced aged stored blood delayed neutrophil apoptosis (a proinflammatory phenomenon), plasma from stored blood that had undergone prestorage leukoreduction did not, in fact, modify this effect. The current study demonstrates a mortality association with older blood despite universal leukoreduction, suggesting that the existence of a clinically relevant mitigating effect of leukoreduction on mortality is doubtful.

Our results must be considered in the context of the study's retrospective design; the analysis is subject to the usual pitfalls of confounding and bias. Data concerning the rate of bleeding and transfusion during the first 24 hours was not available for analysis. It is possible that the evaluation of divergent rates or patterns of transfusion within the first 24 hours could reveal a potential confounder. Similarly, adequately precise data on cause of death was not available to the investigators, and associations between storage age and the incidence of infection, organ failure, or other morbidities were not evaluated. Certainly, prospective confirmation of the effect of blood storage age is now warranted. Schulman et al.[18] attempted such a trial in the setting of a single-center Level I trauma center, randomizing patients to receive exclusively young (<11 days) versus old (>20 days) blood during the first 24 hours of hospitalization. Unfortunately, in 1 year they were only able to enroll a small number of patients secondary to limitations of the blood bank. It is reasonable to expect that other institutions would face a similar challenge given the tight supply of blood nationally. The authors noted that a multi-institutional trial would be necessary to recruit enough patients for a robust analysis.

In summary, the key finding of the present study is that while larger volumes of blood, irrespective of age, are associated with increased odds of mortality, the transfusion of blood stored beyond 2 weeks appears to significantly potentiate this association. For patients who receive relatively smaller transfusion volumes, blood age appears to have no effect on mortality. Although these results support the work of others, it would be premature to recommend any modification of current blood banking practice in the absence of prospective data. Nonetheless, the implication that the transfusion of blood of relatively longer storage age may have negative consequences demands attention and further evaluation of the association between storage age and outcome is warranted.

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DISCUSSION

Dr. Michael D. Grossman (Bethlehem, Pennsylvania): Well, let me start by thanking the association for giving me the opportunity to discuss this manuscript. It's concise. It's

well-written. And, frankly, the presentation was even better. It was very clean and easy to understand.

In preparing for this I had an opportunity to learn a little something about the ubiquitous process of blood transfusion and the practice of blood banking.

Now, clearly there is a great deal known and clearly a great deal that is not known. The authors, as you heard, performed a retrospective cohort study to test the hypothesis that leukoreduced old blood would produce worse outcomes in trauma patients compared to leukoreduced young blood.

They did use a cutoff of 14 days to define old versus young. And they concluded that mortality risk generally increases for patients that receive larger transfusions with more old than young blood as a proportion of their total transfusion. And I think that that simplifies it a bit but you saw the data.

The authors assert that this risk is independent of total transfusion volume and ISS, among some other demographic factors for which the patients were stratified.

I have several observations and a few specific questions. First off, it's clear that the subject of red cell transfusion is a hot topic. There are seven podium papers related to it this year.

Second, a strategy for massive transfusion using one to one component therapy and liberal use of fresh whole blood in forward areas of Iraq has resulted in favorable outcomes and it is suggested few problems with systemic inflammatory response as compared to high volume crystalloid resuscitation.

In many cases this blood is only hours old as there is a good supply of willing donors and blood banking facilities in these forward areas are quite limited.

The difference between this practice and the practice of massive transfusion in civilian areas is substantial. Finally, a review of the civilian literature reveals a host of problems associated with the transfusion of old blood.

I found no fewer than 11 separate postulated mechanisms by which transfusion of old blood harms the trauma patient, including decreased red cell deformity, increased viscosity, up-regulation of proinflammatory cytokines, increased apoptosis of PMNs, and inhabitation of nitric oxide, resulting in vasoconstriction, just to name a few.

So at the bedside I can expect this to result in higher rates of infection, longer ICU stays and increased odds of mortality.

So while I accept the postulates of immuno-modulation associated with stored red cells, it is less clear that the clinical consequences can be so easily separated from those attributed to the exsanguination that prompts high volume transfusion.

Despite efforts to control for variables associated with the need for transfusion, it is simply counterintuitive to me that a patient needing ten units of blood is less sick than one who needs none.

The variables seem inextricably linked, although I am sure they are not. This brings me to the study and a few questions.

When trauma patients receive a lot of blood, they usually receive it pretty quickly. Did you examine the rate or the effect of the rate of blood transfusion within that first 24-hour period?

Two, in civilian practice I would think virtually any massive transfusion will involve some old blood, probably quite a lot. Can you comment on this and whether you made any attempt to control for it?

Three, would a physiologic scoring system be a better scheme for stratification of risk than ISS? And, finally, if blood age is a continuous variable, why evaluate it as a discreet variable, that is, greater or less than 14 days?

It's hard to support that number as an independent risk factor without knowing the age distribution of the old blood in your study. Again, I would like to thank the association for the privilege of membership and congratulate the authors on a really nice study.

Dr. Randall Friese (Dallas, Texas): I'd like to congratulate the authors on a great presentation; however, I did find the stratification and the determination of young versus old a little confusing.

And particularly if a patient had received ten units of blood, five of which were less than 14 days and five of which were over 14 days, what group does that patient fall in?

And I'd like to echo Dr. Grossman's comments, why or please defend your choice of dichotomizing these ages as such.

Why not choose a point estimate of the age of blood, either the mean or the median, probably, preferably, the mean since that will emphasize the extremes of age and analyze your data in that way?

Dr. Michael L. Hawkins (Augusta, Georgia): I guess we all had the same question about why 14 days as the arbitrary cutoff.

I raise another issue. What about the use of plasma? Was there any association with the volume of the plasma which I assume was given to patients given greater than ten units of blood?

Dr. Jeffry L. Kashuk (Denver, Colorado): As per the previous question, did you control for concomitant FFP administration in this group? Our group has recently noted that FFP concurs an even greater risk of MOF related mortality than RBC's alone.

Dr. Jordan A. Weinberg (Birmingham, Alabama): Thank you very much for your questions. I'll start with Dr. Grossman's—with respect to the first question, we did not have data for the rate of transfusion during that first 24-hour period and I agree that that would be interesting to look at. But our data was not detailed enough to provide exactly at what time a transfusion occurred on a given date.

With respect to the issue of adequately controlling for severity of injury, this is important because despite controlling for injury severity by accounting for anatomic and physiologic variables, the fact remains that transfusion volume in the first 24 hours in and of itself is going to be a surrogate for

injury severity. So when you try to evaluate the influence of blood storage age on mortality, it is a challenge to tease out the influence of storage age itself from the effect of transfusion volume. That's why we felt it important to look at both the effect of young blood and the effect of old blood. And we demonstrated that as the volume of young blood transfusion increased, the odds of mortality increased, all the while controlling for total transfusion volume. Greater volumes of old blood were likewise associated with increased odds of mortality, but the association was of a significantly greater magnitude. If storage age was not important, you would expect to see a very similar magnitude of odds ratios in both groups, but in fact the odds ratios associated with old blood were significantly higher. So, the take-home message is that while transfusion volume, irrespective of storage age, is associated with increased odds of mortality, the transfusion of older blood versus younger blood significantly increases those odds.

Why did we choose 14 days as our cutoff? Well, the existing literature concerning this subject—both laboratory work and clinical reports—supports that the adverse effects of blood seem to show up at that 14-day mark. So that's why we choose it. We also looked at a 7-day cutoff and found no statistical differences. And we attempted a 21-day cutoff but our numbers just didn't support that in terms of having enough patients in the particular groups.

Why not look at the mean age of blood? The problem with looking at the mean age is that it makes certain assumptions that are not necessarily correct. For example, if one were to consume five apples, four of them fresh and one rotten, the mean freshness of the five-apple group is going to lean toward fresh rather than rotten. But there is no rationale to make the assumption that the four fresh apples are going to somehow protect you from getting sick from the rotten one. We think the same goes for blood age—there is no reason to assume that younger blood will somehow offset the deleterious effect of older blood. Knowing that others have performed such analyses in this way, we did look at mean and it demonstrated no differences in outcomes. And we think it showed no difference because it's not really the right way to look at this.

With respect to plasma, we did not account for FFP transfusions and that is something that we should definitely look into.