Older Blood Is Associated With Increased Mortality and Adverse Events in Massively Transfused Trauma Patients: Secondary Analysis of the PROPPR Trial

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Study objective: The transfusion of older packed RBCs may be harmful in critically ill patients. We seek to determine the association between packed RBC age and mortality among trauma patients requiring massive packed RBC transfusion.

Methods: We analyzed data from the Pragmatic, Randomized Optimal Platelet and Plasma Ratios trial. Subjects in the parent trial included critically injured adult patients admitted to 1 of 12 North American Level I trauma centers who received at least 1 unit of packed RBCs and were predicted to require massive blood transfusion. The primary exposure was volume of packed RBC units transfused during the first 24 hours of hospitalization, stratified by packed RBC age category: 0 to 7 days, 8 to 14 days, 15 to 21 days, and greater than or equal to 22 days. The primary outcome was 24-hour mortality. We evaluated the association between transfused volume of each packed RBC age category and 24-hour survival, using random-effects logistic regression, adjusting for total packed RBC volume, patient age, sex, race, mechanism of injury, Injury Severity Score, Revised Trauma Score, clinical site, and trial treatment group.

Results: The 678 patients included in the analysis received a total of 8,830 packed RBC units. One hundred patients (14.8%) died within the first 24 hours. On multivariable analysis, the number of packed RBCs greater than or equal to 22 days old was independently associated with increased 24-hour mortality (adjusted odds ratio [OR] 1.05 per packed RBC unit; 95% confidence interval [CI] 1.01 to 1.08): OR 0.97 for 0 to 7 days old (95% CI 0.88 to 1.08), OR 1.04 for 8 to 14 days old (95% CI 0.99 to 1.09), and OR 1.02 for 15 to 21 days old (95% CI 0.98 to 1.06). Results of sensitivity analyses were similar only among patients who received greater than or equal to 10 packed RBC units.

Conclusion: Increasing quantities of older packed RBCs are associated with increased likelihood of 24-hour mortality in trauma patients receiving massive packed RBC transfusion (≥10 units), but not in those who receive fewer than 10 units. [Ann Emerg Med. 2019;73:650-661.]

Please see page 651 for the Editor's Capsule Summary of this article.

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INTRODUCTION

Background

Trauma is a leading cause of death among adults. The primary cause of death in the first 48 hours after injury is hemorrhage.[1] The transfusion of blood components (packed RBCs, platelets, and plasma) is a standard resuscitation intervention in patients with hemorrhagic shock.

The age of transfused packed RBCs may be important in the outcomes of patients transfused because of traumatic hemorrhagic shock. Although current standards permit packed RBC storage for up to 42 days, important changes occur to erythrocytes as the blood ages, including alteration and breakdown of cellular structure; release of oxygen, free hemoglobin, iron, and other proinflammatory microparticles; and lactic acid accumulation from anaerobic metabolism.[2-4] These changes begin almost immediately once donated packed RBCs are collected, with effects worsening over time.

Animal models of trauma-hemorrhage and resuscitation demonstrate significant consequences related to the use of stored blood, such as increased levels of proinflammatory

*All members are listed in the Appendix.

Editor's Capsule Summary

What is already known on this topic

Stored packed RBCs degrade and undergo potentially toxic changes over time that may affect outcomes among trauma patients receiving older stores.

What question this study addressed

The study examined the association of stored blood age and volume of transfusion with outcomes in severely injured patients.

What this study adds to our knowledge

This secondary analysis of a prospective randomized trial does not exclude the possibility that for trauma patients receiving 10 or more units of blood, an increasing proportion of older blood results in higher early mortality rates.

How this is relevant to clinical practice

Future research should prospectively evaluate outcomes of trauma patients likely to require massive transfusion who receive fresh compared with stored blood.

mediators,[5] adherence of RBCs to the microvasculature and limited tissue oxygenation,[6] and increased mortality in the first 4 hours after transfusion of stored packed RBCs.[7] In human beings, clinical signs of stored blood toxicity include thrombosis, infection, multiple organ failure, and death.[8,9]

Importance

Recent prospective randomized clinical research studies (Informing Fresh Versus Old Red Cell Management, Standard Issue Transfusion Versus Fresher Red-Cell Use in Intensive Care, Age of Blood Experiment, and Red Cell Storage Study) found no association between transfused packed RBC age and outcomes.[10-13] However, these studies involved cardiac surgery and general critical care patients.[14] The studies did not include patients with major trauma who often require large-volume transfusions administered during short periods (≥10 packed RBC units in 24 hours).

Patients with traumatic hemorrhagic shock may be particularly susceptible to stored blood toxicity because they require large volumes of packed RBCs administered within a very short period and experience widespread tissue damage and inflammation that animal studies suggest may render these patients vulnerable to stored blood toxicity. In such animal models, although increased end-organ injury, nosocomial infection, and mortality result when injured animals in hemorrhagic shock receive older packed RBCs, similar adverse events do not occur in the setting of hemorrhagic shock without injury.[7,15-20] These observations may help to explain why previous studies have not observed harm from older packed RBC transfusion in nontrauma populations. We suspect that the rapid timing and intensity of massive packed RBC transfusion may exacerbate the inflammation of traumatic hemorrhagic shock and the potential for stored blood toxicity. Understanding the association between packed RBC age and outcomes is important to help optimize outcomes after traumatic hemorrhagic shock. A conceptual model of the interplay between volume and age of packed RBC units transfused to critically ill patients is shown in Figure 1*A*.

Goals of This Investigation

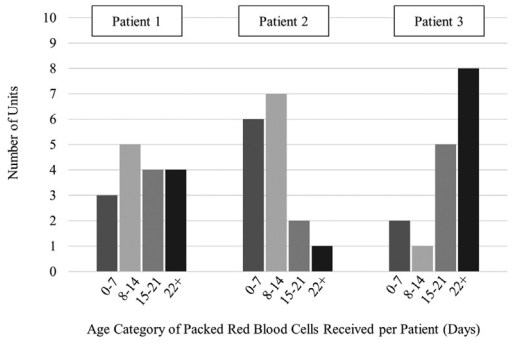
The Pragmatic, Randomized Optimal Platelet and Plasma Ratios (PROPPR) trial is one of the only large-scale clinical trials of massive transfusion in trauma patients.[21] We sought to examine the association among transfused packed RBC age and patient outcomes in the PROPPR trial.

MATERIALS AND METHODS

Theoretical Model of the Problem

In this study, we hypothesized that patient outcomes are influenced by exposure to aged packed RBCs. Exposure to aged blood includes 2 factors: the age of the packed RBC unit (0 to 7, 8 to 14, 15 to 22, and ≥22 days), and the total number of units of each packed RBC age group patients receive. See Figure 1*B* for an example of this categorization.

A



B

Subject	0-7 Days	8-14 Days	15-21 Days	22+ Days	24-Hour Total
1	3	5	4	4	16
2	6	7	2	1	16
3	2	1	5	8	16

Figure 1. Conceptual model of the dynamic relationship between packed RBC age and volume.

Confounders in this relationship include patient factors (age, sex, and race), total amount of blood transfused, and injury severity (measured with the Injury Severity Score and Revised Trauma Score). We therefore conceptualized fitting a multivariable model with death within 24 hours as the primary outcome, and with multiple variables accounting for exposure to different blood age categories.

Study Design and Setting

We performed a secondary analysis of data from the PROPPR trial.[22] This study was approved by the institutional review board of the University of Alabama at Birmingham; because it involved secondary analysis of existing data, no participant consent was needed. The PROPPR trial was a pragmatic, phase 3, multisite, randomized trial completed in 2015. The trial compared the use of a 1:1:1 with a 1:1:2 plasma:platelet:packed RBCs ratio in relation to mortality among severely injured trauma patients predicted to receive massive transfusion. Twelve Level I trauma centers in North America participated in the study. The complete methods of the PROPPR trial have been previously described.[21]

Selection of Participants

Patients enrolled in the PROPPR trial were adults aged ≥15 years or older, admitted to the study site directly from the scene of injury, who met the criteria for highest-level trauma activation, received at least 1 unit of packed RBCs in the first hour of hospitalization, and were predicted to require massive transfusion (≥10 packed RBC units in the first 24 hours), indicated by an Assessment of Blood Consumption score of greater than or equal to 2. We included all patients enrolled in the PROPPR trial in the current analysis.

Methods of Measurement

The primary exposures were the shelf age (days) and volume of transfused packed RBC units. We categorized packed RBC age according to 4 storage time frames: 0 to 7 days, 8 to 14 days, 15 to 21 days, and greater than or equal to 22 days. These time frames were chosen according to documented changes noted as beginning within the first 7 days of storage,[6] to be consistent with previous blood age studies[10-12,23] and to capture the effect of cellular breakdown that occurs in packed RBCs during the first 4 weeks of storage and worsens with prolonged storage. We defined packed RBC volume in terms of packed RBC units. We characterized the number of packed RBC units with both continuous (number of units) and categorical (0, 1 to 10, 11 to 20, and ≥21 units) schemes. Massive transfusion has traditionally been defined as greater than or equal to 10 packed RBC units in 24 hours, with an alternative definition of greater than or equal to 3 packed RBC units transfused in 1 hour.[24] Four transfusion volume categories were selected to reflect the variation in patient transfusion requirements because PROPPR criteria specified that enrolled patients were those "anticipated to require massive transfusion," although not all patients were, in fact, massively transfused.

Outcome Measures

We studied 24-hour mortality as the primary outcome, which was consistent with the original PROPPR trial[21]; secondary outcomes were 30-day mortality and the composite variable of death within 24 hours or the development of at least 2 major adverse events. Adverse events relevant to the analysis included acute lung injury, acute kidney injury, acute respiratory distress syndrome, cardiac arrest, deep venous thrombosis, infection, multiple organ failure, myocardial infarction, pulmonary embolism, sepsis, stroke, systemic inflammatory response syndrome, transfusion-associated circulatory overload, and transfusion-related metabolic complication such as hyperkalemia or hypocalcemia.

Statistical Analysis

We determined the volume and age distribution of packed RBC units transfused in the study. We evaluated the association between the number of transfused packed RBC units in each packed RBC age category and 24-hour survival, using random-effects logistic regression. We accounted for clustering by clinical site and adjusted for age, sex, race, mechanism of injury (penetrating, blunt, and burn), Injury Severity Score, Revised Trauma Score, and PROPPR trial treatment group. We repeated the analysis, characterizing packed RBC volume categorically (0 to 7, 8 to 14, 15 to 21, and ≥22 days). We also adjusted for total volume of packed RBC units transfused in the first 24 hours after hospital admission (1 to 10, 11 to 20, and ≥21 units). In addition, we also stratified the analysis by patients who received fewer than 10 or greater than or equal to 10 packed RBC units in the first 24 hours after hospital admission. Summary measures, such as averages, were not used because this would assume an equal mixing of effects of each blood age (eg, the deleterious effects of the stored blood negated by the protective effects of the fresh blood).

We determined the number of patients experiencing greater than or equal to 2 adverse events. Because death within 24 hours is a competing risk for adverse events, we assessed the association between packed RBC age and

volume and the composite variable (≥2 adverse events or death within 24 hours). We also assessed the association between packed RBC age and volume and 30-day mortality.

Revised Trauma Score was missing for 73 patients (59 had missing respiratory rate, 22 had missing systolic blood pressure, and 1 was missing both parameters); patients with missing Revised Trauma Scores were omitted from the primary analyses; all others were included. In a sensitivity analysis, we repeated the analysis, using multiply imputed Revised Trauma Scores. Because Revised Trauma Score was not normally distributed, we conducted the imputation with predictive mean matching. We added study site as an indicator variable to account for clustering within study site. We carried out the multiple imputation with 20 iterations, combining the estimates by using rules created by Rubin.[25] We conducted all analyses with Stata (version 14.2; StataCorp, College Station, TX).

RESULTS

The PROPPR trial enrolled a total of 680 patients; we excluded 2 patients because of missing packed RBC age data, leaving 678 in the analysis (Figure 2). Patients were primarily white men, with a median age of 34 years (interquartile range [IQR] 25 to 51) (Table 1). Study patients received a total of 8,830 units of packed RBCs in the first 24 hours of treatment. Individual patients received a median of 9 units (IQR 5.5 to 15) of packed RBCs in the first 24 hours of presentation to the hospital. Patients who received greater than or equal to 10 packed RBC units received a median of 17 units (IQR 12 to 25) of packed RBCs, and patients who received fewer than 10 packed RBC units received a median of 6 units (IQR 4 to 7) of packed RBCs in the first 24 hours. Figure 3 depicts the median number of units received by patients in each packed RBC age group (0 to 7, 8 to 14, 15 to 21, and ≥22 days), stratified by 24-hour mortality status. In other words, this graph shows the median number of packed RBC units of each age group that was received for people who survived compared with those who did not. Therefore, each patient may be represented in more than one packed RBC age category, depending on his or her 24-hour mortality status and the ages of packed RBC units received. The median packed RBC unit age was 19 days (IQR 13 to 27); the distribution of packed RBC unit age varied widely across study sites, from 12 days (IQR 8 to 14) in site 1 to

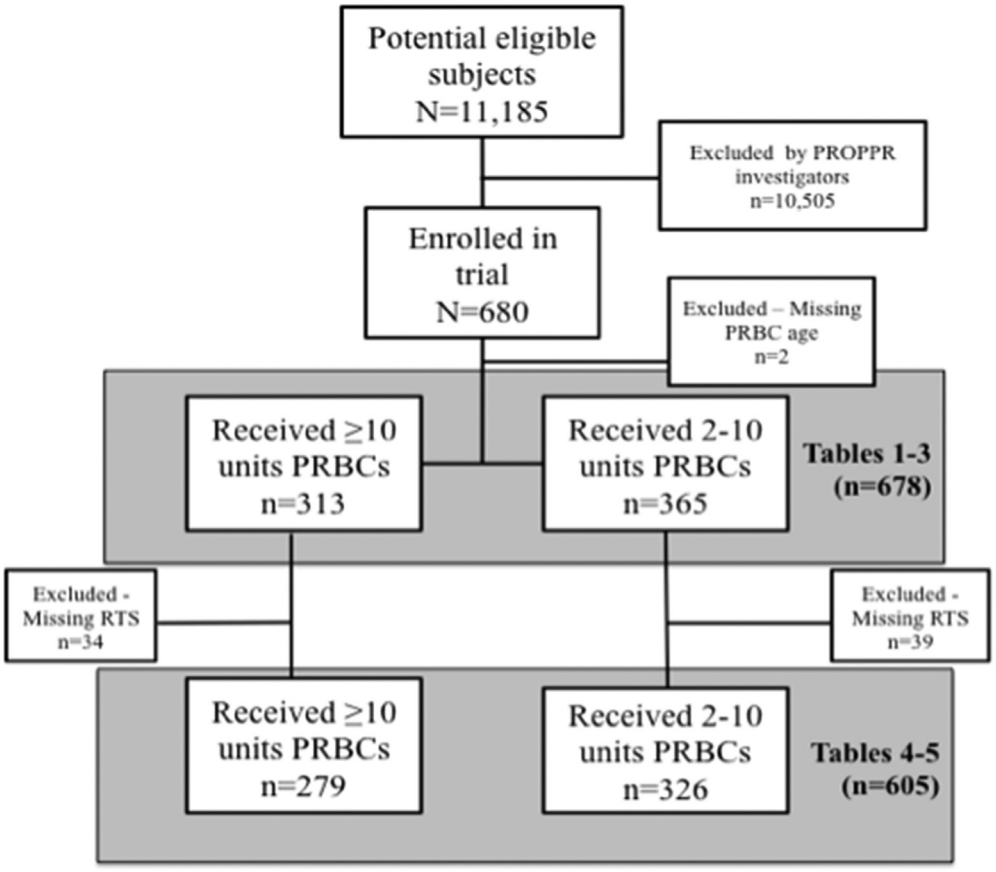


Figure 2. Flowchart of patients included in the analysis. *RTS*, Revised trauma score.

Table 1. Patient and transfusion* characteristics (N=678 subjects).

Characteristic	All Patients	Massively Transfused (n=313)	Nonmassively Transfused (n=365)
Age, median (IQR), y	34 (25–51)	33 (24–52)	35 (25–50)
Male sex, No. (%)	541 (80.5)	251 (80.2)	293 (80.3)
Race, No. (%)			
White	432 (63.7)	200 (63.9)	232 (63.6)
Black	186 (27.4)	86 (27.5)	100 (27.4)
Other	60 (8.9)	27 (8.6)	33 (9.0)
Mechanism of injury, No. (%)			
Blunt	350 (51.6)	174 (55.6)	176 (48.2)
Penetrating	320 (47.2)	135 (43.1)	185 (50.7)
Both	8 (1.2)	4 (1.3)	4 (1.1)
Injury Severity Score, median (IQR)	26 (17–41)	33 (22–42)	24 (14–34)
Revised Trauma Score, median (IQR) (n=605)	6.8 (4.1–7.8)	6.4 (4.1–7.6)	6.9 (4.1–7.8)
Glasgow Coma Scale score, median (IQR)	14 (3–15)	13 (3–15)	14 (3–15)
Systolic blood pressure, median (IQR) (n=656), mm Hg	102 (80–126)	100 (80–126)	104 (82–126)
Diastolic blood pressure, median (IQR) (n=561), mm Hg	70 (51–91)	70 (50–91)	70 (53–91)
Pulse rate, median (IQR) (n=675), beats/min	114 (94–133)	121 (98–139)	110 (93–127)
Respiratory rate, median (IQR) (n=619), breaths/min	20 (17–26)	20 (17–27)	20 (18–26)
Total packed RBCs transfused for all patients (n), units	8,830	6,776	2,054
Packed RBC unit age, median (IQR), days	19 (13–27)	20 (16–26)	19 (14–26)
Total packed RBC units per subject (n), median (IQR)	9 (5–15); min 1, max 115	17 (12–25); min 5, max 115	6 (4–7); min 1, max 9
Total 0- to 7-day-old packed RBC units per subject (n), median (IQR)	3 (2–5); min 1, max 38	0 (0–2); min 0, max 38	0 (0–0); min 0, max 8
Total 8- to 14-day-old packed RBC units per subject (n), median (IQR)	4 (2–7); min 1, max 58	3 (0–7); min 0, max 58	1 (0–1); min 0, max 9
Total 15- to 21-day-old packed RBC units per subject (n), median (IQR)	3 (2–6); min 1, max 35	3 (0–8); min 0, max 56	1 (0–2); min 0, max 8
Total ≥22-day-old packed RBC units per subject (n), median (IQR)	5 (3–10); min 1, max 69	6 (2–12); min 0, max 69	1 (0–3); min 0, max 9
Total packed RBC units per study site (n), median (IQR)	647.5 (499.5–795.0); min 230, max 2,017	469.5 (350.5–583.0); min 150, max 1,682	142 (98.5–260.0); min 76, max 335

CI, Confidence interval.

*Data reflect packed RBCs transfused in the first 24 hours of treatment. All packed RBCs given in the PROPPR trial were stored in 1 of 3 additive solutions. All solutions available in the United States and Canada have equivalent storage durations.[29] Numbers may not equal 100% because of missing data.

28 days (IQR 24 to 31) in site 12. The distribution of ages for packed RBCs transfused by study site is shown in Figure 4. On further analysis, no associations were found among study site and patient mortality.

One hundred patients (14.8%) died within the first 24 hours of hospitalization (Table 2). The most common major adverse events were infection (30.1%), sepsis (28.0%), and acute kidney injury (23.5%). At least 2 serious adverse events occurred in 43.5% of subjects. The composite outcome (death within 24 hours or ≥2 adverse events) occurred in 57.1% of patients. The 30-day mortality rate was 24.2%.

On multivariable analysis, the number of packed RBCs greater than or equal to 22 days old was independently associated with an increase in death within 24 hours (adjusted odds ratio of 1.05 per packed RBC unit; 95% confidence interval 1.01 to 1.08) (Table 3). In other words, transfusion of each additional unit of packed RBCs aged greater than or equal to 22 days was associated with a 5% increase in mortality risk. However, this association was noted only for patients who received greater than or equal to 10 packed RBC units; when packed RBC volume was modeled as a categoric variable (1 to 10, 11 to 20, and ≥21 units), increase in death within 24 hours was limited to patients receiving greater than 21 units of packed RBCs that were greater than or equal to 22 days old (Table 4).

Increased numbers of packed RBC units that were 8 to 14, 15 to 21, and greater than or equal to 22 days old were

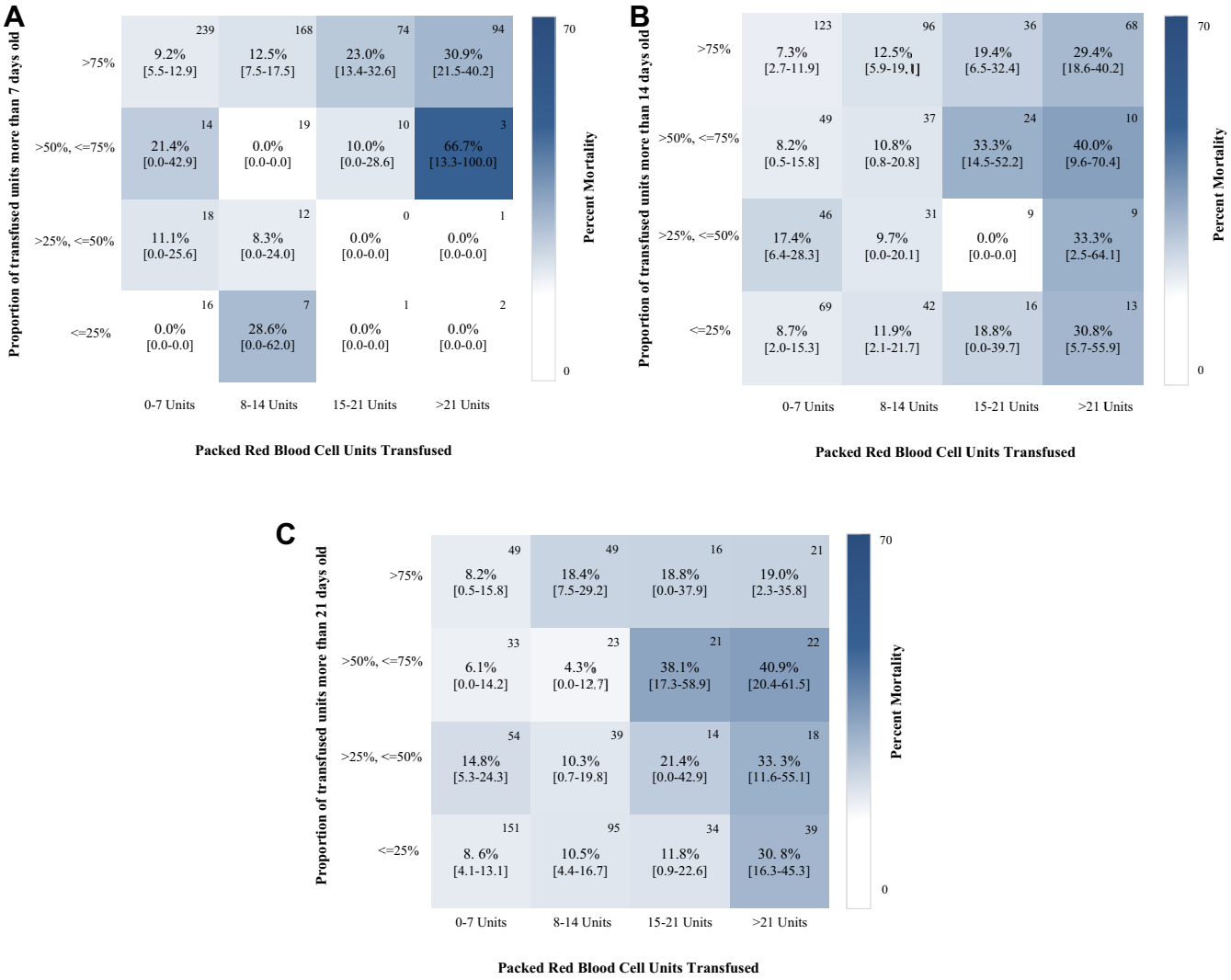


Figure 3. Volume of packed RBCs transfused in the first 24 hours, stratified by packed RBC age group and patient 24-hour mortality status. The number of patients is presented at the top right, and the proportion who died is shown at the center. Numbers in brackets indicate 95% CI.

associated with increased odds of the composite outcome of greater than or equal to 2 adverse events or death within 24 hours (Table 5). When packed RBC volume was characterized categorically, these associations persisted for packed RBC units that were 8 to 14 days old and greater than or equal to 22 days old. The number of packed RBCs that were 15 to 21 and greater than or equal to 22 days old was associated with increased 30-day mortality.

When repeating the analysis using imputed Revised Trauma Scores for the 73 patients with missing respiratory rate, systolic blood pressure, or both, we observed slightly different results (Table E1, available online at http://www.annemergmed.com). With packed RBC volume characterized as a continuous variable, the number of packed RBC units greater than or equal to 22 days old and 8 to 14 days old was significantly associated with death within 24 hours. When packed RBC volume was modeled categorically, the association between the number of packed RBC units greater than or equal to 22 days old and death within 24 hours persisted. In addition, the number of packed RBC units 8 to 14 days old was associated with increased odds of death.

When characterized on a continuous basis, the number of packed RBC units was associated with increased odds of death within 24 hours or greater than or equal to 2 adverse events, regardless of packed RBC unit age (Table E2, available online at http://www.annemergmed.com). However, when packed RBC age was examined categorically, this association seemed to be limited to packed RBC units 8 to 14 days old and greater than or equal to 22 days old. There was also evidence of increased 30-day mortality with the number of packed RBC units

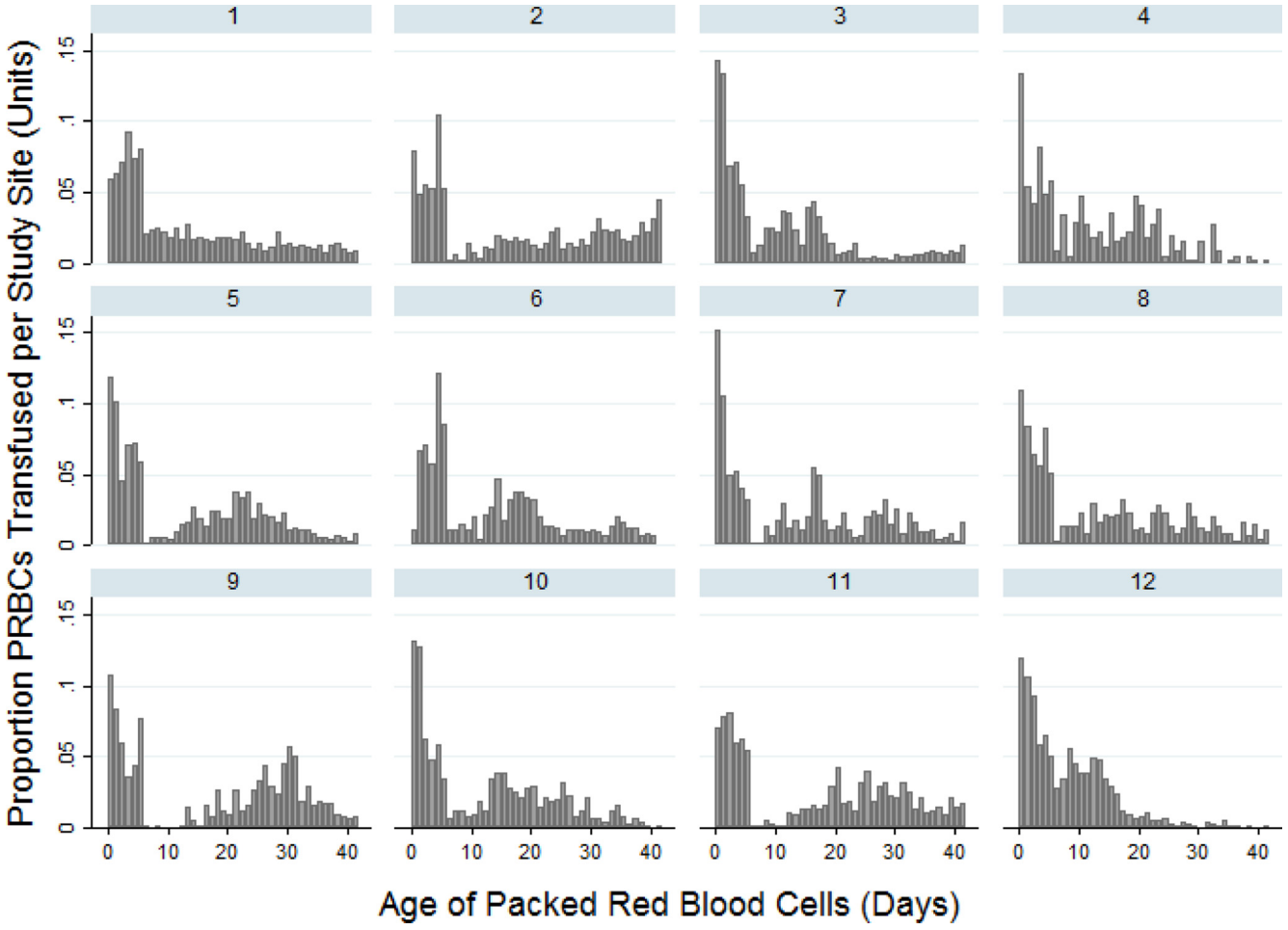


Figure 4. Distribution of packed RBC ages for transfused patients among PROPPR study sites.

that were 8 to 14, 15 to 21, and greater than or equal to 22 days old.

LIMITATIONS

This analysis has important limitations; prospective validation of these results is mandatory before changes in clinical practice are implemented. For example, total transfused packed RBC volume may serve as a proxy of the degree of physiologic damage or injury. Although we accounted for site clustering with random-effects regression, we observed notable variation in the median packed RBC age across the study sites, suggesting that practice variation may influence these results. In terms of allocating packed RBC units according to age, this aspect of care was not incorporated as part of the treatment protocol for patients enrolled in the PROPPR trial. Furthermore, no standard protocols exist to guide blood banks or emergency medical services in the choice of packed RBC units according to storage age. Thus, these factors may have also influenced outcomes on both the patient and study-site level.

Although our sensitivity analyses supported the primary findings, some of the additional findings exhibited uncertain patterns and wide confidence intervals. These results may indeed be attributed to the large number of clinical and demographic variables included in the analysis to properly control for confounding factors; in fact, it is possible that these findings are random. As such, caution should be used when these findings are interpreted; application to clinical settings is not recommended. A randomized controlled trial is the only way to overcome these limitations and to clarify the effect of packed RBC age on traumatic hemorrhagic shock. Additional efforts must also be taken to identify patient factors associated with vulnerability to stored blood toxicity.

The purpose of the PROPPR trial was to compare blood product ratios, not packed RBC age. Although the trial applied strict inclusion and exclusion criteria and used standardized protocols at all study sites, variations in patient mix and clinical protocols used during the course of hospitalization may have influenced the results. Other factors of influence to consider include time from injury to initiation of care, diagnosed and undiagnosed

Table 2. Outcomes and major adverse events, n=678 patients.

Adverse Event	No. (%)
Major adverse events	
Acute lung injury	104 (15.3)
Acute kidney injury	159 (23.5)
Acute respiratory distress syndrome	94 (13.9)
Deep venous thrombosis	49 (7.2)
Infection*	204 (30.1)
Multiple organ failure	35 (5.2)
Myocardial infarction	2 (0.3)
Pulmonary embolism, asymptomatic	22 (3.2)
Pulmonary embolism, symptomatic	27 (4.0)
Sepsis	190 (28.0)
Stroke	19 (2.8)
Ventilator-associated pneumonia	120 (17.7)
Transfusion-associated circulatory overload	1 (0.2)
Transfusion-related metabolic complication†	112 (16.5)
Rhabdomyolysis	18 (2.7)
24-h death	100 (14.8)
≥2 adverse events	195 (43.5)
24-h death or ≥2 adverse events	387 (57.1)
30-day death	164 (24.2)‡

*Includes urinary tract infection, wound infection, line infection, etc.
†Includes hypocalcemia and hyperkalemia.
‡Thirty-day status was not known for 4 subjects.

comorbidities, and variation in care outside of transfusion protocols both within and among study sites. Although we used panel regression techniques to account for clustering by study center, we limited further inference to protect site confidentiality. Finally, we acknowledge the limitations associated with a secondary analysis of a randomized trial. Our findings may suggest a possible risk of adverse outcomes related to the transfusion of stored packed RBCs in the trauma population, but require validation with a Level I prospective study specifically designed to address the issue of blood age in the trauma setting.

Although we focused on the effect of transfused packed RBCs, platelets are also associated with cellular breakdown during storage and increased risk of complications after trauma.[26,27] In addition, practitioners and blood banks may have selected specific aged packed RBCs for massive transfusion. However, given the tempo of packed RBC transfusions in PROPPR, we do not expect systematic bias. Finally, the nature of the PROPPR trial did not allow definitive diagnosis of all injuries before study enrollment. As such, patients with traumatic brain injury associated with coagulopathy and complications after trauma were included in the study sample.[28]

DISCUSSION

In the current analysis, the transfusion of packed RBCs greater than or equal to 22 days old was associated with an increase in death within 24 hours in patients who received greater than or equal to 10 units in the first 24 hours after hospital admission. The magnitude of associated harm was related to the number of such units transfused. Thus, critically injured patients who required transfusion of greater than or equal to 10 packed RBC units experienced a 5% increase in their risk of mortality with transfusion of each additional packed RBC unit aged 22 days or more. The prevalence of adverse events and 30-day mortality was similarly higher for patients receiving older packed RBC units, with hints of these harmful effects with packed RBC units as young as 8 days. These observations highlight the potentially toxic effects of transfusion with older packed RBC units in traumatic hemorrhagic shock. These risks will not necessarily increase linearly, as suggested by the results. In fact, with transfusion of each additional packed RBC unit, patients' status will change and their risk of mortality will be altered according to their current physiologic condition. Therefore, caution must be used in the interpretation of these findings. Application to clinical practice is not warranted according to these results; rather, additional randomized studies are needed to confirm these findings among trauma patients who receive greater than or equal to 10 units of packed RBCs.

Our analysis has important contrasts with a previous trial of transfused packed RBC age.[13] The Informing Fresh Versus Old Red Cell Management trial randomized 20,858 patients requiring packed RBC transfusion to either treatment with freshest or oldest-available packed RBC units, finding no difference in mortality.[23] However, the trial excluded patients requiring uncross-matched or massive-quantity blood transfusion. Similarly, investigators of the Standard Issue Transfusion Versus Fresher Red-Cell Use in Intensive Care, Age of Blood Experiment, and Red Cell Storage Study compared the effect of the freshest available packed RBCs with the standard-issue or oldest available packed RBCs in intensive care patients, critically ill patients, and elective cardiac surgery patients, respectively.[10-12] These studies found no difference in mortality, length of stay, or complications among patients who received fresh packed RBCs versus standard-issue packed RBCs. However, only 15% of patients were injured, and the subjects received a limited number of packed RBC units (median 4 units or fewer).[10-12,23] Our use of the PROPPR data offered perspectives from a large, multicenter series of very high-acuity traumatic hemorrhagic shock patients receiving large volumes of

Table 3. Associations between packed RBC age and 24-hour mortality.

Variable	Unadjusted OR (95% CI)	Multivariable Adjusted, All Patients, OR (95% CI)	Multivariable Adjusted, Patients Receiving ≥10 Units Packed RBCs (n=313), OR (95% CI)	Multivariable Adjusted, Patients Receiving <10 Units Packed RBCs (n=361), OR (95% CI)
Packed RBC age category, days				
0–7	0.96 (0.89–1.06)	0.97 (0.88–1.08)	0.96 (0.86–1.08)	0.95 (0.68–1.32)
8–14	1.06 (1.03–1.10)	1.04 (0.99–1.09)	1.04 (0.99–1.09)	1.00 (0.79–1.27)
15–21	1.02 (0.98–1.05)	1.02 (0.98–1.06)	1.02 (0.98–1.06)	0.86 (0.64–1.14)
≥22	1.04 (1.02–1.07)	1.05 (1.01–1.08)	1.05 (1.01–1.08)	1.05 (0.83–1.32)
Total packed RBC units				
1–10		1 [Reference]		
11–20		1.43 (0.77–2.65)		
≥21		1.09 (0.40–2.98)		
Patient age		1.01 (1.00–1.03)	1.02 (1.00–1.03)	1.02 (0.99–1.04)
Sex (male vs female)		1.23 (0.68–2.21)	1.92 (0.89–4.11)	0.62 (0.22–1.75)
Race				
White		1 [Reference]	1 [Reference]	1 [Reference]
Black		1.52 (0.84–2.79)	1.46 (0.68–3.16)	1.36 (0.46–4.06)
Other		0.55 (0.20–1.56)	0.71 (0.21–2.47)	0.27 (0.03–2.29)
Mechanism of injury				
Blunt vs penetrating		1.41 (0.71–2.78)	2.08 (0.97–4.46)	0.86 (0.30–2.44)
Burn vs penetrating		6.01 (0.98–37.0)	NA	NA
Injury Severity Score		1.03 (1.01–1.05)	1.55 (1.11–2.17)	1.32 (0.80–2.17)
Revised Trauma Score		0.66 (0.56–0.77)	0.52 (0.37–0.72)	0.35 (0.22–0.55)
PROPPR trial treatment group		1.45 (0.85–2.50)	1.03 (0.55–1.94)	2.24 (0.97–5.17)

OR, Odds ratio; *NA*, not applicable.

Data in cells reflect ORs for death per additional packed RBC unit received. ORs were estimated from random-effects models, accounting for clustering by study site. Multivariable models were adjusted for patient age, sex, race, mechanism of injury, Injury Severity Score, Revised Trauma Score, and PROPPR trial treatment group. The model for "All Patients" was additionally adjusted for total packed RBC units transfused in the first 24 hours after admission.

Table 4. Associations between packed RBC age and 24-hour mortality, stratified by packed RBC age and volume.

Packed RBC Age Category, Days	Total Volume (Units) of Each Packed RBC Age Category, Days (95% CI)		
	1–10	11–20	≥21
0–7	0.82 (0.44–1.51)	NA	NA
	(n=196)	(n=4)	(n=6)
8–14	1.62 (0.88–2.99)	0.92 (0.23–3.65)	6.41 (0.88–46.5)
	(n=377)	(n=32)	(n=13)
15–21	0.72 (0.40–1.30)	1.20 (0.36–3.93)	1.85 (0.52–6.53)
	(n=379)	(n=26)	(n=23)
≥22	2.56 (1.19–5.48)	7.80 (2.99–20.34)	4.18 (1.24–14.04)
	(n=384)	(n=64)	(n=36)

Data in cells reflect adjusted ORs for death per additional unit of packed RBCs received. ORs were estimated from a multivariable random-effects model adjusted for patient age, sex, race, mechanism of injury, Injury Severity Score, Revised Trauma Score, total packed RBC units transfused in the first 24 hours after admission, and PROPPR trial treatment group, and accounting for clustering by study site. Patients may be represented in more than one combination of packed RBC age and total volume categories.

blood products rapidly.[14] We observed that the transfusion of blood greater than or equal to 22 days old was most consistently associated with poor outcomes among patients who were massively transfused. However, the odds of death within 30 days appeared to also be associated with the number of 15- to 21-day-old packed RBC units. In addition, the odds of negative outcomes (eg, the composite outcome of ≥2 adverse events or death within 24 hour) also appeared to be associated with the number of packed RBC units that were 8 to 14 and 15 to 21 days old.

Therefore, although our results most clearly suggest avoiding packed RBCs greater than 22 days old for patients who are massively transfused after trauma, omitting or minimizing use of packed RBCs 8 to 14 and 15 to 21 days old in this population may have merit. Though we observed this association, transfusion strategies used in clinical practice must be selected prospectively in response to anticipated transfusion need; post hoc classification of massive transfusion is not clinically useful. Randomized controlled trials that

Table 5. Associations between packed RBC age, major transfusion-related adverse events, and death within 30 days.

Packed RBC Age Category, Days	≥2 Adverse Events or 24-Hour Death, OR (95% CI)	≥2 Adverse Events or 24-Hour Death, OR (95% CI)	30-Day Death, OR (95% CI)	30-Day Death, OR (95% CI)
Packed RBC volume (units) as continuous variable				
0–7	1.08 (0.995–1.17)		1.00 (0.91–1.11)	
8–14	1.13 (1.06–1.19)		1.03 (0.98–1.08)	
15–21	1.08 (1.03–1.14)		1.06 (1.02–1.10)	
≥22	1.11 (1.07–1.16)		1.09 (1.04–1.12)	
Packed RBC volume (units) as categoric variable				
0–7				
0 units		1 [Reference]		1 [Reference]
1–10		0.87 (0.57–1.33)		0.95 (0.56–1.63)
11–20		NA		NA
≥21		1.02 (0.13–8.32)		0.44 (0.01–21.29)
8–14				
0 units		1 [Reference]		1 [Reference]
1–10		1.80 (1.20–2.69)		1.42 (0.81–2.50)
11–20		2.87 (1.07–7.73)		1.98 (0.70–5.59)
≥21		3.90 (0.64–23.84)		2.20 (0.19–25.47)
15–21				
0 units		1 [Reference]		1 [Reference]
1–10		0.94 (0.64–1.37)		1.15 (0.67–1.96)
11–20		3.35 (0.90–12.52)		1.66 (0.49–5.59)
≥21		2.23 (0.58–8.51)		4.49 (1.38–14.65)
≥22				
0 units		1 [Reference]		1 [Reference]
1–10		1.62 (1.05–2.52)		1.90 (1.003–3.58)
11–20		4.48 (2.08–9.68)		12.46 (5.09–30.51)
≥21		6.83 (2.24–20.81)		5.11 (1.59–16.41)

ORs were estimated from random-effects models, adjusted for patient age, sex, race, mechanism of injury, Injury Severity Score, Revised Trauma Score, total packed RBC units transfused in the first 24 hours after admission, and PROPPR trial treatment group, and accounting for clustering by study site.

examine the effect of stored packed RBC units of all ages are therefore still needed in this patient population. Thus, it follows that the natural next step should include a trial that enrolls only adult patients with major trauma who are anticipated to require massive transfusion, and who are randomized to receive all-fresh or all-standard-issue stored packed RBCs. Finally, our findings suggest that development of a "stored blood index" may be useful to clinicians and investigators. Such an index may quantify the risk of adverse outcomes associated with transfusion of each unit of packed RBCs according to storage age.

An important limitation of our analysis is the absence of packed RBC age randomization. Multiple factors may influence the patterns of the age of RBCs given to a patient, including confounding by indication and the packed RBC storage practices of an institution. A randomized trial assigning patients in traumatic hemorrhagic shock to younger versus older packed RBC units may help to clarify this association. An alternate strategy may entail defining the harmful mechanisms of stored blood toxicity, potentially leading to pharmacologic interventions to block the harmful effects of stored packed RBCs.

In summary, the transfusion of older packed RBCs in patients with traumatic hemorrhagic shock who received greater than or equal to 10 packed RBC units was associated with worsened outcomes. Injured patients requiring massive blood transfusion may benefit from receipt of fresh packed RBC units.

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REFERENCES

1. Kauvar DS, Lefering R, Wade CE. Impact of hemorrhage on trauma outcome: an overview of epidemiology, clinical presentations, and therapeutic considerations. *J Trauma*. 2006;60:S3-S11.
2. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. *Anaesthesia*. 2015;70(suppl 1):29-37, e9-12.
3. Oh JY, Stapley R, Harper V, et al. Predicting storage-dependent damage to red blood cells using nitrite oxidation kinetics, peroxiredoxin-2 oxidation, and hemoglobin and free heme measurements. *Transfusion*. 2015;55:2967-2978.
4. Hess JR. Measures of stored red blood cell quality. *Vox Sang*. 2014;107:1-9.
5. Jackman RP, Utter GH, Muench MO, et al. Distinct roles of trauma and transfusion in induction of immune modulation after injury. *Transfusion*. 2012;52:2533-2550.
6. Chin-Yee IH, Gray-Statchuk L, Milkovich S, et al. Transfusion of stored red blood cells adhere in the rat microvasculature. *Transfusion*. 2009;49:2304-2310.
7. Stapley R, Rodriguez C, Oh JY, et al. Red blood cell washing, nitrite therapy, and antiheme therapies prevent stored red blood cell toxicity after trauma-hemorrhage. *Free Radic Biol Med*. 2015;85:207-218.
8. Wang D, Sun J, Solomon SB, et al. Transfusion of older stored blood and risk of death: a meta-analysis. *Transfusion*. 2012;52:1184-1195.
9. Qu L, Triulzi DJ. Clinical effects of red blood cell storage. *Cancer Control*. 2015;22:26-37.
10. Cooper DJ, McQuilten ZK, Nichol A, et al. Age of red cells for transfusion and outcomes in critically ill adults. *N Engl J Med*. 2017;377:1858-1867.
11. Lacroix J, Hebert PC, Fergusson DA, et al. Age of transfused blood in critically ill adults. *N Engl J Med*. 2015;372:1410-1418.
12. Steiner ME, Ness PM, Assmann SF, et al. Effects of red-cell storage duration on patients undergoing cardiac surgery. *N Engl J Med*. 2015;372:1419-1429.
13. Heddle NM, Cook RJ, Arnold DM, et al. Effect of short-term vs long-term blood storage on mortality after transfusion. *N Engl J Med*. 2016;375:1937-1945.
14. Sparrow RL. Red blood cell storage duration and trauma. *Transfus Med Rev*. 2015;29:120-126.
15. Belizaire RM, Makley AT, Campion EM, et al. Resuscitation with washed aged packed red blood cell units decreases the proinflammatory response in mice after hemorrhage. *J Trauma Acute Care Surg*. 2012;73:S128-S133.
16. Mangalmurti NS, Xiong Z, Hulver M, et al. Loss of red cell chemokine scavenging promotes transfusion-related lung inflammation. *Blood*. 2009;113:1158-1166.
17. Nicholson SE, Johnson RA, Craig T, et al. Transfusion-related acute lung injury in a rat model of trauma-hemorrhage. *J Trauma*. 2011;70:466-471.
18. Cortes-Puch I, Wang D, Sun J, et al. Washing older blood units before transfusion reduces plasma iron and improves outcomes in experimental canine pneumonia. *Blood*. 2014;123:1403-1411.
19. Lei C, Yu B, Shahid M, et al. Inhaled nitric oxide attenuates the adverse effects of transfusing stored syngeneic erythrocytes in mice with endothelial dysfunction after hemorrhagic shock. *Anesthesiology*. 2012;117:1190-1202.
20. Baron DM, Beloiartsev A, Nakagawa A, et al. Adverse effects of hemorrhagic shock resuscitation with stored blood are ameliorated by inhaled nitric oxide in lambs. *Crit Care Med*. 2013;41:2492-2501.
21. Holcomb JB, Tilley BC, Baraniuk S, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma: the PROPPR randomized clinical trial. *JAMA*. 2015;313:471-482.
22. Holcomb JB, Tilley BC, Baraniuk S, et al. Pragmatic Randomized Optimal Platelet and Plasma Ratios (PROPPR) Trial. 2015. Available at: https://biolincc.nhlbi.nih.gov/studies/proppr. Accessed October 16, 2018.
23. Cook RJ, Heddle NM, Lee KA, et al. Red blood cell storage and in-hospital mortality: a secondary analysis of the INFORM randomised controlled trial. *Lancet Haematol*. 2017;4:e544-e522.
24. Savage SA, Zarzaur BL, Croce MA, et al. Redefining massive transfusion when every second counts. *J Trauma Acute Care Surg*. 2013;74:396-400; discussion 400-402.
25. Rubin D. *Multiple Imputation for Nonresponse in Surveys. Wiley Series in Probability and Mathematical Statistics: Applied Probability and Statistics*. New York, NY: Wiley & Sons; 1987.

26. Perales Villarroel JP, Figueredo R, Guan Y, et al. Increased platelet storage time is associated with mitochondrial dysfunction and impaired platelet function. *J Surg Res*. 2013;184:422-429.
27. Inaba K, Branco BC, Rhee P, et al. Impact of the duration of platelet storage in critically ill trauma patients. *J Trauma*. 2011;71:1766-1773; discussion 1773-1774.
28. Epstein DS, Mitra B, O'Reilly G, et al. Acute traumatic coagulopathy in the setting of isolated traumatic brain injury: a systematic review and meta-analysis. *Injury*. 2014;45:819-824.
29. Dumont LJ, AuBuchon JP. Evaluation of proposed FDA criteria for the evaluation of radiolabeled red cell recovery trials. *Transfusion*. 2008;48:1053-1060.

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