

Transfusion of Cryopreserved Packed Red Blood Cells Is Safe and Effective After Trauma

A Prospective Randomized Trial

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Objectives: To determine the safety and efficacy of cryopreserved packed red blood cell (CPRBC) transfusion in trauma patients.

Background: Liquid packed red blood cells (LPRBCs) have an abbreviated shelf-life and worsening storage lesion with age. CPRBCs are frozen 2 to 6 days after donation, stored up to 10 years, and are available for 14 days after thawing and washing. CPRBCs can be utilized in diverse settings, but the effect on clinical outcomes is unknown.

Methods: We performed a prospective, randomized, double-blind study at 5 level 1 trauma centers. Stable trauma patients requiring transfusion were randomized to young LPRBCs (≤ 14 storage days), old LPRBCs (> 14 storage days), or CPRBCs. Tissue oxygenation (StO₂), biochemical and inflammatory mediators were measured, and clinical outcomes were determined.

Results: Two hundred fifty-six patients with well-matched injury severity and demographics ($P > 0.2$) were randomized (84 young, 86 old, and 86 CPRBCs). Pretransfusion and final hematocrits were similar ($P > 0.68$). Patients in all groups received the same number of units postrandomization (2 [1–4]; $P > 0.05$). There was no difference in the change in tissue oxygenation between groups. CPRBCs contained less $\alpha 2$ -macroglobulin, haptoglobin, C-reactive protein, and serum amyloid P ($P < 0.001$). Organ failure, infection rate, and mortality did not differ between groups ($P > 0.2$).

Conclusions: Transfusion of CPRBCs is as safe and effective as transfusion of young and old LPRBCs and provides a mechanism to deliver PRBCs in a wide variety of settings.

Keywords: cryopreserved packed red blood cells, hemorrhage, packed red blood cells, tissue oxygenation, trauma

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Injured patients frequently experience sufficient blood loss to necessitate blood transfusion.¹ The goal of liquid packed red blood cell (LPRBC) transfusion is to restore perfusion to patients in shock by expanding intravascular volume and replacing oxygen-carrying hemoglobin (Hb). Approximately 14 million units of blood are transfused annually, making blood transfusion one of the most common medical therapies performed on trauma patients.^{2,3}

For many patients, the transfusion of LPRBCs is a life-saving therapy with the benefits outweighing the associated risks. However, a number of studies have shown that blood transfusion is independently associated with increased mortality.^{4–8} The reason for the increased mortality is unknown. It may be due, in part, to the age of blood. Evidence from nonrandomized and retrospective studies suggests that both blood transfusion and age of blood are independently associated with increased morbidity and mortality.^{9,10} The etiologies of the negative effects of older blood that include increased infection rates, increased organ failure, and increased mortality have not been delineated, but are believed to be related to some elements of the storage defect.^{8–10} Blood stored for up to 42 days undergoes a “storage lesion” over time that consists of both morphologic and biochemical changes.^{11,12}

Progress in maintaining the quality and function of ex vivo hypothermically stored LPRBCs has been slow.^{13,14} The new storage solutions do not fully suppress the metabolic and physical changes associated with aging RBCs.^{11,12} This limitation could be overcome with cryopreservation, which uses the beneficial effects of ultralow temperatures to suppress molecular motion and arrest metabolic and biochemical reactions.^{15,16} In contrast to hypothermic storage, RBC physiology, including Hb structure, membrane and cellular energy, is unaffected by long-term storage in the frozen state.^{17–19}

Continuous maintenance of an adequate blood supply continues to be a major challenge. There are seasonal and disaster-related decreases in blood availability, and it is difficult to maintain a steady national blood supply without excessive waste of this precious resource. These issues are exacerbated by the need to have less common blood types available and the relatively short life span of LPRBCs. The availability of blood for trauma patients is profoundly affected by mass casualty scenarios and by a relatively small number of patients who require massive transfusions. It is also highly affected by region with much less availability in rural areas. Each year approximately 3% of donated blood units expire before use resulting in an estimated cost of more than \$80.0 million.²⁰ The ability to store RBCs for a much longer period could significantly alleviate unpredictable shortages associated with increased use and the waste of unused blood.

Cryopreserved PRBCs (CPRBCs) have the potential to revolutionize the blood banking industry by increasing the life span of RBCs from 42 days to 10 years without a diminution in their efficacy during

the storage period.²¹ We have previously shown that cryopreservation and deglycerolization of RBCs results in a reduction of potentially harmful biochemical mediators, and transfusion of CPRBCs results in improved tissue oxygenation compared with LPRBCs.^{22,23} The current study was performed to examine the therapeutic effectiveness and safety of CPRBC transfusion in trauma patients. We hypothesized that transfusion of CPRBCs is safe and effective in trauma patients and results in equivalent clinical outcomes compared to LPRBCs.

METHODS

We performed a prospective, randomized double-blinded study at Oregon Health & Science University (OHSU), University of Texas Health Science Center at Houston/Memorial Hermann Hospital, UT Southwestern/Parkland Memorial Hospital, University of Texas Health Science Center at San Antonio, and University of Cincinnati Medical Center. The study was approved by the Institutional Review Board at each center, and registered at ClinicalTrials.gov (Trial number NCT01038557), where the full protocol can be accessed. OHSU served as the Data Coordinating Center and central laboratory for research-specific assays. Hemodynamically normal trauma patients more than 15 years old with an injury severity score (ISS) more than 4 and anemia were eligible for the study. Consent was obtained from the patient or a legal representative. An Hb less than 7 mg/dL was utilized as the transfusion threshold. If a blood transfusion was ordered by the patients' primary team, they were enrolled in the study and randomized by the local blood bank to receive young LPRBCs (≤ 14 days old), old LPRBCs (> 14 days old), or CPRBCs. The randomization was generated at OHSU and determined by a random number generating program and patient allocation was determined by filling the next line on the list. Randomization lists were stratified by site in random permuted-block design. Once a patient was randomized to a blood group, the blood bank at each institution assigned patients on the basis of the randomization scheme. Patients exclusively received blood from that group for the remainder of their hospitalization. Exclusion criteria included inability to maintain the blood randomization owing to blood bank limitations, need for emergent transfusion, patients who had a massive transfusion, bilateral hand injuries preventing near infrared spectroscopy measurements, pregnancy, prisoner status, and active use of vasoregulatory medications.

CPRBCs were provided to each site by the Armed Services Blood Program at no charge and stored in a -80°C freezer. These cells are frozen in glycerol within 6 days of donation and stored for up to 10 years. If a patient was randomized to receive CPRBCs, the cells were thawed and deglycerolized utilizing an ACP 215 (Haemonetics Corporation, Braintree, MA). These cells were available for transfusion in approximately 90 minutes. Thawed CPRBCs were prepared for potential use in consented patients who underwent planned surgical procedures and were transfused intraoperatively or postoperatively as ordered by the primary team. LPRBCs were provided by the blood bank at each site. All blood bank personnel were trained on the preparation of CPRBCs before the study. Providers and patients were blinded to the type of transfusion performed.

Laboratory Assays

Blood samples were drawn before transfusion (baseline), at the end of transfusion of each unit of blood for the first 2 units, and 12 hours after completion of transfusion. Samples were also drawn from each transfused unit of RBCs. Samples were collected in sodium citrated tubes, immediately centrifuged, and the plasma or supernatant was stored at -80°C until assayed at OHSU.

Biochemical markers of impaired RBC function were assessed in patient and unit samples. Free Hb was measured by enzyme-linked immunosorbent assay (Bethyl Laboratories, Montgomery, TX). Using the Luminex Bio-Plex 200 (Luminex, Austin, TX), haptoglobin, α_2 -

macroglobin, C-reactive protein, and serum amyloid P were analyzed utilizing the Bio-Plex Pro Human Acute Phase 4-Plex Panel (Bio-Rad Laboratories, Inc, Hercules, CA), and inflammatory cytokines (interleukin [IL]-2, IL-4, IL-6, IL-8, IL-10, granulocyte macrophage colony stimulating factor (GM-CSF), interferon- γ [IFN- γ], tumor necrosis factor- α [TNF- α]) were assayed using the Bio-Plex Pro Human Cytokine 8-Plex Panel (Bio-Rad Laboratories, Inc).

2,3-DPG was quantified in patient and unit samples as a marker of the ability of transfused Hb to deliver O_2 to tissues. Whole blood from patients was collected into ice-cooled heparinized tubes for immediate deproteinization. Samples were neutralized with 3.5 M potassium carbonate solution and centrifuged. The supernatant was stored at -80°C , and analyzed with a commercially available kit (Roche Diagnostics, Mannheim, Germany).

Blood coagulation parameters were assessed in patient samples. Standard coagulation assays (prothrombin time/international normalized ratio [PT/INR], activated partial thromboplastin time [aPTT], fibrinogen, D-dimer, and Protein C) were measured using a STA Compact Hemostasis System (Diagnostica Stago, Inc, Parsippany, NJ). Whole blood samples were analyzed by thrombelastography (TEG 5000; Haemonetics Corporation) to assess the time to clot formation (t time), the rate of clot formation (α -angle), time in minutes to reach 20-mm clot strength (k time), maximal amplitude of the tracing representing platelet contribution, absolute clot strength (G value), and the degree of fibrinolysis (LY30).

Tissue Oxygenation

A near infrared spectroscopy sensor was placed on the thenar eminence of an uninjured upper extremity and tissue oxygenation (StO_2 ; Hutchinson Technology, Inc, Hutchinson, MN) was measured continuously starting 1 hour before the first transfusion of RBCs until 12 hours after completion of the second transfusion.

Clinical Parameters

All patients enrolled in the study underwent daily screening by study personnel for adverse clinical outcomes until hospital discharge. In-hospital mortality was observed. Patients were screened for acute respiratory distress syndrome (American-European Consensus Conference Criteria) and acute renal failure (RIFLE Classification). The combination of a blood stream infection and positive Systemic Inflammatory Response Syndrome score was defined as sepsis. Centers for Disease Control criteria were utilized to define ventilator-associated pneumonia, blood stream infections, surgical site infections, and urinary tract infections.

Screening for thromboembolic disease was determined by each site. Deep vein thrombosis was defined by a positive venous duplex examination, and pulmonary embolus was diagnosed by computed tomographic angiography in symptomatic patients.

Data Management

Study data were collected and managed using REDCap electronic data capture tools hosted at OHSU. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing (1) an intuitive interface for validated data entry; (2) audit trails for tracking data manipulation and export procedures; (3) automated export procedures for seamless data downloads to common statistical packages; and (4) procedures for importing data from external sources.

Statistical Analysis

We based our power analysis on a prior study showing that transfusion of old LPRBCs results in a StO_2 reduction of approximately 5%.²⁴ On the basis of a power of 80% and $\alpha = 0.05$, it was

estimated that a total of 288 patients would be required to complete the study. The study concluded after the enrollment of 254 patients due to the completion of the funding cycle.

Data were analyzed using SPSS version 22 (IBM Corp., Armonk, NY). Descriptive statistics were computed for all study variables, and stratified distribution plots were examined to verify the normality of distribution of continuous variables. Normally distributed, continuous variables were compared by *t* test or analysis of variance (ANOVA) followed by Tukey post hoc analysis, and reported as mean \pm SEM. Nonparametric data were compared with the Mann-Whitney *U* test, Kruskal-Wallis test, or Wilcoxon rank sum test, and reported as median (interquartile range). Dichotomous variables were assessed by χ^2 analysis. Significance was set at $P < 0.05$ for all analyses.

RESULTS

Two hundred fifty-six patients were randomized and received blood (84 young LPRBCs, 86 old LPRBCs, and 86 CPRBCs). Sex, age, and ISS did not differ between groups (Table 1). The population was moderately injured with a mean ISS of 19. There were no differences in hospital length of stay, intensive care unit length of stay, ventilator days, median systolic blood pressure before transfusion or after transfusion between groups (Table 1). The median age of LPRBCs was 32 days in the old group and 7.5 days in the young group. CPRBCs were transfused a median of 1 day after thawing and deglycerolization. Patients in all groups received the same number of RBC units after randomization and there were no differences in the number of patients in each group who received 1 unit or to 5 units

or more. There was also no difference in the day of admission the first randomized unit was given (Table 2). There were no differences in adverse clinical outcomes including mortality between groups (Table 3). There were also no differences in outcomes in the subgroup of patients in each group who received to 5 units or more. No transfusion reactions were reported in this study.

Changes in StO_2 over the course of 3 hours are shown in Figure 1. Utilizing area under the curve measurements, there were no differences in the change in StO_2 from baseline within or between groups.

Biochemical and inflammatory markers that influence RBC function were measured in the units of PRBCs (Table 4). Similar to our previous study,²² CPRBCs contained lower concentrations of $\alpha 2$ -macroglobulin, haptoglobin, c-reactive protein (CRP), and serum amyloid P ($P < 0.001$ vs old and young PRBCs). CPRBCs and young LPRBCs also had higher levels of 2,3-DPG ($P < 0.01$ vs old PRBCs), which facilitates O_2 delivery from RBCs to tissues. Free Hb levels were lower in the young LPRBC group, and similar in CPRBCs and old LPRBCs. Anti-inflammatory cytokines IL-4 and IL-10 were increased in CPRBCs ($P < 0.001$ vs old and new PRBCs). Inflammatory markers GM-CSF and IFN- γ and IL-6 levels were elevated in CPRBCs compared with old LPRBCs ($P < 0.001$); however, IL-6 and IFN- γ were barely detectable in any of the specimens.

Biochemical markers and cytokine levels were also measured in patient specimens (Table 5). Patients in the CPRBC group had lower CRP levels at baseline compared with the old LPRBC group ($P < 0.05$). There were no differences between or within groups with respect to $\alpha 2$ -macroglobulin, haptoglobin, or 2,3-DPG. IL-2

TABLE 1. Patient Demographics, Lengths of Stay, Ventilator Days, and Systolic Blood Pressure

	Old LPRBCs		Young LPRBCs		CPRBCs		P (Between Groups)
	N		N		N		
Age (yr)	86	47.9 (28.1–67.0)	82	52.3 (29.4–62.5)	86	49.3 (30.9–62.6)	0.89
Sex (male/female)	86	46/40	82	50/32	86	57/29	0.27
Injury severity score	86	19 (11–26)	82	19 (10–32)	86	19 (14–29)	0.97
Hospital days	86	13.5 (9–22)	82	13 (8–20.5)	86	18 (10–26)	0.22
ICU days	86	5 (2–11)	82	6 (2–11)	86	7 (3–14.5)	0.12
Ventilator days	86	0 (0–4.25)	82	0 (0–4.50)	86	1 (0–9.5)	0.07
Baseline systolic blood pressure (mm Hg)	86	124 (108–137)	82	120 (107–135)	86	126 (109–142)	0.38
12-h systolic blood pressure (mm Hg)	86	127 (113–143)	82	124 (110–140)	86	128 (115–142)	0.33

Data are presented as median (interquartile range). N values represent the number of patients in each group in whom the measured parameter is available. ICU indicates intensive care unit.

TABLE 2. Hematologic Data

	Old LPRBCs		Young LPRBCs		CPRBCs		P (Between Groups)
	N		N		N		
Patient hematocrit (%)							
Baseline	80	20.9 \pm 2.2	75	21.1 \pm 2.0	81	20.9 \pm 2.2	0.68
Unit 1	74	23.3 \pm 2.1*	67	23.3 \pm 3.0*	76	22.6 \pm 3.4*	0.25
Unit 2	46	26.1 \pm 4.5*	43	25.7 \pm 3.5*	36	24.8 \pm 2.7*	0.30
12 h post	77	24.7 \pm 2.9*	72	24.8 \pm 2.8*	77	24.8 \pm 3.4*	0.99
Patients who received only 1 unit (N)		22		25		19	0.43
Patients who received \geq 5 units (N)		12		7		9	0.54
Hospitalization day first unit was received	86	4 (2–7)	82	4 (2–8)	86	4 (3–8)	0.48
Age of blood (d)	86	32 (23–36)	82	7.5 (5–11)†	86	1 (1–3)†	0.00
Units transfused	86	2 (1–3)	82	2 (1–3)	86	2 (1–4)	0.58
Transfusion time (min)	86	75 (40.5–130)	82	90 (51.25–141.25)	86	86 (37.75–127.25)	0.60

Data are presented as means \pm SE or median (interquartile range). Age of CPRBCs refers to days after thawing and deglycerolization.

* $P < 0.05$ vs baseline within group; † $P < 0.05$ vs old LPRBCs.

TABLE 3. Clinical Outcomes

	Old LPRBCs	Young LPRBCs	CPRBCs	P (Between Groups)
Total patients	85	82	86	
Acute renal failure	8%	9%	12%	0.45
Acute respiratory distress syndrome	2%	6%	5%	0.46
Ventilator-associated pneumonia	11%	13%	16%	0.28
Infection	26%	30%	28%	0.77
Sepsis	7%	6%	9%	0.58
Deep vein thrombosis	15%	17%	15%	0.97
Pulmonary embolism	7%	4%	6%	0.73
Mortality	3%	4%	4%	0.65

Data are presented as percentage of occurrence. Total patients represent the number of patients in whom the outcome was measured daily until discharge from the hospital.

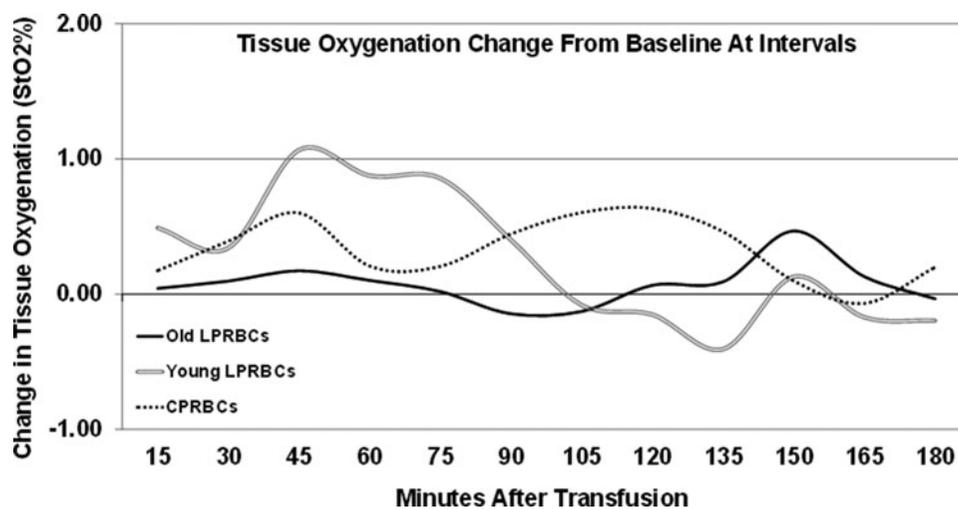


FIGURE 1. Mean percent change in tissue oxygenation over time in patients who received old PRBCs (black line), young PRBCs (gray and white line), or CPRBCs (dashed line).

TABLE 4. Inflammatory Mediators and Biochemical Markers—RBC Units

	N	Old LPRBCs	N	Young LPRBCs	N	CPRBCs	P (Between Groups)
Inflammatory cytokines (pg/mL)							
IL-2	106	0 (0–0)	89	0 (0–0)	92	0 (0–0)	0.48
IL-4	106	0 (0–0)	89	0 (0–0)	92	0.5 (0.3–0.8)*†	<0.001
IL-6	106	0 (0–0)	89	0 (0–0.2)	92	0 (0–0.2)*	0.02
IL-8	106	0.86 (0–2.73)	89	1.24 (0.01–2.87)	92	1.49 (0.47–2.52)	0.20
IL-10	106	0 (0–0)	89	0 (0–0.1)	92	1.3 (0.7–1.6)*†	<0.001
GM-CSF	106	30 (9–67)	89	25 (10–59)	92	103 (78–163)*†	<0.001
IFN- γ	106	0 (0–0)	89	0 (0–0)	92	0 (0–0)*†	<0.01
TNF- α	106	0 (0–0)	89	0 (0–1)	92	0 (0–1)	0.21
Biochemical markers							
α 2-Macroglobulin (ng/mL)	106	130 (78–251)	93	140 (85–222)	100	0.9 (0.9–1.5)*†	<0.001
Haptoglobin (ng/mL)	105	75 (38–130)	93	73 (37–130)	100	2.8 (0.79–3.6)*†	<0.001
C-reactive protein (pg/mL)	105	49 (18–162)	93	100 (30–408)*	100	13.5 (8.4–20.2)*†	<0.001
Serum amyloid P (ng/mL)	105	5.5 (3.6–8.7)	93	5.6 (3.8–10.3)	100	0.06 (0.02–0.08)*†	<0.001
Free hemoglobin (μ g/mL)	114	3508 (2073–4093)	100	2427 (897–3558)*	108	3356 (2178–4171)*†	<0.001
2,3 DPG (g/L)	41	0.09 (0.02–0.17)	80	0.20 (0.8–0.37)*	92	0.27 (0.12–0.44)*	<0.001

Data are presented as median (interquartile range).

* $P < 0.05$ versus old LPRBCs; † $P < 0.05$ versus young LPRBCs.

TABLE 5. Inflammatory Mediators and Biochemical Markers—Patient Samples

	N	Old LPRBCs	N	Young LPRBCs	N	CPRBCs	P (Between Groups)
Inflammatory cytokines (pg/mL)							
IL-2							
Baseline	78	0.10 (0–1.82)	65	0.37 (0–3.00)	71	0.72 (0–2.75)	0.14
12-h	74	0.14 (0–1.62)	63	0.35 (0–2.75)	68	1.05 (0–2.83)*	<0.05
IL-4							
Baseline	77	0.63 (0.15–1.32)	65	0.49 (0–1.36)	70	0.81 (0.21–1.35)	0.37
12-h	74	0.53 (0.09–1.39)	63	0.54 (0–1.31)	68	0.80 (0.27–1.46)	0.31
IL-6							
Baseline	78	36 (18–81)	65	33.36 (15.07–64.54)	71	26.61 (16.99–58.96)	0.35
12-h	74	33 (15–64)	63	34.83 (14.7–57.27)	68	28.55 (18.66–56.72)	0.43
IL-8							
Baseline	78	14 (8–22)	65	14.11 (10.17–20.73)	71	13.02 (7.72–20.61)	0.73
12-h	74	14 (9–25)	63	34.83 (14.7–57.27)	68	14.73 (11.44–22.03)	0.38
IL-10							
Baseline	78	0.15 (0–1.73)	65	0.17 (0–2.05)	71	0.10 (0–14.08)	0.65
12-h	74	0.02 (0–1.5)	63	0.30 (0–1.79)	68	0.55 (0–1.44)	0.85
GMCSF							
Baseline	78	2.55 (0–9.67)	65	5.18 (0–14.32)	71	5.64 (0–14.08)	0.26
12-h	74	1.88 (0–8.64)	63	2.96 (0–7.33)	68	3.14 (0–10.5)	0.88
IFN- γ							
Baseline	78	34.77 (0–71.68)	65	36.94 (0–75.89)	71	7.46 (0–77.09)	0.22
12-h	74	15.50 (0–84.44)	63	20.61 (0–79.66)	68	44.33 (0–94.85)	0.31
TNF- α							
Baseline	78	5.57 (3.04–10.76)	65	6.06 (2.4–14.06)	71	5.67 (2.73–11.85)	0.89
12-h	74	6.12 (2.24–10.14)	63	6.60 (3.29–11.01)	68	9.38 (4.7–14.2)	0.13
Biochemical markers							
α 2-Macroglobulin (ng/mL)							
Baseline	78	637 (404–899)	68	569 (356–826)	72	444 (314–675) *†	<0.01
12 h	73	575 (366–868)	63	567 (324–770)	70	458 (368–786)	0.51
Haptoglobin (ng/mL)							
Baseline	77	1394 (414–2710)	68	1101 (375–1775)	71	1215 (459–1990)	0.33
12 h	75	1470 (494–2360)	62	897 (349–208)	69	1350 (411–2700)	0.30
C-reactive protein (pg/mL)							
Baseline	78	97 (30–192)	68	76 (30–143)	72	52 (22–115)*	<0.05
12 h	73	63 (31–129)	63	66 (24–131)	70	64 (32–112)	0.99
Serum amyloid P (ng/mL)							
Baseline	78	37 (25–54)	68	35 (26–57)	72	33 (17–48)	0.22
12 h	75	33 (23–55)	63	37 (22–54)	70	35 (22–52)	0.83
Free hemoglobin (μ g/mL)							
Baseline	73	155 (66–329)	58	165 (93–340)	72	170 (78–306)	0.79
12 h	75	194 (106–194)	63	201 (86–422)	69	174 (82–287)	0.53
2,3 DPG (g/L)							
Baseline	73	0.30 (0.12–0.47)	60	0.31 (0.22–0.47)	72	0.30 (0.16–0.49)	0.59
12 h	75	0.36 (0.25–0.47)	60	0.33 (0.22–0.47)	70	0.31 (0.23–0.56)	0.94

Data are presented as median (interquartile range).

* $P < 0.05$ vs old LPRBCs; † $P < 0.05$ vs young LPRBCs.

levels were increased in patients who received CPRBCs at 12 hours ($P < 0.05$). There were significant increases in free Hb noted in the old LPRBC group during transfusion, but not in the other groups ($P < 0.05$ vs baseline). Standard coagulation assays (PT/INR, aPTT, fibrinogen, and D-dimers) and thrombelastogram parameters (r value, k , α -angle, maximal amplitude, and LY30) did not differ between or within groups and median values were all within normal limits (data not shown).

DISCUSSION

The current trial is the largest prospective randomized trial ever performed comparing CPRBCs to LPRBCs. The study reveals that transfusion of CPRBCs, old LPRBCs, and young LPRBCs has similar effects on clinical outcomes in trauma patients. These trans-

fusions also have similar effects on tissue oxygenation, biochemical mediators, inflammation, and coagulation parameters.

The findings from our trial are very similar to the recently published ABLE (Age of Transfused Blood in Critically Ill Adults) and RECESS (Effects of Red Cell Storage Duration on Patients Undergoing Cardiac Surgery) trials that revealed no difference in mortality or secondary outcomes in critically ill patients and cardiac surgery patients randomized to receive old LPRBCs or young LPRBCs.^{25,26} Neither of these trials focused on trauma patients.

RBCs frozen with 40% (weight/volume) glycerol and stored at -80°C are washed after thawing to reduce the glycerol content to less than 1%.^{27–29} This step is beneficial as it allows removal of glycerol, cell debris, platelets, leukocytes, anti-A and anti-B isoagglutinins, the anticoagulant used for collection, and other plasma and nonplasma biologically active substances that may have physiologic

significance by playing a role in inflammation, vascular dysfunction, and transfusion reactions.^{30–32} This was confirmed in our study by the markedly reduced quantities of $\alpha 2$ -macroglobulin, haptoglobin, CRP, and serum amyloid P in the CPRBCs.

Other authors have shown that washing the RBCs reduces plasma and nonplasma substances to less than 5% of their original value.^{33–35} Exposure of thawed cells to the saline wash induces a small degree of hemolysis, but this may be a selective hemolysis of “subhemolytically” damaged or senescent cells. “Subhemolytic” damage due to freezing has been shown by Rowe et al to be related to in vivo erythrocyte age, the older cells being more susceptible.³⁰ These damaged cells probably would not survive when transfused. The excellent in vivo survival indicates that more fragile cells are removed during the postthaw processing. This would explain why CPRBCs were equally effective at raising the hematocrit despite the removal of RBCs that occurs with the deglycerolization step.^{18,34,36}

There has been extensive use of CPRBCs in civilian and military practice, especially during the period from 1960 to 1980.^{37–44} The first cryopreserved RBC bank was organized at Chelsea Naval hospital in Boston, MA in 1956, and the first clinical study investigating the safety and efficacy of the allogeneic transfusion of CPRBCs was conducted at the US Navy hospital at Danang, South Vietnam.^{40,45} The blood was collected, processed, and cryopreserved in Naval hospitals in the United States and shipped in styrofoam containers packed with dry ice. Within 6 months, 43 critically wounded military casualties requiring massive transfusion received a total of 347 units of LPRBCs and 307 units of CPRBCs.⁴⁰ Post-transfusion plasma Hb, bilirubin, platelet counts, urine Hb, and serum creatinine levels were not different between patients transfused with CPRBCs or LPRBCs.⁴⁰

A component transfusion therapy program utilizing CPRBCs was developed at Cook County Hospital in Chicago.⁴⁶ CPRBCs have been used for up to 64% of transfusions depending on the availability of erythrocytes for freezing. The institution of CPRBCs was associated with a decrease in the incidence of transfusion reactions from 0.57% to 0.11%.⁴⁶ The New York Blood Center reported a similar experience. This group instituted the use of CPRBCs in children with thalassemia in 1968 and they showed a decrease in the incidence of febrile reactions from 50% to 1%.⁴⁷ Even highly washed LPRBCs produced a 10% rate of febrile reactions in these patients.⁴⁸

The use of CPRBCs was largely abandoned owing to medical and logistical concerns. Prior methods of deglycerolizing the RBCs involved open techniques that were not automated introducing the potential for infection. The ACP 215 provides a closed, automated method of processing thawed CPRBCs that accelerates their availability. Thawed, deglycerolized CPRBCs are approved by the Food and Drug Administration for use up to 14 days after thawing providing the ability to initiate transfusion in emergent settings similar to thawed plasma. Fourteen days were chosen because after this period the percentage of viable cells drops below 80 compared with the original donated unit and not due to any harmful effects. CPRBCs continue to be used in the conflicts in Iraq and Afghanistan and by America's Blood Centers during disasters as a limited resource.

According to the America's Blood Center STOPLIGHT blood availability monitoring program, on average approximately 30% of national reporting sites have an available blood supply of only 2 days or less.⁴⁹ Blood collected after national disasters historically takes 2 days to reach blood banks representing too long of a delay to be of immediate use to victims. Within a month of 9/11, the American Red Cross collected 928,293 units of LPRBCs with an estimated 287,000 of those being extra units of blood. Ultimately, 49,860 of these units were wasted representing an overall wastage rate of 5% and a wastage rate of 17% of the extra units collected.⁵⁰ It is likely that the total number of wasted units was much greater, but the actual number is not available. Owing to limited availability of the ACP

215, only 9500 type O units were frozen after 9/11.⁵⁰ It is clear that current blood banking technology is not amenable to disaster scenarios, and the use of CPRBCs has great potential to provide a viable solution.

The estimated cost of a CPRBC unit is \$600 compared with \$200 for a LPRBC unit. Owing to the increased cost and logistical complexities associated with CPRBCs, it would be ideal to maintain frozen blood banks to supplement standard LPRBC blood banks to increase the flexibility necessary to field compatible RBCs during periods of increased usage, disaster scenarios, in rural areas with limited access and to patients with rare blood types or blood disorders.

Limitations

This study was intentionally performed in stable trauma patients after initial resuscitation to focus on the effects of individual unit transfusions. Patients received a limited number of transfusions, preventing an analysis of the effects of larger volume transfusions on outcomes. The findings of this trial cannot be translated to massive transfusion scenarios and the effects of large volume transfusions of CPRBCs remain unknown. This will become the focus of future studies.

The power analysis for this study was based on a difference in StO₂ observed in our previous study comparing transfusion of LPRBCs less than 21 days old to LPRBCs 21 days old or more. This study was not powered for equivalence of clinical parameters and therefore must be considered a pilot trial for that purpose. Our larger planned study will be powered for equivalence of clinical parameters.

CONCLUSIONS

We have shown, in moderately injured trauma patients, that transfusion with CPRBCs is equally safe and effective compared to transfusion with LPRBCs. Due to the ability to store CPRBCs for up to 10 years and store them thawed for up to 14 days, cryopreservation represents a flexible technology that has the potential to substantially change blood banking in diverse settings. Future studies will focus on comparisons between LPRBC and CPRBC transfusions in emergent settings.

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DISCUSSANTS

R. Maier (Seattle, WA):

The quality of this study as a prospective trial and the completeness of biochemical, physiologic, and outcome data are noteworthy.

This study is based on the recognized potential of the detrimental impact of stored blood transfusion, particularly aged blood, in the critically ill and the lack of consistent access to stored blood, particularly during mass casualties and in the austere environment, both for the military and rural areas. Thus, there is a recognized need to improve our access and the quality of the blood transfusions that we give our patients.

I have several questions.

The results are clear. I think the authors in this patient population, as they defined it, have shown that glycerol-stored cryopreserved blood was noninferior to young or aged cold stored liquid blood and all had similar host, physiologic red cell survival, O₂ delivery, and immune responses. However, there are recent meta-analyses that have also failed to show a difference between these blood products except in the extreme conditions of large-volume transfusion in hypotensive patients and with or without a massive transfusion.

Do the authors have any subset data or insights regarding the potential for deleterious effects in the patients who did have the most blood transfusion and the largest volume of transfusion or were the most critically ill? Was the trial of sufficient size or breadth to answer this question?

Second, many of us in the field of trauma and critical care have long had these concerns regarding the potential deleterious effects of banked blood on tumor recurrence, immunosuppression, increased infections, and higher mortality for decades, similar to the previous comments in the last presentation. In addition, the instability and inconsistent nature of the blood supply has been with us since the first blood bank opened at Cook County Hospital in 1939. Why has your study not been done sooner or why has no one else done your study sooner?

We have been able to cryopreserve red cells, as you pointed out, for over 50 years. What kept us from doing this study? Was it political? Financial? Subversive? Why have we not done this for our patients? The technology was there long before last year.

Third, a major logistic challenge is thawing and washing the glycerol from the cryopreserved blood. Many people who need blood need it now. With the lack of benefit in physiologic responses, do you expect there to be an ongoing significant interest in pursuing this technology except for the very austere environments for both the military and rural, or do you propose that it should be the routine approach for transfusion practice in this country?

Response From M.A. Schreiber:

First of all, Dr Maier asked about massive transfusion patients. We entered patients in this study after they had been stabilized, so some of these patients had received transfusions before they were randomized. These patients were now stabilized and they did not require massive transfusions, so we do not have a subset of patients who received massive transfusion.

The question is an important one. The study that we are now proposing to the National Institutes of Health (NIH) is a study that will include all comers, including massive transfusion patients, and that is the group that we really want to study at this point.

I have utilized the frozen red cells in theater, and we have used massive quantities of frozen red cells. I have not seen personal deleterious effects with these cells. However, I do not have data to support that comment at this time.

Why has not this study been done sooner? Dr Maier, have you spent much time with blood bankers? Blood banking in the United States has not changed probably for about 50 to 60 years. This idea is not well supported by the blood banking community. As I have tried to move this forward, I have faced a lot of resistance in my efforts within the blood banking communities.

Literally tens of thousands of frozen units have been given, but they have not been well studied. That is really the impetus for us to study them. I really think that there is a lot of inertia in this area.

Dr Maier asked the question about the logistical difficulties of using these cells. You have to thaw them before you utilize them. I think an important point that is sometimes lost is once you thaw the cells, you can maintain them for 14 days. You could have an entire frozen red cell bank, of which you maintain a quantity of those thawed, sort of like we do with thawed plasma. Therefore, it is not problematic. In our Level 1 trauma centers, we could maintain 100 or 1000 units thawed, utilize them as we do liquid units, and still maintain a massive quantity frozen.

Now, I do not actually propose that we convert all of our blood banks to frozen blood banks, but I think that our frozen blood bank supply can augment our liquid packed cell use, and we can use this for the various things that it has been used for in the past, rare blood types, patients with thalassemia, surges in trauma patients, and mass casualty situations. The liquid red cell bank that we are currently using is not amenable to these situations, and we can use these in those situations.

P. Rhee (Tucson, AZ):

This is something that has been needed for a long time. When I was in active duty in the Navy, every time I got deployed I had 600 units of red blood cells that were frozen, and I and nobody in the entire ship knew what to do with it or how to process it. We would send our youngest corpsman to go figure out how to use it. When we found out that it was filled with glycerol and it took a while to wash

the glycerol out, we were always afraid of what that process did to the red cells.

I think your study has shown nicely to the trauma surgeons what happens with this process, but a lot of these data actually are available on the blood banker side, because they have been studying this and known about this for a long period of time.

This study, I think, is a very good pilot trial with about 80 people in each arm. The first question is about your intent for future studies that will address efficacy, safety, and equivalency.

The second question was on your data itself, looking at why people who got the frozen red blood cells got lesser units of transfusions.

The third question is about cost.

Response From M.A. Schreiber:

We have proposed to the NIH a study of 685 patients, comparing frozen red blood cells versus standard liquid red cells without differentiating the age of the liquid red cells. Our main outcome measure is change in multiple organ failure score. That study will cost \$25 million, and we will see if I can find the money to do that.

That is our intention. We do want to do a major study, all comers, including massive transfusion patients. That is the goal at this time.

The next question refers to cost. You asked about the cost of these cells. It is difficult to estimate. It depends on who is preparing them, whether it is military or civilians. They cost somewhere between 400 and \$600. A standard unit of liquid red cell costs about \$200. I think that cost has to be interpreted with the life span of the red cells being 10 years versus 42 days, and the ability to avoid waste. Yes, the cells are more expensive, but the question would be if you did an overall analysis, would they actually be more expensive overall to the user and to society?

S. Steinberg (Columbus, OH):

Most of the discussion so far has focused on the frozen cells. I certainly see the utility of building that process into our modern blood banking. I am curious, though, to hear your comments about the relatively minimal differences between the young and old liquid cells. We have been told for years about the horrors of using old blood. Should we not be so concerned, or is this study not really geared to detecting those problems?

Response From M.A. Schreiber:

Steve, that is a great point. I have gone around the country talking about how horrible old red cells are. I have to eat my words. I have learned that you never should be too vitriolic. I think that, again, we were not powered for equivalence, but I think that it is pretty clear that there is not a lot of difference between the old and young red cells in our study.

As many of you probably know, in the April 9, 2015 issue of the *New England Journal of Medicine*, two studies came out—two large, prospective, randomized trials, the ABLE study (ISRCTN44878718) and the RECESS study (NCT00991341.), one performed on critical care patients and one performed on cardiac surgery patients, both of which randomized patients to old and young red cells, and neither study found any differences.

The same thing that we found in our study has also been confirmed in two other patient populations in two other prospective randomized trials. There have been no prior prospective randomized trials comparing old and red cells. I think we need to change our mentality about this and, really, the best data suggest that the older red cells are just as good as the younger red cells.