Effect of red blood cell storage duration on major postoperative complications in cardiac surgery: A randomized trial

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ABSTRACT

Background: Although observational studies suggest an association between transfusion of older red blood cell (RBC) units and increased postoperative risk, randomized trials have not supported this. The objective of this randomized trial was to test the effect of RBC storage age on outcomes after cardiac surgery.

Methods: From July 2007 to May 2016, 3835 adults undergoing coronary artery bypass grafting, cardiac valve procedures, or ascending aorta repair, either alone or in combination, were randomized to transfusion of RBCs stored for ≤14 days (younger units) or for ≥20 days (older units) intraoperatively and throughout the postoperative hospitalization. According to protocol, 2448 patients were excluded because they did not receive RBC transfusions. Among the remaining 1387 modified intent-to-treat patients, 701 were randomized to receive younger RBC units (median age, 11 days) and the remaining 686 to receive older units (median age, 25 days). The primary endpoint was composite morbidity and mortality, analyzed using a generalized estimating equation (GEE) model. The trial was discontinued midway owing to enrollment constraints.

Results: A total of 5470 RBC units were transfused, including 2783 in the younger RBC storage group and 2687 in the older RBC storage group. The GEE average relative-effect odds ratio was 0.77 (95% confidence interval [CI], 0.50-1.19; P = .083) for the composite morbidity and mortality endpoint. In-hospital mortality was lower for the younger RBC storage group (2.1% [n = 15] vs 3.4% [n = 23]), as was occurrence of other adverse events except for atrial fibrillation, although all CIs crossed 1.0.

Conclusions: This clinical trial, which was stopped at its midpoint owing to enrollment constraints, supports neither the efficacy nor the futility of transfusing either younger or older RBC units. The effects of transfusing RBCs after even more prolonged storage (35-42 days) remains untested. (J Thorac Cardiovasc Surg 2020;160:1505-14)
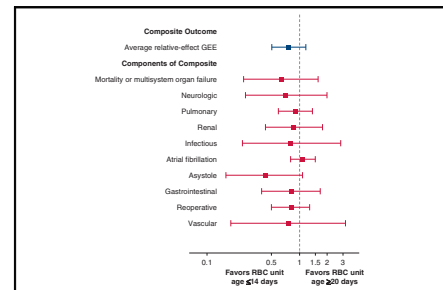
Forest plot of postoperative complications and composite average relative effect.

Central Message

This randomized trial, which was stopped owing to enrollment constraints, supports neither the efficacy nor the futility of transfusing younger versus older red blood cells. The effect of storage nearer the end of shelf life remains untested.

Perspective

Laboratory studies demonstrate blood product degradation beyond 28 days of storage. This randomized trial of red blood cells (RBCs) stored for ≤14 days versus ≥20 days, which was stopped at the midpoint owing to enrollment constraints, supports neither the efficacy nor the futility of transfusing younger versus older RBCs in reducing post–cardiac surgery morbidity and mortality. The effect of even more prolonged RBC storage (35-42 days) remains untested.

Abbreviations and Acronyms
CI = confidence interval
GEE = generalized estimating equation
HR = hazard ratio
ICU = intensive care unit
MODS = multiple organ dysfunction syndrome
OR = odds ratio
RBC = red blood cell
STS = Society of Thoracic Surgeons

Scanning this QR code will take you to the article title page to access supplementary information.

During red blood cell (RBC) storage, a series of interdependent biochemical and morphological changes occur—the storage lesion—leading to RBC product degradation.[1-6] However, the effect of these changes on outcomes after RBC transfusion remains unclear.[1,7-14] Observational clinical studies in high-risk and critically ill patients have linked longer RBC storage with complications of every organ system[15-18]; however, clinical trials of transfusing fresh versus "standard issue" RBCs in adult cardiac surgery and critically ill adults have all been negative.[19-21]

Discordance between observational and clinical trials may be related to RBC age in clinical trials, because standard issue older RBC units typically averaged just over one-half of the Food and Drug Administration's mandated expiration time for stored RBCs (42 days) or because of recruitment challenges or underpowered trials (Appendix E1). Thus, our objective in designing this randomized trial was to determine whether longer RBC storage was related to a composite of morbidity and mortality after adult cardiac surgery, with adequate power to compare outcomes of patients receiving RBC units stored for only ≤14 days throughout the perioperative period versus RBC units stored for ≥20 days. This first and final report from the trial presents results at the time the trial was discontinued for enrollment constraints, which coincided with the planned second interim analysis with one-half the patients enrolled.

METHODS

Trial Design, Intervention, and Oversight

In this single-center, blinded, 2-arm parallel-design randomized trial, we compared younger RBC units versus older RBC units transfused throughout surgery and the postoperative hospitalization. The trial was overseen by the Cleveland Clinic's Cardiothoracic Anesthesiology Department, and surgeons were blinded to the treatment arm. Endpoints were abstracted prospectively by registry nurses in the quality-control Clinical

Investigations group, who had no knowledge of the patients' randomized arm, further blinding the trial.

If transfused, patients randomized to the younger RBC group were to receive only units stored for ≤14 days, and those randomized to the older RBC group were to receive only units stored for ≥20 days. The randomization sequence was generated using the PLAN procedure in SAS (SAS Institute, Cary, NC), using randomly sized blocks. The Department of Outcomes Research randomized patients online within 24 hours of surgery. If the blood bank was unable to ensure that a patient would receive only blood stored within the range to which he or she were randomized, the patient was excluded from the trial. Patients who did not receive transfusions also were excluded, according to protocol (Figure 1). Thus, analytically, the trial analysis was by modified intention to treat. Some patients received 1 or more RBC units contrary to their randomization, primarily from delivery of RBC units of improper age from the blood bank. Patients with these protocol deviations were retained in the trial in their originally randomized group. Transfusion of other blood products was at the discretion of the care team. Subject participation ended at index hospital discharge.

The trial protocol was approved by the Cleveland Clinic's Institutional Review Board (IRB 07-140) and was registered at ClinicalTrials.gov (identifier NCT00458783; Online Data Supplement). All included patients provided written informed consent. A multidisciplinary Data and Safety Monitoring Board, independent of trial investigators, tracked patient accrual and adjudicated clinical events according to definitions of the Society of Thoracic Surgeons (STS) adult cardiac surgery database (www.sts.org/registries-research-center/sts-national-database/sts-adult-cardiac-surgery-database).

Subjects

Our cohort for this trial comprised patients age ≥18 years undergoing scheduled primary or reoperative coronary artery bypass grafting, a cardiac valve procedure, or ascending aorta repair, either alone or in combination, using cardiopulmonary bypass, at Cleveland Clinic from July 2007 to May 2016. Patients unwilling to receive blood for religious reasons or refusing consent were excluded (Figure 1).

Endpoints

The primary composite endpoint was the occurrence of mortality or multisystem organ failure, cardiac events (ventricular tachycardia, fibrillation, or asystole; atrial fibrillation), pulmonary events (pneumonia, prolonged postoperative ventilation, pulmonary embolus), neurologic events (stroke, coma), renal failure, infection (deep sternal wound infection, sepsis), gastrointestinal events (ischemia, infarction), any reoperation (for bleeding, tamponade, cardiac dysfunction), and vascular events (dissection, limb ischemia), as defined for the STS database.

Secondary outcomes included the number and age of RBC units transfused, intensive care unit (ICU) length of stay, and duration of postoperative hospitalization.

Sample Size

Sample size was based on the primary endpoint, with a chi-square test comparing the proportion of the composite outcome between randomized groups. The estimated unadjusted odds ratio (OR) between the younger (≤14 days) and older (>14 days) RBC storage groups was 0.83 (22.4% vs 25.9%), and the adjusted OR was 0.86 (95% confidence interval [CI], 0.75-0.99). However, we anticipated a larger effect—an OR of 0.77—because we defined the older RBC group as storage age of ≥20 days instead of >14 days. Based on our reported STS data, the composite outcome was estimated to be 30%. These 2 assumptions led to an estimated composite event occurrence of 27.3% in the younger RBC storage group and 32.7% in the older group. Setting type I error to 0.05 and power to 0.85 yielded a sample size of 1328 transfused patients per treatment arm. The total sample size could increase to 4810 transfused patients if the OR
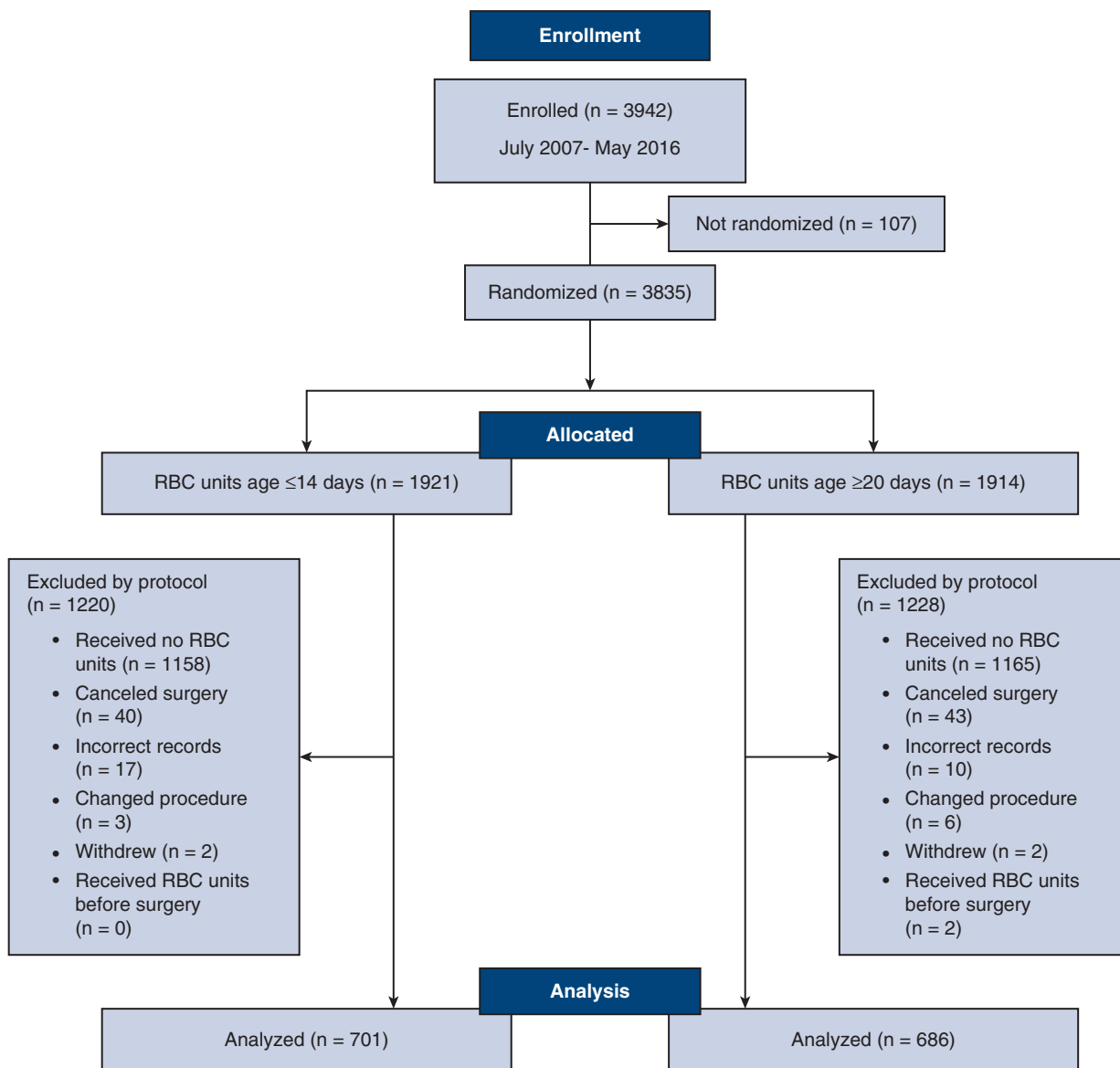
Enrollment

Enrolled (n = 3942)

July 2007- May 2016

Not randomized (n = 107)

Randomized (n = 3835)

Allocated

RBC units age ≤14 days (n = 1921)   RBC units age ≥20 days (n = 1914)

Excluded by protocol
(n = 1220)

- Received no RBC
  units (n = 1158)
- Canceled surgery
  (n = 40)
- Incorrect records
  (n = 17)
- Changed procedure
  (n = 3)
- Withdrew (n = 2)
- Received RBC units
  before surgery
  (n = 0)

Excluded by protocol
(n = 1228)

- Received no RBC
  units (n = 1165)
- Canceled surgery
  (n = 43)
- Incorrect records
  (n = 10)
- Changed procedure
  (n = 6)
- Withdrew (n = 2)
- Received RBC units
  before surgery
  (n = 2)

Analysis

Analyzed (n = 701)   Analyzed (n = 686)

FIGURE 1. CONSORT diagram of study enrollment and randomization. *RBC*, Red blood cell.

was 0.83 (28% vs 32%) or decrease to 1600 if the OR was 0.71 (26.5% vs 33.5%). Thus, to achieve 0.05 type I error and 0.85 power, the trial was designed to enroll 2840 patients, 1420 per treatment arm.

Interim Analyses

Three interim analyses were planned at 25%, 50%, and 75% of planned subject accrual. Our calculations of group sequential boundaries assumed nonbinding stopping rules and accounted for monitoring both the null (efficacy) and alternative (futility) hypotheses (see Appendix E2 for details and Table E1). A critical Z-value of 2.84 was used to estimate interim-adjusted CIs based on the Z-statistic criterion for significance for the second interim analysis. Specifically, CIs were adjusted for group-sequential design (using a confidence coefficient of 2.8409) to maintain an overall study α of .05, significant at $P < .0045$ for efficacy and $P > .742$ for futility.

Endpoint Analyses

All analyses were based on the modified intent-to-treat principle. The significance level for each hypothesis was 0.05. SAS version 9.4 (SAS Institute) and East 5.3 (Cytel, Cambridge, Mass) were used for all analyses. Balance of baseline characteristics between the 2 treatment groups was assessed using the absolute standardized difference.

For the primary composite endpoint analysis, we assessed the average relative-effect OR across its 10 components.[22] A generalized estimating equation (GEE) distinct-effects model was fit, and a separate treatment effect (ie, log-OR) and associated standard error were estimated for each component. Component effects were then averaged to estimate the average relative effect and test whether it was equal to 0, with the standard error of the estimator based on the GEE robust covariance matrix across components. The heterogeneity of treatment effects across components was
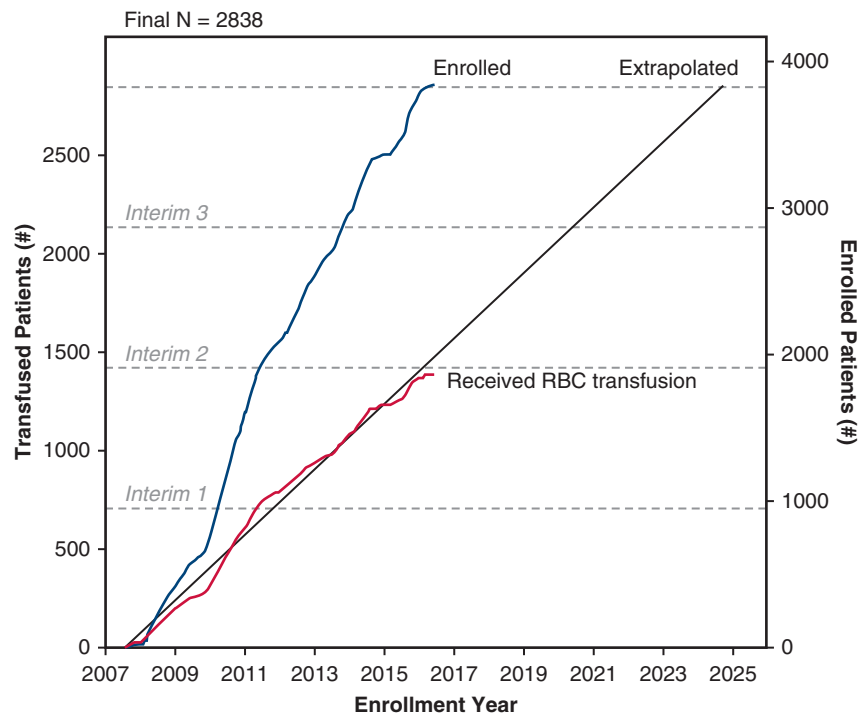
FIGURE 2. Study enrollment over time. The *blue line* indicates patients enrolled. The *red line* indicates patients who received red blood cell (*RBC*) transfusions during the intraoperative or postoperative period and thus were included in analyses. The *black line* is an extrapolated line from the linear regression of the number of patients who received RBC transfusions over time. The *dashed lines* indicate preplanned interim and final analyses.

assessed by a treatment-by-component interaction test in the distinct-effects GEE model.

This analysis departed from the statistical analysis planned before enrollment began in 2007, because E.J.M. subsequently devised the afore-mentioned method for analyzing composite endpoints, which is independent of the unequal number of events occurring for each endpoint. This is a common conundrum encountered when analyzing composite events, which can be driven by the most prevalent event at the expense of possibly more severe, but less commonly occurring, events.

To analyze the effect of RBC age on ICU and postoperative hospital lengths of stay, we used Cox regression to compare groups on time to discharge alive, calculating hazard ratios (HRs) and interim-adjusted 95% CIs. Patients dying in the hospital were considered to never have had the "discharge alive" event and thus, to avoid death as a competing risk, were assigned a duration of 1 day longer than the observed maximum postoperative length of stay. Poisson regression was used to assess the treatment effect on the number of transfusions, and a 2-sample *t* test was used for age of transfusion.

We conducted a per-protocol sensitivity analysis for comparison with the primary modified intent-to-treat analyses. For this, only patients receiving RBC units of the storage age to which they were randomized were included.

Trial Closure

Patient accrual was nearly linear (Figure 2) but logistically could average roughly 1 patient per operating day. Only 36% of the patients received a transfusion over the 9-year study period. Owing to enrollment constraints (Appendix E1), the trial was discontinued without crossing the efficacy or futility boundaries.

RESULTS
Patients

Of the 3835 patients randomized by trial midpoint, 2448 (64%) were excluded, 2323 because they did not receive transfusions and 125 for reasons listed in Figure 1. Thus, 1387 randomized patients receiving RBC transfusions were included in this modified intent-to-treat second interim analysis: 701 randomized to receive RBC units of age ≤14 days and 686 to receive RBC units of age ≥20 days. The 2 groups were generally balanced in terms of baseline characteristics, clinical factors, and surgical procedures performed (Table 1).

Transfusions

A total of 5470 RBC units were transfused: 2783 units in the younger RBC storage group and 2687 in the older RBC storage group. There were some protocol deviations. Thirty-four patients (4.8%) in the younger group received at least 1 RBC unit stored for ≥20 days, and 41 patients (6.0%) in the older group received at least 1 RBC unit stored for ≤14 days. In addition, 15 patients (2.1%) in the younger RBC group and 24 patients (3.5%) in the older RBC group received at least 1 RBC unit stored for 14 to 20 days (Figure 3). The median age of RBC units per patient was

TABLE 1. Baseline patient characteristics and procedure details by RBC age group

| Characteristic | RBC age ≤14 d (N = 701) | | RBC age ≥20 d (N = 686) | | ASD* |
	Missing, n	No. (%) or mean ± SD	Missing, n	No. (%) or mean ± SD	
Demographic data					
Age at surgery, y	0	69 ± 12	0	70 ± 12	0.07
Female	0	381 (54)	0	326 (48)	0.14
Body mass index, kg/m^2	0	28 ± 5.9	0	28 ± 5.9	0.03
Cardiac comorbidities					
New York Heart Association functional class	154		144		0.09
I		101 (18)		115 (21)	
II		268 (49)		270 (50)	
III		168 (31)		148 (27)	
IV		10 (1.8)		9 (1.7)	
Prior myocardial infarction	0	124 (18)	0	136 (20)	0.05
Cardiogenic shock	0	2 (0.28)	0	1 (0.14)	0.03
Intra-aortic balloon pump	0	22 (3.1)	0	26 (3.8)	0.04
Urgent surgery	0	144 (21)	0	158 (23)	0.07
Unstable angina	0	33 (4.7)	0	20 (2.9)	0.09
Atrial fibrillation	0	116 (17)	0	132 (19)	0.07
Left ventricular ejection fraction ≤40%	115	59 (10)	94	68 (11)	0.05
Prior cardiac surgery	0	245 (35)	1	240 (35)	0.02
Noncardiac comorbidities					
Hematocrit, %	5	38 ± 4.5	3	38 ± 4.7	0.04
Chronic obstructive pulmonary disease	0	170 (24)	0	165 (24)	0
Hypertension	0	570 (81)	0	573 (84)	0.06
Diabetes	0	180 (26)	0	177 (26)	0.07
Stroke	0	63 (9.0)	0	68 (10)	0.03
Peripheral arterial disease	0	108 (15)	0	106 (15)	0
Triglycerides, mg/dL	177	126 ± 92	193	115 ± 76	0.12
Creatinine, mg/dL	2	1.1 ± 0.89	3	1.1 ± 0.80	0.01
Bilirubin, mg/dL	14	0.63 ± 0.42	12	0.63 ± 0.43	0.02
Procedure performed	0		0		0.15
CABG only		97 (14)		96 (14)	
Valve only		368 (52)		370 (54)	
Combined CABG and valve		225 (32)		194 (28)	
Other		11 (1.6)		26 (3.8)	

Some data were available only through 2010. *RBC*, Red blood cell; *ASD*, absolute standardized difference; *SD*, standard deviation; *CABG*, coronary artery bypass grafting. *ASD is the absolute difference in means or proportions divided by the pooled standard deviation. An ASD >0.2 suggests imbalance between groups.

11 days (quartiles, 8, 13) in the younger RBC group and 25 days (quartiles, 19, 30) in the older RBC group.

Endpoints
Primary endpoint. Over all components of the composite outcome, the average relative-effect GEE OR was 0.77 (95% CI, 0.50-1.2; P = .08) for the younger RBC group versus the older RBC group (Figure 4). When the study was stopped, an additional 600 to 700 patients would have been needed to assess whether this trend would continue on this trajectory (Figure 5), and the conditional power would be 0.75 to detect efficacy. If instead, the null hypothesis trend (no difference) continued to the end, the conditional power would be 0.14.

Differences between the 2 groups for all individual components were associated with P > .01, compared with the interim-adjusted significance criterion of P ≤ .004. The P value for the treatment-component interaction test was .50, indicating a lack of evidence for heterogeneity of treatment effect across the 10 components of the primary outcome. In-hospital mortality or multisystem organ failure was 2.3% (n = 16) in the younger RBC group and 3.5% (n = 24) in the older RBC group (Table 2). Except for atrial fibrillation, occurrence of all major postoperative morbidities was descriptively lower (ORs <1.0; Figure 4) in the younger RBC group versus the older RBC group.
Secondary endpoints. The median ICU length of stay was 51 hours (quartiles, 28, 113) in the younger RBC group and 54 hours (quartiles, 29, 114) in the older RBC group (HR, 1.1; interim-adjusted 95% CI, 0.94-1.29; P = .9). The median hospital length of stay was 9 days (quartiles, 7, 13) in the younger RBC group and 9 days (quartiles, 7, 13) in the
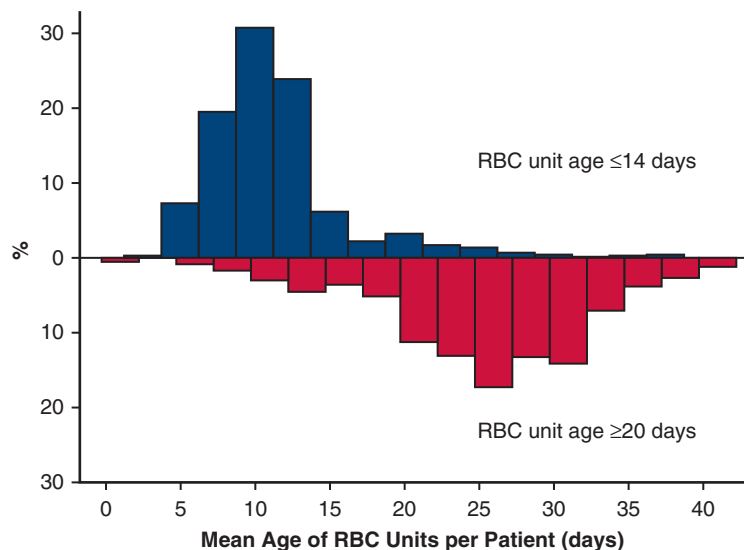
FIGURE 3. Mirrored histogram of distribution of red blood cell (*RBC*) age by RBC unit age randomization group. Note that despite the intention to transfuse only RBC units stored for ≤14 days in one group and for ≥20 days in the other group, some patients received units contrary to their randomization (protocol deviations) but were retained in their original group on the basis of a modified intent-to-treat analysis.

older RBC group (HR, 1.0; interim-adjusted 95% CI, 0.85-1.18; *P* = .07).

Sensitivity analysis. In the per-protocol analysis for the primary outcome, the average relative-effect OR was 0.70 (95% CI, 0.40-1.2; *P* = .06), consistent with the primary result (Figure E2).

DISCUSSION

Principal Findings

In adult cardiac surgical patients randomized to receive younger (≤14 days) versus older (≥20 days) RBC units, at the trial's midpoint, morbidity and mortality did not differ significantly between the 2 study groups; however, this trial supports neither the efficacy nor the futility of transfusing either younger or older RBC units perioperatively in the cardiac surgery setting.

Relationship to Previous Randomized Trials

Previous randomized trials reported no statistically significant benefit of transfusing fresh RBC units over standard issue (14-21 days of storage) units. These trial findings are consistent with laboratory investigations reporting that biological changes do not occur until approximately 28 days of RBC storage.[2] Despite best efforts, our study and other trials have been unable to randomize a sufficient number of patients to an RBC storage duration longer than a mean of 24 to 28 days.[19,20] Thus, we and others have not yet addressed safety issues related to transfusing RBC units near the end of their shelf life of 35 to 42 days,[23] particularly in patients with cardiovascular disease.

Our study is similar to the investigation reported by Steiner and colleagues[19] that exclusively examined patients

undergoing cardiac surgery; however, they included pediatric patients. Their investigation randomized 1098 patients to RBC units with ≤10 days versus >21 days of storage, with a primary outcome of changes in multiple organ dysfunction syndrome (MODS) score at 7 days and 28 days postoperatively. Their investigation was terminated early owing to "time constraints on the funding of the study"; however, they reported a similar change in MODS score at 7 days and 28 days between the fresh and older RBC groups. However, use of the MODS score appears to be limited in the adult cardiac surgery population because most patients are extubated, with invasive line monitoring removed within 24 hours of ICU admission. Thus, the ratio of arterial partial pressure of oxygen to fraction of inspired oxygen and pressure-adjusted heart rate components of the MODS score are not available at 7 and 28 days. The only available outcome metrics beyond 24 to 48 hours would be changes in platelet count, bilirubin, and creatinine.

Heddle and colleagues[24] studied the influence of RBC storage duration on outcomes in a mixed general hospitalized patient population in the Informing Fresh versus Old Red Cell Management (INFORM) trial. Administrative International Classification of Diseases, 10th Revision codes were used to categorize patients into a low-risk group and a high-risk group. Patients were randomized to short-term RBC storage (n = 6936; median, 11 days of storage) and standard issue, which was called long-term storage (n = 13,922; median, 24 days of storage). The number of RBC units transfused was similar in the 2 groups, as was the primary outcome of mortality. The findings of Heddle and colleagues are in agreement with other trials[19,20]
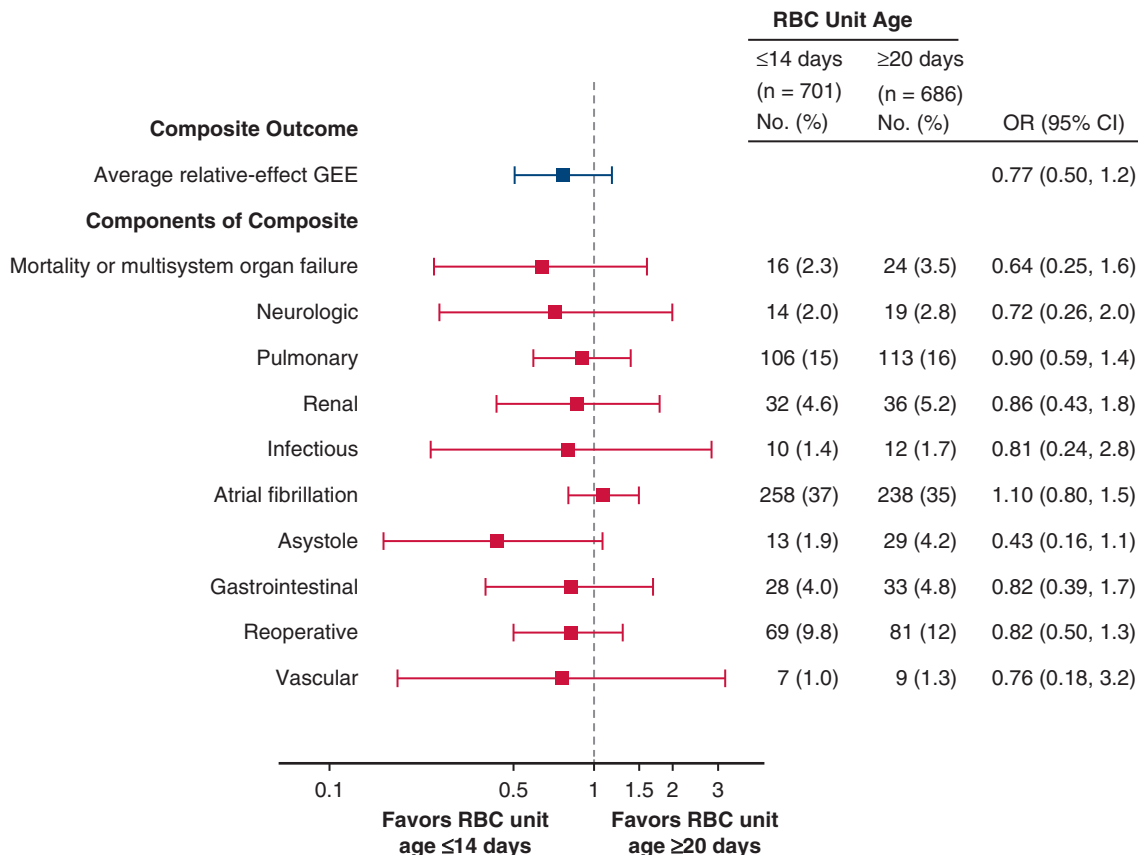
FIGURE 4. Forest plot illustrating the effect of red blood cell (*RBC*) unit age group on major postoperative complications. Estimated odds ratios and 95% confidence intervals of the composite outcome and its individual components in RBC unit age ≤14 days versus ≥20 days were derived from a generalized estimating equation by averaging the 10 distinct treatment effects. *GEE*, Generalized estimating equation.

demonstrating the safety of standard issue (ie, middle-aged) blood, but the trial was limited in terms of the number of RBC units transfused that were near the end of their shelf life.

In a secondary analysis of this trial, Cook and colleagues[25] compared a subgroup receiving RBC units stored for ≤7 days with a subgroup receiving at least 1 RBC unit stored for 8 to 35 days and another group receiving at least 1 RBC unit stored for >35 days. Although the authors attempted to report on RBC units with prolonged storage (>35 days), patients in the prolonged storage group did not exclusively receive RBC units stored for >35 days, but rather received transfusions containing admixtures of RBC units of varying ages.

The Transfusion versus Fresher Red-Cell Use in Intensive Care (TRANSFUSE) trial was a multicenter randomized trial comparing 90-day mortality in 4994 ICU patients who received the freshest available RBCs (mean age, 12 days) or standard issue RBCs (mean age, 22 days).[21] Mortality was 25% (n = 610) in the former group and 24% (n = 594) in the latter group.

The Age of Blood Evaluation (ABLE) trial randomized critically ill adult ICU patients to receive fresh RBC units <8 days old (n = 1211; mean RBC age, 6.1 ± 9 days) or standard issue RBCs (n = 1219; mean age, 22 ± 8.4 days), with the primary outcome of 90-day mortality.[20] Mortality was similar in the 2 groups (37% for fresh and 35% for standard issue), as were secondary outcomes of morbidity and length of stay. The patient population included a diverse group (medical, surgical, and trauma ICU admissions) with differing preadmission comorbidities. The authors were unable to determine whether some patient subgroups were more vulnerable than others to RBCs of longer storage age.

Clinical Implications

RBC storage in the United States is limited to 42 days based on Food and Drug Administration regulations issued decades ago[26-28] before the associations of donor characteristics, storage time and milieu, and interunit variability with the quality of RBC products were established.[1-6] Despite well-described mechanisms of
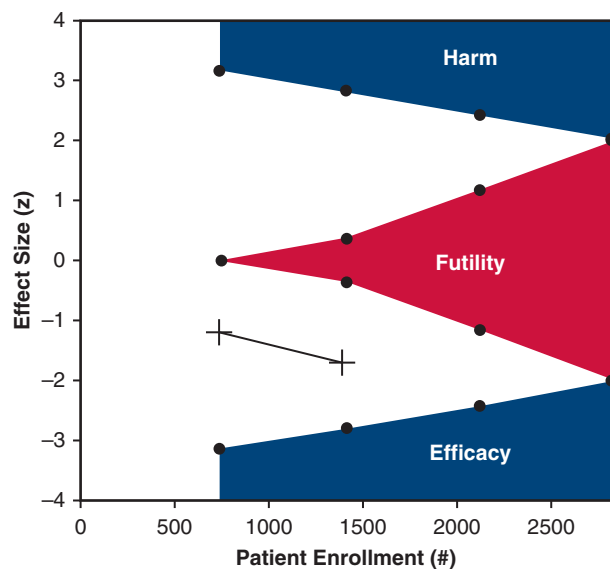
FIGURE 5. Group sequential design with predetermined boundaries for efficacy, harm, and futility. The vertical axis is Z, the standardized effect size (ie, difference in group proportions divided by the standard error of the difference), where Z of 0 means no effect, $Z > 0$ indicates that red blood cell (RBC) age $\leq$14 days has a higher occurrence of major complications, and $Z < 0$ indicates that RBC age $\leq$14 days has a lower occurrence of major complications. The horizontal axis is the cumulative number of planned (n = 2838, rounded to a final target sample size of 2840) and observed (n = 737 at first interim analysis, 1387 at this second interim analysis) patients. Interim analysis results are shown by the solid line (+), with Z-statistics of -1.2 and -1.7, respectively. By the nature of the group sequential design, type I and type II errors were limited to 0.05 and 0.10, respectively. Extrapolating the current straight line, it would intersect the upper efficacy boundary at approximately 2200 patients, roughly 700 more patients than were enrolled in the trial at its midpoint, when it was stopped.

TABLE 2. Postprocedure adverse events contributing to primary endpoint by category for each red blood cell age group

Adverse event	RBC age $\leq$14 d (n = 701), N (%)	RBC age $\geq$20 d (n = 686), N (%)
Mortality or multisystem organ failure	16 (2.3)	24 (3.5)
In-hospital mortality	15 (2.1)	23 (3.4)
Multisystem organ failure	3 (0.42)	3 (0.43)
Neurologic morbidity	14 (2.0)	19 (2.8)
Stroke	7 (1.0)	6 (0.87)
Coma	9 (1.3)	14 (2.0)
Pulmonary morbidity	106 (15)	113 (16)
Pneumonia	9 (1.3)	14 (2.0)
Pulmonary embolus	0 (0)	0 (0)
Prolonged ventilation	99 (14)	107 (16)
Renal morbidity	32 (4.6)	36 (5.2)
Infectious morbidity	10 (1.4)	12 (1.7)
Deep sternal wound infection	3 (0.42)	2 (0.29)
Septicemia/sepsis	7 (1.0)	10 (1.5)
Cardiac arrhythmia		
Atrial fibrillation	258 (37)	238 (35)
Asystole	13 (1.9)	29 (4.2)
Gastrointestinal morbidity	28 (4.0)	33 (4.8)
Reoperative morbidity	69 (10)	81 (12)
Bleeding/tamponade	36 (5.1)	44 (6.4)
Graft occlusion/valve dysfunction, noncardiac reasons	41 (5.8)	47 (6.9)
Vascular morbidity	7 (1.0)	9 (1.3)
Aortic or femoral artery dissection	0 (0)	1 (0.14)
Acute limb ischemia	7 (1.0)	8 (1.2)

RBC, Red blood cell.

degradation of RBCs with storage time, many of which are interdependent and some irreversible, it has been difficult for clinical trials to identify increased morbidity with longer RBC storage.[1,7-14]

Prudent and colleagues[29] have highlighted key features of our current state of knowledge and shed light on why basic science studies are incongruent with results of clinical trials: (1) clinical trials have studied RBCs in the time frame of reversible storage lesions (changes related to metabolism) for both fresh and what are termed standard issue or old groups, and (2) irreversible lesions generally occur after 28 to 35 days of storage and include shape changes, microvesiculation, and hemolysis. No clinical trial, including ours, has addressed the association between storage of RBCs beyond 35 days and patient outcomes. Such a trial would likely require "aging" of RBC units to near the approved limit of storage duration, raising ethical questions.

In contrast to clinical trials, observational clinical studies have been consistent with laboratory investigations, providing biological plausibility for a link between increasing duration of RBC storage and complications.[15-18] Methodology may explain these differences.[15,19,20,24] Observational studies have been larger and have had a longer median RBC storage age compared with randomized trials.[30] In previous work, we reported higher in-hospital mortality, more postoperative morbid events, and lower long-term survival in patients transfused with older RBC units.[15] An observational study of >13,000 critically ill patients by Goel and colleagues[16] reported higher mortality in patients receiving RBC units stored for >35 days. Other observational studies have similarly reported higher risks of postoperative infections, renal and wound complications, and mortality with prolonged RBC storage.[17,18]

Two meta-analyses have reported higher risk for patients transfused with prolonged-storage RBCs.[23,31] A

meta-analysis of 21 studies conducted by Wang and colleagues[31] found that transfusion of older stored RBC units was associated with higher risk of death (OR, 1.16; 95% CI, 1.07-1.24). Remy and colleagues reported that storage age was longer in observational studies than in randomized trials ($P = .01$), with observational studies reporting a higher risk of death in patients exposed to increasingly older RBC units (OR, 1.13; 95% CI, 1.01-1.24; $P = .01$).[23] The equivalent ORs from our trial are 1.3 (95% CI, 0.42-2.0) for the primary composite endpoint and 1.6 (95% CI, 0.61-4.0) for mortality or multisystem organ failure.

What randomized trials do not address is the safety of RBC units stored for 35 to 42 days. This was highlighted in the recent publication from the ABLE trial, in which the authors noted the need for further study of morbidity related to transfusion of RBCs near the end of shelf life.[32] Reducing the upper limit of RBC storage to 35 days has been suggested.[10,33] Ireland, Germany, the United Kingdom,[10] and US National Institutes of Health[30] limit blood storage to 35 days.[30] The argument that adopting a 35-day storage limit would be disruptive does not take into account 2 important trends: (1) dissemination and implementation of national blood management programs have been associated with decreased RBC use, and (2) some regions in the United States have excess RBC inventory.[34,35]

Strengths and Limitations

The primary limitation of our trial is the cessation of subject accrual at the halfway point because of enrollment constraints. This resulted in an inconclusive trial that supports neither efficacy nor futility. It also highlights the inherent challenges of a randomized transfusion trial in cardiac surgery (Appendix E1). Ideally, randomization would be performed at the first call for a unit of RBCs; however, that is unrealistic, because blood must be set up in anticipation of transfusion, but blood of the appropriate age often must be procured before surgery. Thus, it must be assumed that randomized patients will be transfused at random, leading to a modified intent-to-treat analytic strategy. The standardized differences between groups in our study demonstrate the reasonableness of this assumption.

Other limitations include protocol deviations, inadvertent or for reasons of patient safety, which meant that some patients received an admixture of RBC units of different ages, which would be expected to dilute any difference in trial endpoints. Low mortality and morbidity limit the ability to analyze individual endpoints.

Although our clinical trial was stopped at midpoint, it was robust in terms of being well designed with nationally defined clinical endpoints and rigorous analysis with a contemporary approach to composite endpoints that can overcome many of the recognized pitfalls of such endpoints. For example, our group sequential design allowed monitoring of efficacy and futility over the course of the study and protected against type I (false-positive) and type II (false-negative) conclusions in the process. We used an advanced, flexible statistical methodology for the primary endpoint analysis, the average relative-effect OR.[22] This method first estimates a treatment effect for each component of the composite, then averages over them, giving the same weight to each, while accounting for within-subject correlation among the components in the variance. We chose this method over the simpler collapsed composite (any vs none) method or a common-effect global OR GEE method, because treatment effect estimates obtained with those methods are driven by components with the highest frequency, unlike the average relative-effect method that weighs each component equally. Finally, our sensitivity analysis reached the same conclusion as our modified intent-to-treat analysis.

CONCLUSIONS

This randomized clinical trial of RBC units stored for ≤ 14 days versus ≥ 20 days supports neither the efficacy nor futility of transfusing either younger or older RBC units perioperatively in the cardiac surgery setting. What remains untested, and is possibly untestable by a clinical trial, is whether prolonged storage of RBCs at or near the end of their shelf life is associated with elevated risk of adverse postoperative events. Because such a trial may be unethical, it raises the question of whether in the absence of human trial data, laboratory data related to degradation over time should instead be adopted to guide the conversation about whether the limit of RBC storage duration should be reduced to <42 days.

Conflict of Interest Statement
Authors have nothing to disclose with regard to commercial support.

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Key Words: transfusion, postoperative outcomes, distinct-effects generalized estimating equation model

APPENDIX E1. LESSONS LEARNED FROM A RANDOMIZED CLINICAL TRIAL OF TRANSFUSION PRACTICES IN CARDIAC SURGERY

A trial of blood transfusions in the cardiac surgery setting faces a number of challenges, and many lessons have been learned from the trial reported in this article.

Safety of Cardiac Surgery

Because of the safety of cardiac surgery, the number of endpoints is small. In this study, the challenge was met by a composite morbidity/mortality endpoint. However, it was unknown whether or not each complication in the composite would respond to RBC unit age in the same direction. We found that atrial fibrillation did not follow the same pattern as other complications and occurred most frequently. This problem was obviated by a statistical method that equally weights the components. Another solution could have been a multi-institutional trial, with its high logistical complexity and expense.

Uncertainty of Transfusion

With cardiac surgical programs focused on blood conservation, it is uncertain which patients will require a transfusion—or, more accurately, receive a transfusion. (Some trials have used an algorithm to somewhat more precisely target patients at a higher likelihood of receiving transfusion.) A major enrollment constraint for the present trial was the institution of stringent blood conservation quality improvement initiatives that reduced RBC use from the 52% used in the trial design to near 20% by trial discontinuation. What blood banks do is set up blood for every patient before surgery in anticipation that all patients will undergo transfusion, even if only a small fraction actually do. This has an important effect on trial design. Ideally, when the first unit of blood is called for, eligible and consented patients would be randomized. That would work if the type or age of blood units were always available in the quantity needed; however, blood banks need prior notice, particularly to retrieve older blood. Thus, having the requisite number of units available may require obtaining units from other blood banks.

The consequence of this logistic challenge is that randomization must occur before it is known whether a patient will require transfusion. A true intent-to-treat analysis is inappropriate, because most patients in each arm would not be exposed to the treatment. Thus, an assumption must be made that transfusion will be a random event in each study arm.

Effectiveness of Blood Banks

Particularly in a trial that extends over many years, during which blood bank employees will likely change, constant education, reeducation, alertness, and continual buy-in to the trial are needed to avoid protocol deviations. When these occur, a corrective action plan must be in place to avoid contaminating the trial.

APPENDIX E2. SEQUENTIAL MONITORING

We planned on 3 interim analyses at 25%, 50%, and 75% of the total accrual. Our calculations of group sequential boundaries assumed nonbinding stopping rules and accounted for monitoring both the null (efficacy) and alternative hypotheses (futility). We used the gamma family spending function with gamma -4 for efficacy and gamma -2 for futility, which is between the Pocock and O'Brien-Fleming approaches (Figure E1). We were thus spending beta somewhat faster than alpha during the trial, allowing for early termination if there was a small treatment group difference. The stopping boundaries are shown in Figure 5 (on the scale of the Z-statistic with a 2-sample proportions test) and Table E1. The total sample size had to be modified up a bit (n = 2838, rounding to 2840 for the final target sample size) to properly account for the interim analyses. Note that this was the maximum sample size for this study. Because the group sequential design makes it possible to terminate the study early for either efficacy or futility, the expected sample size would be 2004 if the null hypothesis of no difference were true and 2063 if the alternative hypothesis (27.3% vs 32.7%) were true.
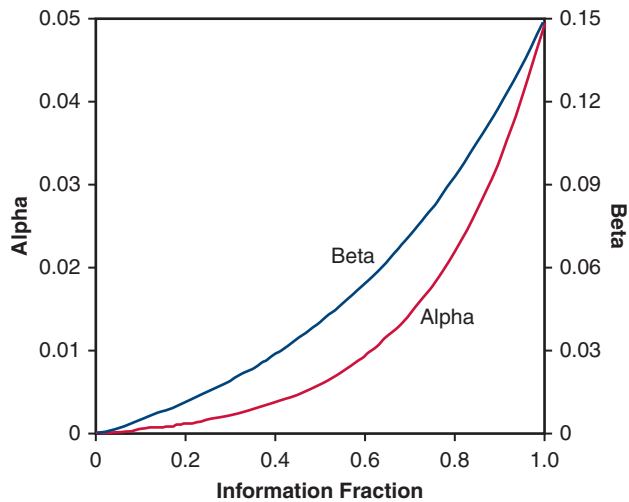
FIGURE E1. Alpha and beta spending functions used in calculating group sequential boundaries. The beta (futility) is spent faster than alpha (efficacy), allowing for early termination if there is little difference between treatment groups.
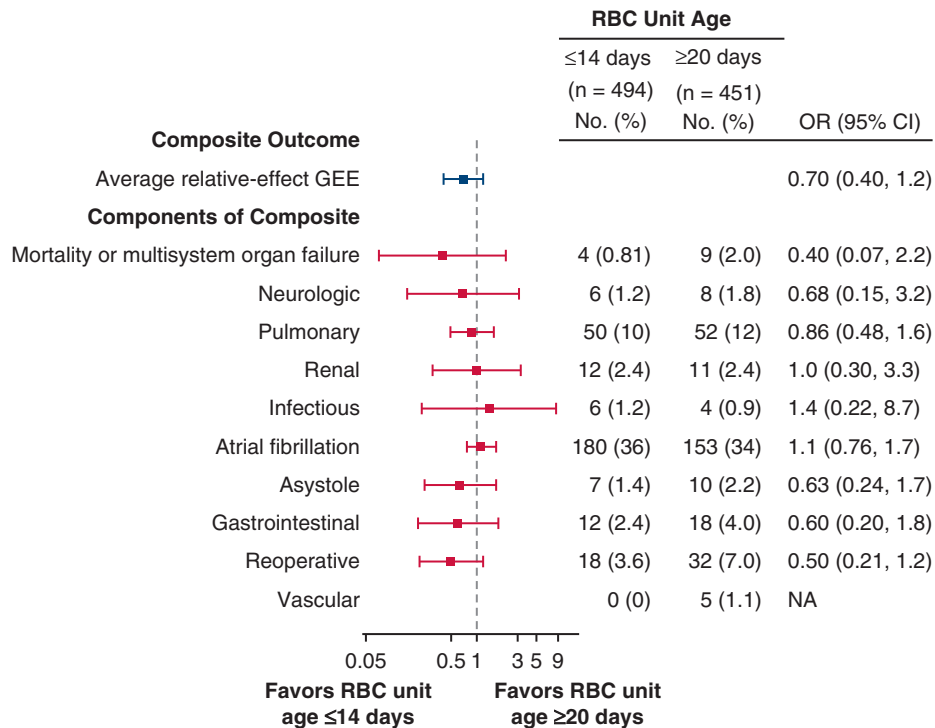

	RBC Unit Age		
	≤14 days (n = 494) No. (%)	≥20 days (n = 451) No. (%)	OR (95% CI)
Composite Outcome			
Average relative-effect GEE			0.70 (0.40, 1.2)
Components of Composite			
Mortality or multisystem organ failure	4 (0.81)	9 (2.0)	0.40 (0.07, 2.2)
Neurologic	6 (1.2)	8 (1.8)	0.68 (0.15, 3.2)
Pulmonary	50 (10)	52 (12)	0.86 (0.48, 1.6)
Renal	12 (2.4)	11 (2.4)	1.0 (0.30, 3.3)
Infectious	6 (1.2)	4 (0.9)	1.4 (0.22, 8.7)
Atrial fibrillation	180 (36)	153 (34)	1.1 (0.76, 1.7)
Asystole	7 (1.4)	10 (2.2)	0.63 (0.24, 1.7)
Gastrointestinal	12 (2.4)	18 (4.0)	0.60 (0.20, 1.8)
Reoperative	18 (3.6)	32 (7.0)	0.50 (0.21, 1.2)
Vascular	0 (0)	5 (1.1)	NA

FIGURE E2. Per-protocol analysis. Patients included are those who received only the red blood cell (*RBC*) storage age to which they were randomized. *CI*, Confidence interval; *GEE*, generalized estimating equation.

TABLE E1. Stopping boundaries on the *P* value scale and probabilities of crossing the boundaries

P value threshold	Interim 1 (n = 708)	Interim 2 (n = 1418)	Interim 3 (n = 2128)	Final (N = 2838)
To reject H0, *P* must be less than or equal to	.0016	.0048	.0147	.044
To reject H1, *P* must be greater than or equal to	.9478	.7128	.2424	.044
Boundary crossing probability under H0	.054	.271	.472	.203*
Boundary crossing probability under H1	.071	.251	.376	.302*

H0: there is no difference in the proportions of composite outcome between the treatment groups; H1: there is a difference. *Probability of reaching the final analysis without early boundary crossing.