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The Effects of Storage Age of Blood in Massively Transfused Burn Patients: A Secondary Analysis of The Randomised Transfusion Requirement in Burn Care Evaluation (TRIBE) Study.

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Abstract

Objective: Major trials examining storage age of blood transfused to critically-ill patients administered relatively few blood transfusions. We sought to determine if the storage age of blood affects outcomes when very large amounts of blood are transfused.

Design: A secondary analysis of the multicenter randomized Transfusion Requirement in Burn Care Evaluation (TRIBE) study which compared restrictive and liberal transfusion strategies.

Setting: Eighteen tertiary-care burn centers.

Patients: TRIBE evaluated 345 adults with burns ≥ 20% of the body surface area. We included only the 303 patients that received blood transfusions.

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All statistical analyses were performed by Dr. Sandra L. Taylor PhD.

Interventions: The storage ages of all transfused red-cell units were collected during TRIBE. *A priori* measures of storage age were the the mean storage age of all transfused blood and the proportion of all transfused blood considered very old (stored ≥ 35 days).

Measurements and Main Results: The primary outcome was the severity of multiple organ dysfunction (MOD). Secondary outcomes included time to wound healing, the duration of mechanical ventilation, and in-hospital mortality.There were 6786 red-cell transfusions with a mean (±SD) storage age of 25.6 ± 10.2 days. Participants received a mean of 23.4 ± 31.2 blood transfusions (range 1–219), and a mean of 5.3 ± 10.7 units of very old blood. Neither mean storage age nor proportion of very old blood had any influence on MOD severity, time to wound healing, or mortality. Duration of ventilation was significantly predicted by both mean blood storage age and the proportion of very old blood but this was of questionable clinical relevance given extreme variability in duration of ventilation (adjusted $r^2 \leq 0.01$).

Conclusion: Despite massive blood transfusion, including very old blood, the duration of red-cell storage did not influence outcome in burn patients. Provision of the oldest blood first by Blood Banks is rational, even for massive transfusion.

Trial Registration: Clintrials.gov NCT01079247.

Keywords

burns; blood; transfusion; storage age; TRIBE

Introduction

Hospital blood banks store red-blood cell (RBC) units for up to 42 days because at least 75% of transfused erythrocytes are still circulating 24 hours after transfusion (1,2). However, with longer storage, RBC units undergo changes that might cause older blood to be less effective and potentially harmful compared to fresher blood (3–14). Retrospective and prospective observational studies have reached differing conclusions on the effects of blood storage age (15–22). In contrast, large pragmatic randomized controlled trials (RCTs) demonstrated that clinical outcomes were not improved with transfusion of fresher blood as compared to older “standard issue” blood among critically ill patients, (the ABLE and TRANSFUSE studies) (23,24), hospitalized patients, (the INFORM study) (25) , or patients undergoing complex cardiac surgery, (the RECESS study) (26) .

Major burn patients have substantial transfusion requirements that greatly exceed those of patients studied in trials such as ABLE (23), TRANSFUSE (24), INFORM (25), and RECESS (26). Some studies suggest that adverse effects related to longer blood storage only become apparent when larger amounts of blood are transfused (27–29). Major burn patients therefore provide an ideal model in which to examine this question.

The recently completed Transfusion Requirement in Burn Care Evaluation (TRIBE) study was a multicenter prospective RCT that compared outcomes between patients transfused with a restrictive or liberal transfusion target (30). The storage age of over 7000 RBC units administered during this study was also collected. The purpose of this secondary analysis of the TRIBE study was to determine if the storage age of transfused blood has any effect on outcome in major burn patients with major transfusion requirements.

Methods

This was a secondary analysis of TRIBE (30), an institutional review board-approved phase III multicenter open-label registered clinical trial, (Clintrials.gov NCT01079247), in which adults with major burns were randomized to receive RBC transfusions to maintain a hemoglobin level approximately 10–11 g/dL (liberal group) or a hemoglobin level approximately 7–8 g/dL (restrictive group).

The storage age of each RBC unit was determined by subtracting the recorded days to expiry of the unit from 42 days, the maximum storage age of transfused blood. We chose two *a priori* measurements to assess blood storage age for each subject: the mean storage age of all transfused RBC units administered, and the proportion of all transfused RBC units that were very old (≥ 35 days of storage). We considered only blood administered up to the time of the outcomes of interest, and not blood transfused on the day of, or following the outcome of interest.

We utilized outcome measures employed in the original TRIBE study (30). The primary outcome was the severity of multiple organ dysfunction (MOD) measured by the highest (worst) daily MOD score (31), following initiation of blood transfusion(s). We selected MOD as the primary outcome because prolonged blood storage is believed to contribute to impaired oxygen delivery and heightened inflammation (3) which would be expected to directly result in organ damage. If multiple values were recorded on any day for any component of the MOD score, the worst level of dysfunction was selected to calculate the MOD score. Since the same highest MOD score could occur on multiple days, the date of the chronologically last highest MOD score was used as the end-point for determining the number and age characteristics of all the preceding blood transfusions.

Secondary outcomes were the duration of mechanical ventilation, time to wound healing, and in-hospital mortality. Ventilation duration was assessed among survivors and we considered all RBC transfusions administered between admission up to the termination of mechanical ventilation. The day of wound healing was defined as occurring 7 days from the final excision and grafting operation, considering only RBC transfusions given before the reported day of wound healing. For in-hospital mortality, we analyzed all the blood transfusions up to, but not including those administered on the day of death or discharge from the hospital.

Statistical Analyses

Simple descriptive statistics, [means ± standard deviations (SD) and/or medians (interquartile range)], were used to summarize distributions of quantitative variables. Proportions were used for categorical variables. Nine transfusions without identified start dates were dropped from the analyses because we could not evaluate their effects relative to the time of an outcome of interest. Transfused units with missing, negative, or storage age > 42 days were dropped for calculation of the descriptive statistics, but were imputed as the overall mean blood age of all transfused units for the regression analyses. To evaluate any relationship between transfusion number and blood storage age, we used a mixed effect linear regression model and modeled blood age versus transfusion number using Proc Mixed in SAS® , (Version 9.4, SAS Institute Inc., Cary, NC, USA). A random effect was included for each center to account for center-specific effects and a random intercept and slope included for each patient.

Because of potential non-independence of outcomes for subjects at the same center, general linear regression models fit using generalized estimating equations (GEE) were used to evaluate relationship between each outcome measure and our blood storage age metrics (mean blood age, and proportion of transfused units ≥ 35 days old). An exchangeable working correlation structure was assumed and robust standard errors used for hypothesis testing and confidence intervals. For each outcome we used multivariable analyses to evaluate the effect of each blood storage age metric with inclusion of the number of transfused RBC units, patient age, gender, percent of the total body surface area burned (%TBSA), inhalation injury, and admission APACHE score as covariates. Logistic models were used for in-hospital mortality and linear models used for MOD score, days to wound healing and ventilation days. Ventilation days and days until wound healing were log transformed to meet model assumptions of Gaussian errors and homogeneity of variances. A quadratic term for the number of transfusions was included in the linear models because model diagnostics indicated non-linear relationships; squared values of the number of transfusions were divided by 100 to improve precision of parameter estimates. Generalized Estimation Equation (GEE) models were fit using Proc Genmodin in SAS® or using the GEE library in R Statistical Computing Language Version 3.4.1 (32,33). A p value < 0.05 was considered statistically significant.

Results

Patient and Transfusion Characteristics

There were 345 subjects in the TRIBE study and the 303 subjects (87.8%) who received at least one RBC transfusion were included (Table 1). A total of 7054 individual RBC transfusions were administered. Leukoreduced blood was used in 86.6% of the transfusions. Transfusions with missing storage age (236), negative values for days until expiration (27), or a recorded storage age > 42 days (5) were dropped from the descriptive statistical analysis leaving 6768 RBC transfusions (96% of all transfusions), (Table 1). Blood storage age was not statistically significantly associated with transfusion number, (slope ± se = 0.002 ± 0.0154, t_{16} = 0.16, p = 0.876), i.e. receiving more blood transfusions was not significantly related to receiving either older or younger blood.

Outcomes

Multiple Organ Dysfunction—There were 292 cases with MOD scores reported after commencing blood transfusion(s) available for the regression analyses. The mean maximum MOD score following the start of blood transfusion(s) was 6.6 ± 4.5 [median 7 (3,10)], and was not significantly related to either the mean storage age of transfused blood (p=0.078), or the proportion of very old blood transfused (p=0.213). The maximum MOD score was significantly related to the number of RBC units transfused, the patient's age, % TBSA, presence of smoke inhalation injury, and APACHE score (Table 2).

Wound Healing—Time to wound healing was recorded in 253 transfused subjects who had surgery. After dropping two subjects who received blood transfusions on or after the reported day of healing, there were 251 subjects available for analysis. The mean number of days to wound healing was 40.5 ± 46.9 days [median 27 (15, 47) days]. Neither the mean storage age of transfused blood nor the proportion of very old transfused RBC units were significantly related to the days to wound healing (Table 3). However, the time to wound healing was significantly associated with the number of transfused RBC units, ($p<0.0001$).

Duration of Ventilation—There were 165 survivors who were mechanically ventilated and transfused blood available for the regression analysis. The mean number of days on mechanical ventilation was 30.5 ± 38.2 [median 18 (8,36)]. Both the mean blood storage age and the proportion of very old transfused units were significantly related to the duration of ventilation ($p=0.023$ and 0.006, respectively, Table 3). Although statistically significant, mean blood storage age and proportion of very old RBC units explained little of the substantial clinical variability in the duration of ventilation as evidenced by adjusted r^2 values of 0.01 and 0.007, respectively (Figure 1). Further, the size of any effect of blood age on duration of ventilation was small. For every day of increase in mean blood storage age, duration of ventilation increases by an estimated 2.2% [95% CI: 0.24%, 4.2%] and for every percentage point increase in the percentage of very old transfused units, duration of ventilation increases by 0.7% [95% CI: 0.1%, 1.3%]. Ventilation duration was also significantly related to the number of RBC units transfused, patient age, % TBSA burn, and inhalation injury (Table 3).

Mortality—Analysis of in-hospital mortality was conducted among 298 subjects after dropping five cases with either insufficient data to identify all transfusions given prior to death or discharge, or patients that received transfusions only on the day of death. The odds of in-hospital death were not significantly increased by either the mean storage age or proportion of very old transfused blood. However, patient age, % TBSA burn, and admission APACHE score all significantly raised the odds of in-hospital death (Table 4).

Discussion

In this secondary analysis of 303 adult burn patients receiving at least one RBC transfusion in the TRIBE study (30), we found that neither the mean storage age of transfused blood nor the proportion of transfused very old blood (≥ 35 days old) affected multiple organ dysfunction, time to wound healing, or in-hospital mortality. A weak positive statistical association, of unknown clinical significance, was observed between the duration of mechanical ventilation, the mean storage age of transfused blood, and the proportion of very old transfused RBC units.

Recent large trials such as ABLE (23), TRANSFUSE (24) , INFORM (25), and RECESS (26) reported that transfusion of the freshest available blood was not advantageous compared to transfusion of older "standard issue" blood. Our investigation adds important new information to the study of blood storage age because of the substantial and unprecedented volume of blood administered in this trial. Some studies have suggested that blood storage age worsens outcomes only when a large amount of blood is transfused (27–29). Our subjects each received a mean of 23 units of blood (median 13 units). In contrast, in the ABLE (23) and TRANSFUSE (24) studies, subjects received a mean of only 4 RBC units while in the INFORM trial (25) patients received a median of only 2 units of blood. In the RECESS study (26) of cardiac surgery patients, a median of only 3 RBC units was transfused and the 75th percentile for the number of transfusions was six RBC units. Consequently, it was uncertain whether the results of these important studies applied to our burn population, whose transfusion requirement was more than five times larger, or to other patients that receive large volumes of transfused blood. However, despite the huge number of transfusions, we found that the mean storage age of transfused blood had no effect upon our primary and secondary outcomes. This was also despite the fact that the mean storage age of blood was 26 days, which is older than the mean storage age of blood given to subjects in the “older blood-standard issue” arms of the ABLE (23) and TRANSFUSE (24) trials (22 days of storage), or the INFORM trial (24 days of storage) (25). Therefore, our findings suggest that transfusion of even very large amounts of relatively older blood does not appear to be harmful.

The ABLE (23), INFORM (25) and TRANSFUSE (24) studies were designed to address the practical question of whether fresh blood is better than old blood. But these studies left unanswered the question of whether very old blood (i.e. 35–42 days old), could be harmful. This is an important question because blood banks preferentially issue their oldest compatible blood first to minimize wastage. The degenerative and pro-inflammatory changes that occur in banked blood, (collectively referred to as the “storage lesion”), increase with time and would be expected to be most severe when stored blood reaches the end of its shelf life (3). Animal studies have found that transfusion of blood at the termination of its shelf life led to increased lung dysfunction and higher mortality (34) and to heightened systemic inflammation (35) compared to transfusion of fresh blood. In healthy human volunteers, autologous transfusion of 6-week old blood led to significantly increased and potentially harmful extravascular hemolysis compared to autologous transfusion of blood stored less than 7 days (36). However, a secondary analysis of the INFORM trial of 24 736 general hospitalized patients found that transfusion of blood stored longer than 35 days had no influence on in-hospital mortality (37). Our observations are in agreement with this. Almost one-quarter of the blood transfused to our subjects was very old, and a median of 2 units of very old blood was transfused per patient (compared to a median of only 1 unit of very old blood per patient in the INFORM secondary analysis). Yet, we found that the proportion of very old transfused blood had no effect on multiple organ dysfunction, wound healing, or in-hospital mortality, among severely burned patients. Our findings suggest that the current strategy whereby blood banks issue the oldest blood first is rational, even for patient populations that have very high transfusion needs, such as major burn patients.

Pulmonary outcomes are particularly relevant to the study of the effects of blood storage age (14) . Transfusion Related Acute Lung Injury (TRALI) is also recognized as an important complication of blood product transfusion (38). In animal studies, transfusion of blood at the end of its storage life has produced TRALI and other lung dysfunction (34,38). In humans, retrospective and observational studies have reached inconsistent conclusions on whether blood storage age is related to worse respiratory outcomes (15,20,39), whereas small prospective randomized trials have not shown any effect of blood storage on lung injury (31,40). Although we found a statistically significant positive relationship between blood storage age and a longer duration of mechanical ventilation, we are uncertain of the clinical relevance of this finding. The highly variable relationship cannot be explained by blood storage age alone. This relationship was weak as evidenced by the widely ranging and inconsistent duration of ventilation among the study subjects, and the very low r^2 values in the regression analyses (Figure 1). Therefore it is unlikely that blood storage age alone exerted an effect on the duration of ventilation. Consequently, our findings do not support use of fresher blood in mechanically ventilated burn patients.

Our study has some limitations. Although we had complete data on nearly 7000 blood transfusions, this was not primarily an investigation of blood storage age. Patients invariably received a mixture of older and fresher blood and use of the mean storage age as a metric assumes that fresher blood somehow offsets possible adverse effects of older blood; an assumption that may not be true. We did not control for possible effects caused by platelet or plasma transfusion, and it is possible that sicker patients could have received more of these blood products. Although the vast majority of units were leukoreduced, some were not. In addition, we did not obtain data on storage solutions. Both factors could potentially affect expression of the storage lesion (3) . We did not consider ABO blood type as a variable. The need for commonly requested (and therefore newer) type O blood tends to promote the release of fresher blood, whereas the need for infrequently required (and therefore older) type B and AB blood tends to promote the release of blood with a longer storage age.

In conclusion, we found that among major burn patients who had major transfusion requirements, neither the mean storage age of transfused blood nor the proportion of very old blood transfused had any clinically meaningful effects. The current approach of administering the oldest compatible blood for transfusion is acceptable, even for patient populations with very high transfusion needs, such as patients with major thermal injuries.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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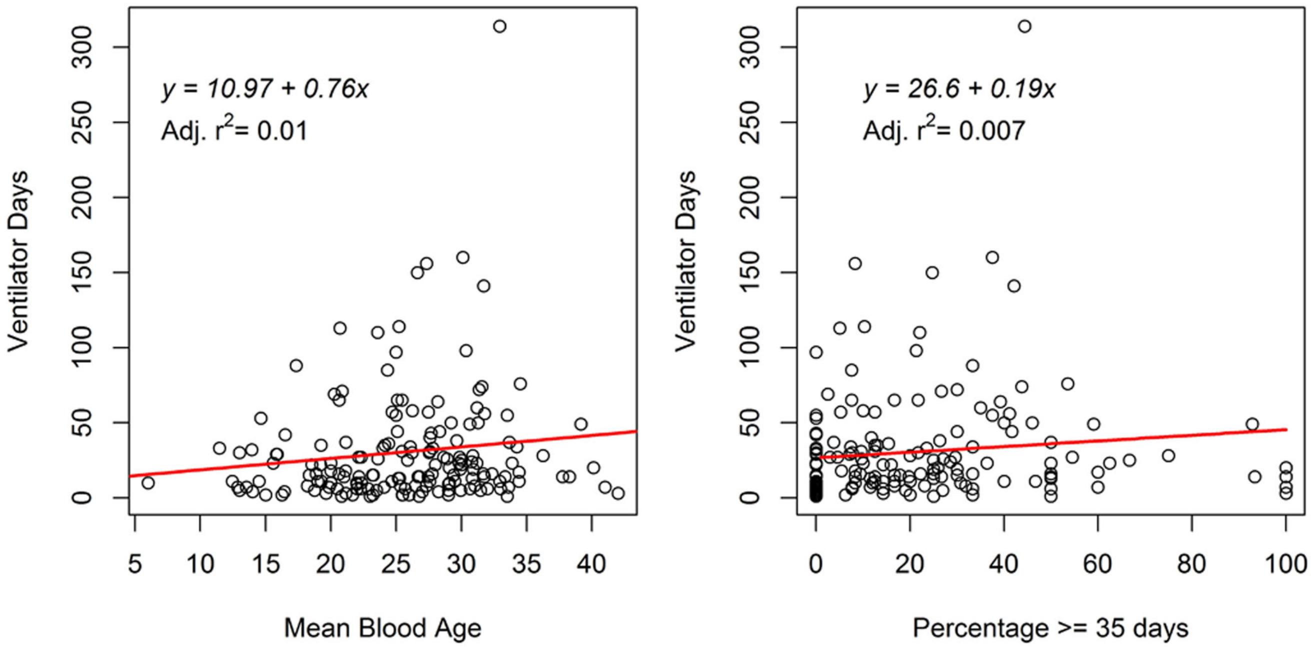

Figure 1:
Scatter plot and fitted line of the relationship between days on mechanical ventilation and mean blood age (left panel) and percentage of units ≥ 35 days (right panel). Solid line indicates the fitted regression line from a simple linear regression of ventilator days versus mean blood age or percentage of units ≥ 35 days. The adjusted r^2 values for these regressions are 0.01 for mean blood age and 0.007 for percentage of units ≥ 35 days.

Table 1:

Patient and Transfusion Characteristics.

Patient Characteristics		**Value**
Age in years		42 (30, 55.5)
% female sex		23.1 (n=70)
% TBSA burn		33 (26, 48.5)
% with Inhalation Injury		22.8 (n=69)
Admission APACHE score		18 (13, 24)
Earliest MOD score		5 (1, 7)
Median length of stay in days		34.5 (22, 62)
30-day mortality		9.6% (N=29)
In-hospital mortality		13.2% (N=40)
Transfusion Characteristics		
Mean number of RBC transfusions per patient		23 ± 31
	Median	13 (6, 27)
	Range	1-219
Mean storage age of transfused RBC in days		25.6 ± 10.2
	Median	26 (17, 42)
% of transfused RBC units that were very old		23.9
Median number of very old RBC units transfused per patient		2 (0, 6)
	Mean	5.3 ± 10.7
% of transfused RBC units ≤ 8 days old		3.3
Number of patients (%) who received exclusively ≤ 8 day-old blood		0 (0)
Number of patients (%) who received exclusively very old * blood		15 (5)
% of RBC transfusions given in the Burn Unit		69.5
% of RBC transfusions given in the Operating Room		30.5

Patient and transfusion characteristics. Values are shown as median (25^{th}, 75^{th} quartiles), mean ± standard deviation, or percentage and number. TBSA: Total Body Surface Area, MOD: Multiple Organ Dysfunction, RBC: red-blood cell.

* very old is defined as ≥ 35 days of storage.

Table 2:

Multivariate Regression Analysis for Effect of Mean Storage Age and Proportion of Very Old Transfused Blood Units on Maximum MOD Score

Maximum MOD score						
	Mean Storage Age			**Proportion ≥ 35 days**		
	Estimate ± SE	**Z**	**P value**	**Estimate ± SE**	**Z**	**P value**
Number of RBC	0.116 ± 0.029	3.98	0.0001	0.112 ± 0.029	3.88	0.0001
(Number of RBC)^2/100	−0.059 ± 0.023	−2.54	0.011	−0.056 ± 0.023	−2.44	0.015
Mean Blood Storage Age	0.052 ± 0.03	1.76	0.078			
Prop35				0.009 ± 0.008	1.24	0.213
Age	0.052 ± 0.013	3.94	0.0001	0.052 ± 0.013	3.92	0.0001
Gender Male	0.073 ± 0.434	0.17	0.867	0.043 ± 0.435	0.1	0.921
%TBSA burn	0.042 ± 0.013	3.15	0.002	0.042 ± 0.014	3.11	0.002
Inhalation Injury	1.381 ± 0.486	2.85	0.004	1.448 ± 0.485	2.99	0.0028
APACHE.Score	0.14 ± 0.032	4.37	<0.0001	0.137 ± 0.032	4.31	<0.0001

Results of multivariable linear regressions of the effect of mean blood storage age or proportion of units with age ≥ 35 days (Prop 35), number of RBC transfusions, patient age, gender, burn size, inhalation injury, and admission APACHE score on maximum MOD score. RBC: red blood cell units, TBSA: total body surface area burn, MOD: multiple organ dysfunction.

Table 3.

Multivariate Regression Analysis for Effect of Mean Storage Age and Proportion of Very Old Transfused Blood Units on Time to Wound Healing and Duration of Ventilation

Wound Healing						
	Mean Blood Storage Age			**Proportion ≥ 35 days**		
	Estimate ± SE	**Z**	**P value**	**Estimate ± SE**	**Z**	**P value**
Number of RBC	0.035 ± 0.003	12.87	<0.0001	0.034 ± 0.003	12.94	<0.0001
$(\text{Number of RBC})^2/100$	−0.011 ± 0.001	−11.15	<0.0001	−0.01 ± 0.001	−11.17	<0.0001
Mean blood storage age	0.004 ± 0.004	1.05	0.296			
Prop35				0 ± 0.001	0.03	0.976
Age	0.003 ± 0.002	1.82	0.069	0.003 ± 0.002	1.84	0.066
Gender Male	−0.023 ± 0.062	−0.38	0.707	−0.025 ± 0.06	−0.41	0.683
%TBSA burn	0.002 ± 0.002	0.99	0.324	0.002 ± 0.002	0.91	0.365
Inhalation Injury	0.005 ± 0.069	0.07	0.941	0.007 ± 0.069	0.1	0.92
APACHE Score	−0.001 ± 0.004	−0.29	0.771	−0.001 ± 0.004	−0.31	0.757
Duration of ventilation						
	Mean Blood Storage Age			**Proportion ≥ 35 days**		
	Estimate ± SE	**Z**	**P value**	**Estimate ± SE**	**Z**	**P value**
Number of RBC	0.035 ± 0.003	10.79	<0.0001	0.035 ± 0.003	12.27	<0.0001
$(\text{Number of RBC})^2/100$	−0.014 ± 0.001	−9.79	<0.0001	−0.014 ± 0.001	−9.63	<0.0001
Mean blood storage age	0.022 ± 0.01	2.27	0.023			
Prop35				0.007 ± 0.003	2.78	0.006
Age	0.019 ± 0.004	4.91	<0.0001	0.019 ± 0.004	4.54	<0.0001
Gender Male	0.093 ± 0.103	0.90	0.366	0.099 ± 0.1	0.99	0.323
%TBSA burn	0.014 ± 0.004	3.92	0.0001	0.013 ± 0.004	3.56	0.0004
Inhalation Injury	0.242 ± 0.113	2.14	0.033	0.256 ± 0.115	2.24	0.025
APACHE Score	0.002 ± 0.008	0.23	0.821	0.002 ± 0.008	0.25	0.806

Results of multivariable linear regressions of the effect of mean blood storage age or proportion of units with age ≥ 35 days (Prop35), number of RBC units, patient age, gender, burn size, inhalation injury, and admission APACHE score on days to wound healing (log transformed) among 251 patients that required surgery and who were transfused, and on duration of ventilation (log transformed) among 165 patients that were mechanically ventilated and transfused. RBC: red blood cell units, TBSA: total body surface area burn.

Table 4:

Multivariate Regression Analysis for Effect of Mean Storage Age and Proportion of Very Old Transfused Blood Units on Mortality

Mortality								
	Mean Blood Storage Age				Proportion ≥ 35 days			
	Estimate ± SE	Z	P value	OR [95% CI]	Estimate ± SE	Z	P value	OR [95% CI]
Number of RBC units	−0.008 ± 0.007	−1.07	0.284	0.993 [0.979, 1.006]	−0.007 ± 0.008	−0.99	0.3229	0.993 [0.978, 1.007]
Mean Blood Storage Age	−0.011 ± 0.038	−0.29	0.772	0.989 [0.919, 1.065]				
Prop35					−0.175 ± 1.22	−0.14	0.885	0.839 [0.077, 9.177]
Age	0.075 ± 0.012	6.28	<0.0001	1.078 [1.053, 1.103]	0.075 ± 0.013	5.83	<0.0001	1.078 [1.051, 1.105]
Gender Male	−0.871 ± 0.559	−1.56	0.119	0.419 [0.14, 1.252]	−0.873 ± 0.557	−1.57	0.117	0.418 [0.14, 1.243]
TBSA	0.05 ± 0.013	3.82	0.0001	1.051 [1.024, 1.078]	0.05 ± 0.014	3.62	0.0003	1.051 [1.023, 1.08]
Inhalation Injury	−0.247 ± 0.296	−0.84	0.404	0.781 [0.437, 1.395]	−0.252 ± 0.311	−0.81	0.4178	0.777 [0.422, 1.43]
APACHE Score	0.119 ± 0.032	3.85	0.0001	1.127 [1.06, 1.197]	0.119 ± 0.032	3.74	0.0002	1.126 [1.058, 1.199]

Results of multivariable logistic regressions of the effect of mean blood storage age or proportion of units with age ≥ 35 days (Prop 35), number of RBC transfusions, patient age, gender, burn size, inhalation injury, and admission APACHE score on in-hospital mortality. RBC: red blood cell units, TBSA: total body surface area burn.