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Total plasma heme concentration increases after red blood cell transfusion and predicts mortality in critically ill medical patients

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Abstract

BACKGROUND: Relationships between red blood cell (RBC) transfusion, circulating cell-free heme, and clinical outcomes in critically ill transfusion recipients are incompletely understood. The goal of this study was to determine whether total plasma heme increases after RBC transfusion and predicts mortality in critically ill patients.

STUDY DESIGN AND METHODS: This was a prospective cohort study of 111 consecutive medical intensive care patients requiring RBC transfusion. Cell-free heme was measured in RBC units before transfusion and in the patients' plasma before and after transfusion.

RESULTS: Total plasma heme levels increased in response to transfusion, from a median (interquartile range [IQR]) of 35 (26–76) $\mu\text{mol/L}$ to 47 (35–73) $\mu\text{mol/L}$ ($p < 0.001$). Posttransfusion total plasma heme was higher in nonsurvivors (54 [35–136] $\mu\text{mol/L}$) versus survivors (44 [31–65] $\mu\text{mol/L}$, $p = 0.03$). Posttransfusion total plasma heme predicted hospital mortality (odds ratio [95% confidence interval] per quartile increase in posttransfusion plasma heme, 1.76 [1.17–2.66]; $p = 0.007$). Posttransfusion total plasma heme was not correlated with RBC unit storage duration and weakly correlated with RBC unit cell-free heme concentration.

CONCLUSIONS: Total plasma heme concentration increases in critically ill patients after RBC transfusion and is independently associated with mortality. This transfusion-associated increase in total plasma heme is not fully explained by RBC unit storage age or cell-free heme content.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Additional studies are warranted to define mechanisms of transfusion-related plasma heme accumulation and test prevention strategies.

Red blood cell (RBC) transfusion is associated with increased risk of death in critically ill patients.¹ This risk may be explained by storage-related changes in banked RBCs. Such changes include depletion of adenosine triphosphate, nitric oxide, and 2,3-diphosphoglycerate, excess cellular stiffness and fragility, and low-grade hemolysis.^{2–5} The resulting cell-free hemoglobin (Hb; free in solution and contained in RBC microparticles)^{3,4,6–8} can undergo oxidation and release cell-free heme.^{9,10} These mediators may cause toxicity when transfused because cell-free Hb promotes circulatory dysfunction by scavenging nitric oxide^{4,7,11,12} and cell-free heme promotes inflammation, immunosuppression, and infection.^{13–16} The risks of transfusion may be particularly harmful during acute illnesses that may aggravate hemolysis and impair clearance of circulating cell-free Hb and heme.^{12,13,17}

We hypothesized that the concentration of total plasma heme would increase in critically ill patients after transfusion in proportion to the storage age and total cell-free heme concentration within the RBC units. We further hypothesized that total cell-free heme concentration in RBC units and posttransfusion plasma would be associated with hospital mortality. To test these hypotheses, we conducted a prospective cohort study of medical intensive care unit (ICU) patients requiring RBC transfusion. We measured total cell-free heme in stored RBC units before transfusion and in patient plasma before and after RBC transfusion. We then analyzed relationships between these measurements and hospital mortality. The objectives of this observational investigation of critically ill patients were: 1) to determine whether total plasma heme increases after RBC transfusion, 2) to determine whether posttransfusion total plasma heme increases with increasing RBC unit storage duration and RBC unit total cell-free heme concentration, and 3) to determine whether total cell-free heme in stored RBC units and posttransfusion plasma predict hospital mortality.

MATERIALS AND METHODS

Study design

This was a prospective cohort study conducted in a 12-bed medical ICU (MICU) at the University of Rochester between October 2011 and June 2013. Consecutive patients requiring RBC transfusion were enrolled before their first RBC transfusion of the hospital stay. Study patients were identified when a request for RBC transfusion was received in the blood bank from the MICU. The blood bank technologist then determined whether the patient had received prior blood transfusions during their hospital stay. If not, the patient was enrolled. A waiver of informed consent was provided by the University of Rochester Research Subjects Review Board.

A sample of each RBC unit transfused during the first transfusion episode (defined below) was removed from the storage bag before transfusion. These samples of ABO group-identical, leukoreduced RBCs were collected with sterile technique by the staff of the transfusion medicine service just before release of the unit from the blood bank for transport to the recipient. A 30-mL BD syringe transfer set (Charter Medical) was connected to the

RBC unit using a sterile tubing welder (TSCD SC-201A, Terumo). After 10 mL of blood was withdrawn from the unit, the tubing was sealed with a heat sealer (Sebra Omni 2600, Vante BioPharm). Syringe contents were dispensed into 15-mL conical vials and centrifuged at 4°C and $3000 \times g$ for 15 minutes. Supernatant aliquots were then stored at -80°C until analysis.

Patient blood samples obtained before and after the first RBC transfusion episode were retrieved from the clinical laboratory. These samples were obtained for routine clinical care in EDTA-containing vacutainer tubes and were stored in the clinical laboratory. The blood sample closest to initiation of the first transfusion episode was used for pretransfusion analysis. Likewise, the first blood sample after completion of the first transfusion episode was used for posttransfusion analysis. These samples were centrifuged at room temperature at $3645 \times g$ for 10 minutes. Supernatant aliquots were frozen at -80°C until analysis.

The first transfusion episode was defined as the total number of RBC units transfused consecutively without interruption on a single day and could include 1 or more RBC units as determined by the clinical team. Although patients may have received additional transfusion episodes later in their ICU or hospital stay, our analysis was restricted to the first transfusion episode. Patients were excluded if they had already received RBC transfusion during their hospital stay before MICU admission or if pre- or posttransfusion blood samples were not available within 24 hours of the RBC transfusion.

Cell-free heme levels in RBC unit supernatants and patient plasma samples were measured using a heme assay kit (QuantiChrom, BioAssay Systems). This assay measures the intensity of color at 400-nm wavelength and quantifies protein-bound and free heme and Hb.^{18,19} RBC-derived microparticles contain both cell-free heme and Hb^{18,20} and probably contribute to the total signal detected by these assays.^{2,8} Therefore, although the relative proportions are unknown, this assay should provide an index of the combined concentrations of free and microparticle-contained Hb and heme (referred to as total heme). Each patient's transfused RBC unit total cell-free heme was calculated as the sum of concentrations measured in each RBC unit transfused during the first transfusion episode. Haptoglobin concentrations were measured in the pre- and posttransfusion plasma samples using magnetic bead assays (Luminex, EMD Millipore Corporation) per manufacturer instructions.

Statistical analysis

Results are expressed as mean $\pm$ standard deviation (SD) or median (interquartile range [IQR]), as appropriate. The primary exposure variables were posttransfusion total plasma heme concentration and transfused RBC unit total cell-free heme concentration from the first transfusion episode. The primary outcome variable was hospital mortality.

The t test, the Wilcoxon rank-sum test, the Kruskal-Wallis test, and the Wilcoxon matched-pairs signed-ranks test were used to compare continuous or discrete variables between groups, and between serial measurements from the same subjects, as appropriate. The chi-square test or Fisher's exact test was used to compare categorical variables, as appropriate. Spearman's rank correlation coefficients between continuous variables were calculated.

Logistic regression analysis was used to assess the associations between total plasma heme and hospital mortality while controlling for potential confounding variables. We considered age and APACHE II score the most clinically important potential confounders. Medical records were reviewed for abstraction of the necessary variables for calculation of APACHE II scores based on the first 24 hours of ICU admission. We also evaluated serum bilirubin as a potential confounder since it may interfere with precision of the heme assay (QuantiChrom, BioAssay Systems).¹⁸

Statistical analyses were performed with computer software (Stata statistical software, Release 14, StataCorp LP). Results were considered statistically significant if the p value was not more than 0.05 unless otherwise specified.

Our sample size of 110 transfused critically ill patients with 30% estimated hospital mortality provides 90% power to detect a difference in posttransfusion total plasma heme concentrations between survivors and nonsurvivors of 25% with alpha error of 0.05, presuming a baseline total plasma heme concentration of 8 $\mu\text{mol/L}$ with SD of 3 $\mu\text{mol/L}$.¹⁹

RESULTS

We enrolled 120 consecutively transfused MICU patients. Nine subjects were excluded (Fig. 1) leaving 111 patients for analysis (Table 1). Seventy-six patients (68%) received 1 RBC unit, 33 patients (30%) received 2 RBC units, and two patients (2%) received 4 RBC units during the first transfusion episode. The median age of the RBC units transfused was 24 days (IQR, 18–30 days; range, 5–42 days). Pretransfusion plasma samples were collected a median of 1.6 (IQR, 0.9–3.0; range, 0.51–15.2) hours before RBC issue from the blood bank, and posttransfusion plasma samples were collected a median of 4.8 (IQR, 3.5–8.7; range, 0.55–21.8) hours after RBCs were issued.

Total plasma heme concentration increased significantly after RBC transfusion (pretransfusion 35 [26–76] $\mu\text{mol/L}$ vs. posttransfusion 47 [35–73] $\mu\text{mol/L}$; $p < 0.001$; Fig. 2). There was a dose response between the number of units transfused and the rise in total plasma heme after transfusion (Fig. S1, available as supporting information in the online version of this paper).

The age and total cell-free heme concentration of RBC units were directly correlated (correlation coefficient, 0.57; $p < 0.001$). There was a weak correlation between transfused RBC unit total cell-free heme and posttransfusion total plasma heme (Spearman's correlation coefficient (ρ) = 0.21, $p = 0.03$). We examined this relationship by quartiles of the time interval between transfusion and acquisition of the posttransfusion sample. The correlation coefficient was strongest for patients in the first quartile (0–3.5 hr, $\rho = 0.54$, $p = 0.004$). It weakened with longer time delays from transfusion to posttransfusion sampling (Quartile 2 [3.6–5 hr] $\rho = 0.38$, $p = 0.04$; Quartile 3 [5.1–9 hr] $\rho = 0.17$, $p = 0.41$; Quartile 4 [>9 hr] $\rho = -0.32$, $p = 0.12$). Despite this weakening relationship between posttransfusion total plasma heme and the amount directly transfused from RBC unit(s) over time, the posttransfusion total plasma heme concentration was similar when comparing patients with longer intervals between transfusion and blood sampling to those with shorter intervals (Fig.

3). Posttransfusion total plasma heme was not correlated with RBC unit storage duration ($\rho = 0.03$, $p = 0.78$).

Posttransfusion total plasma heme concentrations were significantly higher in nonsurvivors compared to survivors (Table 2, Fig. 4). The relationship between posttransfusion total plasma heme and mortality remained significant after adjustment for age and APACHE II score using multivariable logistic regression analysis (odds ratio [OR] for hospital mortality [95% confidence interval] per quartile increase in posttransfusion total plasma heme, 1.76 [1.17–2.66]; $p = 0.007$; Table 3). Pretransfusion total plasma heme, RBC unit storage age, RBC unit total cell-free heme, and plasma haptoglobin concentrations were similar in survivors and nonsurvivors (Table 2). Patients with lower pretransfusion plasma haptoglobin levels accumulated higher posttransfusion total plasma heme (Fig. 5 and Table S1).

Serum bilirubin concentrations were highly correlated with total plasma heme concentrations but did not significantly differ between survivors and nonsurvivors (Fig. S2). Posttransfusion total plasma heme remained a significant predictor of mortality after adjusting for serum bilirubin (OR for hospital mortality per quartile increase in posttransfusion total plasma heme, 1.79 [1.07–3.00]; $p = 0.027$; Table S2).

The presence of sepsis did not influence levels of total plasma heme, either pre- or posttransfusion (Table S3). In contrast, we found that total plasma heme concentrations were higher in patients with more severe organ failure (sequential [sepsis-related] organ failure assessment [SOFA] score²¹) at the time of RBC transfusion (Table S4). Both pre- and posttransfusion total plasma heme levels were significantly correlated with SOFA score (Spearman correlation coefficients, 0.37 and 0.47, respectively; $p < 0.001$ for both). Nevertheless, the RBC transfusion-associated increase in total plasma heme was observed in patients with lower SOFA scores and in those with higher SOFA scores (Table S5).

DISCUSSION

The main findings of this study of critically ill medical patients were that total plasma heme concentration increased after transfusion and higher posttransfusion total plasma heme independently predicted hospital mortality. These findings support the concept that RBC transfusion is linked with toxic accumulation of plasma heme in vulnerable patient populations.

Implications of study findings

We observed an independent association between posttransfusion total plasma heme and mortality. The toxicity of cell-free heme is supported by in vitro studies,⁶ animal models,¹³ and human subjects.^{6,22–24} The adverse effects include nitric oxide scavenging and vasoconstriction,^{4,22} increased vascular permeability,¹³ oxidative stress,⁶ inflammation, and immune dysregulation with increased risk of infection.¹⁶ Our study does not provide insight on the specific mechanisms involved, but provides practical evidence of a concerning association between circulating cell-free heme and vital outcomes in critically ill patients.

We found that posttransfusion total plasma heme levels were similar in those patients with longer time intervals between transfusion and plasma sampling, even though the correlation between RBC unit total cell-free heme and posttransfusion total plasma heme declined as the time interval lengthened. There are at least three interrelated explanations for transfusion-related total plasma heme accumulation, apart from the quantity directly transfused: 1) posttransfusion hemolysis of storage-damaged RBCs,^{13,25,26} 2) transfusion-associated damage and disintegration of host RBCs,¹³ and 3) impairments in the critically ill host's capacity to clear heme.^{14,17}

In support of posttransfusion hemolysis of storage-damaged RBCs, a fraction of transfused RBCs undergo rapid extravascular hemolysis within 24 hours after transfusion, and this population of cleared RBCs is higher in long-storage versus short-storage units.^{25–27} Extravascular hemolysis of transfused and damaged RBCs may also produce an increase in plasma non-transferrin-bound iron (NTBI),²⁷ promoting inflammation and oxidative stress.^{6,25,28,29} This could damage both transfused and host RBCs, further contributing to total plasma heme accumulation. Extracellular heme directly injures RBCs and produces hemolysis,³⁰ perpetuating damage to both transfused and host RBCs. Finally, the excess supply of plasma cell-free Hb and heme consumes haptoglobin and hemopexin, respectively, providing an additional positive feedback loop that may maintain excessive heme concentrations for sustained periods after a single transfusion episode. This latter mechanism is supported by our finding that patients with the lowest pretransfusion haptoglobin levels had the highest posttransfusion total plasma heme.

These mechanisms for heme accumulation are accentuated in septic models.^{13,14,25} Our results suggest that these concerted relationships track more precisely with the severity of critical illness than the presence of sepsis per se. Supporting this conclusion, we found that haptoglobin levels were higher in septic compared to nonseptic individuals, perhaps because it is an acute-phase reactant. In contrast, haptoglobin levels were lower in higher SOFA score compared to lower SOFA score individuals (Table S4). These findings suggest that severe organ failures (stemming from infectious or noninfectious insults) impair host defense against the total plasma heme accumulation that is stimulated by RBC transfusion and other factors.

Our results also suggest that clinically relevant storage duration may not be the major determinant of transfusion toxicity, since RBC unit age did not predict posttransfusion total plasma heme or hospital mortality, consistent with previous studies.^{13,31–34} The mean storage time of RBC units transfused in the United States is 18 days,³⁵ and in our study the median storage time was 24 days with a minimum of 5 days. Pathological changes in RBCs begin in the first week of storage,^{3–5,36} so even our freshest units may have contained storage-damaged RBCs. Our findings support development of mitigation strategies to prevent transfusion-associated accumulation of total plasma heme, including therapeutic use of haptoglobin³⁷ or hemopexin,¹³ improved RBC storage conditions, or pretransfusion RBC washing.³⁸

Comparison to previous studies

The effects of RBC transfusion on cell-free plasma Hb have been studied in healthy volunteers. Berra and colleagues^{39,40} found that cell-free plasma Hb levels increase after autologous blood transfusion in healthy volunteers and patients with endothelial dysfunction, more so in long-storage RBCs. Similar findings were observed by Risbano and colleagues¹¹ when measuring forearm venous cell-free Hb after brachial artery autologous RBC transfusion.

There are few studies measuring the effects of transfusion on plasma Hb or heme in clinically relevant adult patients. Wagener and coworkers¹³ found that transfused critically ill trauma patients have elevated levels of cell-free heme and Hb after completion of resuscitation, but did not report pretransfusion levels. Vermeulen Windsant and coworkers³¹ studied medically stable hematology/oncology patients requiring transfusion and found that cell-free plasma Hb concentration increased significantly after 2-unit RBC transfusions, but did not change after single unit transfusions. Neuman and colleagues⁴¹ found that plasma Hb levels increased slightly 1 hour after transfusion of more than 21-day-old RBC units in non-ICU patients, 80% of whom received 2 RBC units, but these levels declined to less than the baseline value by 24 hours. Nishiyama and Hanaoka³² serially measured plasma Hb during massive transfusion and did not detect an increase in cell-free Hb until at least 2 L of blood was administered. In contrast to these reports, we found a convincing and significant increase in total plasma heme, with most of our patients receiving only a single RBC unit.

Differences in the measurement techniques may play a role in these discrepant findings. We measured total plasma heme, likely including contributions from both free and microparticle-contained Hb and heme, whereas these prior studies used assays to specifically measure cell-free plasma Hb or heme. The discrepancies may also relate to differences in patient population. To our knowledge, ours is the first study examining RBC transfusion-associated changes in cell-free heme in an unselected consecutive sample of critically ill patients. Half of our transfused patients were septic, a condition previously shown to be associated with endogenously high cell-free Hb and low haptoglobin and hemopexin levels.^{14,17,42,43} Taken together, these observations suggest that our patient population was endogenously primed to accumulate total plasma heme after only modest RBC transfusion.

Strengths and limitations

The strengths of our study include the consecutive and unselected sample of medical ICU patients, plasma sampling before and after transfusion, sampling from the RBC storage bag rather than the bag segment appendage,^{44,45} and a patient sample size adequate for multivariable analysis. Our study has several limitations. Most importantly, our study is observational, so the observed association between posttransfusion total plasma heme and hospital mortality does not imply causality, despite our adjustment for age, APACHE II score, and bilirubin. Unmeasured confounders could explain this association.

Resource limitations prevented plasma hemopexin and NTBI measurements. This is a substantial weakness, since additional insights about the risk factors for total plasma heme accumulation could have been gained by analysis of relationships between hemopexin,

NTBI, total plasma heme, and haptoglobin. These analyses should be performed in future studies.

Our spectrophotometric heme assay is not specific for cell-free heme alone. This assay also detects cell-free Hb, other heme species, and bilirubin.¹⁸ Two statistical analyses provide evidence that the association between total plasma heme and mortality was not confounded by the serum bilirubin concentration. First, bilirubin levels were similar in survivors and nonsurvivors. Second, our multivariable logistic regression model indicates that posttransfusion total plasma heme remains independently predictive of mortality after controlling for serum bilirubin. It is possible that the metabolic breakdown of heme to bilirubin contributes to the strong correlation between total plasma heme and serum bilirubin.^{10,39} This could be evaluated in future studies using assays specific for cell-free heme, Hb, and bilirubin.

The delay in posttransfusion total plasma heme measurements is another study limitation. Our posttransfusion plasma samples were obtained a median of 5 hours and maximum of 22 hours after RBC units were issued from the blood bank for transfusion. It is possible that the correlation between RBC unit cell-free heme and posttransfusion total plasma heme would have been stronger if plasma samples were obtained more promptly after transfusion. We were also unable to control for the possibility of patient sample hemolysis in vitro during storage in the clinical laboratory. However, it is unlikely that this preferentially affected pre-versus posttransfusion samples or samples from survivors versus nonsurvivors.

Another limitation is that we restricted our analysis to the first transfusion episode. Many of our patients received additional RBC transfusions later during their hospital stay (Table 1) and the effects of these later transfusions on total plasma heme were not analyzed. However, the importance of posttransfusion total plasma heme is emphasized by its strong association with hospital mortality after just the first transfusion episode, despite the complexity of later hospital care, often including additional RBC transfusion. The relatively small sample size and our single-center study design are additional study limitations.

In conclusion, total plasma heme increases after RBC transfusion in critically ill patients, and the posttransfusion concentration is independently associated with hospital mortality. Posttransfusion total plasma heme is directly correlated with total cell-free heme in the RBC units, but other transfusion-related factors probably contribute to sustained elevations over time. Additional work is required to understand mechanisms of transfusion-related total plasma heme accumulation and develop prevention and mitigation strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS:

ICU	intensive care unit
IQR	interquartile range
MICU	medical intensive care unit
NTBI	non-transferrin- bound iron
SOFA	sequential (sepsis-related) organ failure assessment

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Fig. 1.
Number of patients enrolled and analyzed and reasons for exclusion.

Fig. 2. Total plasma heme concentration before versus after transfusion. Box plots show the median (horizontal line) and 25th and 75th percentiles (lower and upper limits of the box). The dots represent outliers beyond the whiskers that designate the 10th and 90th percentiles. Comparison was made with Wilcoxon matched-pairs signed-ranks test.

Fig. 3.

Relationship between posttransfusion total plasma heme concentration and the time between transfusion and acquisition of posttransfusion plasma sample for heme measurement. Quartiles of the time interval between transfusion and plasma sampling are shown on the horizontal axis. Box plots show the median (horizontal line) and 25th and 75th percentiles (lower and upper limits of the box). The dots represent outliers beyond the whiskers that designate the 10th and 90th percentiles. Comparison was made with the Kruskal-Wallis test.

Fig. 4. Total plasma heme concentration in survivors versus nonsurvivors. Box plots show the median (horizontal line) and 25th and 75th percentiles (lower and upper limits of the box). The dots represent outliers beyond the whiskers that designate the 10th and 90th percentiles. Comparison was made with the Wilcoxon rank-sum test.

Fig. 5.

Relationship between posttransfusion total plasma heme concentration and the pretransfusion plasma haptoglobin concentration. Box plots show the median (horizontal line) and 25th and 75th percentiles (lower and upper limits of the box). The dots represent outliers beyond the whiskers that designate the 10th and 90th percentiles. Comparison was made with Kruskal-Wallis test.

Table 1.

Characteristics of study population *

Variable	Study population			p value
	Overall (n = 111)	Survivors (n = 67)	Nonsurvivors (n = 44)	
Age (years)	59 (49–74)	57 (44–70)	62 (55–76)	0.07
Female sex	51 (46)	33 (49)	18 (41)	0.39
Race				
Caucasian	79 (71)	46 (69)	33 (75)	0.18
African American	27 (24)	16 (24)	11 (25)	
Other	5 (4)	5 (7)	0	
Hispanic or Latino ethnicity	1 (2)	5 (7)	0	0.06
Primary ICU admission diagnosis category				
Sepsis and/or pneumonia	56 (50)	30 (45)	26 (59)	0.29
Respiratory failure	8 (7)	6 (9)	2 (4)	
Congestive heart failure	7 (6)	4 (6)	3 (7)	
GI/hepatobiliary process	17 (15)	11 (16)	6 (14)	
Acute hematologic process	7 (6)	3 (4)	4 (9)	
Acute neurological process	6 (5)	6 (9)	0	
Cardiac arrest	6 (5)	3 (4)	3 (7)	
Acute renal failure	3 (3)	3 (4)	0	
Overdose or withdrawal syndrome	1 (1)	1 (1)	0	
Sepsis-3 criteria met at time of RBC transfusion [†]	79	43 (64)	36 (82)	0.04
APACHE II score	25 (20–31)	24 (18–28)	28 (22–32)	0.002
ICU length of stay (days)	9 (4–16)	9 (4–18)	8 (4–15)	0.58
Hospital length of stay (days)	17 (– 38)	21 (11–42)	12 (6–24)	0.007
Reason for transfusion				
Hb < 7 g/dL or Hct 21%	64 (58)	36 (54)	28 (64)	0.64
Active bleeding with clinical instability	12 (11)	7 (10%)	5 (11)	
Hb < 8.6 g/dL or Hct < 26% with clinical instability	19 (17)	14 (21)	5 (11)	
Hb < 8.6 g/dL or Hct < 26% with comorbidity	10 (9)	7 (10)	3 (7)	
Other	6 (5)	3 (4)	3 (7)	

Variable	Study population			p value
	Overall (n = 111)	Survivors (n = 67)	Nonsurvivors (n = 44)	
Pretransfusion Hb (g/dL)	6.9 (6.4–7.5)	6.8 (6.3–7.6)	7.0 (6.6–7.4)	0.73
Pretransfusion Hct (%)	21 (20–24)	21 (20–23)	21 (20–24)	0.96
RBC units during first transfusion episode				
1	76 (68)	47 (70)	29 (66)	0.88
2	33 (30)	19 (28)	14 (32)	
4	2 (2)	1 (2)	1 (2)	
Total RBC units transfused during entire hospital stay	3 (2–6)	3 (2–6)	3 (2–6)	0.90
Additional RBC transfusions during hospital stay [‡]	76 (68)	47 (70)	29 (66)	0.64

* Data are reported as median (IQR) or number (%).

[‡] Determined by chart review of presence of infection combined with SOFA score of ≥ 2 .

[‡] Number (%) of patients who received additional RBC units after the first transfusion episode some time later in their hospital stay.

Table 2.

Comparison of transfused survivors versus nonsurvivors^{*}

Variable	Survivors (n = 67)	Nonsurvivors (n = 44)	p value
Number of RBC units transfused in first transfusion episode	1 (1 to 2)	1 (1 to 2)	0.627
Total RBC cell-free heme (μmol/L)	98 (71 to 178)	97 (69 to 169)	0.575
Mean RBC unit age (days)	24 (19 to 31)	23 (16 to 32)	0.471
Max RBC unit age (days)	26 (20 to 32)	24 (18 to 32)	0.413
Prettransfusion total plasma heme (μmol/L)	35 (24 to 61)	34 (27 to 113)	0.221
Posttransfusion total plasma heme (μmol/L)	44 (31 to 65)	54 (35 to 136)	0.031
Transfusion-related change in total plasma heme (μmol/L)	6 (−1 to 19)	12 (2 to 30)	0.061
Prettransfusion plasma haptoglobin (μg/mL)	2,046 (894 to 3,444)	1,562 (554 to 3,459)	0.706
Posttransfusion plasma haptoglobin (μg/mL)	2,156 (641 to 4,903)	1,252 (368 to 3,462)	0.169
Serum bilirubin (mg/dL) [‡]	0.5 (0.3 to 1.1)	0.6 (0.25 to 2.7)	0.372

^{*} Data are reported as median (IQR).

[‡] This is the serum bilirubin measurement closest to the transfusion episode. Serum bilirubin was not measured during the ICU stay in three survivors.

Table 3.

Multivariable logistic regression analysis *

Dependent variable	Independent variables	OR (95% CI)	p value
Hospital mortality	Cell-free total plasma heme quartiles	1.76 (1.17–2.66)	0.007
	Age quartiles	1.83 (1.20–2.80)	0.005
	APACHE II score quartiles	1.86 (1.23–2.84)	0.004

* The multivariable model had good discrimination (C statistic = 0.75) but poor calibration (H-L $\chi^2 = 16.20$; $p = 0.040$).