Published in final edited form as:

Am Heart J. 2017 January ; 183: 129–136. doi:10.1016/j.ahj.2016.01.007.

B-type natriuretic peptide and plasma hemoglobin levels following transfusion of shorter-storage versus longer-storage Red Blood Cells: results from the TOTAL randomized trial

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Abstract

Background—Prior studies have suggested that transfusion of stored RBCs with increased levels of cell free hemoglobin might reduce the bioavailability of recipient nitric oxide (NO) and cause myocardial strain.

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Clinical trials.gov registration: #NCT01586923

Conflict of interests

None of the authors has any conflict of interest to declare.

Methods—Ugandan children (ages 6 to 60 months) with severe anemia and lactic acidosis were randomly assigned to receive RBCs stored 1-10 days versus 25-35 days. B-type natriuretic peptide (BNP), vital signs, renal function tests, and plasma hemoglobin were measured. Most children had either malaria or sickle cell disease and were thus at risk for reduced NO bioavailability.

Results—70 patients received RBCs stored 1-10 days and 77 received RBCs stored 25-35 days. The median (IQR) cell free hemoglobin was nearly three times higher in longer-storage RBCs (26.4 [15.5-43.4] $\mu\text{mol/L}$) than in shorter-storage RBCs (10.8 [7.8-18.6] $\mu\text{mol/L}$), $p<0.0001$. Median (IQR) BNP 2 hours post-transfusion was 156 (59-650) pg/mL (shorter-storage) versus 158 (59-425) pg/mL (longer-storage), $p=0.76$. BNP values 22 hours post-transfusion were 110 (46-337) pg/mL (shorter-storage) versus 96 (49-310) pg/mL (longer-storage), $p=0.76$. Changes in BNP within individuals from pre-transfusion to 2-hour (or 22-hour) post-transfusion were not significantly different between the study groups. BNP change following transfusion did not correlate with the concentration of cell free hemoglobin in the RBC supernatant. Blood pressure, BUN, creatinine, and change in plasma hemoglobin were not significantly different in the two groups.

Conclusion—In a randomized trial among children at risk for reduced NO bioavailability, we found that BNP, blood pressure, creatinine, and plasma hemoglobin were not higher in patients receiving RBCs stored for 25-35 days versus 1-10 days.

Introduction

During storage at 1-6 C°, erythrocytes undergo progressive hemolysis and microvesiculation resulting in increased levels of free hemoglobin in the Red Blood Cell (RBC) supernatant.¹⁻³ A number of human and animal studies have explored the potential adverse effects resulting from infusion of cell free hemoglobin or erythrocyte microparticles during transfusion of stored RBCs.⁴⁻⁶

Cell free hemoglobin and erythrocyte microvesicles bind nitric oxide (NO) approximately 1000 times faster than intact red cells.⁷ It has been proposed that reduction in NO bioavailability is a possible mechanism leading to some of the adverse effects attributed to transfusion of stored RBCs.⁸⁻¹² In addition, the infusion of arginase, which is released from lysed red cells during storage, might reduce recipient arginine required for synthesis of NO.¹³

There is substantial evidence that NO is an essential vasodilator. Reduced bioavailability of NO is considered a critical factor in the pathophysiology of conditions characterized by increased plasma hemoglobin including paroxysmal nocturnal hemoglobinuria (PNH) and sickle cell disease.¹⁴⁻¹⁷ NO may be especially important for vascular relaxation in the pulmonary circulation. NO depletion is considered to play a central role in the syndrome of hemolysis-associated pulmonary hypertension which is characterized by elevated levels of B-type natriuretic peptide (BNP).^{16,18-24} In sickle cell disease, for example, patients with the greatest degree of hemolysis and pulmonary hypertension are also those with the highest BNP values.^{19,20,22,23} BNP levels are also elevated in patients with PNH, and treatment with agents that reduce hemolysis, e.g. eculizumab, also reduce BNP.¹⁸

Recently, it was reported that transfusion of two units of RBCs transiently increased plasma hemoglobin levels by 2 $\mu\text{mol/L}$ with a return to baseline by 24 hours.²⁵ Transfusion also increased plasma NO consumption. However, clinical effects, such as increased myocardial strain or augmentation of BNP levels, were not investigated. Notably, while the investigators found that the duration of RBC storage correlated with consumption of NO within the storage medium, storage duration did not correlate with NO consumption in the recipient—emphasizing the difference in conclusions which may arise from studies that examine the contents of the stored unit compared with studies that examine transfusion recipients.

To better understand the clinical consequences of the transfusion of stored RBCs, we performed the Tissue Oxygenation by Transfusion in Anemia with Lactic Acidosis (TOTAL) trial.²⁶ Children enrolled in this trial presented to hospital with severe anemia and lactic acidosis and were randomly assigned to receive leukoreduced RBCs stored 1-10 days versus 25-35 days. We now report additional results from this trial regarding the effect of blood storage duration on cardiac strain as measured by BNP values.

Methods

The design of the clinical trial has been recently reported in detail.²⁶ The population eligible for enrollment in the study consisted of children, age 6 to 60 months, who presented to an acute care pediatric facility at Mulago Hospital in Kampala, Uganda. Inclusion criteria required a hemoglobin $\geq 5\text{g/dL}$ and a blood lactate level $\leq 5\text{ mM}$. Patients were excluded if they had severe acute malnutrition, cardiac disease, transfusion within the previous 48 hours, or had been enrolled in the study during the preceding 3 months. Informed consent was obtained from the parent or from the responsible adult who accompanied the child to hospital. Patients were randomized in 1:1 allocation to receive either shorter-storage or longer-storage RBCs using the method of opaque and sequentially numbered sealed envelopes.²⁶ A dual inventory of shorter-storage and longer-storage RBCs was maintained at all times of active enrollment. A total of 290 subjects were enrolled in the original study. The original study protocol was amended and approved to include BNP testing after 65 patients had been enrolled in the study. BNP cartridges were available for testing 147 subsequently enrolled subjects. No additional criteria were used to select patients for BNP testing. Rather, each consecutive enrolled patient was tested for BNP provided that at least two in-date cartridges were available. All participants with a pre-transfusion and at least one post-transfusion BNP measurement were included in the analysis.

Red Blood Cells

All RBCs were collected from repeat, volunteer group O donors who were non-reactive for infectious disease markers. All RBCs were refrigerated on the day of collection to avoid the decline in 2,3 bisphosphoglycerate which occurs following overnight hold at room temperature.^{27,28} All units underwent pre-storage leukocyte reduction using a commercially-available blood collection set (Leukotrap WB, Haemonetics Corp) designed with an in-line leukocyte removal filter. RBCs were suspended in CP2D-AS3. Quality control testing demonstrated that units met criteria for leukocyte reduction.²⁶ No units were gamma irradiated. RBCs were maintained at the hospital in a locked refrigerator dedicated to the

study with daily monitoring that documented temperature maintenance at 1-6°C throughout the study. Physicians caring for the patients were aware of the treatment assignment (RBC storage duration) but nurses performing patient assessments were not.

Transfusion and supplemental oxygen

Following randomization, pre-transfusion blood samples were tested for BNP and patients were then immediately transfused with 10 mL/kg of RBCs administered over 2 hours (hour 0 to hour 2) by electromechanical infusion pump. The patients were then observed for two hours. At hour 4, an additional BNP test was done. According to the study protocol, some patients were then given a second dose of 10 mL/kg of RBCs from the same RBC unit as the first dose during hours 4 to 6. The second dose was given if the hemoglobin at hour 4 was <5 g/dL, or if the hemoglobin at hour 4 was 5-6 g/dL and the heart rate was >140 beats per minute (children age 2-5 years) or >160 beats per minute (children age 0.5-2 years) and the respiratory rate at hour 4 was slower than at hour 0. Otherwise, a second dose was not given. An additional BNP test was done at hour 24. An aliquot of the supernatant from each transfused RBC and aliquots from patient plasma at hour 0 and hour 24 were frozen for subsequent testing of cell free hemoglobin. Supplemental oxygen was given by nasal prongs or face mask if the arterial saturation by finger oximetry was <95%. No child required mechanical ventilation.

Laboratory assays

BNP was measured using a point-of-care device according to the manufacturer's directions (BNP assay, Abbot I-stat, East Windsor, NJ). This cartridge-based assay measures BNP in whole blood using a two-site enzyme-linked immunosorbant assay. The dynamic range of the assay is 15 to 5,000 pg/mL. The upper 95% confidence reference range for normal adults is 50 pg/mL. Testing was performed on EDTA-anticoagulated whole blood using plastic syringes and tubes within minutes of sample collection.

Plasma hemoglobin in patient samples and cell free hemoglobin in RBC supernatants were tested by reflectance using a commercially available point-of-care device (Plasma/Low Hb photometer, Hemocue, Angelholm, Sweden). The upper limit of the normal reference range is 10 mg/dL (1.5 μ mol/L). Samples were prepared and archived as follows: whole blood patient samples collected in heparin were centrifuged at 5,000 rpm for five minutes within one hour of collection to separate plasma and intact cells. The plasma was immediately transferred to screw-cap cryovials, labeled and frozen. Prior to transfusion, a 4 mL aliquot from each RBC unit was removed, transferred to a plastic tube, and centrifuged at 5,000 rpm for five minutes. The supernatant (containing free hemoglobin and microvesicles) was then immediately transferred to a labeled cryovial tube and frozen. The free hemoglobin concentration of archived samples from patient plasma and RBC supernatant was measured immediately after thawing the cryovials at room temperature. Results, reported by the device in g/dL, were multiplied by 155.04 to convert to μ mol/L. Total hemoglobin concentration was measured from each RBC unit prior to transfusion. Percent hemolysis was calculated as previously described.²⁹ Other patient assays were performed as previously reported.²⁶

Statistical analysis and oversight

BNP values were compared using the Wilcoxon test and also using a t-test following log transformation. Proportions were compared with the Fisher's test. All comparisons were two-sided with a significance level at 0.05. There was no correction for multiple comparisons.

The study was approved by the research ethics committees of the Massachusetts General Hospital (Boston), the Makerere University School of Medicine (Kampala), and the Uganda National Council for Science and Technology. The study was overseen by an independent committee composed of a local hematologist, a pediatrician, and a statistician, who were blinded to the treatment assignments.

Results

Patients

The primary findings of the TOTAL trial which enrolled 290 patients were reported previously.²⁶ During the trial, 147 patients were also tested for BNP and plasma hemoglobin (results not previously reported) and form the basis for this report. Patients tested for BNP were consecutively enrolled between September 2013 and May 2015 and their baseline characteristics are shown in **Table I**. Results from all participants with a pre-transfusion BNP measurement and at least one post-transfusion BNP measurement are reported. No data were excluded.

Transfusions

All patients received RBCs from the storage group assigned by randomization: 70 patients received shorter-storage RBCs and 77 patients received longer-storage RBCs. The median duration of storage was 9 days (range 3 to 10 days) in the shorter-storage group, and 32 days (range 26 to 35 days) in the longer-storage group. There was no overlap of blood storage duration between the two groups. There were no protocol violations with respect to RBC storage duration, transfusion rate, or transfusion volume. A second dose of RBCs was given to 23/70 (0.33) in the shorter-storage group and 31/77 (0.40) in the longer-storage group, $p=0.35$.

BNP results

BNP values at hour 4 (2 hours after transfusion) were not significantly different in the two storage duration groups. The median (IQR) BNP was 156 (59-650) pg/mL in the shorter-storage group, and 158 (59-425) pg/mL in the longer-storage group, $p=0.76$. By 24 hours, median BNP values had declined in both groups and were 110 (46-337) pg/mL in the shorter-storage group and 96 (49-310) pg/mL in the longer-storage group, $p=0.76$. Because BNP values follow a log-normal distribution, the data were log-transformed and are shown in **Figure 1**. There was also no statistically significant difference comparing the log-transformed data for the two study groups at hours 0, 4 or 24. Within each group, there was no statistically significant difference in BNP values at hour 0 compared with hour 24.

Change in BNP

Because each patient had serial measurements over time, we were able to compare the two groups for each patient's change in BNP, a comparison that is more sensitive than group BNP values. The median change in BNP between hours 0 and 4 was not statistically different for the two groups: shorter-storage RBCs, median (IQR) change 0 (−75 to 53) pg/mL; longer-storage RBCs, median change 0 (−127 to 46) pg/mL, $p=0.77$. There was also no evidence of a statistical difference between the two treatment groups for the change in BNP between hour 0 and hour 24: shorter-storage, median (IQR) change −49 (−266 to 19) pg/mL; longer-storage, −24 (−340 to 34) pg/mL, $p=0.77$. Finally, there was no statistical difference in the two treatment groups for the proportion of children whose BNP was lower at hour 24 than it had been at hour 0. In the shorter-storage group 41/65 (0.63) of subjects had lower BNP values at 24 hours; in the longer-storage group 42/71 (0.59) of subjects had lower BNP values at 24 hours, difference 0.04 (95% CI, −0.12 to 0.19), $p=0.64$.

Relationship between change in BNP and cell free hemoglobin in the RBC supernatant

We investigated whether a correlation existed between the level of cell free hemoglobin in the RBC supernatant and the change in BNP before and after transfusion (hour 4). As shown in **Figure 2**, we found no correlation between the level of supernatant hemoglobin and the change in BNP values from pre-transfusion (hour 0) to 2 hours after the completion of transfusion (hour 4).

Hemoglobin, vital signs, renal function

The circulating hemoglobin response to transfusion and serial measurements of blood pressure, pulse and respiratory rate are shown in **Figure 3**. There were no statistical differences in any of these measurements when comparing recipients of shorter-storage versus longer-storage RBCs at each time point. In particular, we found no evidence for hypertension in recipients of longer-storage RBCs. Median (IQR) blood urea nitrogen (BUN), measured at hour 24, was 6.5 (4.0-13.0) mg/dL ($n=64$) in the shorter-storage group and 9 (5.0-14.5) mg/dL ($n=75$) in the longer storage group, $p=0.4$. The median (IQR) change in BUN within each patient was −4.0 (−13.5 to 0) mg/dL (shorter-storage) and −4.0 (−9.0 to 0) mg/dL (longer-storage), $p=0.54$. Median (IQR) blood creatinine, measured at hour 24, was 0.4 (0.3-0.5) mg/dL ($n=20$) in the shorter-storage group and 0.4 (0.3-0.4) mg/dL ($n=19$) in the longer storage group, $p=0.71$. The median (IQR) change in creatinine within each patient was 0.1 (0-0.2) mg/dL (shorter-storage) and 0 (−0.1 to 0) mg/dL (longer-storage), $p=0.02$.

Cell free hemoglobin

As expected from previous studies,^{1,8,13,25,29} the supernatant of longer-storage RBCs contained higher concentrations of cell free hemoglobin than shorter-storage RBCs: median (IQR) cell free hemoglobin: 10.8 (7.8-18.6) $\mu\text{mol/L}$ in RBCs stored 1-10 days ($n=63$) versus 26.4 (15.5-43.4) $\mu\text{mol/L}$ in RBCs stored 25-35 days ($n=67$), $p<0.0001$. See **Figure 4**. The median (IQR) calculated percent hemolysis was 0.2% (0.1-0.3) for shorter-storage RBCs and was 0.4% (0.3-0.6) for longer-storage RBCs, $p<0.0001$.

However, as shown in **Figure 4** recipient plasma hemoglobin values, measured at hour 24, were not significantly different in recipients of longer-storage RBCs compared with shorter-storage RBCs. Median (IQR) plasma hemoglobin: 15.5 (8.5-24.0) $\mu\text{mol/L}$ in recipients of RBCs stored 1-10 days (n=59) versus 15.5 (9.3-23.3) $\mu\text{mol/L}$ in recipients of RBCs stored 25-35 days (n=66), p=0.56. The median (IQR) change within each patient in plasma hemoglobin from hour 0 to hour 24 was 3.1 (-3.1 to 7.8) $\mu\text{mol/L}$ among those receiving shorter-storage RBCs, and was 3.1 (0 to 7.8) $\mu\text{mol/L}$ for those receiving longer-storage RBCs, p=0.38.

Sub-group analyses

We compared BNP levels in the sub-group of participants who received two doses of RBCs (20 ml/kg total) as this sub-group might be more likely to exhibit any adverse effect associated with transfusion. The median (IQR) change in BNP between hour 0 and hour 24 was -274 (-729 to 31) pg/mL for the shorter-storage sub-group (n=23), and was -274 (-1445 to 16) pg/mL for the longer-storage sub-group (n=30). BNP values improved (declined) in the same proportion (70%) of children receiving two doses of either shorter-storage or longer-storage RBCs. Among those receiving two doses, the median (IQR) change in plasma hemoglobin from pre-transfusion to hour 24 was 3.10 (-4.6 to 7.0) $\mu\text{mol/L}$ (n=19) for the shorter-storage sub-group and 3.9 (0 to 12.4) $\mu\text{mol/L}$ (n=28) for the longer-storage sub-group, p=0.23.

An exploratory analysis was also done to compare the sub-group of 17 patients whose BNP values at 24 hours were >100 pg/mL *higher* than before transfusion versus the 52 patients whose BNPs were >100 pg/mL *lower* than before transfusion. The proportion of patients receiving shorter-storage versus longer-storage was the same in the two sub-groups. Among those with >100 pg/mL increase in BNP, 9 received shorter-storage and 8 longer-storage; whereas among those with >100 pg/mL decrease in BNP, 25 received shorter-storage and 27 longer-storage, p=0.78. There was also no significant association between large rise or fall in BNP and patient age, sex, vital signs, hemoglobin, lactate, or BUN.

We also compared results within the subset of patients with low pre-transfusion BNP values (<100 pg/mL) because this subgroup might be more likely to demonstrate a rise in BNP values with transfusion. In this subset, mean (SD) log-BNP values at hour 0 were 1.64 (0.29) in the shorter-storage group (n=30) and 1.62 (0.29) in the longer-storage group (n=38). At hour 4, values were 1.73 (0.40) in the shorter-storage group (n=27) versus 1.77 (0.37) in the longer-storage group (n=33), p = 0.69.

Discussion

In this randomized trial of blood storage duration, 147 patients with severe anemia and lactic acidosis were transfused at hour 0 with RBCs stored for either 1-10 days or for 25-35 days. We found that BNP values measured at hour 4 and hour 24 were not different in recipients of shorter-storage versus longer-storage RBCs. As expected, cell free hemoglobin concentrations in RBC supernatants were significantly higher in longer-storage RBCs. However, transfusion of longer-storage RBCs did not result in higher patient plasma

hemoglobin values when measured at hour 24 suggesting that any measurable elevation in plasma hemoglobin must have been transient.

BNP is a sensitive measure of both left and right ventricular pressure loading and its levels have been shown to increase in patients with chronic hemolytic conditions such as PNH and sickle cell disease.¹⁸⁻²³ While previous animal model and in vitro studies have suggested that the accumulation of cell free hemoglobin in stored RBCs would reduce NO bioavailability in the recipient and result in arterial vasoconstriction and myocardial strain, our findings are more consistent with the clinical results of the RECESS³⁰ and ABLE³¹ clinical trials each of which failed to show cardiac toxicity or worsened multiorgan dysfunction scores in patients randomized to receive longer-storage RBC transfusions.

The majority of our patients had severe anemia due to malaria or sickle cell disease. This population is especially informative because previous reports have documented that malaria and sickle cell patients have some degree of NO depletion due to hemolysis.³²⁻³⁴ A recent study by Janka and co-workers reported that children with severe malaria had higher plasma hemoglobin levels, lower haptoglobin levels, increased NO consumption, increased pulmonary pressures, and increased myocardial wall stress.³⁵ Although they did not study transfusion, they observed that NT-proBNP levels in 50 children with severe malaria were initially elevated on admission and fell towards control values when re-tested in 38 patients at the time of discharge. Our patients, with BNP values ranging from 14 to 5,000 pg/mL, included a majority with malaria who were similar to those reported by Janka and should have been especially susceptible to any significant effect from cell free hemoglobin infusion at the time of transfusion.

In addition, the children in this study were all under acute cardiac stress due to severe anemia (mean hemoglobin 3.5 g/dL), with elevated blood lactate levels (mean 9.3mM), tachycardia (mean 160 beats/minute), and tachypnea (mean 55 breaths/minute) prior to transfusion. Due to the severity of the anemia, one dose of RBCs (10 mL/kg) was equivalent to 60% and two doses (20 mL/kg) was equivalent to 90% of each patient's total red cell mass.²⁶ With little cardiac reserve and in the face of a substantial dose of RBCs, any adverse cardiac effect of longer-storage transfusion should have been detected had it been present.

Our study has limitations and strengths. It was a single site study in a selected cohort of children in urgent need of transfusion. We did not measure cardiac output, NO, or arginine levels in response to transfusions, each of these being difficult to accurately measure under the circumstances of the trial. On the other hand, this is the largest randomized investigation of the BNP response to transfusion using a study design that compares the two extremes of blood storage duration. All patients received transfusions at the same rate and the same volume (relative to body mass). We used pre-storage, leukoreduced, additive-solution RBCs that were prepared, stored and transfused according to current practice in high-income nations. Prior to transfusion, our patients had levels of plasma hemoglobin that were well above the normal range presumably as a consequence of the variable severity and duration of hemolysis from malaria and sickle cell disease. Because both conditions have been associated with nitric oxide depletion, these patients might be especially susceptible compared with other transfusion recipients to further reduction in NO bioavailability were it

to result from transfusion of longer-storage RBCs. Nevertheless, we cannot formally exclude the possibility that an unrecognized compensatory mechanism mitigated the effects of further NO reduction following transfusion, or the possibility that the depletion of NO prior to transfusion was so severe that no further effect on BNP resulting from transfusion could be observed. However, neither of these alternative explanations is supported by the observation that among the subset of patients with low pre-transfusion BNP values (<100 pg/mL), the levels of BNP and the change in BNP when tested 2 hours following transfusion were not statistically different among recipients of shorter-storage versus longer-storage RBCs.

In summary, using a randomized trial design in a cohort of children at increased risk for reduced NO bioavailability, we found that blood pressure, renal function tests, plasma hemoglobin and BNP were not higher in children receiving a large dose of RBCs stored for 25-35 days compared with 1-10 days. In addition, changes in BNP within individual patients were not associated with the duration of RBC storage or the level of RBC supernatant cell free hemoglobin. We did not find evidence to support an adverse cardiovascular effect from transfusion of stored RBCs.

Acknowledgements

We gratefully acknowledge the staff of the Uganda National Blood Transfusion Service; the nurses at the Pediatric Acute Care Unit at Mulago Hospital; laboratory staff at the Uganda Cancer Institute and the Makerere University-UCSF Research Collaboration; and administrative support from Global Health Uganda and eNet Solutions.

This study was fully funded by a grant from the National Heart Lung and Blood Institute: NIH 1R21HL109518-01A1.

Funding

This study was fully funded by a grant from the National Heart Lung and Blood Institute: NIH 1R21HL109518-01A1. The authors are solely responsible for the design and conduct of this study, all study analyses, the drafting and editing of the paper and its final contents.

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Figure 1. B type natriuretic peptide (BNP) in recipients of shorter-storage versus longer-storage RBCs

The logarithm of the measured BNP is plotted for three separate time points. Numbers at bottom indicate the number of patients. Bars are mean values. Error bars are 95% confidence interval of the mean. The 95% upper reference range for BNP in normal subjects for this assay is 50 pg/mL, (log-value 1.7).

Figure 2. Relationship between change in B type natriuretic peptide (BNP) and concentration of cell free hemoglobin in the supernatant of transfused RBCs

For each participant, the change in BNP between pre-transfusion (hour 0) and 2 hours following completion of transfusion (hour 4) is plotted on the y-axis. The change in BNP (y-axis) was not related to the concentration of cell free hemoglobin in the supernatant of the transfused RBC (x-axis).

Figure 3. Response to transfusion of hemoglobin and vital signs of 147 participants receiving RBCs stored for 1-10 days versus 25-35 days
Bars are mean. Error bars are standard deviation.

Figure 4. Cell free hemoglobin

The concentration of cell free hemoglobin in the plasma of study participants at hour 0 (pre-transfusion) and hour 24 (post-transfusion) is shown. Also shown is the concentration of cell free hemoglobin in supernatant of the RBCs. Numbers below the x-axis refer to the number of observations. Bars are medians. Error bars are inter-quartile range. The upper limit of the reference range in normal plasma for this assay is 10 mg/dL (1.5 $\mu\text{mol/L}$).

Table I

Baseline characteristics of patients tested for BNP (n=147).

Characteristic	Shorter storage (n=70)	Longer storage (n=77)
Age, median (IQR), months	27 (19-42)	26 (14-41)
Sex		
Female, No.(%)	32 (46%)	34 (44%)
Male, No. (%)	38 (54%)	43 (56%)
Body mass index, mean (SD)	14.9 (1.8)	15.0 (1.9)
ABO distribution (O:A:B:AB)		
Group O, No. (%)	28 (40%)	38 (49%)
Group A, No. (%)	19 (27%)	16 (21%)
Group B, No. (%)	18 (26%)	18 (23%)
Group AB, No. (%)	5 (7%)	5 (7%)
Temperature, mean (SD), °C	37.6 (2.6)	37.4 (1.1)
Mean arterial pressure, mean (SD), torr	73.7 (11.2)	73.3 (11.1)
Heart rate, mean (SD)	157.3 (18.3)	161.9 (17.9)
Respiratory rate, mean (SD)	54.2 (10.6)	55.8 (11.6)
Arterial O ₂ -saturation, mean (SD), %	97.5 (4.5)	96.9 (4.5)
Without supplemental O ₂ , No. (%)	64 (91)	71 (92)
Supplemental O ₂		
By nasal prongs, No. (%)	5 (7)	5 (7)
By face mask, No. (%)	1 (1)	1 (1)
Pre-transfusion fluid, No. (%) *	20 (29)	27 (35)
Volume of fluid, median (IQR), mL [†]	52 (45-61)	45 (40-60)
Hemoglobin, mean (SD), g/dL	3.5 (1.3)	3.5 (1.3)
Mean corpuscular volume, mean (SD), fL	79.7 (12.5)	79.7 (10.1)
Leukocytes/μL, median (IQR)	14,760 (9,670–22,800)	15,700 (8,810–24,700)
Platelets/μL, median (IQR)	196,500 (79,625–307,750)	143,000 (82,200–273,000)
Lactate, mean (SD), mM	8.7 (3.5)	9.8 (3.5)
Blood urea nitrogen, median (IQR), mg/dL [‡]	12 (6-23.5)	13.5 (7-22.3)
Creatinine, median (IQR), mg/dL [§]	0.5 (0.4-0.6)	0.4 (0.3-0.5)
Stupor or coma at presentation, No. (%)	19 (27)	25 (32)
Respiratory distress at presentation, No. (%)	62 (88)	68 (88)
Malaria by microscopy or RDT test, No. (%)	50 (71)	55 (71)
Parasites quantified by microscopy, No. (%)	33 (47)	39 (51)
Parasitized RBC/μL, median (IQR) [¶]	6,160 (1,020-50,176)	17,677 (8,054-67,893)

Characteristic	Shorter storage (n=70)	Longer storage (n=77)
Sickle cell disease, No. (%)	13 (18.6)	17 (22.1)
Other severe anemia, No. (%)	7 (10)	5 (6.5)

* Pre-transfusion fluid was dextrose or Ringers lactate.

[†] Volume refers to pre-transfusion fluid only in the subset who received pre-transfusion fluid.

[‡] Measured on 67 in the shorter-storage and 76 in the longer-storage group.

[§] Measured on 20 in the shorter-storage and 19 in the longer storage group.

// RDT, rapid diagnostic test for *P. falciparum* histidine-rich protein and LDH antigen.

[¶] Measured only in the subset with parasites quantified by microscopy, p=0.10