Original Investigation

Effect of Transfusion of Red Blood Cells With Longer vs Shorter Storage Duration on Elevated Blood Lactate Levels in Children With Severe Anemia
The TOTAL Randomized Clinical Trial

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IMPORTANCE Although millions of transfusions are given annually worldwide, the effect of red blood cell (RBC) unit storage duration on oxygen delivery is uncertain.

OBJECTIVE To determine if longer-storage RBC units are not inferior to shorter-storage RBC units for tissue oxygenation as measured by reduction in blood lactate levels and improvement in cerebral tissue oxygen saturation among children with severe anemia.

DESIGN, SETTING, AND PARTICIPANTS Randomized noninferiority trial of 290 children (aged 6-60 months), most with malaria or sickle cell disease, presenting February 2013 through May 2015 to a university-affiliated national referral hospital in Kampala, Uganda, with a hemoglobin level of 5 g/dL or lower and a lactate level of 5 mmol/L or higher.

INTERVENTIONS Patients were randomly assigned to receive RBC units stored 25 to 35 days (longer-storage group; n = 145) vs 1 to 10 days (shorter-storage group; n = 145). All units were leukoreduced prior to storage. All patients received 10 mL/kg of RBCs during hours 0 through 2 and, if indicated per protocol, an additional 10 mL/kg during hours 4 through 6.

MAIN OUTCOMES AND MEASURES The primary outcome was the proportion of patients with a lactate level of 3 mmol/L or lower at 8 hours using a margin of noninferiority equal to an absolute difference of 25%. Secondary measures included noninvasive cerebral tissue oxygen saturation during the first transfusion, clinical and laboratory changes up to 24 hours, and survival and health at 30 days after transfusion. Adverse events were monitored up to 24 hours.

RESULTS In the total population of 290 children, the mean (SD) presenting hemoglobin level was 3.7 g/dL (1.3) and mean lactate level was 9.3 mmol/L (3.4). Median (interquartile range) RBC unit storage was 8 days (7-9) for shorter storage vs 32 days (30-34) for longer storage without overlap. The proportion achieving the primary end point was 0.61 (95% CI, 0.52 to 0.69) in the longer-storage group vs 0.58 (95% CI, 0.49 to 0.66) in the shorter-storage group (between-group difference, 0.03 [95% CI, −0.07 to ∞], $P < .001$), meeting the prespecified margin of noninferiority. Mean lactate levels were not statistically different between the 2 groups at 0, 2, 4, 6, 8, or 24 hours. Kaplan-Meier analysis and global nonlinear regression revealed no statistical difference in lactate reduction between the 2 groups. Clinical assessment, cerebral oxygen saturation, electrolyte abnormalities, adverse events, survival, and 30-day recovery were also not significantly different between the groups.

CONCLUSIONS AND RELEVANCE Among children with lactic acidosis due to severe anemia, transfusion of longer-storage compared with shorter-storage RBC units did not result in inferior reduction of elevated blood lactate levels. These findings have relevance regarding the efficacy of stored RBC transfusion for patients with critical tissue hypoxia and lactic acidosis due to anemia.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01586923

JAMA. 2015;314(23):2514-2523. doi:10.1001/jama.2015.13977
Published online December 5, 2015.

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Each year approximately 100 million blood donations are made worldwide.[1] Regulatory agencies use radiolabeled cell survival and in vitro markers of hemolysis rather than measures of oxygen delivery to establish the maximal duration of blood storage, which is usually 5 or 6 weeks depending on the jurisdiction. During storage, however, red blood cells (RBCs) undergo changes that might impair the capacity for tissue oxygenation by transfused RBCs.[2-7]

Recently, randomized trials have examined the frequency of adverse events after transfusion of shorter-storage vs longer-storage RBC units. The Age of Red Cells in Premature Infants (ARIPI) study[8] found no statistical difference in the frequency of 5 major adverse outcomes among premature low-birth-weight infants, and the Age of Blood Evaluation (ABLE) trial[9] found no statistical effect of blood storage on 90-day mortality among critical care patients. The Red Cell Storage Duration Study (RECESS)[10] found no statistical difference according to blood storage duration for the change in the Multiple Organ Dysfunction Score following cardiac surgery. However, none of these studies was specifically designed to examine the effect of blood storage duration on oxygen delivery to tissues. Thus, the clinical effect of blood storage on the efficacy of RBC transfusion remains unresolved.

Severe anemia results in insufficient global tissue oxygenation and lactic acidosis. We designed the Tissue Oxygenation by Transfusion in Severe Anemia With Lactic Acidosis (TOTAL) study to compare longer-storage vs shorter-storage RBC units using reduction of blood lactate levels as an objective in vivo measure of transfusion efficacy. We focused on children presenting with elevated blood lactate levels due to severe anemia who were without other causes of lactic acidosis, such as impaired left ventricular function, uncorrectable hypoxia, bacterial sepsis, or trauma. With severe anemia as the primary basis for their lactic acidosis, these patients represented an informative cohort to study the effect of blood storage on global tissue oxygenation.

Methods

The study was approved by the Human Research Committee of the Massachusetts General Hospital, the School of Medicine Research Ethics Committee of Makerere University College of Health Sciences, and the Uganda National Council for Science and Technology. An independent data monitoring committee composed of a hematologist, a pediatrician, and a statistician who were blinded to the treatment assignments reviewed adverse events and outcomes after every 50th enrollment according to preestablished rules for monitoring the study. Written informed consent was obtained for all patients from the parent or guardian who brought the child to the hospital.

We conducted a randomized, single-center, parallel-group, noninferiority study with 1:1 allocation at the pediatric acute care unit of Mulago Hospital in Kampala, Uganda. Children aged 6 through 60 months who presented with a hemoglobin level of 5 g/dL or lower and a blood lactate level of 5 mmol/L or higher (to convert to mg/dL, divide by 0.111) were eligible. Children were excluded for severe acute malnutrition, cardiac disease, enrollment within the preceding 3 months, or transfusion within the prior 48 hours. Physicians or nurses assigned to the study screened consecutive patients for enrollment. Patients were assigned to receive RBC units stored 25 to 35 days (longer-storage) vs 1 to 10 days (shorter-storage) using 300 consecutively numbered, otherwise identical, opaque, sealed envelopes containing a paper indicating the treatment group. There were equal numbers of envelopes for each treatment group. Envelopes were prepared in 2 blocks of 150. The sealed envelopes were thoroughly mixed for 20 minutes by 3 individuals. The envelopes were then consecutively numbered on the outside and stored in a locked cabinet. Preparation of the envelopes was assigned to an investigator who was not responsible for patient selection, enrollment, or treatment allocation. Nurses responsible for clinical observations during the study did not participate in the preparation of randomization envelopes. Only 1 envelope was opened after each enrollment in consecutive order with the envelope number matching the unique patient number. There was no tampering with the envelopes. The sequence of treatment assignment could not be determined in advance of enrollment. A dual inventory of RBCs (both shorter-storage and longer-storage) was maintained at all times of active enrollment. Physicians caring for the patients were aware of the treatment assignment, but nurses performing patient assessments were not. Although there were no concerns regarding blinding during the first half of the study, permission was granted during the second half of the study to mask the expiration date on the blood label.

RBC Characteristics

The Uganda National Blood Transfusion Service collected blood from repeat volunteer donors confirmed to be blood group O and nonreactive for infectious disease markers according to national policy, and prepared prestorage leukoreduced RBC concentrates suspended in citrate, phosphate, dextrose, and adenine (CP2D-AS3) using commercially available blood collection sets (Leukotrap WB, Haemonetics) designed with an in-line leukocyte reduction filter. RBC units were not gamma irradiated. In Uganda, RBC units may be stored for up to 35 days, and there is no standard policy regarding selection of RBC units for transfusion according to blood storage duration. All RBC units were refrigerated on the day of collection to maintain 2,3-diphosphoglycerate (DPG) levels.[11,12] RBC units were stored in a locked, dedicated refrigerator with daily monitoring to document 1°C to 6°C temperature storage throughout the study.

Clinical Protocol

The original study protocol is given in Supplement 1. All RBC transfusions (10 mL/kg) were given by peripheral vein over 120 minutes using an electromechanical pump. Hour 0

is the start of the first transfusion. At hours 0, 2, 4, 6, 8, and 24, level of consciousness, vital signs, arterial pulse oximetry, use of supplemental oxygen, hemoglobin concentration, and capillary blood lactate concentration (Lactate Pro LT-1710, ARKRAY) were assessed. At hour 4, patients were given a second dose of RBCs—10 mL/kg—from the original unit of RBCs over 120 minutes if the hemoglobin level was lower than 5 g/dL or if the hemoglobin level was 5 to 6 g/dL accompanied by persistent tachycardia (>15% above the upper limit of normal for age) without worsened respiratory distress. Supplemental oxygen was given to patients with an arterial oxygen saturation less than 95% (finger oximetry). A complete blood cell count was done at time 0. Glucose, electrolytes, blood urea nitrogen (BUN), and creatinine were measured at hours 0, 4, and 24. Cerebral tissue oxygen saturation was measured noninvasively during transfusion using near-infrared spectroscopy. Adverse outcomes were recorded during the initial 24 hours by a study physician or a nurse present in the treatment area. Adverse events, not prespecified, included a decline in level of consciousness, new seizures, respiratory distress, pain, vomiting, diarrhea, edema, rash, and death. Clinical data collection ended 24 hours from the start of the first transfusion. Beginning with the 51st enrollment, permission was given to contact families of surviving patients by telephone at 30 days following discharge. Parents who could be contacted by telephone answered a short questionnaire at day 30 categorizing the patient into 1 of 3 groups: died, remains ill or receiving treatment, or returned to good health.

Primary Outcome

The primary outcome was the proportion of participants whose blood lactate level was 3 mmol/L or lower at hour 8 of the trial. Hour 8 was selected because this time point occurred after completion of all transfusions and prior to the expected restoration of 2,3-DPG levels in longer-storage RBCs,[13] thus maximizing the opportunity to observe a difference between the 2 groups. A 25% absolute difference for the primary outcome was selected as the margin of noninferiority balancing the following considerations. Too broad a margin for noninferiority risked the use of RBCs with diminished capacity to resolve elevated blood lactate levels. Based on our pilot study,[14] we estimated this delay to be on the order of minutes to hours. However, too narrow a margin for noninferiority risked barring the use of longer-storage RBCs, thus reducing the inventory of blood available for urgent transfusion.[15] Because a prior study[16] found that an 8-hour delay in transfusion is associated with a 13-fold higher risk ratio for fatal outcomes among children with severe anemia, a practice change that decreases availability of RBCs carries a substantial risk of adverse outcomes.

To further compare the decline in blood lactate following transfusion in the 2 groups, we prespecified 3 additional analyses. Mean lactate levels were compared at hour 2, 4, 6, 8, and 24 for the 2 study groups. The decline in blood lactate was also compared using nonlinear regression by a global test of the difference in the slope of lactate decline after fitting the data to a 1-phase exponential decay curve. Kaplan-Meier survival analysis was used to estimate the time to achieve a blood lactate of 3 mmol/L or lower, and the hazard ratio and CIs were estimated using the log-rank test.

Secondary Outcomes

We compared the longer-storage group with the shorter-storage group for each of the following secondary outcomes: presence of stupor, coma, or respiratory distress at 0, 4, 8, and 24 hours; mean arterial pressure, heart rate, respiratory rate, and arterial oxygen saturation at 0, 2, 4, 6, 8, and 24 hours; electrolytes at 0, 4, and 24 hours; BUN and creatinine at 0 and 24 hours; time from hospital admission until discharge or death; and cerebral tissue oxygen saturation (Sto_2) during the first transfusion (hours 0-2). Cerebral Sto_2 was measured noninvasively with a commercially available device (EQUANOX model 7600, Nonin) using a pediatric near-infrared probe placed on the left forehead.[17] At 30 days, the proportion returning to good health was compared between groups.

Subgroup Analysis

We prespecified that patients receiving 2 doses (20 mL/kg) of RBCs would undergo subgroup analysis for the same primary and secondary end points as the full study cohort. This subgroup was selected because any difference in treatment effect would be more pronounced in those receiving 2 doses of RBCs.

Post Hoc Analysis

After completion of the study, we did bivariable analyses of patient factors associated with achieving a lactate level of 3 mmol/L or lower at hour 8. We also examined the primary and secondary study outcomes comparing patients with malaria vs without malaria.

Statistical Analysis

The proportion of patients achieving a lactate level of 3 mmol/L or lower at hour 8 was tested for noninferiority using a 1-sided test. All other comparisons were tested for superiority using a 2-tailed comparison with a significance threshold of $P < .05$. Statistical analysis was conducted using GraphPad Prism (GraphPad Software), version 6.4. Continuous variables were compared using either a *t* test or a Mann-Whitney test. Categorical variables were compared using a χ^2 test. All comparisons, except those done in post hoc analysis, were specified prior to the completion of the study and were based on intention to treat. No participants dropped out of the study. There was no data imputation. For patients who died during the observation period, all available data were included.

Sample Size

In a pilot study of 74 patients,[14] the proportion of patients achieving a blood lactate level of 5 mmol/L or lower at 2 hours after transfusion of RBCs stored 1 to 10 days was 0.75. The study sample size was originally based on the ability to detect a 25% relative reduction in the proportion achieving

lactate clearance (ie, 75% of 0.75) for longer-storage RBCs. Assuming equal treatment allocation, with a 1-tailed α of .05 as appropriate for a noninferiority hypothesis and a power of 80%, we estimated a minimum sample size of at least 90 participants in each group would be needed have 80% power to demonstrate noninferiority if the true success rates were the same in the 2 groups. The study was approved for enrollment of up to 150 participants in each group to ensure adequate statistical power.

Results

Patients were enrolled between February 2013 and May 2015; follow-up was completed June 2015. Of 827 children assessed, 290 met inclusion criteria and consented to participate; 145 were randomized to each group (**Figure 1**). Patients presented with tachycardia, tachypnea, and respiratory distress consistent with severe anemia complicated by lactic acidosis. No patient was treated with vasopressors, required mechanical ventilation, or underwent dialysis. There were no differences in the baseline clinical characteristics of the 2 groups (**Table 1**). Most children had malaria (81%) or sickle cell disease (13%).

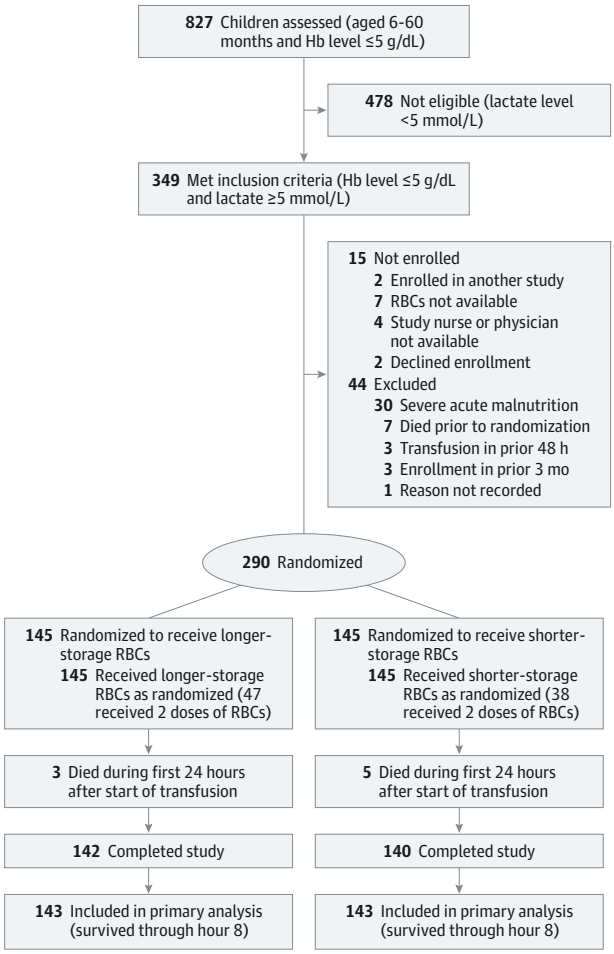
RBC Characteristics

All RBCs underwent prestorage leukoreduction. Median (interquartile range [IQR]) residual leukocyte concentration among 55 randomly selected units following prestorage leukodepletion was 4 leukocytes per microliter (0.5-10/μL). The mean hemoglobin concentration of the RBC units measured at the time of transfusion was 17.5 g/dL (95% CI, 17.1 to 17.9) for the longer-storage group (n = 144) vs 17.7 g/dL (95% CI, 17.4 to 18.0) for the shorter-storage group (n = 145); between-group difference, −0.2 g/dL (95% CI, −0.71 to 0.31), P = .78. The median (IQR) storage duration was 32 days (30-34) for longer-storage RBC units vs 8 days (7-9) for shorter-storage RBC units, P < .001. The distribution of storage duration at the time of transfusion is shown in **Figure 2**. With 2 exceptions, all units were within the storage age defined by the protocol. All patients received the RBCs from the duration group assigned by randomization. No other blood components (plasma or platelets) were administered.

Duration and Volume of Transfusion and Use of Supplemental Oxygen

All patients received an initial transfusion of 10 mL/kg by infusion pump over 120 minutes during hours 0 through 2. Based on criteria of hemoglobin, pulse, and respiratory rate at hour 4, a second dose of RBCs (10 mL/kg given during hours 4 through 6) from the original unit was administered to 47 of 143 patients (32.9% [95% CI, 25.2% to 41.2%]) receiving longer-storage RBCs and to 38 of 144 patients (26.4% [95% CI, 19.4% to 34.4%]) receiving shorter-storage RBCs; between-group difference, 6% (95% CI, −4% to 17%), P = .23. There were no protocol violations of transfusion volume or rate. Given the severe degree of anemia in these patients, the red cell content of each transfusion represented a substantial proportion of their red cell mass (eAppendix 1 in Supplement 2). Transfusion doubled the patients' hemoglobin concentrations: mean hemoglobin level was 3.7 g/dL (95% CI, 3.5 to 3.9) pretransfusion vs 7.1 g/dL (95% CI, 6.9 to 7.3) after transfusion. The 24-hour mean increment in hemoglobin concentration was not statistically different between the 2 study groups: 3.4 g/dL (95% CI, 3.2 to 3.6) for the longer-storage group vs 3.5 g/dL (95% CI, 3.2 to 3.8) for the shorter-storage group; between-group difference, −0.1 (95% CI, −0.45 to 0.25), P = .71 (**Figure 3**). Supplemental oxygen was used in a minority of patients (11% at hour 0; 24% at hour 2; 17% at hour 8; and 8% at hour 24). There was no statistical difference in use of supplemental oxygen between the 2 study groups. (eAppendix 2 in Supplement 2).

Figure 1. Participant Flow in the TOTAL Study



Hb indicates hemoglobin; RBC, red blood cell; TOTAL, Tissue Oxygenation by Transfusion in Severe Anemia With Lactic Acidosis.

Primary Outcome

At presentation, the mean (SD) blood lactate level for all patients was 9.3 mmol/L (3.41). Lactate levels declined to

Table 1. Baseline Characteristics of Children Enrolled in the TOTAL Study (N=290)

Characteristics	Longer RBC Storage (n = 145)	Shorter RBC Storage (n = 145)
Age, median (IQR), mo	25.5 (15.0-39.9)	26.5 (18.5-39.6)
Sex, No. (%)		
Girls	67 (46)	71 (49)
Boys	78 (54)	74 (51)
BMI, mean (SD)	15.1 (1.9)	15.2 (1.9)
Blood group distribution, No. (%)		
O	72 (50)	57 (39)
A	31 (21)	39 (37)
B	33 (23)	40 (28)
AB	9 (6)	9 (6)
Temperature, mean (SD), °C	37.4 (1.2)	37.6 (2.2)
Mean arterial pressure, mean (SD), mm Hg	72.4 (11.7)	73.6 (11.8)
Heart rate, mean (SD)	163.0 (17.9)	157.8 (17.5)
Respiratory rate, mean (SD)	55.5 (11.5)	53.8 (11.0)
Arterial oxygen saturation, mean (SD), %	97.5 (3.7)	97.2 (5.2)
Not receiving supplemental oxygen, No. (%)	132 (91)	126 (87)
Receiving supplemental oxygen, No. (%)		
By nasal prongs	12 (8.3)	17 (11.7)
By face mask	1 (0.7)	2 (1.4)
Pretransfusion fluid, No. (%)[a]	42 (29.0)	46 (31.7)
Volume of fluid, median (IQR), mL[b]	45 (40-60)	50 (43-60)
Hemoglobin, mean (SD), g/dL	3.7 (1.3)	3.6 (1.3)
Mean corpuscular volume, mean (SD), fL	80.8 (11.7)	79.8 (12.5)
Leukocytes, median (IQR), /μL	13 900 (8850-22 500)	14 950 (8890-25 330)
Platelets, median (IQR), /μL	133 000 (83 000-274 000)	128 000 (68 100-283 000)
Lactate, mean (SD), mmol/L	9.5 (3.4)	9.2 (3.4)
Stupor or coma at presentation, No. (%)	41 (28.2)	51 (35.1)
Respiratory distress at presentation, No. (%)	129 (89)	129 (89)
Malaria by microscopy or RDT, No. (%)	116 (80.0)	118 (81.4)
Malaria parasites by microscopy, No. (%)	84 (58)	84 (58)
Parasitized RBC, median (IQR), /μL[c]	21 535 (8322-92 791)	29 860 (5110-106 879)
Sickle cell disease, No. (%)	21 (14.5)	18 (12.4)
Other severe anemia, No. (%)	10 (6.9)	14 (9.6)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); IQR, interquartile range; RBC, red blood cell; RDT, rapid diagnostic test; TOTAL, Tissue Oxygenation by Transfusion in Severe Anemia With Lactic Acidosis.

Conventional unit conversion: To convert lactate to mg/dL, divide by 0.111.

[a] Pretransfusion fluid was dextrose or Ringers lactate.

[b] Volume refers to pretransfusion fluid only in the subset who received pretransfusion fluid.

[c] Measured only in the subset with parasites seen by microscopy.

3 mmol/L or lower at hour 8 in 87 of 143 patients (0.61 [95% CI, 0.52 to 0.69]) in the longer-storage group vs 83 of 143 patients (0.58 [95% CI, 0.49 to 0.66]) in the shorter-storage group; between-group difference, 0.03 (95% CI, −0.07 to ∞), $P < .001$. The 7% upper CI of inferiority was less than the 25% prespecified margin. There were no significant differences in mean lactate concentration between the 2 groups at 0, 2, 4, 6, 8, or 24 hours (**Figure 4**A). Kaplan-Meier analysis (Figure 4B) estimated the median time to achieve a blood lactate of 3 mmol/L or lower at 4 hours in both groups with a nonsignificant hazard ratio of 0.99 (95% CI, 0.77 to 1.26), $P = .92$ (Mantel-Cox log-rank test). The clearance of lactate over 24 hours was compared between the 2 groups using global nonlinear regression with the data fit to a single-phase exponential decay curve with a rate constant of 0.652 hours^{-1}. In this model the slope and half-disappearance time of lactate values in the 2 groups were not statistically different, $P = .18$.

Secondary Outcomes

Cerebral StO_2 rose significantly during transfusion (eAppendix 2 in Supplement 2) in both groups and to the same degree (71.6% at the start of transfusion to 78.9% at completion of transfusion in the longer-storage group vs 72.8% at the start of transfusion to 77.0% at completion of transfusion in the shorter-storage group) (**Figure 5**). The median (IQR) area under the curve of cerebral StO_2 during transfusion was 679 (334-1156) for the longer-storage group vs 521 (303-835) for the shorter-storage group, $P = .25$. The cerebral StO_2 area under the curve rose by more than 1000 units in 19 of 61 patients (31% [95% CI, 20% to 49%]) in the longer-storage group vs 13 of 59 patients (22% [95% CI, 12% to 35%]) in the shorter-storage group; between-group difference, 9.1% (95% CI, −6.7% to 24.3%), $P = .26$. The greatest increase in cerebral StO_2 occurred during the initial 60 minutes of transfusion.

Stupor or coma was present prior to transfusion in 31.7% of patients; and, after 8 hours, persisted in 18 of 143 patients in the longer-storage group (12.6% [95% CI, 7.6% to 19.2%]) vs 28 of 143 patients (19.6% [95% CI, 13.4% to 27.0%]) in the shorter-storage group; between-group difference, −7% (95% CI, −2% to 16%), $P = .11$. Respiratory distress, present in 89% of patients prior to transfusion, persisted at 8 hours among 41 of 143 patients (28.7% [95% CI, 21.4% to 36.8%]) in the longer-storage group vs 43 of 143 patients (30% [95% CI, 22.7% to 38.3%]) in the shorter-storage group; between-group difference, −1.3% (95% CI, −11.9% to 9.1%), $P = .79$ (eAppendix 3 in Supplement 2). Following transfusion, vital signs improved in both study groups to the same degree (**Table 2** and eAppendix 4 in Supplement 2). At presentation, the patients demonstrated low plasma bicarbonate (mean, 15.6 mEq/L [SD, 5.51]) and elevated anion gap (mean, 18.3 mmol/L [SD, 4.89]) consistent with lactic acidosis. Changes in electrolytes, BUN, and creatinine are shown in Table 2 and eAppendix 4 in Supplement 2. Median (IQR) hospital length of stay was 4 days (2-6) in the longer-storage group vs 4 days (3-7) in the shorter-storage group.

Death occurred in 8 patients (3 in the longer-storage group; 5 in the shorter-storage group) during the 24 hours from the start of transfusion. Four additional patients (2 in the longer-storage group; 2 in the shorter-storage group) died in the hospital after the initial 24-hour observation period. New seizures occurred in 3 patients in the longer-storage group and 4 patients in the shorter-storage group. Other adverse events during the initial 24 hours of observation included vomiting (4 in the longer-storage group; 6 in the shorter-storage group); hives (1 patient); and facial puffiness (1 patient) (eAppendix 5 in Supplement 2). At 30 days, follow-up was obtained from the majority of families available for phone contact (100 of 115 patients [87%] for the longer-storage group; 85 of 114 patients [75%] for the shorter-storage group). The proportion of patients who had returned to good health by 30 days was 86 of 100 patients (86% [95% CI, 77.6% to 92.1%]) in the longer-storage group vs 79 of 85 patients (93% [95% CI, 85.3% to 97.4%]) in the shorter-storage group; between-group difference, −7% (95% CI, −16 to 2), P = .13.

Subgroup Analysis

Two doses of the RBC unit (20 mL/kg total) were given to 85 patients. There was no statistical difference in the 2 storage subgroups for the proportion of patients with a lactate level of 3 mmol/L or lower at 8 hours: 28 of 47 patients (0.60 [95% CI, 0.44 to 0.73]) in the longer-storage subgroup vs 20 of 38 patients (0.53 [95% CI, 0.36 to 0.69]) in the shorter-storage subgroup; between-group difference, 0.07 (95% CI, −0.14 to 0.27), P = .52. Mean lactate levels were not statistically different between those receiving 2 doses of longer-storage RBCs vs 2 doses of shorter-storage RBCs at any time point following transfusion. There was also no statistically significant difference between the subgroup receiving shorter-storage vs longer-storage RBCs for improvement in cerebral tissue oxygen saturation, proportion of patients with persistent stupor or coma at hour 8, correction of vital signs, resolution of acidosis, or changes in electrolytes, BUN, or creatinine. The proportion of children reported to have returned to good health at 30-day follow-up was not statistically different between the 2 subgroups: 30 of 36 patients (83.3% [95% CI, 67.2% to 93.6%] in the longer-storage group vs 20 of 23 patients (87% [95% CI, 66.4% to 97.2%] in the shorter-storage group; between-group difference, −4% (95% CI, −21% to 17%), P = .59 (eAppendix 6 in Supplement 2).

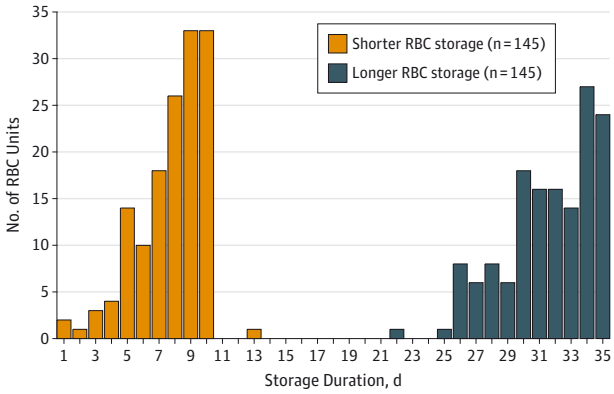
Post Hoc Analyses

In post hoc analyses, we did not find any patient characteristic significantly associated in bivariable analyses with achieving a blood lactate level of 3 mmol/L or lower at hour 8. We also did not find any difference in reduction in blood lactate levels when comparing patients with malaria vs those without malaria (eAppendix 7 in Supplement 2).

Discussion

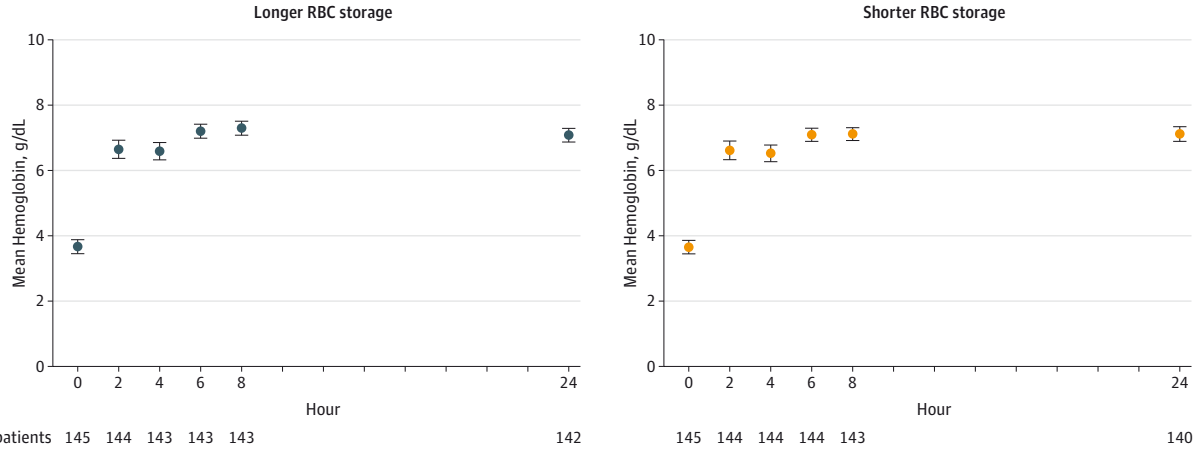
In this randomized trial among Ugandan children with severe anemia and lactic acidosis, leukoreduced RBC units

Figure 2. Duration of Red Blood Cell Storage by Study Group



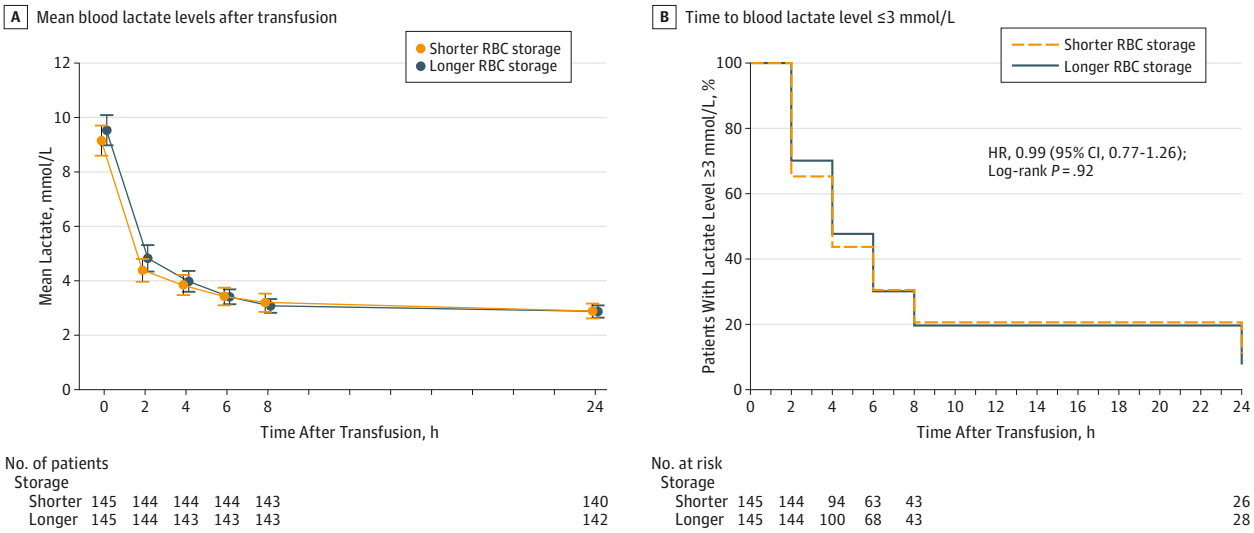
RBC indicates red blood cell. Because each patient received blood from 1 donor unit, the n value refers to both patients and units.

Figure 3. Mean Hemoglobin Levels Before and After Transfusion



RBC indicates red blood cell. Error bars indicate 95% CIs. Hour 0 is the start of the first transfusion.

Figure 4. Lactate Clearance in Response to Transfusion

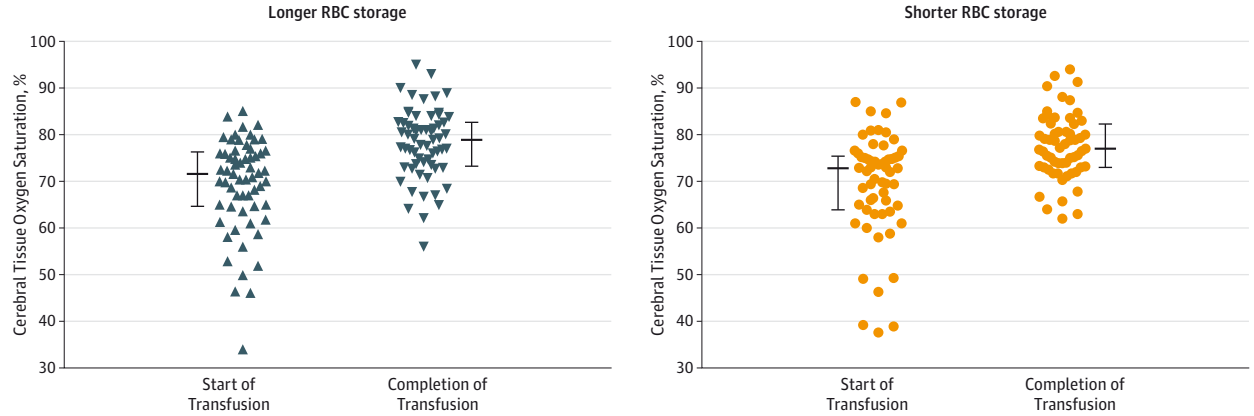


No. of patients							
Storage							
Shorter	145	144	144	144	143		140
Longer	145	144	143	143	143		142

No. at risk							
Storage							
Shorter	145	144	94	63	43		26
Longer	145	144	100	68	43		28

RBC indicates red blood cell. Conventional unit conversion: To convert lactate to mg/dL, divide by 0.111. Error bars indicate 95% CIs. Hour 0 is the start of the first transfusion.

Figure 5. Cerebral Tissue Oxygen Saturation in Response to Transfusion



	Longer RBC storage		Shorter RBC storage	
No. of patients	61	61	59	59
No. of transfusions	61	61	59	59

RBC indicates red blood cell. Error bars indicate interquartile range. The thick horizontal line indicates the median. For longer RBC storage, the *P* value was <.001 for the comparison of cerebral tissue oxygen saturation pretransfusion vs after transfusion. For shorter RBC storage, the *P* value was <.001 for the comparison of cerebral tissue oxygen saturation pretransfusion vs after transfusion.

maintained under standard storage conditions for 25 to 35 days were not inferior to RBC units stored for up to 10 days as measured either by resolution of lactic acidosis at 8 hours (primary outcome measure) or by secondary outcomes defined by improvement in clinical symptoms, normalization of vital signs, correction of laboratory abnormalities, and improvement in cerebral tissue oxygen saturation. This study has 6 important and distinguishing design features: (1) patients had severe anemia to levels dependent on RBC transfusion for restoration of tissue oxygenation; (2) the extremes of storage duration were compared with no overlap between the 2 groups; (3) patients in both groups received the same volume and rate of transfusion, which, given the severity of anemia, averaged 59% of their pretransfusion red cell mass for those receiving 1 dose and averaged 92% for those receiving 2 doses; (4) study participants did not have confounding conditions adversely affecting tissue oxygen delivery such as impaired cardiac output, sepsis, tissue injury, hemorrhage, shock, or vasopressors; (5) the primary end point (lactate level) was not subject to observer bias; and (6) the study used RBCs collected, processed, stored, and administered according to standards used in countries with high gross national income.

Table 2. Secondary Outcomes: Vital Signs, Electrolytes, and Renal Function Tests at Various Time Points Among Children in the TOTAL Study[a]

	Longer RBC Storage		Shorter RBC Storage		Between-Group Difference (95% CI)
	Mean (95% CI)	No.	Mean (95% CI)	No.	
Vital Signs					
MAP, mm Hg					
0 h	72.4 (70.5 to 74.3)	145	73.6 (71.7 to 75.5)	145	−1.18 (−3.89 to 1.53)
4 h	77.3 (75.5 to 79.1)	143	75.8 (74.1 to 77.5)	144	1.47 (−1.03 to 3.97)
24 h	74.0 (72.4 to 75.6)	141	73.9 (72.3 to 75.5)	140	0.14 (−2.15 to 2.44)
Heart rate					
0 h	163.0 (160.1 to 165.9)	145	157.8 (155.0 to 160.6)	145	5.12 (1.04 to 9.21)
4 h	144.1 (141.0 to 147.2)	143	138.2 (135.4 to 141.0)	144	5.92 (1.73 to 10.12)
24 h	131.9 (129.2 to 134.6)	142	127.9 (124.9 to 130.9)	140	4.02 (−0.08 to 8.12)
Respiratory rate					
0 h	55.5 (53.6 to 57.4)	145	53.8 (52.0 to 55.6)	145	1.71 (−0.89 to 4.31)
4 h	43.8 (42.1 to 45.5)	143	41.7 (40.2 to 43.2)	144	2.10 (−0.20 to 4.40)
24 h	36.6 (35.1 to 38.1)	142	36.6 (35.2 to 38.0)	140	0.08 (−1.20 to 2.13)
Electrolytes					
Potassium, mEq/L					
0 h	4.3 (4.2 to 4.4)	137	4.2 (4.1 to 4.3)	125	0.11 (−0.08 to 0.30)
4 h	3.8 (3.6 to 4.0)	63	3.7 (3.5 to 3.9)	52	0.13 (−0.17 to 0.44)
24 h	3.9 (3.8 to 4.0)	133	3.9 (3.8 to 4.0)	117	0.05 (−0.11 to 0.21)
Carbon dioxide, mmol/L					
0 h	15.1 (14.2 to 16.0)	136	16.2 (15.2 to 17.2)	125	−1.11 (−2.45 to 0.23)
4 h	16.2 (14.8 to 17.6)	62	19.1 (17.4 to 20.8)	52	−2.87 (−5.07 to −0.70)
24 h	22.1 (21.5 to 22.7)	133	22.4 (21.7 to 23.1)	117	−0.28 (−1.19 to 0.63)
Anion gap, mmol/L					
0 h	18.3 (17.6 to 19.0)	134	18.1 (17.4 to 18.8)	124	0.16 (−0.84 to 1.16)
4 h	17.9 (15.8 to 20.0)	57	16.1 (14.8 to 17.4)	52	1.80 (−0.79 to 4.39)
24 h	12.8 (12.4 to 13.3)	130	13.4 (12.8 to 14.0)	117	−0.63 (−1.37 to 0.11)
Renal Function					
BUN, mg/dL					
0 h	17.8 (15.4 to 20.3)	136	19.3 (15.4 to 23.2)	125	−1.55 (−6.07 to 2.98)
4 h	14.9 (11.2 to 18.6)	62	14.5 (9.7 to 19.3)	52	0.40 (−5.69 to 6.49)
24 h	11.8 (9.7 to 13.9)	133	13.1 (9.4 to 16.9)	117	−1.31 (−5.50 to 2.88)
Creatinine, mg/dL					
0 h	0.4 (0.3 to 0.5)	64	0.4 (0.3 to 0.5)	61	−0.02 (−0.13 to 0.09)
24 h	0.4 (0.4 to 0.5)	63	0.4 (0.3 to 0.5)	59	−0.02 (−0.13 to 0.09)

Abbreviations: BUN, blood urea nitrogen; MAP, mean arterial pressure; RBC, red blood cell; TOTAL, Tissue Oxygenation by Transfusion in Severe Anemia With Lactic Acidosis.

SI conversion: To convert blood urea nitrogen to mmol/L, multiply by 0.357; creatinine to µmol/L, multiply by 88.4.

[a] Time points: 0 h indicates hour 0 of the study and pretransfusion; 4h, hour 4 of the study and 2 hours after completion of transfusion; 24 h, hour 24 of the study. Additional data are presented in eAppendix 4 in Supplement 2.

Lactic acidosis is a serious disorder associated with fatal outcomes.[18] The prompt decline in blood lactate levels, in the absence of other interventions shared by these patients, suggests that RBC transfusion ameliorated lactic acidosis by improving tissue oxygenation. Consistent with this, cerebral Sto_2 levels rose before the reduction in blood lactate. The improvement was not likely due to the volume effects of transfusion because a previous multicenter study documented that either volume loading[19] or delays in transfusion[16] are associated with a significantly higher rate of fatal outcomes in severe pediatric anemia. Of interest, among patients receiving longer-storage RBCs, cerebral Sto_2 and lactate both improved prior to the expected restoration of 2,3-DPG in transfused cells.[13] In addition, despite acidosis, patients receiving longer-storage RBCs did not demonstrate hyperkalemia at hours 4 or 24.

Although there is no prior published benchmark to define noninferiority of lactate resolution by transfusion, our study found no suggestion of a clinically important difference between the 2 storage groups. The upper confidence limit of 7% in the proportion of patients achieving a lactate level of 3 mmol/L or lower does not represent a clinically important difference in lactate clearance. In fact, lactate clearance rates at each time point for both study groups were well above clinical target goals previously reported,[18,20-22] even when using data for the longer-storage group corresponding to slowest lactate clearance based on 95% CIs. Moreover, the 3 additional analyses of lactate clearance over time met clinical target goals. The majority of our patients had either malaria or sickle cell disease, conditions that can contribute to microvascular ischemia.[23,24] Because erythrocytes from longer-storage blood demonstrate in vitro

evidence of reduced cell deformability,[25] any degree of recipient microvascular blockade would be expected to favor the shorter-storage group. Nevertheless, no clinical disadvantage following transfusion of longer-storage RBCs was observed, and lactate clearance was the same for those with or without malaria.

Our study has the limitations of any single-site study conducted in a selected cohort of patients. Although randomization did not use a computer-generated sequence, the 2 study groups were completely balanced in all measured respects and a dual inventory of shorter-storage and longer-storage RBCs was maintained throughout the study to insure that each enrolled patient received the treatment assigned by randomization. Although the findings provide information on the in vivo performance of erythrocytes following storage and transfusion, the kinetics of lactate clearance would differ in patients with cardiac, vascular, and perfusion abnormalities. Further studies in other patient cohorts are necessary to determine lactate clearance and tissue oxygenation following transfusion of RBCs of different storage durations. Finally, although the study was powered to compare differences in lactate clearance, it was not designed to detect differences in survival or other adverse outcomes that were the focus of other studies.[8-10]

This study provides biological evidence that longer-storage RBCs correct lactic acidosis and increase cerebral tissue oxygenation as effectively as shorter-storage RBCs. Prior studies in Europe and North America have found it challenging to measure the specific contribution of transfused stored RBCs to oxygen delivery because transfusions are routinely given to patients before the development of anemia severe enough to impair global tissue oxygenation.[26-31] Our observations are particularly informative because, unlike most randomized clinical trials, this study assigned patients to the 2 extremes of blood storage duration and administered a high dose of transfused cells. As a result, the study design provided the greatest opportunity to observe an effect of RBC storage duration.

Conclusions

Among children with lactic acidosis due to severe anemia, transfusion of longer-storage compared with shorter-storage RBCs did not result in inferior reduction of elevated blood lactate levels. These findings have relevance regarding the efficacy of stored RBC transfusion for patients with critical tissue hypoxia and lactic acidosis due to anemia.

ARTICLE INFORMATION

Published Online: December 5, 2015. doi:10.1001/jama.2015.13977.

Author Contributions: Drs Dzik and Cserti-Gazdewich had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.
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Obtained funding: Stowell, Dzik.
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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Stowell reports serving on the scientific review committee for Haemonetics Corporation. No other disclosures were reported.

Funding/Support: The study was fully funded by grant 1R21HL109518-01A1 from the National Institutes of Health. Nonin Corporation supplied the equipment used for noninvasive cerebral oxygen measurements.

Role of the Funder/Sponsor: The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Contributions: We thank Annet Akiror, Joan Adyek, and John Kasirye (all from Uganda National Blood Transfusion Service) for blood collection and processing; Judith Mutesi, Robinah Asaba, Eva Kagoya, and Sophie Chemutai (all from Mulago Hospital, Kampala) for patient care services; Samuel Nsobya, PhD (Makerere University–UCSF Research Collaboration), for laboratory assays; David Mpiima (Uganda Cancer Institute) for laboratory assays; Esther N. Ssebyala (Global Health Uganda, Kampala) for administrative support; Eileen Selogie (eNet Solutions) for database support; Sarah Kiguli-Walube, MD (Makerere University), for administrative support; Henry Ddungu, MD, Jane Achan, MD, and Albert Maganda (all from the data and safety monitoring board). These contributors received compensation according to their usual salary for their contributions.

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