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Effect of Fresh Red Blood Cell Transfusions on Clinical Outcomes in Premature, Very Low-Birth-Weight Infants

The ARIPI Randomized Trial

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Context Even though red blood cells (RBCs) are lifesaving in neonatal intensive care, transfusing older RBCs may result in higher rates of organ dysfunction, nosocomial infection, and length of hospital stay.

Objective To determine if RBCs stored for 7 days or less compared with usual standards decreased rates of major nosocomial infection and organ dysfunction in neonatal intensive care unit patients requiring at least 1 RBC transfusion.

Design, Setting, and Participants Double-blind, randomized controlled trial in 377 premature infants with birth weights less than 1250 g admitted to 6 Canadian tertiary neonatal intensive care units between May 2006 and June 2011.

Intervention Patients were randomly assigned to receive transfusion of RBCs stored 7 days or less (n=188) vs standard-issue RBCs in accordance with standard blood bank practice (n=189).

Main Outcome Measures The primary outcome was a composite measure of major neonatal morbidities, including necrotizing enterocolitis, retinopathy of prematurity, bronchopulmonary dysplasia, and intraventricular hemorrhage, as well as death. The primary outcome was measured within the entire period of neonatal intensive care unit stay up to 90 days after randomization. The rate of nosocomial infection was a secondary outcome.

Results The mean age of transfused blood was 5.1 (SD, 2.0) days in the fresh RBC group and 14.6 (SD, 8.3) days in the standard group. Among neonates in the fresh RBC group, 99 (52.7%) had the primary outcome compared with 100 (52.9%) in the standard RBC group (relative risk, 1.00; 95% CI, 0.82-1.21). The rate of clinically suspected infection in the fresh RBC group was 77.7% (n=146) compared with 77.2% (n=146) in the standard RBC group (relative risk, 1.01; 95% CI, 0.90-1.12), and the rate of positive cultures was 67.5% (n=127) in the fresh RBC group compared with 64.0% (n=121) in the standard RBC group (relative risk, 1.06; 95% CI, 0.91-1.22).

Conclusion In this trial, the use of fresh RBCs compared with standard blood bank practice did not improve outcomes in premature, very low-birth-weight infants requiring a transfusion.

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ALTHOUGH RED BLOOD CELL (RBC) transfusions are used routinely in acutely ill patients, including those in neonatal intensive care units, the clinical consequences of the prolonged storage of RBCs have not been firmly established. Reported adverse consequences have been attributed to the generation of cytokines in the storage medium.[1] Changes to RBC mem-

See also Patient Page.

CME available online at www.jamaarchivescme.com and questions on p 1485.

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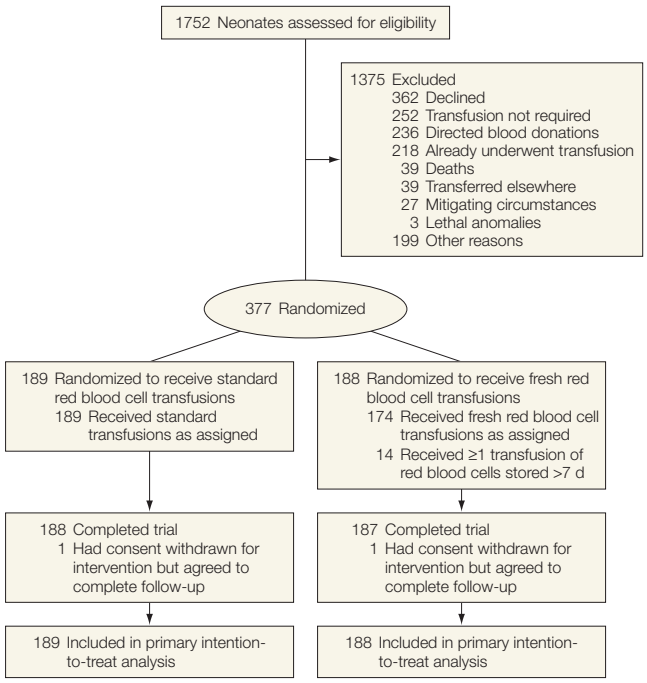
Figure 1. Participant Flow



branes that alter deformability of RBCs and an inability to scavenge nitric oxide and biochemical changes such as decreased levels of 2,3-diphosphoglycerate may be even more important than the generation of cytokines because they impair the ability of RBCs to deliver oxygen to meet tissue needs.[2,3] In vulnerable patients such as critically ill premature infants, transfusing older RBCs may result in higher rates of organ dysfunction and morbidity because of the deleterious oxygen deficits or the proinflammatory effects of bioactive materials that accumulate during RBC storage.

In recent years, several observational studies conducted primarily in adults have demonstrated that prolonged RBC storage is associated with increased rates of infection, organ failure, death, and increased lengths of stay.[4-14] Unfortunately, it is extremely difficult to disentangle cause-effect relationships in observational studies, wherein sicker patients often receive more RBC transfusions and, frequently, a greater number of older units.

Premature infants requiring multiple transfusions are routinely exposed to older RBCs because of a "dedicated" donor policy introduced in the 1980s to decrease the risk of viral transmission through transfusions of blood from multiple donors. The dedicated donor policy designates a specific unit of donated RBCs for use by 1 infant exclusively over the course of his/her transfusion needs up to the expiration date of the unit. Despite the inherent significant decrease in risk of viral transmission with dedicated units, by design this approach increases rates of transfusion of older RBCs. The policy remains a standard of practice in most neonatal intensive care units in Canada and the United States.[15,16] In a previous study examining RBC characteristics and their impact on outcomes, we documented that transfusion of leukoreduced RBCs was associated with a decrease in organ injury in premature infants[17]; therefore, the type of RBCs transfused may matter. It is possible that prolonged cell storage may have exacerbated the observed effect of nonleukoreduction given the possible interaction between length of storage and presence of white blood cells.[18]

In neonatal intensive care, the dedicated donor policy provided an ideal setting in which to evaluate the effects of prolonged RBC storage in vulnerable patients. Therefore, our objective in the Age of Red Blood Cells in Premature Infants (ARIPI) trial was to evaluate whether RBCs stored for 7 days or less decreased serious neonatal morbidity and mortality compared with standard blood bank issue.

METHODS

In 6 Canadian tertiary neonatal intensive care units, we enrolled infants in a double-blind, randomized controlled trial comparing the clinical consequences of transfusing RBCs stored for 7 days or less vs standard practice between May 2006 and June 2011.[19] The study was approved by the research ethics boards both at the central coordinating center and at each of the participating sites.

Study Population

We assessed the eligibility of all premature infants admitted to each of the 6 participating neonatal intensive care units who had a birth weight of less than 1250 g and required 1 or more RBC transfusion for the treatment of anemia. We excluded premature infants who had already received an RBC transfusion, were scheduled to undergo an exchange transfusion or to receive a directed donation, had rare blood types that would lead to difficulty with crossmatching, were moribund on admission to the neonatal intensive care unit, were not expected to survive because of a severe congenital anomaly, or whose attending clinical team specifically requested fresh RBCs.

A representative in each infant's health care team approached families to ask if they would be willing to have a research coordinator speak to them about the study. If yes, the coordinator described the study (risks/benefits, voluntary participation, procedures). Families were given adequate

time to reflect on the information and had any questions answered. Parents or legal guardians then provided written informed consent. We did not approach parents or guardians of infants when the health care team thought it was inappropriate (eg, extreme family crisis, child protection issues). Race/ethnicity of the infants was not ascertained; however, research coordinators ascertained parental race/ethnicity through direct parent interview or by chart review because race/ethnicity has been associated with variation in neonatal intensive care outcomes.[20]

Study Interventions

Premature infants were randomly assigned to receive either RBCs stored 7 days or less (the fresh RBC group) or current standard-issue RBCs with storage time ranging from 2 to 42 days depending on site (the standard RBC group). For all neonatal RBC transfusions, standard-issue RBC units were divided into aliquots to increase usage and decrease waste because of the smaller volume of RBCs required for premature infants.

Adherence to the 7 days or less rule was monitored by local blood bank personnel to maintain blinding, and blood bank log statistics were collated by the blinded statistician at the coordinating center and provided to the data and safety monitoring board. In the standard RBC group, each aliquot was designated for use in a single infant up to its expiration date.[21] A summary of the standard blood bank practices is provided in eTable 1 (available at http://www.jama.com).

All blood products used in this trial were collected by Canadian Blood Services or Héma-Québec and prepared by the local hospital blood bank according to local institutional practice. Red blood cells and other blood products were administered in accordance with the standard clinical practice at each site. Transfusion triggers were not protocolized and there were no other controlled interventions in keeping with the trial's pragmatic design.

Randomization

Research coordinators at each center randomly assigned eligible infants using an interactive voice response system. Following eligibility screening by the research coordinator, the system generated a unique number. The research coordinator then telephoned the hospital blood bank staff and reported the number. In turn, blood bank staff referred to a manual of unique numbers generated by an independent statistician prior to study activation to determine the study intervention allocated to the randomized patient.

The randomization schedule was stratified by site in variable blocks of 4 and 6. Allocation occurred only after an order to transfuse was received and only if a supply of RBCs stored for 7 days or less was available at the time of allocation. Randomized patients received all transfusions during the study period according to the intervention they were allocated. Study investigators, research coordinators, attending care teams, and the infants' families were blinded to treatment allocation.

Study Outcomes

The primary outcome was a composite outcome composed of mortality and major neonatal morbidities associated with acute organ dysfunction or fail-

Table 1. Baseline Characteristics[a]

Characteristics	Standard Red Blood Cell Group (n = 189)	Fresh Red Blood Cell Group (n = 188)
Mothers		
Racial/ethnic group		
White	123 (65.1)	138 (73.4)
Black	24 (12.7)	17 (9.1)
Latin American	1 (0.5)	4 (2.1)
Asian	8 (4.2)	6 (3.2)
Aboriginal	20 (10.6)	10 (5.3)
Filipino	4 (2.1)	0
Arab	1 (0.5)	3 (1.6)
Other/unknown	8 (4.2)	10 (5.3)
Antenatal corticosteroids	157 (83.1)	163 (86.7)
Cesarean delivery	126 (66.7)	98 (52.1)
Vaginal breech delivery	5 (2.6)	23 (12.2)
Infants		
Male	94 (50.0)	109 (58.0)
Multiple birth	60 (31.7)	56 (29.9)
Born at study hospital	166 (44.0)	170 (45.1)
Antibiotics administered	170 (89.9)	171 (91.0)
Age, mean (SD), d	9.9 (9.5)	10.0 (9.7)
Gestational age, mean (SD), wk	26.8 (1.8)	26.38 (1.5)
Birth weight, mean (SD), g	831.8 (188.1)	838.3 (201.4)
5-min Apgar score, mean (SD)	6.4 (2.1)	6.3 (2.1)
CRIB score, median (IQR)[b]	5.0 (2.0-8.0)	5.0 (2.0-8.3)
SNAP-II day 1 score, median (IQR)[c]	14.0 (8.0-24.0)	14.0 (6.5-24.3)
SNAP-II day 3 score, median (IQR)[c]	5.0 (5.0-12.0)	5.0 (0.0-14.0)
Hemoglobin level at neonatal intensive care unit admission, mean (SD), g/L	149.5 (27.4)	147.1 (24.1)

Abbreviation: IQR, interquartile range.
[a] Data are expressed as No. (%) unless otherwise indicated.
[b] The Clinical Risk Index for Babies (CRIB) score assesses the initial (first 12 hours of life) clinical severity in preterm infants based on birth weight, gestational age, congenital malformation, base excess, and fraction of inspired oxygen. The higher the score, the higher the risk of mortality (score range, 0 to 23).
[c] The Score for Neonatal Acute Physiology, version 2 (SNAP-II) is a neonatal illness severity score that evaluates 6 empirically weighted, physiology-based items during a 12-hour time frame, including lowest blood pressure, lowest temperature, Po_2/Fio_2 ratio, lowest serum pH, seizures, and urine output. SNAP-II has a score range of 0 (low severity) to 115 (high severity).

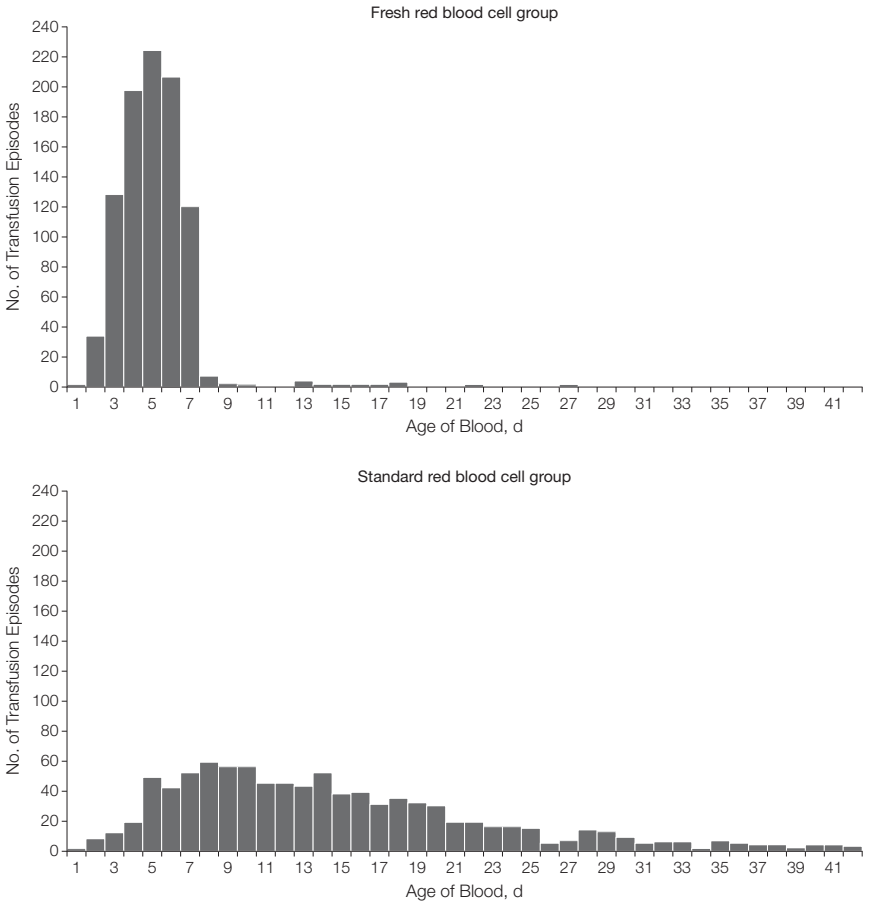
ure. In addition to death, the 4 major morbidities comprising the composite outcome were bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, and intraventricular hemorrhage. Red blood cell transfusions have been associated with the morbidities included in our composite outcome.[22-24] Bronchopulmonary dysplasia was defined as oxygen dependency for at least 28 days at 36 weeks of postmenstrual age.[25] The presence of retinopathy of prematurity (presence of extraretinal fibrovascular tissue on ophthalmological examination) of stage 3 or greater was recorded as an outcome. A diagnosis of necrotizing enterocolitis was based on stage 2 or greater using Bell criteria,[26] and a diagnosis of intraventricular hemorrhage was based on grade III or greater (blood in ventricles with evidence of ventricular enlargement) using Papile criteria.[27]

All relevant major morbidities comprising the primary outcomes that were present on the day of randomization were recorded. We monitored infants for up to 90 days of their stay in the neonatal intensive care unit to ascertain whether they met the threshold and definition for one of the complications included in the composite outcome. Individual complications had to occur after the point of randomization (the receipt of the initial transfusion) to be included as part of the primary outcome. We also assessed worsening of outcomes over the study duration. Individual elements of the composite outcome were adjudicated independently by 2 neonatologists blinded to the study group allocation.

As secondary outcomes, we recorded rates of individual complications comprising the composite outcome and rates of nosocomial infections. Nosocomial infections were categorized as clinically suspected and positive cultures. Clinically suspected infections implied that attending teams observed signs suggestive of infection. This information was derived from medical progress notes and included administration of antibiotics. We also recorded information on the site of infection and the organism identified by culture.

As tertiary outcomes, we examined length of mechanical ventilation and supplemental oxygen use, need for vasopressors, other blood products, and invasive vascular access as well as length of stay in the neonatal intensive care unit. Rates of minor and major interventions were also examined. Major interventions included all major surgical procedures such as laparotomies and thoracotomies. Minor interventions included laser therapy for retinopathy of prematurity, tracheostomy, endoscopic procedures such as bronchoscopy, and all transcutaneous procedures such as nephrostomy and cardiac catheterizations.

Figure 2. Distribution of Age of Red Blood Cell Transfusion Episodes in Fresh and Standard Groups



Sample Size and Statistical Analysis

We estimated that a total of 450 infants would be needed to detect a difference between groups, with a 2-tailed α of .05 and 80% power, for a comparison of 2 independent proportions if there was an absolute decrease of 15% in the composite outcome measure. Our initial estimate of sample size included an assumption of blood bank noncompliance of 10%. The data safety monitoring board, blinded to treatment group, reviewed 2 formal interim analyses and regular reports of our primary composite outcome as well as serious adverse events. With data safety monitoring board approval, we readjusted our sample size calculation to 372 infants after the first interim analy-

sis demonstrated an actual noncompliance rate of less than 4%.

Our primary analysis was conducted using an intention-to-treat approach and, therefore, included all randomized infants. Baseline characteristics of patients in the 2 treatment groups were reported using frequency distributions and descriptive statistics including measures of central tendency and dispersion. The principal analysis of our composite measure of major neonatal morbidities and mortality was an unadjusted χ^2 test comparing the proportion of events in each treatment group. Further logistic regression analyses examined the effect of adjustment for clinically relevant covariates that were known strong predictors of the outcome (sex, birth weight, gestational age) or that reflected imbalances at baseline. We measured the average storage using the age of each individual transfusion episode as well as weighting the average age by the volume of each transfusion episode. We conducted prespecified subgroup analyses by birth weight, gestational age, and neonatal acuity (Score for Neonatal Acute Physiology and Clinical Risk Index for Babies scores). A per-protocol analysis of infants adherent to their allocated treatment was also conducted to examine the robustness of our primary estimates. All analyses were conducted using SAS version 9.2 (SAS Institute Inc). All statistical tests were 2-sided and significance was set at $P<.05$.

RESULTS

Study Population

A total of 1752 neonates were screened for eligibility and 377 met eligibility criteria and were randomized between May 2006 and June 2011 (FIGURE 1). All randomized infants completed the trial follow-up (1 infant in each group had consent for intervention withdrawn). Fourteen patients randomized to the fresh RBC group (n=188) received at least 1 transfusion of RBCs stored for greater than 7 days. Specifically, 23 of the 151 RBC transfusions administered to the 14 infants were of RBCs stored greater than 7 days. Baseline demographic and clinical characteristics were similar in both groups, with the exception of more male infants allocated to the fresh RBC group (TABLE 1).

The mean age of blood in the fresh RBC group was 5.1 (SD, 2.0) days (median, 5 [interquartile range, 4.0-6.0] days) compared with 14.6 (SD, 8.3) days (median, 13 [interquartile range, 8.0-19.0] days) in the standard RBC group (FIGURE 2). The mean and median volumes transfused were similar in both groups, as were pretransfusion hemoglobin values (TABLE 2). The mean number of donor exposures in the fresh RBC group was 3.7 compared with 2.1 in the standard RBC group (Table 2). Postrandomization cointerventions including modes of ventilation, insertion of lines and catheters, other blood products, and major surgical and diagnostic procedures did not differ between the 2 groups. (TABLE 3)

Major Complications and Death

A total of 199 infants (53.0%) experienced our composite primary outcome of major complications or death. Among infants in the fresh RBC group, 99 (52.7%) had the primary outcome compared with 100 (52.9%) in the standard RBC group (relative risk, 1.00; 95% CI, 0.82-1.21) (TABLE 4). Ninety-seven infants representing 107 events of intraventricular hemorrhage (n=35 events), necrotizing enterocolitis (n=37 events), or retinopathy of prematurity (n=35

Table 2. Transfusion Data

	Standard Red Blood Cell Group (n = 189)	Fresh Red Blood Cell Group (n = 188)	Mean Difference (95% CI) or Wilcoxon *P* Value for Comparison of Medians
No. of donor exposures, mean (SD)	2.08 (1.64)	3.70 (2.70)	1.61 (1.16 to 2.07)
Age of red blood cells, d			
Mean (SD)	14.58 (8.26)	5.10 (2.05)	–9.48 (–10.02 to –8.93)
Median (IQR)	13.00 (8.00-19.00)	5.00 (4.00-6.00)	<.001
Weighted mean (SD)[a]	13.91 (5.65)	5.08 (1.07)	–8.83 (–9.61 to –8.05)
Weighted median (IQR)	14.00 (10.50-17.04)	5.00 (4.42-5.53)	<.001
Pretransfusion hemoglobin level, mean (SD), g/L	95.50 (11.41)	96.20 (11.30)	0.70 (–1.61 to 3.01)
Volume per transfusion episode, mean (SD), mL	14.18 (7.84)	14.05 (6.82)	–0.14 (–0.81 to 0.53)
Total volume of transfusion episodes, mL			
Mean (SD)	59.78 (42.60)	60.35 (40.54)	0.56 (–7.86 to 8.98)
Median (IQR)	51.00 (28.00-76.00)	57.00 (29.00-78.50)	.65
No. of transfusion episodes			
Mean (SD)	4.94 (3.88)	5.01 (4.00)	0.06 (–0.73 to 0.86)
Median (IQR)	4.00 (2.00-6.00)	4.00 (2.00-7.00)	.97
No. (%) with 1 to ≥10 episodes			
1	32 (16.93)	29 (15.43)	.53[b]
2	30 (15.87)	35 (18.62)	
3	24 (12.70)	17 (9.04)	
4	22 (11.64)	31 (16.50)	
5	19 (10.05)	17 (9.04)	
6	15 (7.94)	11 (5.85)	
7	9 (4.76)	12 (6.38)	
8	7 (3.70)	2 (1.06)	
9	6 (3.17)	4 (2.13)	
≥10	25 (13.23)	30 (15.96)	

Abbreviation: IQR, interquartile range.
[a]Weighted by volume of transfusion received.
[b]χ^2 *P* value.

Table 3. Postrandomization Cointerventions

	No. (%)		
	Standard Red Blood Cell Group (n = 189)	Fresh Red Blood Cell Group (n = 188)	Relative Risk (95% CI)
Cointerventions			
Supplemental oxygen	179 (94.7)	182 (96.8)	1.02 (0.98-1.07)
Nasal continuous positive airway pressure	152 (80.4)	149 (79.3)	0.99 (0.89-1.09)
Mechanical ventilation	153 (80.9)	151 (80.3)	0.99 (0.90-1.10)
High-frequency ventilation	74 (39.2)	75 (39.9)	1.02 (0.79-1.31)
Surfactant	35 (18.5)	33 (17.6)	0.95 (0.62-1.46)
Intravenous steroids	63 (33.3)	70 (37.2)	1.11 (0.85-1.46)
Antibiotics	170 (90.0)	171 (91.0)	1.01 (0.95-1.08)
Cardiovascular pressors	71 (37.6)	75 (39.9)	1.06 (0.82-1.36)
Peripheral intravenous line	157 (83.1)	158 (84.0)	1.01 (0.92-1.10)
Peripheral arterial line	49 (25.9)	42 (22.3)	0.86 (0.60-1.23)
Central venous line	12 (6.4)	11 (5.9)	0.92 (0.41-2.03)
Peripherally inserted central catheter	132 (69.8)	125 (66.5)	0.95 (0.83-1.09)
Umbilical vein catheter	75 (39.7)	80 (42.6)	1.07 (0.84-1.36)
Umbilical artery catheter	80 (42.3)	82 (43.6)	1.03 (0.81-1.29)
Albumin, 5%	26 (13.8)	31 (16.5)	1.19 (0.74-1.93)
Albumin, 25%	21 (11.1)	20 (10.6)	0.95 (0.53-1.70)
Freshly frozen plasma	19 (10.1)	20 (10.6)	1.05 (0.58-1.91)
Pentastarch	0	2 (1.1)	Not estimable
Platelets	39 (20.6)	41 (21.8)	1.05 (0.71-1.55)
Immunoglobulin	9 (4.8)	10 (5.3)	1.12 (0.46-2.69)
Surgical procedures			
Cardiothoracic surgery	38 (20.1)	48 (25.5)	1.27 (0.87-1.85)
Neurosurgery	0	1 (0.5)	Not estimable
Abdominal surgery	18 (9.5)	17 (9.0)	0.95 (0.51-1.79)
Laser eye surgery	17 (9.0)	23 (12.2)	1.36 (0.75-2.46)
Orthopedic/vascular surgery	1 (0.5)	1 (0.5)	1.01 (0.06-15.95)
Inguinal hernia repair	7 (3.7)	6 (3.2)	0.82 (0.30-2.52)
Central line insertion	2 (1.1)	2 (1.0)	1.01 (0.14-7.06)
Diagnostic procedures			
Laryngoscopy/bronchoscopy	0	1 (0.5)	Not estimable
Urologic	0	1 (0.5)	Not estimable

Table 4. Primary Outcomes

	No. (%)		
Primary Outcomes	Standard Red Blood Cell Group (n = 189)	Fresh Red Blood Cell Group (n = 188)	Relative Risk (95% CI)
Necrotizing enterocolitis (Bell criteria stage ≥2)	15 (7.9)	15 (8.0)	1.00 (0.48-2.12)
Intraventricular hemorrhage (Papile criteria grade ≥3)	11 (5.8)	18 (9.6)	1.65 (0.80-3.39)
Retinopathy of prematurity (stage ≥3)	26 (13.8)	23 (12.2)	0.89 (0.53-1.50)
Bronchopulmonary dysplasia	63 (33.3)	60 (31.9)	0.96 (0.72-1.28)
Death	31 (16.4)	30 (16.0)	0.97 (0.61-1.54)
Composite primary outcome: any of above	100 (52.9)	99 (52.7)	1.00 (0.82-1.21)

events) were adjudicated for presence and severity. Analysis of the individual components of our composite outcome did not identify any clinically significant difference between groups except for a statistically nonsignificant increase in rates of grade III to IV intraventricular hemorrhage in the fresh RBC group (relative risk, 1.65; 95% CI, 0.80-3.39). Analysis of a composite measure of patients experiencing any progression of intraventricular hemorrhage, necrotizing enterocolitis, or retinopathy of prematurity yielded a relative risk of 1.04 (95% CI, 0.89-1.22) and a relative risk of 1.01 (95% CI, 0.90-1.13) with the addition of bronchopulmonary dysplasia or death.

Infection and Length of Stay

A total of 292 infants (77.4%) had at least 1 clinically suspected infection, while 248 infants had at least 1 confirmed infection. The rate of clinically suspected infection in the fresh RBC group was 77.7% (n=146) vs 77.2% (n=146) in the standard RBC group (relative risk, 1.01; 95% CI, 0.90-1.12). Rates of confirmed infections were 67.5% (n=127) in the fresh RBC group vs 64.0% (n=121) in the standard RBC group (relative risk, 1.06; 95% CI, 0.91-1.22) (TABLE 5). Among confirmed cases, rates of bacterial, fungal, and viral infections were similar between the 2 groups. Major sequelae of infections including rates of pneumonia, meningitis, and osteomyelitis were also similar (Table 5). The median length of neonatal intensive care unit stay was 77 days (interquartile range, 50-104 days) in the standard RBC group and 84 days (interquartile range, 50-104 days) in the fresh RBC group (Wilcoxon $P=.55$).

Subgroup and Sensitivity Analyses

Prespecified subgroup analyses by birth weight, gestational age, and sex did not document any appreciable differences between the fresh and standard RBC groups (eTable 2). A per-protocol analysis did not alter the observed effect on our primary outcome (relative risk, 0.92; 95% CI, 0.61-1.40).

Transfusion-Associated Adverse Events

There were no transfusion reactions observed in either group. One serious adverse event potentially related to transfusion was a diagnosis of cytomegalovirus infection in an infant randomized to the standard RBC group.

COMMENT

Among critically ill premature infants, fresh RBC transfusions compared with standard RBC transfusion practice did not decrease or increase rates of complications or death in our composite measure. We did not find any clinically meaningful or statistically significant differences in individual complications, in secondary or tertiary outcomes, or in the prespecified subgroup analyses.

Premature infants with birth weights less than 1250 g represent a population frequently exposed to transfusions and extremely susceptible to complications and mortality. We considered these infants to be at high risk of complications from the adverse effects of older RBCs. With an immature circulation, limited physiologic reserve, immature immune responses, and enhanced susceptibility to oxygen damage, we would have expected to be able to find evidence of benefit if fresh RBCs had favorable biological properties.

Infants who participated in the ARIPI trial were exposed to a significant volume and frequency of RBC transfusions. Infants were given a mean of 5 transfusions (median, 4.0), each of 14 mL. Given an estimated total blood volume of 100 mL/kg,[28] this represents a significant transfusion exposure.

Only a few small studies have compared the consequences of RBC storage times.[29-31] None of these studies evaluated clinically important consequences and none were conducted in vulnerable premature infants. In an unblinded randomized trial, Gruenwald et al[32] compared the use of fresh reconstituted whole blood to standard blood products in 64 newborns undergoing cardiac surgery. The investigators documented that transfusion of fresh reconstituted whole blood decreased chest tube blood loss, improved bleeding scores, and shortened periods of ventilation and hospital lengths of stay. However, the role of fresh RBCs in this population remains unclear.

We did not find any clinically meaningful or statistically significant differences and, therefore, the many laboratory changes that occur with prolonged RBC storage may not be as important as once thought. Alternatively, a mean RBC storage time of 2 weeks in the standard RBC group may not have been sufficient to detect biological effects attributed to storage or clinically significant storage lesions occurring toward the end of the accepted RBC shelf life. Our choice of a 7-day threshold for fresh RBCs was based primarily on feasibility, as well as on limited laboratory evidence and precedence in other clinical studies rather than on a strong biological rationale. Similarly, our choice of standard-issue RBCs as a comparator was primarily based on ethical considerations. Choosing a specific

Table 5. Infection Outcomes

Outcome Measures	Standard Red Blood Cell Group (n = 189)	Fresh Red Blood Cell Group (n = 188)	Relative Risk (95% CI)
Clinically suspected infections			
No. (%) with ≥1 infection	146 (77.2)	146 (77.7)	1.01 (0.90 to 1.12)
No. of infections			
Mean (SD)	3.7 (4.1)	3.9 (4.3)	0.23 (–0.62 to 1.08)[a]
Median (IQR)	2.0 (1.0-5.0)	3.0 (1.0-6.0)	.65[b]
Culturally confirmed infections			
No. (%) with ≥1 infection	121 (64.0)	127 (67.5)	1.06 (0.91 to 1.22)
No. (%) with ≥1 infection confirmed in			
Blood	91 (48.1)	95 (50.5)	1.05 (0.86 to 1.29)
Central nervous system	12 (6.3)	11 (5.9)	0.92 (0.42 to 2.04)
Lungs	56 (29.6)	69 (36.7)	1.24 (0.93 to 1.65)
No. of infections			
Mean (SD)	2.5 (3.0)	2.6 (2.9)	0.1 (–0.50 to 0.70)[a]
Median (IQR)	2.0 (0.0-4.0)	1.0 (0.0-4.0)	.75[b]
Bacterial infections			
Any bacterial infection, No. (%)	121 (64.0)	123 (65.4)	1.02 (0.88 to 1.19)
No. (%) with ≥1 infection confirmed in			
Blood	91 (48.1)	93 (49.5)	1.03 (0.84 to 1.26)
Central nervous system	12 (6.3)	11 (5.9)	0.92 (0.42 to 2.04)
Lungs	56 (29.6)	68 (36.2)	1.22 (0.91 to 1.63)
No. of infections			
Mean (SD)	2.3 (2.8)	2.3 (2.6)	0.0 (–0.55 to 0.55)[a]
Median (IQR)	2.0 (0.0-4.0)	1.0 (0-4.0)	.98[b]
Viral infections			
Any viral infection, No. (%)	4 (2.1)	5 (2.7)	1.26 (0.34 to 4.61)
No. of infections			
Mean (SD)	0.03 (0.2)	0.04 (0.2)	0.01 (–0.03 to 0.05)[a]
Median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.73[b]
Fungal infections			
Any fungal infection, No. (%)	18 (9.5)	22 (11.7)	1.23 (0.68 to 2.22)
No. of infections			
Mean (SD)	0.2 (0.5)	0.2 (0.7)	0.0 (–0.12 to 0.12)[a]
Median (IQR)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.48[b]
Infection sequelae, No. (%)			
Pneumonia	21 (11.1)	19 (10.1)	0.91 (0.51 to 1.64)
Meningitis	1 (0.5)	4 (2.1)	4.02 (0.45 to 35.65)
Osteomyelitis	0	0	Not estimable

Abbreviation: IQR, interquartile range.
[a] Mean difference (95% CI).
[b] Wilcoxon *P* value.

range with much older RBCs would have more deliberately evaluated the clinical consequences of more extreme storage lesions but would have constituted a deviation from standard of practice and raised recruitment concerns given the lack of clinical benefit anticipated from transfusion of exclusively older RBCs. Very premature infants receiving multiple RBC transfusions with various storage ages present difficulties for analysis and interpretation because the biochemical, physiological, and clinical effects of interactions among the various storage times are not known. As such, exploratory analysis must compare patients in exclusive storage time categories (eg, infants with RBC transfusions only of blood stored <14 days vs infants with RBC transfusions only of blood stored ≥14 days). Unfortunately, there were too few infants (n=12) who received RBC transfusions only of blood stored longer than 14 days to permit meaningful analysis.

Our study population was limited to premature, very low-birth-weight infants who required at least 1 transfusion. Results may not be generalizable to more mature or less ill infants.

We tried to ensure that our choice of complications included in the composite primary outcome had a plausible biological relationship to outcomes. Three suggested mechanisms have been postulated to cause deleterious effects attributed to stored RBCs. First, several alterations in cell membranes and in depletion of 2,3-diphosphoglycerate adversely affect oxygen transport, thereby impairing oxygen delivery to target organs.[33-36] Second, older RBCs may induce a greater inflammatory response than fresh RBCs because of the buildup of RBC supernatant volume.[28,37,38] Finally, RBCs elicit an immunosuppressive effect in transfusion recipients. This immunomodulatory effect in critically ill or compromised infants may result in increased rates of nosocomial infections, in turn leading to organ dysfunction and death. Previous studies have focused on physiological and laboratory effects of the dedicated donor policy rather than clinically important outcomes.[39-42]

In conclusion, the transfusion of fresh RBCs did not improve clinical outcomes in high-risk, premature, very low-birth-weight infants. We thus do not recommend any changes to storage time practices for the provision of RBCs to infants admitted to neonatal intensive care.

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