Transfusion-Related Acute Gut Injury: Necrotizing Enterocolitis in Very Low Birth Weight Neonates after Packed Red Blood Cell Transfusion

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Objective This is a repeat cohort study in which we sought to determine whether an association of necrotizing enterocolitis (NEC) <48 hours of a packed red blood cells (PRBC) transfusion was a prior sampling artifact.

Study design All very low birth weight neonates with NEC Stage ≥IIB admitted over an 18-month period were categorized for NEC: (1) <48 hours after a PRBC transfusion; (2) unrelated to the timing of PRBCs; and (3) never transfused.

Results Eight hundred eighty-three admissions over 18 months were reviewed; 256 were very low birth weight that resulted in 36 NEC cases and 25% were associated with PRBC (n = 9). PRBC-associated cases had lower birth weight, hematocrit, and rapid onset of signs (<5 hours). The timing of association of PRBC transfusion and NEC differed from random, showing a distribution that was not uniform over time ($\chi^2 = 170.7$, df = 40; $P < .000001$) consistent with the possibility of a causative relationship in certain cases of NEC. Current weight at onset of NEC did not differ; however, the more immature the neonate the later the onset of NEC creating a curious centering of occurrence at a median of 31 weeks postconceptual age.

Conclusions We conclude that PRBC-related NEC exists. Transfusion-related acute gut injury is an acronym we propose to characterize a severe neonatal gastrointestinal reaction proximal to a transfusion of PRBCs for anemia. The convergence at 31 weeks postconceptual age approximates the age of presentation of other O_2 delivery and neovascularization syndromes, suggesting a link to a generalized systemic maturational mechanism. *(J Pediatr 2011;158:403-9)*.

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Necrotizing enterocolitis (NEC) occurs in approximately 10% of the very low birth weight (VLBW) population and is considered a multifactorial disorder converging on a common final clinical presentation associated with several etiologic mechanisms, including ischemia (eg, reperfusion), infection (eg, gut colonization), mechanical injury (eg, viscosity, embolic), iatrogenic factors (eg, catheters, excessive enteral feeding), and immunological barrier dysfunction.[1-4] To date, there is no single, unifying consensus on causation.[5]

In 2006, our institution reported a temporal association between packed red blood cell (PRBC) transfusions and the development of NEC in VLBW neonates.[6] This report found that 35% of patients with NEC developed disease within 22 ± 5 hours of receiving a "booster" PRBC transfusion for anemia; an event that occurred >28 days postnatal age in low acuity convalescing neonates—well beyond the classic period of risk factors.[1-3] Clinical features of that subset of patients with NEC differed from classic presentations showing later postnatal onset, lower birth weight, less acuity of current illness, and more significant anemia than those VLBW patients who had NEC unrelated to timing of transfusion.[6]

The phenomenon of transfusion-associated gut injury might well be the neonatal manifestation of the well-characterized transfusion-associated acute lung injury (TRALI), the leading cause of transfusion-related death in adults.[7] In the current report, transfusion-related acute gut injury (TRAGI) is the acronym we propose to characterize severe gastrointestinal reactions in premature neonates after an infusion of PRBCs.

To determine whether our prior report was a sampling error, we sought to replicate the review using another cohort of VLBW patients. We hypothesized that a late-onset form of NEC may involve injury occurring shortly after a blood transfusion.[6]

NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PRBC	Packed red blood cells
PDA	Patent ductus arteriosus
TH_1, TH_2	T-helper cell type (type 1, type 2)
TRAGI	Transfusion-related acute gut injury
TRALI	Transfusion-related acute lung injury
VLBW	Very low birth weight

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Methods

This review of practice outcomes was Health Insurance Portability and Accountability Act compliant and approved by the New York Medical College Institutional Review Board. All VLBW admissions whether inborn or transferred to our regional neonatal intensive care unit (NICU) over an 18-month period from January 2007 to June 2008 were reviewed for the diagnosis of NEC and its relationship to the timing of PRBC transfusion.

Fluids and nutrition were provided according to guidelines for feeding and fluid management.[8] Strategies to ensure similar clinical practices such as treatment of symptomatic Patent ductus arteriosus (PDA) with ibuprofen,[9] diagnosis and management of sepsis, and use of permissive hypercapnia through judicious application of ventilatory pressure support to minimize volutrauma to immature lungs were followed as we and others have described.[10]

All patients who received PRBC transfusions were given irradiated, leukoreduced, PRBCs stored in Adsol-3 storage media (15 mL/kg PRBC transfusion provides: sodium chloride 7.5 mg/kg, dextrose 23 mg/kg, adenine 0.6 mg/kg, citrate 12.6 mg/kg, phosphate 5.6 mg/kg; New York Blood Center policy). PRBC transfusions were initiated within 30 minutes of release from the blood bank and infused intravenously via a peripheral catheter over no longer than 4 hours. No other intravenous solutions were administered simultaneously with PRBCs through the same peripheral intravenous port. To limit donor exposure, PRBC transfusions were aliquoted from a single red cell unit dedicated to a single patient for the shelf life of that product (maximum of 42 days from collection).

Indications for "booster" transfusion included symptomatic anemia (failure to gain weight [10 to 15 g/kg/d over 7 days], apnea >5 to 6 episodes per 8-hour interval, lethargy, unexplained tachycardia, or tachypnea) or failure to observe an increase in reticulocyte counts above 5% at or below a hematocrit of 25%.[6]

Gestational age was based on the best estimate of the attending neonatologist using obstetrical parameters and physical examination. A diagnosis of NEC was given if the neonate developed symptoms consistent with NEC (eg, abdominal distention, emesis, bloody stools) and there was evidence of pneumatosis intestinalis on abdominal radiograph, Bell stage ≥IIb, or surgical confirmation.

Table I. Maternal and neonatal characteristics of study patients before NEC

	TRAGI (n = 9)	Non–transfusion-related NEC (n = 15)	Never-transfused NEC (n = 12)
Birth weight (g)	770 ± 57*	815 ± 67	1114 ± 62
	748; 590-1130	696; 550-1370	1098; 750-1370
Gestational age (wk)	26 ± 0†	27 ± 1	29 ± 1
	25; 24-28	26; 23-36	30; 27-33
Female sex	78% (7/9)	40% (6/15)	33% (4/12)
Small for gestational age	11% (1/9)	33% (5/15)	25% (3/12)
Maternal age (y)	29 ± 3	31 ± 2	30 ± 2
	27; 17-47	34; 18-39	34; 17-40
Gravida	2 ± 0	2 ± 0	2 ± 0
	2; 1-5	2; 1-5	2; 1-4
Preeclampsia toxemia	0% (0/9)	7% (1/15)	33% (4/12)
Cesarean section	44% (4/9)	60% (9/15)	75% (9/12)
Outborn during onset of NEC	11% (1/9)	13% (2/15)	33% (4/12)
Apgar score at 5 min	7 (4-9)	7 (3-9)	8 (2-9)
Delivery room Resuscitation	89% (8/9)	67% (10/15)	58% (7/12)
Patent ductus arteriosus >2 wk before NEC	89% (8/9)	60% (9/15)	25% (3/12)‡
Ibuprofen treatment >2 wk before NEC	67% (6/9)	53% (8/15)	25% (3/12)
Positive blood culture at onset of NEC	22% (2/9)	7% (1/15)	17% (2/12)
% Nutrition provided as enteral feeds at onset of NEC	67 ± 17	67 ± 12	88 ± 9
	100; 0-100	100; 0-100	100; 0-100
Receiving only enteral feeds at onset of NEC	67% (6/9)	60% (9/15)	83% (10/12)
Receiving only TPN at onset of NEC	33% (3/9)	27% (4/15)	8% (1/12)
Receiving both enteral feeds and total parenteral nutrition at onset of NEC	0% (0/9)	13% (2/15)	8% (1/12)
Central lines at onset of NEC	33% (3/9)	40% (6/15)	17% (2/12)
Ventilator dependence at onset of NEC	44% (4/9)	40% (6/15)	0% (0/12)†
Receiving oxygen at onset of NEC	89% (8/9)	87% (13/15)	42% (5/12)‡
Nasal cannula at onset of NEC	22% (2/9)	27% (4/15)	17% (2/12)
Nasal continuous positive airway pressure at onset of NEC	11% (1/9)	20% (3/15)	17% (2/12)
Peak inspiratory pressure at onset of NEC	20 ± 3	17 ± 2	None intubated
	20; 12-28	18; 12-22	
% Oxygen at onset of NEC	36 ± 4	34 ± 6	22 ± 0
	35; 21-58	25; 21-100	21; 21-25

Data are reported as mean ± standard error of the mean, median; range, or % (n).
*$P < .004$ versus other two groups.
†$P < .04$ versus other two groups.
‡$P < .02$ versus other two groups.

Exclusively breast-fed neonates were defined as those patients receiving greater than 80% of all enteral feeds from expressed breast milk. Hyaline membrane disease required ground-glass appearance of the lungs on radiograph, grunting, retracting, and an elevated oxygen requirement to keep oximeter saturations ≥88% to 90%, chronic lung disease as oxygen requirement at 36 weeks corrected gestational age, apnea if respirations ceased for >20 seconds and were associated with a fall in oximeter values <90% with a heart rate <100 beats per minute according to bedside monitoring records, and sepsis was diagnosed if the patient received antibiotics for >5 days and had a culture of body fluid from a sterile compartment that grew bacteria. Retinopathy of prematurity was diagnosed using AAP guidelines.[11] The age of transfused blood was the number of days since the donor gave blood until an individual patient received that product during the most recent transfusion before the development of NEC.

Patients were divided into three groups: (1) transfusion-associated NEC (history of PRBC transfusion 48 hours before the onset of symptoms of NEC); (2) non–transfusion-associated NEC (PRBC transfusion >48 hours before the onset of symptoms of NEC); and (3) NEC, but never having received a transfusion before the onset of abdominal signs. Our cutoff of 48 hours was based on our prior observation of a temporal association between PRBC transfusion and the development of NEC.[6]

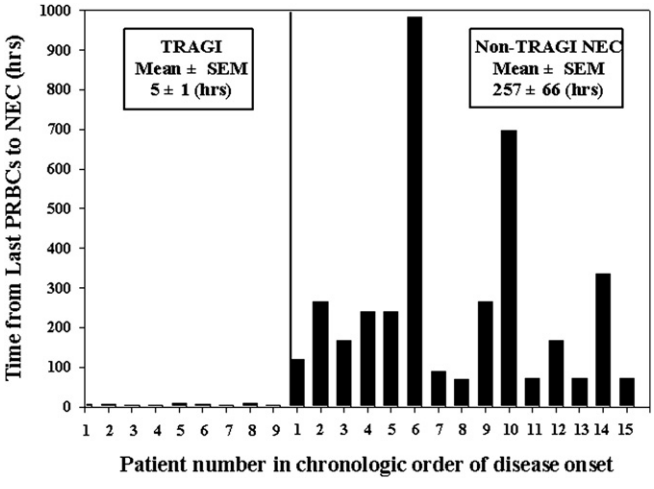

Figure 1. The temporal onset of NEC differs between the two groups. Patients with transfusion-related NEC (left panel; n = 9) show little variability in timing of presentation occurring within a maximum of 9 hours of presentation. The neonates with non-transfusion-related NEC (right; n = 15) are also organized chronologically and show no temporal relationship relative to prior transfusions. We also observed a third group of patients with NEC who never received an antecedent PRBC transfusion (n = 12). There was a maximum of 4 cases in 1 month and 2 months with no cases during the 18-month study period.

Statistical Analysis

Demographic data were compared between the three groups using ANOVA followed by the Student Newman-Keuls post hoc analysis or χ^2 analysis for dichotomous variables. Goodness-of-fit tests were done using the exact χ^2 test (StatXact, v7; Cytel Inc, Cambridge, Massachusetts). A value of $P < .05$ was considered statistically significant for the primary hypothesis, and a Bonferroni correction for up to 10 comparisons on secondary clinical correlates between the groups would require a value of $P < .005$.

Results

Of 883 regional NICU admissions originating from a catchment area of 5000 square miles, 23 000 annual births, six level II and three level III affiliates, 256 (29%) cases were VLBW neonates in whom a diagnosis of NEC occurred in 14% of this population. These neonates included 7 outborn patients (19%) transferred for surgical consultation. All transfused patients met our center's requirements for receiving PRBC transfusion. Twenty-five percent of all NEC cases (n = 9) developed clinical signs <48 hours of a PRBC transfusion, and 75% (n = 27) developed disease either unrelated to timing of transfusion (n = 15) or were never previously transfused (n = 12; **Table I**). Erythropoietin was not used in any patient.

Relation to Transfusion

To ascertain whether the timing of association of PRBC transfusion and NEC differed from random, we reasoned that if there was no relation between NEC and blood transfusions, then occurrences of NEC would have been expected to occur uniformly with time after transfusion. However, the actual distribution of NEC after transfusion (**Figure 1**) differed significantly from uniform ($\chi^2 = 170.7$, df = 40; $P < .000001$), consistent with the possibility of a causative relationship in some cases of NEC.

Clinical Correlates

We found no differences in 5-minute Apgar scores, delivery room resuscitation, ibuprofen treatment, positive blood cultures, use of total parenteral nutrition, or central lines or number of apneic episodes 48 hours before the onset of NEC. We did observe fewer patients with a dependence on mechanical ventilation and supplemental oxygen in patients with NEC who were never transfused (**Tables I** and **II**). Prophylactic ibuprofen and postnatal steroids were not used and no cases of idiopathic spontaneous intestinal perforation occurred. Once the diagnosis of NEC was established, the clinical course did not differ between the two groups (**Table II**). This suggested that the NEC after TRAGI did not represent a novel clinical entity distinct from non-TRAGI disease or from NEC found unrelated to transfusion.

TRAGI neonates also had significantly lower hematocrit and lower birth weights but similar current weight and postnatal age at onset of NEC <5 hours after the transfusion event (**Figure 1** and **Tables I** and **II**).[6] The number of transfusions, single donor exposure, and age of transfused blood did not

Table II. Characteristics at presentation of clinical signs of stage IIb NEC

	TRAGI (n = 9)	Non–transfusion-related NEC (n = 15)	Never-transfused NEC (n = 12)
Weight at onset of NEC (g)	1089 ± 98 1160; 660-1560	1210 ± 76 1210; 790-1730	1168 ± 59 1178; 740-1530
Postnatal age at onset of NEC (d)	30 ± 5 39; 4-46	34 ± 3 32; 18-58	14 ± 2* 15; 6-24
Postconceptual age at onset of NEC (wk)	30 ± 1 31; 25-33	32 ± 1 32; 27-39	31 ± 1 31; 28-34
Hematocrit before NEC	26 ± 2† 25; 20-33	33 ± 2 32; 24-46	38 ± 3 38; 23-58
Number of PRBC transfusions before NEC	5 ± 1 5; 2-12	7 ± 1 5; 1-22	N/A
Hours from most recent transfusion to onset of NEC	5 ± 1‡ 6; 3-9	257 ± 66 168; 70-984	N/A
Single donor blood	100% (9/9)	100% (15/15)	N/A
Age of blood (d)	10 ± 3 6; 1-31	13 ± 2 12; 6-31	N/A
Made NPO during transfusion§	83% (5/6)	69% (9/13)	N/A
Breast milk as enteral feeds 48 h before most recent transfusion	17% (1/6)	45% (5/11)	33% (4/12)
Respiratory deterioration 24 h before NEC onset	0% (0/9)	7% (1/15)	0% (0/12)
PIP 24 h after onset of NEC (cm H_2O)	29 ± 5 25; 18-60	26 ± 3 24; 18-44	26 ± 3 21; 16-42
% Oxygen 24 h after onset of NEC	56 ± 11 40; 25-100	56 ± 9 40; 21-100	54 ± 11 30; 21-100
Bloody stools	67% (6/9)	53% (8/15)	25% (3/12)
Emesis	11% (1/9)	7% (1/15)	25% (3/12)
Abdominal distention	67% (6/9)	60% (9/15)	58% (7/12)
Free air	22% (2/9)	40% (6/15)	50% (6/12)
Surgery	33% (3/9)	47% (7/15)	50% (6/12)
Death	22% (2/9)	47% (7/15)	42% (5/12)

NPO, nothing by mouth; *N/A*, not applicable.
Data are reported as mean ± standard error of the mean, median; range.
*$P < .004$ versus other two groups.
†$P < .006$ versus other two groups.
‡$P < .008$.
§If feeding, made NPO 2 to 4 hours before, during, and 2 to 4 hours after the most recent transfusion.

differ between patients with NEC and TRAGI and those without TRAGI (Table II). All groups developed NEC at a median postconceptual age of 31 weeks (Table II and Figure 2).

In the TRAGI group, 83% of cases were kept NPO 2 to 4 hours before, during, and 2 to 4 hours after PRBC transfusion compared with 69% in the non-TRAGI group (P = NS, Table II). No differences were noted for use of breast milk versus formula (Table II), or percentage of maximum feeds at onset of disease (Table I).

Discussion

We concluded that our previous observation of a temporal association (<48 hours) between PRBC transfusion and the development of NEC in VLBW neonates representing 25% of all NEC cases persists in our service.[6] Affected neonates were significantly more anemic than nonaffected or patients with NEC who had never received a transfusion, were lower birth weight, were older, and had a higher prevalence of treated PDA >2 weeks before onset of signs. In contrast to our previous report, NEC occurred even sooner after transfusion (average of 5 versus 22 hours), and the antecedent severity of illness, though still relatively mild, was no longer just stable growing VLBWs.[6] We acknowledge that other centers may not experience this phenomenon, or at least not to the same degree. This phenomenon may be limited to centers with similar patient populations and transfusion practices as ours.

The rapidity of onset of NEC (<5 hours) after PRBC transfusion is of serious concern and suggests a causal relationship based on: (1) the clustering of cases occurring shortly after transfusion; (2) an analogous pattern of lung injury after PRBC known as TRALI; and (3) previously characterized pathophysiologic processes that might underlie this phenomenon in patients with antecedent risks.

The most widely accepted theory to explain the pathogenesis of TRALI is a "two hit" model in which host neutrophils are "primed" by an antecedent illness followed by the passive transfusion of biological response mediators.[7,12,13] Biological response mediators are either donor antibodies (eg, HLA or neutrophil antigen) or another class of activators such as bioactive lipids, free hemoglobin, red blood cell membrane fragments, cytokines, and so forth, accumulated in stored blood products. By analogy to the lung, as the body's largest immune organ, the naïve neonatal gut mucosa, is rich in leukocytes and neutrophils, and, on first exposure to gut flora or nutrient antigens after birth, is prone to exaggerated immune responses.[1,14] This immunological conversion from a TH_2 to TH_1 phenotype involving primed neutrophils,[1,15] offers a credible explanation for the apparent predilection of this clinical entity in VLBWs compared with TRALI in more

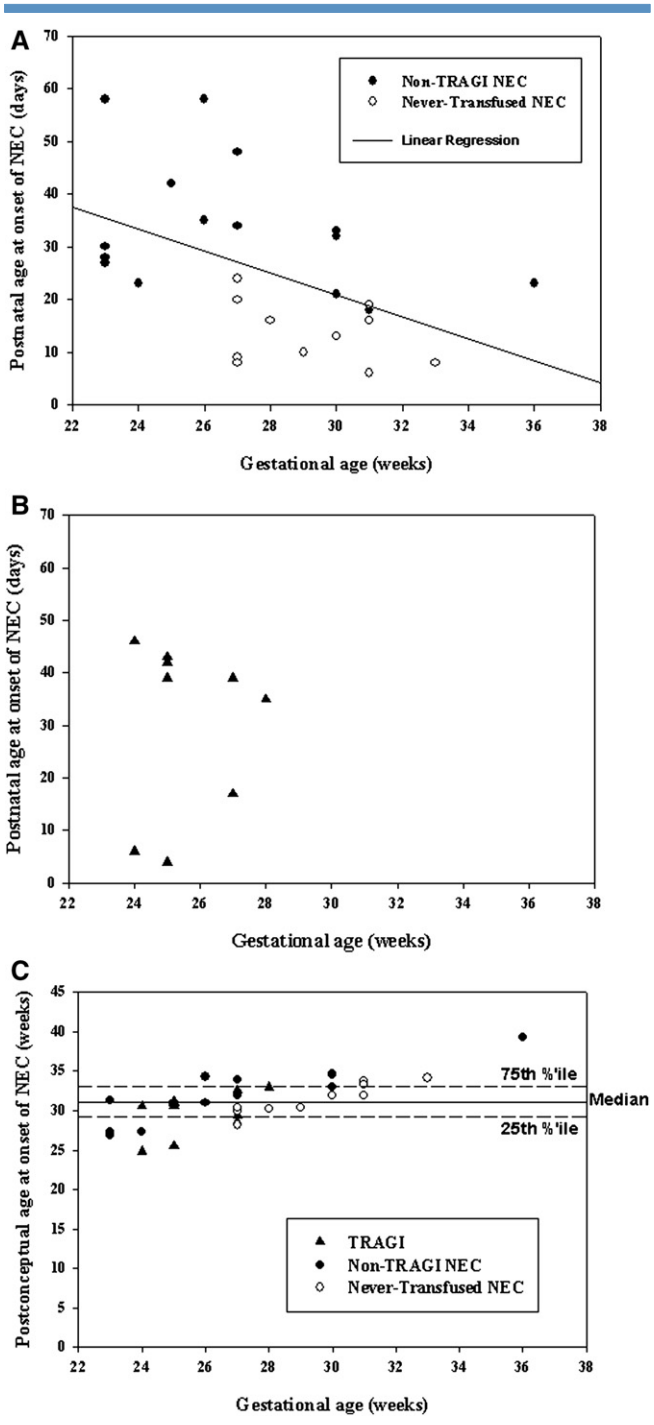

Figure 2. **A,** The earlier the gestational age, the later the onset of NEC in cases not associated with PRBC transfusions (n = 27; linear regression: $r = 0.47$; $P < .01$ for $\log_{10}$-transformed postnatal age versus gestational age). **B,** Cases with TRAGI showed no correlation with postnatal age. **C,** In the combined groups, NEC occurs at a median postconceptual age of 31 weeks (31 ± 0.5, 25 to 39 weeks; mean ± standard error of the mean, range) independent of gestational age or other attributed factors.

mature patients. To determine whether this analogy held true, we sought evidence of a prior published relationship between transfusions and NEC.

At least one anecdotal report documented the occurrence of NEC shortly after PRBC transfusion in a 3-week-old extremely low birth weight neonate; however, the limited evidence and method of analysis did not support a direct causal link.[16] McGrady et al[17] described an outbreak of NEC in which cases were significantly more likely to have had a history of PRBC transfusion; but precise relational timing was not reported. Similarly, Valieva et al[18] found an association between more frequent transfusions within 96 hours of NEC. In a review of clinician practice patterns, Bednarek et al[19] showed that NICUs with more liberal transfusion practices had a higher risk-adjusted occurrence of NEC, and, more recently, two reports by Christiansen et al[20] found that approximately one-third of surgical NEC cases as well as 38% of NEC Bell stage III cases[21] were temporally associated with PRBC transfusion. In the only large-scale prospective trials, the PINT study showed a nonsignificant trend for fewer NEC cases in the liberal transfusion group, but the occurrence of NEC was not discussed in a report by Bell et al.[22,23]

Could anemia per se have been the cause of NEC? Animal models demonstrate that anemia can impair gut blood flow and increase oxygen extraction as a compensatory mechanism,[24,25] yet, no compelling clinical evidence exists linking anemia as a causal factor to NEC. The TRAGI cases in this and our previous report were hemodynamically stable, and neither exhibited signs or symptoms nor laboratory evidence suggesting impaired cardiac output, ischemia, or hypoxemia before the occurrence of NEC. Whether transfusions at low hematocrits are more dangerous than those given for less moderate anemia represents the basis of a first "hit" in a neonatal two-hit interaction can only be answered by prospective studies of outcomes as they relate to differing transfusion thresholds. Nevertheless, there are a variety of other putative and credible mechanisms to consider in this association in our patients.

Preterm infants receiving a blood transfusion in the presence of a significant PDA show a reduction in mesenteric flow 4 hours after transfusion, an effect proportional to the volume of packed cell transfusate;[26] however, none of our patients with TRAGI had an untreated PDA at the time of transfusion. This negative finding does not preclude all vascular responsive issues because transfusions per se are associated with a transient reduction in mean flow velocity, an increase in viscosity, and a lower fractional extraction of oxygen.[27-29]

Feeding may affect oxygen balance near the critical O_2 point[8] by contributing to reduced oxygen availability arising from red blood cell deficits in 2,3 DPG, abnormal red blood cell rheology, and changes in nitric oxide availability.[7,30] Most patients in our TRAGI group (83%) were not fed for 2 to 4 hours before or during the transfusions; their treatment in this respect, did not differ from the non-TRAGI (but previously transfused) group.

Krimmel et al[24] showed that if bolus feedings were preceded by blood transfusions, only anemic neonates weighing

<1250 grams failed to develop a postprandial rise in mesenteric artery blood flow velocity; all of our cases were below this weight. In related work, Szabo et al[25] showed that bolus-fed hypoxemic piglets had reduced postprandial gastric blood flow. In contrast to these two reports, the majority of our subjects were previously being fed formula as a continuous (not bolus) infusion in both groups when signs of NEC presented, thereby abrogating feeding mode as a causal relationship. Whether breast milk would mollify our observed risk-relationships could only be inferred from the report by Christensen.[20] Taken together, the issue of whether to feed during PRBC transfusions is unresolved.[31]

Blood transfusions alter the maturation pattern of T-cells 10 to 14 days later,[32,33] allowing for either the possibility of sensitization from repeated exposure to small quantities of allogeneic white blood cells from the same donor blood or perhaps also accounting for the later postnatal age of onset of TRAGI compared with never transfused cases (Table II). Although cytokine levels in leukoreduced transfused patients do not differ from controls,[34] the supernatant from stored red blood cells can "prime" unstimulated allogeneic PMNs in vitro and induce expression of CD11b, release of IL-8, and alter chemotactic properties.[34,35] Because all of our subjects had at least one prior transfusion from the same donor, the possibility of an inducible immunomodulatory effect remains.[33,36,37]

Wang-Rodriguez[32] reported anti-HLA antibodies in extremely low birth weight neonates who were transfused and developed NEC. In one TRAGI case who died, we found no anti-HLA or anti-neutrophil antibodies in the donor blood, suggesting that other biological response mediators existed in the transfused product. For example, another mechanistic consideration in our patients would invoke the presence of free hemoglobin (hemolysis of the transfusate) interacting with an additional humoral mediator introduced via serum similar to reports of intravenous immunoglobulin G given to prevent hemolytic disease that later resulted in NEC; none of our subjects had washed PRBCs.[38,39]

Last, we noted that all cases of NEC occurred coincident with a median postconceptual age of 31 weeks, a time when other conditions associated with neovascularization and oxygen toxicity begin as a clinical issue. This suggests an intriguing hypothesis that expression of angiogenic factors in the anemic gut vascular bed may be subject to similar developmental irregularities, as is vascular endothelial growth factor expression in the retina or with anemia.[40] Certain gut flora (ie, *Bacteroides thetaiotaomicron*) are already known to affect gut vascularization by inducing production of angiogenins (ie, Ang4) from Paneth cells.[41,42]

In summary, we again observed a temporal association between PRBC transfusion and the development of NEC in moderately anemic VLBW neonates, a phenomenon that is now corroborated in other reports[20,21] and more compellingly, by the existence of TRALI, an analogous phenomenon in older patients. We believe that our data are primarily useful for hypothesis-generating purposes, as a much larger sample size is necessary to decisively link one or more of the proposed mechanisms we reviewed. The interplay between the contribution of anemia per se and transfusion in TRAGI can only be assessed in humans by a trial that provides an unbiased comparison between relevant risk factors and different hematocrit thresholds for transfusion. ■

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