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Anemia, Red Blood Cell Transfusions, and Necrotizing Enterocolitis

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Abstract

In the past 15 years, multiple clinical studies have identified a temporal association between red blood cell (RBC) transfusions and necrotizing enterocolitis (NEC). With some variability, most of these studies indicate that up to one-third of all cases of NEC involving very-low-birth weight infants may occur within 24–48 hours after receiving a RBC transfusion. There is also evidence that the risk of such transfusion-associated NEC may be higher in infants transfused with the greatest severity of anemia. In this article, we summarize the clinical evidence pertaining to these issues; specifically, the contribution of RBC transfusions, and the contribution of severity of underlying anemia, to the pathogenesis of a type of NEC potentially termed, “Transfusion/Anemia-associated NEC”.

Keywords

NEC; anemia; transfusion; RBC; intestinal injury

Necrotizing enterocolitis is a leading cause of death among extremely premature infants with case-fatality rates of 20–30%.¹ These infants also are a heavily-transfused population, and in

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recent years, there has been renewed interest in potential adverse events following the administration of blood products, particularly in the context of the reported association between RBC transfusions and necrotizing enterocolitis (NEC).^{2, 3} In the last 15 years, several case reports and retrospective studies show that up to one-third of all VLBW infants who develop NEC may have received one or more RBC transfusions in the 24–72 hours prior to onset of NEC.^{4–26} However, two prospective studies^{12, 27} have not shown such an association between RBC transfusion and NEC; one of these two studies highlighted that it may be the severity of the underlying anemia, not the transfusion event, that may increase the risk of NEC.¹² In the following sections, we review current evidence and the biological plausibility of these associations. A literature search was performed using the databases PubMed, EMBASE, and Scopus. To avoid bias in identification of existing studies, key words were carefully short-listed prior to the actual search from anecdotal experience and from PubMed's Medical Subject Heading (MeSH) thesaurus.

The association between RBC transfusions and NEC in premature infants

There is increasing evidence to support this association, although the issue of causality remains unresolved. McGrady *et al.*²⁸ were the first to notice the temporal association between RBC transfusions and NEC. They investigated an outbreak of 33 cases of NEC in their neonatal intensive care unit (NICU) in 1987 and found increased risk of NEC following transfusions [odds ratio (OR) 15.1, 95% confidence interval (CI) 2.59–92.51]. In subsequent years, this temporal association was noted again in a few case reports.^{29, 30} Bednarek *et al.*³¹ also found some supportive evidence; while comparing transfusion practices in 6 NICUs, they noted that NICUs with fewer transfusions had a lower incidence of NEC (OR 0.3, CI 0.1–0.8) than the NICUs that transfused premature infants more frequently (OR 1.1, CI 0.5–2.2).

In recent years, the association between RBC transfusions and NEC has received significant investigative attention. Since 2006, several retrospective studies^{5, 11, 13–16, 21, 23, 25, 26, 32–38} have consistently found this association with several common findings: (a) 25–40% of all cases developed NEC 2–48 hours after having received a RBC transfusion;^{5, 11, 13, 21, 23, 25, 26, 28, 31, 33–38} (b) neonates with transfusion-associated NEC were more premature and had a lower birth weight than those who develop NEC unrelated to transfusion;^{5, 11, 13, 21, 23, 25, 26, 28, 31, 33–38} (c) transfusion-associated NEC has a delayed onset, usually beyond 4 weeks of postnatal age;^{5, 13, 21, 23, 25, 26, 28, 31, 33–38} (d) neonates with transfusion-associated NEC may have had one or more previous RBC transfusions;^{5, 25} and (e) the risk of NEC may have been higher in infants who were severely anemic before the transfusion.²¹ Most of these studies did not find an effect of the storage age of RBCs.^{5, 13, 25} There was conflicting evidence on the severity of illness in infants who developed NEC following RBC transfusions; Mally²³ reported NEC in relatively-well, convalescing infants, whereas others found higher risk of transfusion-associated NEC in infants who had a patent ductus arteriosus (OR 2.68, CI 1.81–3.97)^{6, 13, 23, 26, 33, 37} and/or were on respiratory support at NEC onset (OR 3.16, CI 1.60–6.22).^{6, 11, 13, 23, 26, 33}

Three studies have reported findings conflicting with the aforementioned studies. Harsono *et al.*³⁹ found that RBC transfusions were actually protective against NEC. In a retrospective

review of the medical records of 2123 infants, they asked whether VLBW infants who receive RBC transfusions beyond 28 days were at increased risk of NEC in a 48-hour period following the transfusion. In their cohort, 43 (2%) infants developed NEC, and 26 of these 43 infants (60%) developed NEC within 48 hours after a transfusion. After controlling for birth weight, gender, and a history of umbilical artery catheter insertion, they found that RBC transfusions had a protective effect against NEC (OR= 0.30; 95% CI: 0.15–0.60). The authors posited that this protective effect of RBC transfusions may be mediated through the correction of tissue hypoxia related to chronic anemia. In a prospective case-control study, Sharma *et al.*²⁷ compared 42 NEC cases with matched, equally-transfused controls and did not find an association of transfusion with NEC in either a 48-hour or 7-day time period before the onset of NEC. In a recent, prospective study, Patel *et al.*¹² analyzed a multicenter cohort of 598 VLBW infants. They evaluated exposure to RBC transfusion in a given week and found no association between RBC transfusion and the subsequent development of NEC (adjusted cause-specific hazard ratio 0.44, 95% CI: 0.17–1.12). In contrast, the study reported that severe anemia (defined as a hemoglobin < 8 g/dL) in a given week was associated with a higher risk of NEC (adjusted cause-specific hazard ratio 5.99, 95% CI 2.00–18.0).

Mohamed and Shah⁶ performed meta-analysis of observational data from studies on transfusion-associated NEC and found increased odds of NEC within 48 hours after receiving a RBC transfusion. Meta-analysis of 5 studies^{5, 11, 21, 25, 32} showed increased odds of NEC after recent transfusion (OR 3.91, CI: 2.97–5.14). Meta-analysis of 4 of these studies^{25, 37–39} that reported adjusted estimates revealed a similar but lower risk of NEC (pooled adjusted OR 2.01, CI: 1.61–2.50). A more recent meta-analysis of 13 studies found no association between RBC transfusion and NEC occurring within 48 hours of transfusion (OR 1.13, 95% CI: 0.99–1.29) with high statistical heterogeneity among studies ($I^2=93%$).⁴⁰ The differences between these two meta-analyses may be due to a positive-result or publication bias, with earlier studies all reporting an association between RBC transfusion and NEC vs. the lack of such an association in the more recent ones. In addition, a meta-analysis of randomized trials comparing restrictive vs. liberal RBC transfusion strategies in preterm infants found no effect of more RBC transfusions (higher hemoglobin transfusion thresholds), compared to fewer RBC transfusions, on the risk of NEC (OR 0.6, CI 0.3–1.21).⁴⁰ The pooled point-estimate of effect from these randomized trials are similar to the point-estimates of studies by Patel *et al.*¹² and Sharma *et al.*,²⁷ with each study favoring a lower risk of NEC associated with exposure to RBC transfusion.

Effects of RBC transfusions and anemia on gut perfusion and injury

In premature infants, both anemia and RBC transfusions can theoretically alter intestinal perfusion and cause/augment injury. Plausible mechanisms include immaturity of the splanchnic vascular bed, re-oxygenation injury in the anemic gut, and immune mechanisms similar to those seen in transfusion-related lung injury (TRALI). Current clinical evidence does not support a major contribution of the storage age of transfused RBCs to NEC,^{5, 13, 25} but older RBCs could accentuate microvascular ischemia and tissue hypoxia.

Host immaturity

The immaturity of the vascular autoregulatory responses in the neonatal gastrointestinal tract is well-known, and could potentially place the infant at increased risk of gut mucosal injury. Szabo *et al.*⁴¹ showed that hypoxia impaired the normal post-prandial hyperemic response and decreased the gut oxygen delivery in newborn piglets. Krimmel and coworkers⁴² showed that RBC transfusions can dampen the normal postprandial increase in mesenteric blood flow in premature infants with a birth weight <1250 grams. In another study, Gupta *et al.*⁴³ demonstrated that RBC transfusions reduced the mesenteric arterial blood flow in infants with a patent *ductus arteriosus* 4 hours after the transfusion. Blau *et al.*²⁶ suggest that NEC may peak at the post-menstrual age of 31–32 weeks^{44, 45} because of a developmental susceptibility to neovascularization and oxygen toxicity during this period, possibly related to increased expression of angiogenic factors in the anemic gastrointestinal tract.⁴⁶

Anemia

Anemia can impair splanchnic perfusion,⁴¹ resulting in tissue hypoxia, anaerobic metabolism, and accumulation of its by-products such as lactic acid.⁴⁷ Anemia can also impair the normal maturation of vascular autoregulation in the premature intestine,⁴⁸ predisposing to ischemic injury, and possibly, NEC.⁴⁷

Singh *et al.*²¹ reviewed the medical records of 111 preterm infants with confirmed NEC and 222 matched controls, and found lower hematocrit to be associated with NEC (OR 1.10, $p=0.01$) in multivariate models. They showed that RBC transfusions within the preceding 24 hours (OR 7.6, $p=0.001$) and 48 hours (OR 5.55, $p=0.001$) were associated with NEC. However, other retrospective studies led by Josephson,¹³ Christensen,⁵ Paul,²⁵ and Blau²⁶ did not find a significant effect of anemia.

In convalescing preterm infants, anemia is a frequent, near-universal event. The most common reason for low hematocrits in this subgroup is anemia of prematurity. However, in infants with NEC, other etiologies may need to be carefully excluded. Some studies have shown an association of NEC with activation of the Thomsen-Friedenreich cryptic T antigen (T-antigen activation) on RBCs, causing low-grade hemolysis and anemia in multi-transfused patients who have previously received blood from adult donors carrying anti-T antibodies.⁴⁹ Although the pathophysiological significance of T-activation in NEC remains unresolved,⁵⁰ the presence of low-grade, smoldering intestinal inflammation prior to transfusion may be difficult to exclude in some patients. This scenario may involve reverse causation with intestinal injury causing anemia and not otherwise. Growing preterm infants who are anemic but otherwise stable are usually monitored with an expectation of spontaneous improvement in hematocrits. Because anemia could manifest in premature infants with myriad presentations,⁵¹ non-specific symptoms of early NEC such as tachycardia, feeding intolerance, and irritability could be ascribed to anemia and treated with a transfusion. In these infants, later onset of NEC could be associated erroneously with the RBC transfusion.

In the study by Patel *et al.*,¹² severe anemia in a given week was associated with a 6-fold higher risk of NEC. In additional analyses among RBC transfused infants, each 1 g/dL

decrease in the lowest measured hemoglobin in a given week was associated with a 65% increase in the risk of NEC ($P < 0.01$). Between 49 to 90 days of age, infants with NEC, compared to those without NEC, tended to have a lower hemoglobin (mean difference: -1.5 g/dL, $p = 0.06$). However, the study did not evaluate for the interaction between severe anemia and RBC transfusion, although several cases of NEC occurred following exposure to both within the same week. A recent case-crossover study by Le *et al.*⁵² found no association between RBC transfusion and NEC (OR 1.80, CI 0.60–5.37). A subgroup analysis reported that infants with anemia (Hb < 9.3 g/dL) were at increased risk (OR for RBC transfusion and NEC = 6, CI 0.7–50) compared to those who were not anemic (OR = 1, CI 0.2–5.0), although there was no statistical evidence of heterogeneity (test for subgroup difference $p = 0.19$).

Direct effects of RBC transfusions

Blau *et al.*²⁶ hypothesized that transfusion-associated NEC may also share immunological mechanisms with TRALI. TRALI is speculated to result from a two-hit insult in which host neutrophils are primed by an antecedent illness, followed by passive transfusion of biological response mediators such as donor antibodies such as those directed against the human leukocyte antigens (HLA), biologically-active lipids, free hemoglobin, red cell membrane fragments, and inflammatory cytokines present in stored blood⁵³. Similar factors may cause mucosal injury in the premature intestine, which displays a developmentally-regulated pro-inflammatory bias with exaggerated immune responses to bacterial and/or nutritional antigens.^{54, 55} Anti-HLA antibodies have been detected in some,⁵⁶ but not all,²⁶ studies on premature infants who were transfused and developed NEC. Paul *et al.*²⁵ reported that 83% donors in their cases of transfusion-associated NEC were male, who were less likely to carry anti-HLA antibodies in their blood than female donors. Another mechanistic consideration would invoke the presence of free hemoglobin and/or free heme (released from RBC lysis during storage and/or washing), interacting with additional humoral mediator(s) introduced via plasma.⁵³ Ho *et al.*⁵⁷ measured fecal calprotectin (FC) before and after RBC transfusions in VLBW infants, and showed that FC increased faster than baseline after RBC transfusions and were higher in multiple-transfused infants. In this cohort, FC was the highest in infants with the lowest hematocrits and in those who received RBCs that had been in storage for >21 days.

Stored blood

Stored RBCs show reduced deformability, increased aggregation, and the loss of nitric oxide. Nitric oxide stored in RBCs is covalently-bound to cysteine residues of hemoglobin and its gradual release helps maintain microvascular perfusion and tissue oxygen delivery. In stored blood, RBCs are rapidly depleted of nitric oxide, which correlates with loss of RBC function, and can be associated with a paradoxical reduction in oxygen delivery, vasoconstriction, and ischemic injury to the intestine (and other organs).⁵⁸ Stored RBCs may also have direct pro-inflammatory effects such as activation of neutrophils to produce interleukin (IL)-8 and phospholipase A2. Transfused blood contains high levels of IL-1, IL-6 and IL-8, which may rise further with increasing duration in storage.⁵⁹

Feeding and transfusion-related NEC

Three studies have addressed the issue of withholding feeds around the time of transfusion.^{11, 32} After a cluster of cases of transfusion-associated NEC, Perciaccante *et al.*³² changed from a practice of no disruption of feeding schedules to a practice of withholding feedings from 4 hours before the start of the transfusion until 4 hours after the completion of the transfusion. Before introducing the practice of withholding feedings, they recorded a history of an RBC transfusion in the preceding 48 hours prior to onset of NEC in 7 out of 18 (38.9%) cases of NEC. Following the change in feeding practices, they did not record any cases of NEC that occurred within 48 hours of a RBC transfusion. These findings are similar to those reported by El-Dib *et al.*,¹¹ who also used a pre- vs. post-practice change design and detected a significant reduction in the incidence of transfusion-associated NEC from 5.3% to 1.3% after instituting the feeding policy change. As part of a multicenter quality improvement (QI) initiative in 8 NICUs, Talavera *et al.*⁶⁰ implemented withholding of feedings during transfusions (but not before or after) and allowed no changes in fortification or volume advancement on the day of transfusion coupled with standardized human-milk feeding. The study reported a decrease in NEC incidence from 8% to 3.1% ($p = 0.001$), with continued decline to 0.9% after additional interventions over a 3-year period as part of the QI effort. The observation that intestinal perfusion is increased by bolus feeding compared to continuous feeding or fasting suggests that any benefit from withholding feedings is multi-factorial.^{61, 62}

Role of clinical initiatives to prevent anemia, such as delayed cord clamping

Potential means of preventing anemia, among premature neonates, include delayed clamping of the umbilical cord or the somewhat more rapid technique of cord stripping (also called cord milking), and methods for limiting phlebotomy losses. A Cochrane review of 15 randomized controlled trials of delayed cord clamping or milking vs. immediate cord clamping, indicated that 38% fewer patients in the cord transfusion groups developed NEC (241 infants, RR 0.62, CI 0.43–0.9).⁶³ Also, there were 39% fewer RBC transfusions among the cord transfusion recipients, but it was not possible to determine whether the lower NEC risk was due to fewer transfusions, or due to higher hematocrits (thus less anemia), or whether the lower risks of NEC and fewer transfusions were unrelated to each other.

Outcome of transfusion-associated vs. transfusion-independent NEC

Josephson *et al.*¹³ showed that infants with transfusion-associated NEC had a higher frequency of surgical intervention and longer duration of stay than those with non-transfusion-associated NEC. The overall mortality in NEC/RBC-transfused and NEC/non-RBC-transfused groups was similar (10/47, 21% vs. 7/46, 15%, respectively; $p=0.45$). These results contrast with the findings of Stritzke *et al.*,³⁷ who reported mortality and morbidity data from 927 infants with NEC vs. 2781 VLBW controls from Canadian Neonatal Network. After adjusting for confounding factors, no significant differences in mortality/neonatal morbidity were found between the two groups. Of all the studies on transfusion-associated NEC, 6 reported unadjusted estimates of mortality.^{5, 11, 26, 32, 33, 37} In their meta-

analysis, Mohamed and Shah⁶ detected increased mortality in patients who had transfusion-associated NEC than those with NEC not associated with transfusion (pooled OR 1.88, CI 1.35–2.61). None of the studies reported adjusted estimates.

Clinical outcomes in transfusion-associated NEC need careful evaluation because these infants are usually more premature and may have had a higher severity of illness prior to transfusion,^{5, 9, 11, 13, 21, 26, 32, 34, 35} both of which are independent predictors of morbidity and mortality in NEC.⁵⁴ Josephson *et al.*¹³ reported that patients who developed NEC after receiving one or more transfusions also had a higher incidence of PDA and intraventricular hemorrhage, and more frequent use of central vascular catheters. In this particular study,¹³ and in several others,^{11, 26, 33} infants who developed NEC after a transfusion were more likely to have been on respiratory support (supplemental oxygen/assisted ventilation) in the 48 hour-period preceding the onset of NEC.

Conclusion

Based on current clinical evidence, “Transfusion/Anemia-associated NEC” appears to be a plausible clinical entity. However, there is a need for cautious interpretation of data because studies reporting these associations have been susceptible to bias and to the effect of confounding variables or reverse causation.⁶⁴ There is a critical need for a developmentally-relevant animal model to determine whether severe anemia or RBC transfusions could cause inflammatory injury in the premature intestine, or if injury results from an interplay between these risk factors. We also need large, prospective, multi-center trials to evaluate the effect of RBC transfusion and anemia on NEC and other outcomes, as well as the scientific merit of interventions such as withholding feedings before, during, and/or after RBC transfusion. Such large, multicenter trials are ongoing or planned (<https://clinicaltrials.gov/ct2/show/NCT01702805>, <http://neoepoch.com/wheat-trial/>). In addition, clinical and pre-clinical studies to understand the potential interaction between anemia and RBC transfusion on the outcome of NEC are needed to provide a biologic basis for the reported epidemiologic associations. Strategies to decrease RBC transfusions, such as the use of recombinant erythropoietin or its synthetic, longer-acting homologues such as darbepoietin may also be relevant to these issues. Other potentially relevant approaches that require additional study include repletion of nitric oxide (NO) in stored RBCs before transfusion or administration of inhaled NO to at-risk neonates during transfusion.⁵

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