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Do Red Cell Transfusions Increase the Risk of Necrotizing Enterocolitis in Premature Infants?

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Abstract

BACKGROUND—Recent studies have detected an association between red blood cell (RBC) transfusions and NEC. We hypothesized that RBC transfusions increase the risk of NEC in premature infants, and investigated whether the risk of 'transfusion-associated' NEC is higher in infants with lower hematocrits and advanced postnatal age.

METHODS—Retrospective comparison of NEC patients and controls born at <34 weeks gestation.

RESULTS—The frequency of RBC transfusions was similar in NEC patients (47/93, 51%) and controls (52/91, 58%). Late-onset NEC (> 4 weeks of age) was more frequently associated with a history of transfusion(s) than early-onset NEC (adjusted OR=6.7; 95% CI=1.5–31.2; $p=0.02$). Compared to non-transfused patients, RBC-transfused patients were born at an earlier gestational age, had greater intensive care needs (including at the time of onset of NEC), and longer hospital

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stay. A history of RBC transfusions within 48-hours prior to NEC onset was noted in 38% of patients, most of whom were extremely low birth weight (ELBW) infants.

CONCLUSIONS—In most patients, RBC transfusions were temporally unrelated to NEC and may be merely a marker of overall severity of illness. However, the relationship between RBC transfusions and NEC requires further evaluation in ELBW infants using a prospective cohort design.

Keywords

RBC transfusion; premature infant; necrotizing enterocolitis

INTRODUCTION

Necrotizing enterocolitis (NEC) is an inflammatory bowel necrosis of preterm infants (1). In population studies, the incidence of NEC has been estimated to be approximately 1 to 3 per 1000 live births (2), with more than 90% of all cases occurring in premature infants. In preterm and very low birthweight (VLBW) infants, the incidence of NEC may be as high as 4 to 11% (2). In the absence of clear information on its etiopathogenesis, NEC is treated mainly with supportive measures and continues to be a major source of morbidity and mortality. Up to 40% of all cases may develop severe disease with bowel necrosis and/or perforation(s), a subset that remains at a high risk of adverse gastrointestinal, hepatic, and neurodevelopmental outcome or death (3).

Premature infants are a heavily-transfused population, with more than half of all VLBW infants requiring one or more red blood cell (RBC) transfusions during the course of their stay in the neonatal intensive care unit (NICU) (4). The most common indications for a neonatal RBC transfusion include volume expansion, cardiorespiratory compromise, anemia of prematurity, growth failure, apnea, and frequently, a hematocrit value below institutional RBC transfusion thresholds (5). In many infants with NEC, a review of antecedent events often reveals a history of red cell transfusions in the preceding 1–2 days prior to the onset of symptoms, and at least three studies have now described an association between NEC and RBC transfusions. McGrady and coworkers (6) reported an association of NEC and RBC transfusions during an outbreak of 20 cases of NEC. Similarly, Mally and colleagues (7) noted that six of their 17 cases had a history of red cell transfusion in the 48 hours preceding the onset of symptoms. More recently, Christensen and colleagues (8, 9) reviewed 118 patients with stage III NEC and noted that 38% of their patients had received a RBC transfusion 18 ± 12 hours prior to onset of symptoms. These studies did not include a non-NEC control group in the analysis and the mechanistic basis of this association remains unclear.

In this study, we reviewed data from two level III NICUs to investigate the association between RBC transfusions and NEC. We hypothesized that RBC transfusions are a risk factor for the onset of NEC and investigated whether transfusion events are temporally related to NEC. We also investigated whether the risk of NEC following RBC transfusions is higher in infants with lower hematocrits and advanced postnatal age. We argued that the risk of NEC following RBC transfusions was related to underlying anemia because (a) RBC

transfusions can trigger gut mucosal injury in patients with severe anemia during cardiopulmonary bypass (10); (b) NEC is associated with diverse conditions that are marked by anemia, including glucose-6-phosphate dehydrogenase deficiency, hemolytic disease of newborn, and in donor twins in twin-to-twin transfusion syndrome (11, 12); and (c) many 'stable,' growing premature neonates with severe anemia may be in a high cardiac output state with restricted gut perfusion, potentially at risk of mucosal injury (13). Based on these data, we speculated that RBC transfusions may increase the risk of NEC in infants with severe anemia of prematurity. Since anemia of prematurity evolves as a function of postnatal age, we further hypothesized that the risk of NEC following RBC transfusions would be most evident in older premature infants.

STUDY DESIGN AND METHODS

Study Design and Patient Population

A retrospective, case-control study was performed under appropriate oversight by the Emory University Institutional Review Board. All NICU admissions between January 2004 and April 2007, at two Level III centers in Atlanta, GA (Grady Memorial Hospital and Emory University Hospital Midtown) were reviewed. Infants born at ≥ 34 weeks gestation and with a history of NEC were identified from the discharge diagnoses in the perinatal database and were included in the study if they met criteria for Bell stages II or III (14). Eligible controls were identified for each case based on date of admission date (± 1 month), gestational age (± 1 week), and birth weight (± 200 grams). If several controls were identified with a similar admission date, the infant with the closest admission date to the NEC case was included. The exclusion criteria were extramural birth, major congenital anomalies, spontaneous bowel perforations (in first week of life), and (for controls) death prior to discharge.

Definitions, Data Collection and Transfusion Guidelines

Antenatal history, birth weight, gestational age, patent ductus arteriosus (PDA; defined on routine color flow Doppler echocardiography), intraventricular hemorrhage (IVH; identified and classified by sonographic features), weight, age, and hematocrit at birth and onset of NEC, and signs of NEC were recorded. The transfusion protocol in the two hospitals recommended the administration of 15 mL/kg leukoreduced, irradiated, CPDA-1-preserved RBCs, which were stored for ≤ 14 days, according to standardized guidelines that are generally similar to the recommendations of the American Association of Blood Banks (15) and are accepted at both hospitals (supplemental table 1). The same guidelines were also used to define anemia in an individual infant. Time elapsed between the last RBC transfusion received prior to NEC and the onset of NEC was recorded for all infants. Patients who developed NEC and had a history of RBC transfusion (NEC/RBC-transfused) were compared with those who did not receive any transfusions prior to NEC (NEC/non-RBC transfused). The age of blood, total RBC transfusion events, total volume of transfused RBCs, and number of donor exposures were also recorded.

Statistical Analysis

Sample size calculations for a 1:1 case-control study were performed with NCSS PASS 2005 using the logistic regression module. We assumed that approximately 60% of all

neonates would require a RBC transfusion. A logistic regression of the outcome (NEC or no NEC control) on the predictor (RBC transfusion – yes or no) with a sample size of 200 neonates (assuming 60% of the 200 neonates would require transfusion) provided approximately 90% statistical power at the 0.05 significance level to detect an odds ratio (OR) of 3.0 where the $OR = [p_1/(1-p_1)]/[p_0/(1-p_0)]$ and the proportion of infants with NEC when a RBC transfusion was required, $p_1 = 0.82$ and the proportion with NEC when a RBC transfusion was not required, $p_2 = 0.60$. The potential association between RBC transfusion and NEC was evaluated using a chi-square test. An odds ratio plus the 95% confidence interval was calculated to measure the degree of association between RBC transfusions and NEC. Other potential risk factors were compared between the NEC and non-NEC control group using a chi-square test or Fisher's exact test for categorical variables. Continuous variables such as gestational age and birth weight were compared between the two groups using the Wilcoxon rank-sum test. All statistical tests were 2-sided. A p -value < 0.05 was considered statistically significant. Similar analyses were performed for infants with NEC who received one or more RBC transfusions (NEC/RBC-transfused) versus infants with NEC who did not receive a RBC transfusion (NEC/non-RBC-transfused). Finally, based on data on the association of RBC transfusions and NEC, we classified patients according to their postnatal age at the time of onset of NEC into those with 'early-onset' (< 4 weeks of age) vs. 'late-onset' disease that occurred beyond 4 weeks of age. The demographic and clinical characteristics of these patients were compared using similar statistical tests. We next performed a multivariable analysis on characteristics that (1) showed significant differences between various groups at least at the level of $p < 0.10$, and (2) had a known, biologically-plausible effect on gut mucosal injury (7, 16–18). Covariates associated with late-onset NEC were identified by multivariable logistic regression using bootstrap bagging (19). Covariates retained in at least 50% of the models at $p < 0.10$ were considered reliable (20). The odds ratio and its 95% confidence interval were calculated for each factor in the presence of the other covariates in the final model. The goodness-of-fit of the model was evaluated using the Hosmer-Lemeshow chi-square statistic.

The Cochran-Armitage test for linear trend was used to determine whether the percentage of VLBW infants who had received one or more RBC transfusions prior to the development of NEC increased with postnatal age at the time of diagnosis of NEC. The association between the number of RBC transfusions and postnatal age at the time of onset of NEC was quantified using the Spearman rank correlation coefficient (r_s). Parametric tests were used to compare the hematocrits in various subgroups; temporal changes were analyzed by repeated-measures analyses with a means model using the software SAS Proc Mixed (version 9; SAS Institute, Cary, NC) providing separate estimates of the means by times prior to NEC diagnosis (1 week, 72 hours, 48 hours, 24 hours before NEC and at the time of NEC) for three separate subgroups (1: early-onset NEC vs. late-onset NEC; 2: NEC/RBC-transfused vs. NEC/non-RBC-transfused; 3: early-onset NEC/RBC-transfused, late-onset NEC/RBC-transfused, early-onset NEC/non-RBC-transfused, and late-onset NEC/non-RBC-transfused). A compound-symmetry form was assumed for repeated measurements of hematocrit and robust estimates of the standard errors were used to perform statistical tests and construct 95% confidence intervals (21). The model-based means are unbiased with unbalanced and

missing data, provided the missing data are non-informative and are randomly distributed (22).

RESULTS

Demographic characteristics of infants with NEC and non-NEC controls

Medical and laboratory records were reviewed from all 3725 neonatal admissions to two Level III NICUs in Atlanta, Georgia from the period January 2004 – April 2007. The overall incidence of NEC in this population was 2.5% (93/3725) and the incidence of NEC in neonates with a birth weight of ≥ 1500 g was 8.6% (75/868). There were 18 cases of NEC in patients with birth weights ≥ 1500 grams, two of whom weighed ≥ 2000 grams at birth. Eighty-one (87%) neonates had definite NEC (stage II), while 12/93 (13%) had advanced disease (stage III). The overall mortality in this patient population was 18% (17/93). Ninety one neonates, matched as previously described, were included in the study as controls. No eligible controls were identified for two NEC cases. Demographic data (Table 1) did not reveal significant differences between the NEC and the non-NEC control groups. The number of infants with a history of RBC transfusions was also similar between the two groups: 51% (47/93) in the NEC group and 58% (53/91) among the non-NEC controls had received RBC transfusions (odds ratio 0.73; 95% CI=0.41–1.31; $p=0.29$).

NEC and history of RBC transfusions

We next classified patients in the NEC group according to their transfusion status into NEC/RBC-transfused (47/93, 51%; stage II 40/47, 85%; stage III 7/47, 15%) and NEC/non-RBC transfused subgroups (46/93, 49%; stage II 41/46, 89%; stage III 5/46, 11%). NEC/RBC-transfused patients had lower birth weights and gestational age, more frequent history of indomethacin prophylaxis against IVH, a higher incidence of PDA, a higher frequency of intraventricular hemorrhage, and more frequent use of central vascular catheters (Table 2). Compared to the NEC/non-RBC-transfused group, NEC/RBC-transfused patients were receiving higher FiO_2 , ($p<0.001$) and were more frequently receiving respiratory support (supplemental oxygen or assisted ventilation; $p<0.0001$) in the 48 hours preceding the onset of NEC. A similar percentage of patients in both groups were receiving full enteral feeds at the time of NEC diagnosis (47.8% vs. 47.7%, $p=0.55$). The overall mortality in NEC/RBC-transfused and NEC/non-RBC-transfused groups was 10/47 (21%) and 7/46 (15%), respectively ($p=0.45$).

The median age at onset of NEC in the NEC/RBC-transfused group was 37 days (interquartile range 23–55 days), which was later than in the NEC/non-RBC transfused group (median 13 days, interquartile range 7–24; $p<0.0001$). At the time of diagnosis of NEC, NEC/RBC-transfused patients weighed less than those in the NEC/non-RBC-transfused subgroup ($p=0.0002$). The two subgroups were not significantly different in their clinical presentation of NEC, although we did notice a trend towards a higher incidence of pneumatosis (90.0% vs. 71.7%; $p=0.19$), a greater need for surgery (34.0% vs. 21.7%, $p=0.19$), and a lower frequency of bloody stools in the NEC/RBC-transfused group than in the non-transfused patients (19.2% vs. 32.6%, $p=0.14$).

NEC, history of RBC transfusions, and postnatal age

Since NEC occurred in our NEC/RBC-transfused patients at a later postnatal age than in the NEC/non-RBC transfused subgroup, we next investigated the relationship of NEC and postnatal age. The association of NEC with previous RBC transfusions strengthened with increasing postnatal age at the time of onset of NEC and most infants who developed NEC after 4 weeks of age had a history of previous RBC transfusions. Among patients who developed NEC at < 4 weeks of age, 17/58 (29%) had received RBC transfusion(s) prior to onset of NEC, whereas 30/35 (86%) patients who developed NEC at ≥ 4 weeks of age had a previous history of transfusions ($p < 0.0001$). All patients who developed the disease at ≥ 7 weeks of age had received RBC transfusions. Based on these data, we sub-classified our NEC patients into those with ‘early-onset’ disease occurring at < 4 weeks of age and a ‘late-onset’ subgroup with onset of disease beyond 4 weeks of age. In the late-onset NEC subgroup, patients were born at an earlier gestational age (25.7 weeks, interquartile range 24.6–27.3 *vs.* 30 weeks, range 27.7–32 in early-onset, $p < 0.0001$) with a lower birth weight (735 grams, interquartile range 680–1005 grams *vs.* 1240g, 960–1550 grams in early-onset, $p < 0.0001$), had a higher frequency of PDA (15/35, 42.9% *vs.* 3/35, 5%, $p < 0.0001$), IVH (10/35, 28.6% *vs.* 5/35, 8.6%, $p = 0.011$), and blood culture-proven sepsis (22/35, 62.9% *vs.* 14/58, 24.1%, $p = 0.0002$), and received central lines (31/35, 88.6% *vs.* 29/58, 50%, $p = 0.0002$) more frequently than those with early-onset disease. In the 48-hour period prior to NEC, more patients in the late-onset group were receiving supplemental oxygen or assisted ventilation than those with early-onset disease (30/35, 85.7% *vs.* 23/58, 39.6%, $p = 0.02$). Compared to the early-onset subgroup, RBC transfusions were associated with increased risk of NEC in patients with late-onset disease (odds ratio = 14.5; 95% CI = 4.8–43.6; $p < 0.0001$). Patients in the late-onset NEC/RBC-transfused subgroup were most premature, were receiving the most support (central lines, respiratory support), and had the longest length of hospital stay (data not depicted).

We next performed a multivariable logistic regression analysis to identify the covariates associated with late-onset NEC. Nine characteristics were shortlisted that were significant at $p < 0.10$ between various groups and with a known, biologically-plausible relationship with intestinal injury: gestational age, birthweight, 5-min Apgar score < 6, PDA, FiO₂ at 48 hours prior to onset of NEC, ventilatory status 48 hours prior to onset of NEC, history of positive blood culture(s), hematocrit at the time of onset of NEC, and history of RBC transfusion(s). In our bootstrap algorithm, RBC transfusion(s) (included as a positive/negative response) and blood culture results (included as a positive/negative response) were associated with late-onset NEC (Table 3). In the final model, the requirement of 1 or more RBC transfusions (adjusted odds ratio = 6.7; 95% CI = 1.5 to 31.2, $p = 0.02$) was associated with late-onset NEC even after adjustment for the blood culture result and gestational age.

Temporal change in hematocrits

Compared to the NEC/non-RBC-transfused subgroup, NEC/RBC-transfused patients had lower hematocrits at birth, 1 week prior to the onset of NEC, and at the time of onset of NEC ($p < 0.01$; supplemental fig. 1). We also noted lower hematocrits in infants with late-onset NEC than in those with early-onset disease ($p = 0.01$). We next compared hematocrits prior to the onset of NEC in our four sub-groups classified by early- *vs.* late-onset and the

RBC transfusion status. Early-onset NEC/non-RBC-transfused patients had the highest hematocrits among the four subgroups ($p=0.02$). To determine whether lower hematocrits in infants with late-onset NEC merely reflected anemia of prematurity (developing as a function of age) or actually increased the risk of NEC following RBC transfusions, we also compared the hematocrits in late-onset NEC/RBC-transfused patients with the late-onset NEC/non-RBC-transfused infants; these two subgroups did not differ from each other (supplemental fig. 1).

Transfusion histories of NEC/transfused patients and Non-NEC/transfused controls

Both NEC/RBC-transfused and non-NEC/RBC-transfused groups received similar total RBC volumes, a similar numbers of RBC transfusions, and were exposed to a similar number of RBC donors. Data on the storage age of RBCs was available for 34 patients (161 transfusion episodes). The average storage age of RBCs administered in the NEC/RBC-transfused and non-NEC/RBC-transfused groups was 9 and 8 days, respectively ($p=0.009$).

In our NEC/RBC-transfused group, patients with late-onset disease received more RBC transfusions, a larger total RBC volume, and were also exposed to more donors. We also detected a significant correlation between the number of transfusion events and the postnatal age at onset of NEC ($r_s = 0.37$, $p=0.044$). The median interval from the last RBC transfusion to the onset of NEC in NEC/RBC-transfused patients was 84 hours (range 2 – 1190 hours). When analyzed as a function of time elapsed following the transfusion, the frequency of NEC in the sub-groups with early- and late-onset disease was not different (Table 4). The number of infants who developed NEC within 24 hours after a transfusion (13/47) was apparently larger than in subsequent 24 hour intervals, but the statistical significance of the small numbers was difficult to ascertain. Infants who developed NEC in the initial 24 hours, 24–48 hours, and subsequent days following transfusions had similar hematocrits at birth and at onset of NEC (data not depicted).

We analyzed the demographic and clinical characteristics of the 18 infants in NEC/RBC-transfused group who developed NEC within 48 hours of receiving a RBC transfusion. When compared to the 75 infants with NEC who had either not received any RBC transfusions ($n=46$) or received an RBC transfusion > 48 hours prior to the onset of NEC ($n=29$), these infants had lower birth weights (median 735 vs. 1160 grams, $p=0.0003$) and gestational age (25.9 vs. 28.4 weeks, $p=0.001$), were older at onset of NEC (30 days vs. 22 days, $p=0.045$), weighed less at the time of NEC (1062 vs. 1450 grams, $p=0.001$), and were receiving more respiratory support ($p<0.0001$). The two groups had similar hematocrits at birth (41.5 ± 6.4 vs. $45.3\pm7.3\%$ in NEC <48h and >48h post-transfusion groups, respectively) and at onset of NEC (30.7 ± 6.1 vs. $32.1\pm8.2\%$, respectively). We also did not detect differences in the clinical features of NEC, including emesis, bloody stools, abdominal distention, pneumatosis, free air, and surgical intervention. These data are summarized in supplemental table 2.

DISCUSSION

We present a detailed investigation on the role of RBC transfusions in NEC. Although a similar number of infants received RBC transfusions in both NEC as well as in the non-NEC

control groups, RBC transfusions were associated with an increased risk of NEC in patients who developed the disease beyond 4 weeks of age. This association strengthened as a function of postnatal age, and all patients who developed NEC beyond 7 weeks of age (n=20) had received RBC transfusions. Infants in the NEC/RBC-transfused group generally developed the disease at a later postnatal age than in the NEC/non-RBC-transfused group (median 37 vs. 13 days), which is consistent with previous observations of Mally (7) and Christensen (8), who also noted a delayed onset of NEC in patients with a history of RBC transfusions. In our cohort, NEC/RBC-transfused patients were born at an earlier gestational age with lower birth weights, had lower hematocrits, higher incidence of ductal patency, higher needs for respiratory support (supplemental oxygen or assisted ventilation) in the 48 hours preceding the onset of NEC, a higher frequency of surgery, and a longer duration of hospital stay than the NEC/non-RBC transfused group. The high intensive care needs of our NEC/RBC-transfused group contrast with the previous reports (7), where patients with 'RBC transfusion-associated' NEC were stable, growing preterm infants who were not ventilated and had no other active medical problems except anemia. The 'need' for RBC transfusions in most patients in our series may be merely a surrogate of their overall severity of illness that may reflect delayed recovery from early neonatal complications of prematurity. Furthermore, RBC transfusions were temporally separated prior to the development of NEC in most patients by several days and therefore, were difficult to connect to NEC in a pathophysiological context.

In our series, the delayed onset of NEC and the higher intensive care needs of our NEC/RBC-transfused patients were consistent with their lower gestational age and birth weight than the non-transfused patients. The inverse relationship between gestational age and the time of onset of NEC is well-known (23). Unlike premature neonates who develop NEC after the first 3–4 postnatal weeks, NEC in near-term or term neonates tends to occur earlier, is frequently associated with obvious risk factors such as intra-uterine growth restriction, chorioamnionitis, perinatal asphyxia, congenital heart disease, gastroschisis, or Hirschsprung's disease, and may involve the colon more often than in preterm infants (24). While these differences in the timing of NEC and associated risk factors between the two populations are well recognized, a clear time-based definition of early vs. delayed NEC is not available. In this context, the 4-week time-point we identified in our series merits further investigation as a watershed to differentiate 'early-' and 'late-onset' NEC as two distinct entities with different pathophysiological mechanisms.

A third of our NEC/RBC-transfused patients (18/47; 38%) received a RBC transfusion in 48 hours preceding the onset of NEC, a proportion that is remarkably similar to previous reports by Mally (6/17; 35%) and Christensen (40/112; 35%) (8, 9). In our series as well as in these previous two reports (7, 9), patients with 'transfusion-associated' NEC were born at earlier gestation ages and had lower birth weights. Krimmel and colleagues (25) showed that RBC transfusions can dampen the normal postprandial increase in mesenteric blood flow in premature infants, particularly in those with a birth weight <1250 grams. This immaturity of vascular autoregulation in extremely premature infants is likely related to low endothelial NO synthesis (26), and could explain a higher risk of mucosal injury following transfusions

in this patient population. Prospective studies on the role of transfusions in NEC may need to focus specifically on these patients.

We did not detect specific evidence to indicate that the risk of NEC following RBC transfusions was higher in infants with lower hematocrits. Although our NEC/RBC-transfused patients had lower hematocrits than the non-transfused patients at birth and at onset of NEC, these differences likely reflected the lower gestational age and related severity of anemia (of prematurity) in the RBC-transfused patients. Infants who developed NEC within 24–48 hours after a transfusion did not have lower hematocrits prior to/at onset of NEC than other patients who developed NEC without an apparent antecedent temporal relationship to RBC transfusions. Similarly, infants with late-onset NEC had lower hematocrits than those with early-onset disease, but infants with late-onset NEC who received RBC transfusions had similar hematocrits to other late-onset patients who did not receive RBC transfusions.

While anemia of prematurity remains the most likely cause of anemia in convalescing preterm infants, alternative etiologies may need careful exclusion in infants with NEC. Some studies have shown an association of NEC with activation of the Thomsen-Friedenreich cryptic T antigen (T-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 (27). The pathophysiological significance of T-activation in NEC remains unresolved (28), yet a finite possibility remains that some patients with “transfusion-associated” NEC could have had a low-grade smoldering illness prior to the transfusion. Growing preterm infants who are anemic but otherwise stable are usually monitored with an expectation of spontaneous improvement in hematocrits. Since manifestations of anemia in premature infants can be highly variable (29), nonspecific 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 development of more obvious clinical manifestations of NEC could be then erroneously associated with the RBC transfusion.

We recognize significant limitations and pitfalls of this study. The retrospective nature of this investigation limited us to information in the medical records. Variability in recognition of early signs of NEC such as feeding intolerance or abdominal distension by care-providers is well-recognized (8). While we did not detect significant differences in clinical signs in NEC/RBC-transfused and NEC/non-RBC transfused groups, subtle changes could have been overlooked. The present study also does not provide insights into the effects of storage age of RBCs as we exclusively use RBC units that are 14 days old. Although we did detect a 1-day difference in the mean storage age of RBCs received by NEC/RBC-transfused patients vs. the non-NEC/RBC-transfused controls, the clinical importance of this small difference is unclear (30). We also do not have detailed information on potential confounders such as feeding practices, human milk intake, and antibiotic use. Finally, we note that our study population was an urban, predominantly African-American cohort, the results from which may not be readily generalized to the US population as a whole and the effect of ethnicity cannot be tested.

In conclusion, we have identified an association of RBC transfusions with ‘late-onset’ NEC, which is similar to previous reports by Mally (7) and Christensen (8). However, in contrast to these previous reports, NEC/RBC-transfused patients in our series were apparently sicker and were receiving more support. While RBC transfusions prior to the onset of NEC appear to be a surrogate of the overall severity of illness in most patients, further study is needed to determine whether RBC transfusions, by triggering splanchnic vasoconstriction, could trigger intestinal injury in extremely premature infants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

RBC	red blood cells
NICU	neonatal intensive care unit
NEC	necrotizing enterocolitis
PDA	patent ductus arteriosus
IVH	intraventricular hemorrhage

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Table 1

Demographic and Clinical Characteristics of NEC Cases and Non-NEC Controls

Characteristic	NEC (Cases) (n=93)	Non-NEC (Controls) (n=91)	p-value
Transfusion – no. (%)	47 (50.5)	53 (58.2)	0.29
Male sex – no. (%)	57 (61.3)	53 (58.2)	0.67
Race – no. (%)			
Black	74 (79.6)	75 (82.4)	
Caucasian	10 (10.8)	5 (5.5)	0.46
Hispanic	9 (9.6)	9 (9.9)	
5 min Apgar <6 – no. (%)	12 (12.9)	16 (17.6)	0.38
PDA* – no. (%)	18 (19.4)	16 (17.6)	0.76
Indomethacin – no. (%)	36 (39.6) [§]	36 (39.6)	1.00
IVH [£] Grade 2 – no. (%)	15 (16.1)	11 (12.1)	0.43
Central line [†] – no. (%)	60 (64.5)	60 (65.9)	0.84
Positive blood culture [§] – no. (%)	36 (38.7)	24 (26.4)	0.074
Mode of delivery			
Cesarean section	57 (61.3)	60 (65.9)	
Vaginal	36 (38.7)	31 (34.1)	0.51
Birth weight, g			
Median	1030	1060	
Interquartile range	740–1410	755–1440	0.81
Gestation, wks			
Median	27.9	28.0	
Interquartile range	25.7–30.7	26.0–30.6	0.71
Hematocrit at birth			
Mean ± sd	44.6 ± 7.2	45.0 ± 7.7	
Median	44.7	44.5	0.47
Interquartile range	40.2–48.0	41.0–50.3	
Hematocrit at NEC			
Mean ± sd	31.8 ± 7.8		
Median	29.5	–	–
Interquartile range	26.9–36.4		

* PDA, Patent ductus arteriosus.

[£] IVH, Intraventricular hemorrhage.[§] Data unavailable for two patients.[†] Placed prior to diagnosis of NEC.[§] Developed prior to diagnosis of NEC

Table 2

Demographic and Clinical Characteristics of NEC Patients by RBC Transfusion Status

Characteristic	NEC/RBC (n=47)	NEC/non-RBC (n=46)	p-value
Male sex – no. (%)	29 (61.7)	28 (60.9)	0.93
Race – no. (%)			
Black	34 (72.3)	40 (87.0)	0.16
Caucasian	6 (12.8)	4 (8.7)	
Hispanic	7 (14.9)	2 (4.3)	
5 min Apgar <6 – no. (%)	9 (19.2)	3 (6.5)	0.070
PDA – no. (%)	16 (34.0)	2 (4.4)	0.0003
Indomethacin – no. (%)	33 (70.2)	3 (6.8)	<0.0001
IVH* Grade 2 – no. (%)	13 (27.7)	2 (4.4)	0.002
Central vascular catheter – no. (%)	46 (97.8)	14 (30.4)	<0.0001
Positive blood culture – no. (%)	24 (51.1)	33 (71.7)	0.041
Mode of delivery			
Cesarean section	33 (70.2)	24 (52.2)	0.074
Vaginal	14 (29.8)	22 (47.8)	
Birth weight, g			
Median	760	1415	<0.0001
Interquartile range	660–950	1180–1680	
Gestation, wks			
Median	25.9	30.7	<0.0001
Interquartile range	24.6–27.4	29.0–32.4	
Length of stay, days			
Median	100	43.5	<0.0001
Interquartile range	56–114	28–56	
Age at NEC, days			
Median	37	13	<0.0001
Interquartile range	23–55	7–24	
Weight at NEC, g			
Median	1230	1578	0.0002
Interquartile range	892–1540	1325–1810	
Hematocrit at birth			
Mean ± sd	42.6 ± 6.8	46.6 ± 7.2	0.022
Median	42.7	45.7	
Interquartile range	37.7–47.3	42.0–49.7	
Hematocrit at NEC			
Mean ± sd	29.6 ± 5.0	34.1 ± 9.4	0.023
Median	28.5	30.6	
Interquartile range	26.5–32.6	27.5–39.5	
Vent status 48hrs prior to onset of NEC – no. (%)*			

Characteristic	NEC/RBC (n=47)	NEC/non-RBC (n=46)	p-value
Ventilator	9 (21.4)	1 (2.6)	<0.0001
Continuous positive airway pressure	12 (28.6)	2 (5.1)	
Oxygen	15 (35.7)	8 (20.5)	
Room air	6 (14.3)	28 (71.8)	
Full feeding at diagnosis of NEC – no. (%) [*]	22 (47.8)	21 (47.7)	0.55
FiO ₂ at 48hrs prior to onset of NEC – no. (%) [*]			<0.0001
0.23 [§]	11 (26.2)	32 (82.1)	
> 0.23	31 (73.8)	7 (17.9)	
Number of apnea episodes at 48hrs prior to onset of NEC – no. (%) [*]			0.002
Zero	27 (64.3)	36 (92.3)	
One or more	15 (35.7)	3 (7.7)	

^{*} Data unavailable for 5 NEC/RBC patients and 7 NEC/non-RBC patients

[§] 0.23 is the median value for FiO₂ at 48hrs prior to onset of NEC for all NEC patients

Table 3

Multivariable Logistic Regression Analysis of Factors Associated with Late-Onset Disease (>4 weeks of Age) based on Bootstrap Results

<i>Variable</i>	<i>Frequency of Occurrence in 10,000 Bootstrap Multivariable Analysis</i>	<i>Odds ratio/Significance</i>
Positive blood culture	6725	3.9 (1.2, 12.7); $p=0.02$
Transfusion	5767	6.7 (1.5, 31.2); $p=0.02$
Gestational Age	5144	Not significant
PDA	4911	Not significant
Vent status 48hrs prior to onset of NEC	3026	Not significant
Hematocrit at NEC	2339	Not significant
Birth Weight (g)	1994	Not significant
FiO ₂ at 48hrs prior to onset of NEC	1117	Not significant
5 min Apgar < 6	985	Not significant

Table 4**Blood Transfusion History of NEC/RBC-Transfused Patients**

Characteristic	Early-onset NEC/RBC-transfused (n=17)	Late-onset NEC/RBC-transfused (n=30)	p-value
No. of RBC transfusion episodes Median (Interquartile range)	2 (1–3)	6 (4–11)	0.0002
No. of RBC donors used Median (Interquartile range)	2 (1–2)	5 (3–7)	<.0001
Total volume of RBCs transfused, ml ^{&} Median (Interquartile range)	25.8 (14.4–34.4)	75.0 (44.4–115.1)	0.0003
No. of patients with 48 hours from last RBC transfusion to onset of NEC – no. (%)	8 (47.1)	10 (33.3)	0.35
No. of patients from last RBC transfusion to onset of NEC (%)			0.053
<24 hrs	5 (29.4)	8 (26.7)	
24–48 hrs	3 (17.6)	2 (7.0)	
48–72 hrs	2 (11.8)	2 (7.0)	
72 hrs – 1 week	5 (29.4)	3 (10.0)	
> 1 week	2 (11.8)	15 (50.0)	

[§]Only patients from EUHM (Emory University Hospital Midtown) center have the age of blood information, which has 16 NEC/RBC-transfused and 18 non-NEC/RBC-transfused patients

[&]Based on 15ml/kg for each RBC transfusion