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Packed red blood cell transfusion is not associated with increased risk of necrotizing enterocolitis in premature infants

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Abstract

OBJECTIVE—Recent reports have posited a temporal association between blood transfusion with packed red blood cells (BT) and necrotizing enterocolitis (NEC). We evaluated the relationship between BT and NEC among infants at three hospitals who were consented at birth into a prospective observational study of NEC.

STUDY DESIGN—We used a case–control design to match each case of NEC in our study population of infants born at <33 weeks postmenstrual age (PMA) to one control infant using hospital of birth, PMA, birth weight and date of birth.

RESULT—The number of transfusions per infant did not differ between 42 NEC cases and their controls (4.0 ± 4.6 vs 5.4 ± 4.1 , mean $\pm$ s.d., $P = 0.063$). A matched-pair analysis did not identify an association of transfusion with NEC in either the 48-h or 7-day time periods before the onset of NEC. Stratifying on matched-sets, the Cox proportional hazard model did not identify any difference in the total number of BTs between the two groups (hazard ratio 0.78, 95% confidence interval 0.57 to 1.07, $P = 0.11$).

CONCLUSION—In contrast to previous studies, our case–control study did not identify a significant temporal association between BT and NEC. Additional large prospective randomized studies are needed to clarify the relationship between BT and NEC.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating complication of prematurity that afflicts 5% to 7% of premature infants.^{1,2} Only a few factors—prematurity, aggressive feedings and pathogenic intestinal microbes—are well accepted as risk factors for NEC.^{1–4} NEC presents with systemic signs of illness and intestinal signs of infection and inflammation^{5–7} and can rapidly progress to result in hemorrhagic–ischemic intestinal necrosis and multi-organ failure.^{1,2,6} In recent years, multiple cohort and case–control studies have presented evidence to support a temporal association between blood transfusion with packed red blood cells (BT) and NEC.^{8–20} Many of these studies abstracted data retrospectively, which may introduce bias.²¹

Proving a temporal association between BT and NEC is especially challenging, because in some infants, BT may be performed in response to a prodromal sign of NEC before a diagnosis of NEC is certain. For instance, pallor and anemia are clinical features of NEC² and other life-threatening illnesses. Anemia may precede overt NEC in many patients.⁸ Based on the analysis of the temporal association of BT and NEC, most recent studies have implied a causal relationship between these two events in a subset of cases of NEC. We tested the null hypothesis that there is no association between the number and/or timing of BT and NEC in a population of infants enrolled in a prospective investigation of the relationship of intestinal microbiota and NEC.⁷ Our analysis used a rigorous case–control methodological design that accounted for all BTs and adjusted for major potential confounding variables.

METHODS

We recruited all infants born at <33 weeks postmenstrual age (PMA) at three university hospitals into a longitudinal prospective investigation of intestinal microbiota and NEC. The institutional review boards of all three hospitals approved the research project, and informed consent was obtained before any prospective data collection. Perinatal events, treatments, timing of all BTs and neonatal outcomes were abstracted. All study terms and conditions were predefined. PMA was defined as the best obstetrical estimate or by modified Ballard examination²² by the attending neonatologist when an obstetrical estimate was not available. Infants with chromosomal or severe congenital malformations were excluded. A diagnosis of NEC (Bell's stage II)²³ required unambiguous radiographic evidence of pneumatosis or hepatobiliary air and/or documentation of intestinal necrosis on laparotomy or laparoscopy. In accord with the methodology used by Wan-Huen *et al.*,¹⁹ for each case of confirmed NEC, we defined the onset of NEC to have been the time at which an infant demonstrated a change in clinical status (for example, new or markedly increased gastric residuals; abdominal distention; respiratory deterioration) consistent with the prodrome of NEC, before the diagnosis of NEC. A diagnosis of spontaneous intestinal perforation was made in an infant with an isolated pneumoperitoneum in whom intestinal necrosis was not present on laparotomy or laparoscopy. We considered a diagnosis to be indeterminate in an infant with an isolated pneumoperitoneum if neither direct visualization of the intestine nor subsequent development of intestinal obstruction occurred during hospitalization. Each infant for whom we confirmed a diagnosis of NEC was subsequently matched to a single control infant using

hospital, PMA (± 1 week), birth weight (± 100 g) and date of birth (± 3 months). We attempted but were not able to match two control infants to every case of NEC, because the total study population spanned three hospitals. We calculated the time interval between BT and onset of NEC using the time of initiation of BT.

All transfusions used packed red cells preserved in AS-5 (Optisol, Terumo Medical Corporation, Somerset, NJ, USA) or in AS-1 (Adsol, Baxter Health care, Deerfield, IL, USA). Both preservatives are SAGM based (saline, adenine, glucose and mannitol), with only minor differences. Preserved units were leukoreduced. In one hospital, irradiated units were available upon request but were not administered routinely.²⁴ The other two hospitals used irradiated preserved cells for premature infants by protocol. To limit exposure to multiple donors, designated preserved units that were < 7 days old were used for the same recipient for the allowed duration (maximum 42 days) based on criteria published by the American Association of Blood Banks.²⁵ In general, all three hospitals followed guidelines for BTs in the premature infant (Appendix) that were more restrictive than those recommended by the American Association of Blood Banks.²⁵ However, clinicians were allowed to exercise discretion and order BT if an infant unexpectedly deteriorated and was judged to be likely to benefit even if strict transfusion criteria had not been met. The usual volume of transfusion was 10 to 20 ml kg⁻¹ over 2 to 4 h or over a shorter duration if the infant was in hemorrhagic shock. Feedings were not routinely withheld during transfusions.

Statistical analysis

Paired samples were tested by Wilcoxon rank test or by McNemar's test for nominal or ordinal variables. Paired-sample *t*-test was used for continuous variables. Models of Cox proportional hazard were constructed to analyze the relationship between each BT and the risk of developing NEC. The cumulative count of BTs (not the amount of blood transfused) was used as a time-dependent covariate (using the day of life on which the transfusion occurred) in a Cox proportional hazard model. The time to onset of NEC was the outcome of interest, and the analysis was stratified by matched sets. Because there were more females in the control than in the NEC group, and because of differences ($P = 0.026$) in the type of enteral feedings between the two groups (Table 1), gender and type of enteral milk (maternal milk, formula or both) were added to the above model to assess these variables as potential confounders. The difference in the number of BTs between cases and controls through the age of onset of NEC was compared with zero using a Wilcoxon's signed-rank test.

In all analyses, $P < 0.05$ was considered significant. Results are expressed as odds ratio (OR) or hazard ratio (HR) with 95% confidence intervals (CIs). Analyses were carried out using SAS for Windows, version 9.3.²⁶

RESULTS

During the study period from 15 January 2009 to 12 October 2011, a total of 585 infants born at < 33 weeks PMA were enrolled in the prospective study. Of these infants, 42 (or 7.2%) developed NEC and 8 developed spontaneous intestinal perforation. In three infants with an isolated pneumoperitoneum, the diagnosis was indeterminate. The age of onset of NEC was 23 ± 18 days (mean $\pm$ s.d.). The age of onset of spontaneous intestinal perforation

was 6 ± 2 days (range, 1 to 7 days). Including 42 control infants, the PMA of all 84 infants was 27 ± 2 weeks and the birth weight was 982 ± 326 g (Table 1). Forty-three percent of the infants in the NEC group were female compared with 62% in the control group. Among these 84 infants, 55 (or 65%) received 393 BTs. NEC infants received 168 and control infants received 225 BTs (mean $\pm$ s.d. = 4.0 ± 4.6 vs 5.4 ± 4.1 ; mean difference = -1.4 , CI -2.8 to 0.1 , $P = 0.063$). There was a significant difference in the distribution of types of enteral feedings between the two matched groups before the age of onset of NEC (Table 1). Paired comparisons showed no differences with respect to the need for resuscitation, continuous positive airway pressure or mechanical ventilation in the delivery room, ethnic background; maternal treatment with betamethasone or other neonatal morbidities (for example, mechanical ventilation after delivery, hypotension, patent ductus arteriosus (diagnosed by color doppler echocardiography), chronic lung disease (oxygen supplementation at 36 weeks PMA) and intraventricular hemorrhage (using Papile's classification²⁷)).

Among the 42 matched case-control pairs, in 26 pairs neither the case nor the control infant had been transfused and in 4 pairs both infants had been transfused within 48 h of the age of onset of NEC (Table 2). Among the 12 discordant pairs, there were 5 in which only the control infant had been transfused compared with 7 in which only the NEC infant had been transfused (OR 1.4, CI 0.38 to 5.59, $P = 0.77$). Reanalysis using an interval of 7 days before the onset of NEC showed that there were 18 pairs in which no BT was administered and 8 pairs in which both infants received BT. Among the 16 discordant pairs, there were 11 in which only the control infant vs 5 in which only the NEC infant had received BT (OR 0.45, CI 0.12 to 1.42, $P = 0.21$, Table 2).

The fifth of nine Bradford Hill criteria for assessing a putative causal relationship asks 'Is there a biological gradient as seen in a dose-response relationship?'.²¹ Hence, we evaluated a potential dose-response relationship in two ways. A cumulative dose-response relationship was assessed using a Cox proportional hazard model to stratify the matched sets, with the total number of days on which any BT was administered from birth as the time-dependent covariate. In this analysis, the total number of BT days did not differ between the two groups (HR = 0.78, CI 0.57 to 1.06, $P = 0.11$). We also sought evidence for an acute dose-response by determining within each matched pair whether each infant had received BT on 2 or 3 consecutive days (no infant received BT on >3 consecutive days). We then compared the proportion of NEC infants with control infants who had received consecutive day BTs. Two patients in this study received BTs on 3 consecutive days and six received BTs on 2 consecutive days, yet only one of these eight infants was in the NEC group.

Although the difference in the number of BTs (number in NEC case – number in control case) through the age of onset of NEC in each matched set was negative (-0.74), this was not statistically significant ($P = 0.08$). There were 8 matched pairs in which the NEC patient had more transfusions and 18 pairs in which the control had more transfusions. After adjusting for gender and type of enteral feedings, our analysis did suggest (Table 3) that both female gender (HR 0.33, $P = 0.047$) and, interestingly, an increased number of BTs (HR 0.69, $P = 0.056$) may offer some protection against NEC.

Post-hoc power analysis

Using an exact conditional approach, the power of a McNemar's test was estimated for a reference value of 12% (corresponding to 5 of 42 discordant pairs in which the control infant received a transfusion within the 48-h window) and a difference of 5% (corresponding to two additional discordant pairs, for a total of 7, in which the NEC case had received a transfusion within 48 h of onset of disease). We conducted this power analysis *post-hoc*, because it required knowledge of the percentage of discordant pairs in which the control but not the NEC case had received a transfusion in the 48-h window before the age of onset of NEC. This percentage was not estimable before the study. Figure 1 graphically depicts additional estimates of power for differences up to 35%.

The table within Figure 1 summarizes the power for the observed difference in discordant probabilities (5%) and increasing differences in steps of 5%. The approximate OR is also provided. Our sample size of 42 matched pairs with 12% and 17% in the two discordant cells has limited power (5%). On the other hand, our sample size did have 80% power to detect an absolute difference between the percentages in discordant cells of 35% (corresponding to an OR of about 4). In consideration of the results reported in two earlier investigations,^{16,17} our sample size had more than adequate power. These two case-control studies^{16,17} each demonstrated positive associations between BT and NEC with ORs in excess of 5. In one of these investigations,¹⁷ the ORs were 5.6 for BT within 48 h of NEC and 7.6 for BT within 24 h of NEC. The estimated pooled OR for cohort studies is even larger.

DISCUSSION

Multiple recent cohort^{8–14} and case-control^{15–20} studies have presented evidence to support a temporal association between BT and NEC in at least a subset of infants with NEC. We endeavored to determine whether this association occurred in infants cared for at our three university hospitals by analyzing a detailed data set that had been compiled during a prospective investigation of the relationship between intestinal microbiota and NEC and sepsis.⁷ In contrast to the above cited studies that abstracted key clinical data retrospectively, our study personnel identified and thoroughly characterized the clinical course of infants with NEC in near real time through meticulous concurrent review of clinical, laboratory, radiological and pathological findings. Furthermore, we used a rigorous case-control methodology, captured all BTs and adjusted for known potential confounding variables. We used proper matched sample statistical analyses, including Cox proportional hazard modeling, that allowed robust statistical assessment for acute and cumulative dose-response relationships between BTs and NEC. In contrast to most existing studies, none of our analyses identified a statistically significant temporal or dose-response relationship between BT and NEC. In fact, our data suggest that an increased number of transfusions may protect against NEC.

In their review of both cohort and case-control studies of the association between BT and NEC, Kirpalani *et al.*²¹ articulated several design and methodological characteristics that introduce potential bias into existing cohort and case-control studies, including our own. Most importantly, these include the difficulty in excluding prodromal NEC before BT,

imperfect or absent risk adjustment for known confounding factors, the inability to adjust for unknown but potential confounders and the absence of adjustment for the severity of illness at the time of BT. We also observed other design or analytical weaknesses among some of the published case-control studies, including imperfect assignment of matched controls, application of an unmatched analytic technique to a matched sample and/or declination of the opportunity to analyze information about all transfusions.

It is important to highlight that none of the studies that has found a relationship between BT and NEC can completely refute the alternative explanation that, in some cases of NEC, BT was given in the prodromal phase before a firm clinical diagnosis of NEC could have been made. Pathological samples obtained at laparotomy have shown patterns of repair and regeneration of gut tissue that indicates that initial gut injury took place hours to days before the clinical diagnosis.² At laparotomy, surgeons often visualize areas of necrotic intestine that are hours to days older than other areas with fresh or evolving NEC.^{1,2} Both fulminant and insidious presentations of NEC have been well described. Pallor, apnea, tachycardia and worsening respiratory status^{1,2} often precede a definitive diagnosis of NEC and may prompt BT. Hence, retrospective studies that ascertain the timing of a firm clinical diagnosis of NEC often do not reflect the nature or duration of a clinical prodrome.²⁸

The link between anemia and NEC found in some studies is not unexpected. Depending on the rapidity and severity of this disease, sequestration of a large volume of blood in the diseased gut is an inherent component of the pathology of NEC.^{1,2} Until a readily available biomarker to diagnose NEC becomes accessible, the nature of NEC is such that intestinal pathology will invariably precede clinical presentation. BT for worsening anemia may occur before the clinical diagnosis of NEC and lead to the erroneous conclusion that BT caused NEC. This situation is analogous to other morbidities of premature infants such as intraventricular or pulmonary hemorrhage that initially may present with pallor and sudden deterioration. Although an acute drop in hematocrit may readily be ascertained, the diagnosis is delayed pending cranial ultrasound or chest radiograph study. To infer a causal relationship between BT and intraventricular hemorrhage would be a flawed conclusion.²⁹

In this light, the recent case-control study by Wan-Huen *et al.*¹⁹ is particularly noteworthy, because it is the only published study that attempted to define the time of onset of NEC. In this report, 97 controls were matched to 49 cases of NEC at a single hospital. Through meticulous review, the authors established the time of onset (as opposed to the time of diagnosis) of each case of NEC based on increased apnea or bradycardia episodes, increased oxygen requirement, abdominal distention or gastric aspirates. Hence, if a BT occurred after the onset of signs of NEC but yet 48 h before a certain diagnosis of NEC could be made, NEC was not considered to be associated with BT (This represents an important methodological refinement compared with the cohort studies by Paul *et al.*¹⁰ and by Blau *et al.*¹¹ in which some cases of NEC were considered to be associated with BT, even though BT was given in response to prodromal signs of NEC). Although Wan-Huen *et al.*¹⁹ argued that their definition of association might have biased the study toward the null hypothesis, the adjusted OR for NEC associated with a BT 48 h before onset of NEC was statistically significant (2.97; $P = 0.003$). We cannot explain why our study results are discrepant. We could not apply our matched sample analytic techniques to the results of Wan-Huen *et al.*¹⁹

owing to unreported data elements. And although Wan-Huen *et al.* conducted an unmatched analysis in a matched study and may have artificially expanded sample size by creating multiple 48-h epoch periods and considering them as independent observations, these nuances would not seem to be fully explanatory of the difference. It is possible that other unidentified confounders existed for which adjustment could not be made.

Recent meta-analyses have also concluded that acceptance of causality between BT and NEC would be premature without the benefit of prospective and more careful methodological studies that adequately control for potential confounders.^{21,30} Large and well-designed randomized controlled trials will provide the most definitive answer to the question of whether BT is associated with NEC in a subset of infants. Such trials will reduce bias inherent to all observational studies that is introduced by the difficulty of ruling out prodromal NEC. And while randomized controlled trials do not guarantee that all known and unknown confounding factors are eliminated, they surely provide better protection against bias than do observational studies. In this setting, the availability of biomarkers that can reliably predict NEC before or during its clinical prodrome would be of enormous value.³¹ Of note is that the relatively small randomized controlled trials that have evaluated the clinical practices and outcomes of BTs have not found an increased risk of NEC related to BT. One such study concluded that a permissive practice of BT was neuroprotective.³² A larger randomized controlled trial that compared a restrictive (low threshold hematocrit) with a liberal (high threshold hematocrit) practice for BTs in infants with birth weights <1000 g identified fewer cases of NEC increased in the more liberally transfused group.³³

CONCLUSION

In summary, we did not find a significant temporal relationship between BT and NEC as has been reported in other studies. Severe restrictions on BT due to concern about BT-associated NEC may result in other unanticipated morbidities. Our results may help clinicians to maintain equipoise about BTs until the results of appropriate randomized controlled studies adequately adjudicate this important potential relationship. Nevertheless, our study does not invalidate the general principle that the decision whether and when to transfuse a premature infant should be made thoughtfully in consideration of the balance of risks and benefits pertinent to each individual patient.

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APPENDIX TRANSFUSION GUIDELINES IN THE STUDY POPULATION

Hematocrit <35% and any of the following conditions:

Patient receiving 0.4 supplemental fractional inspired oxygen concentration by hood or continuous positive airway pressure or receiving fractional inspired oxygen concentration supplementation 0.35 on mechanical ventilation with mean airway pressure 9 or hypotension.

Hematocrit <28% and any of the following conditions:

Receiving 0.4 fractional inspired oxygen concentration by nasal cannula or 0.35 by mechanical ventilation;

Apnea or bradycardia 2 episodes in 24 h requiring bag and mask ventilation without any definite cause;

Heart rate 175 beats min⁻¹ or respiratory rate 70 breaths-min⁻¹ for 24 h without any definite cause; and

Weight gain <10 g kg⁻¹ day⁻¹ for 4 days, while receiving 110 cal kg⁻¹ day⁻¹.

Hematocrit <20% asymptomatic with reticulocyte count <2%.

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Discordant Proportion Difference	Approximate Odds Ratio	Power
0.05	1.4	0.051
0.10	1.8	0.133
0.15	2.2	0.265
0.20	2.7	0.424
0.25	3.1	0.582
0.30	3.5	0.720
0.35	3.9	0.828

Figure 1.

Post-hoc power analysis demonstrates >80% power to detect a difference of 35% in a discordant proportion.

Table 1

Characteristics of infants

<i>Characteristics</i>	<i>NEC</i>	<i>Controls</i>	<i>P</i>
PMA at birth, weeks (mean $\pm$ s.d.)	27 $\pm$ 2	27 $\pm$ 2	0.79 ^a
Birth weight, g (mean $\pm$ s.d.)	983 $\pm$ 333	981 $\pm$ 321	0.95 ^a
Females (%)	18 (43)	26 (62)	0.089 ^b
Feedings (%)			0.026 ^b
Formula	4 (12)	5 (12)	
Human milk	12 (29)	21 (50)	
Both	26 (59)	16 (38)	
Maternal steroid (%)	20 (48)	17 (40)	0.581 ^b
Apgar score at 1 min (median)	4.5	5	0.48 ^c
Apgar score at 5 min (median)	7	7.5	0.47 ^c
Resuscitation at birth (%)	35 (83)	33 (79)	0.53 ^b
Continuous positive airway pressure at birth (%)	12 (29)	7 (17)	0.454
Positive pressure ventilation at birth (%)	30 (71)	30 (71)	1.00 ^b
Intubation and mechanical ventilation (%)	31 (74)	29 (69)	0.56 ^b
Hypotension (%)	11 (26)	8 (19)	0.37 ^b
Patent ductus arteriosus (%)	18 (43)	23 (55)	0.19 ^b
Chronic lung disease (%)	17 (41)	11 (26)	0.08 ^b
Intraventricular hemorrhage (%)	15 (36)	10 (24)	0.17 ^b
Retinopathy of prematurity (%)	10 (24)	21 (50)	0.53 ^b
Periventricular hemorrhage (%)	3 (7)	0	<i>d</i>
Duration of hospitalization, days (mean $\pm$ s.d.)	75.6 $\pm$ 45.6	77 $\pm$ 36	0.80 ^a

Abbreviations: NEC, necrotizing enterocolitis; PMA, postmenstrual age.

^a Paired sample t-test.

^b McNemar chi-square analysis.

^c Wilcoxon's signed-rank test.

^d Unable to calculate test statistic due to zero cell counts.

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Table 2

Paired comparisons of BT within 48 h and within 7 days of onset of NEC

Matched-pair (NEC: control) analysis							
BT within 48 h of NEC				BT within 7 days of NEC			
NEC	Controls		Total	NEC	Controls		Total
	Yes	No			Yes	No	
Yes	4	7	11	Yes	8	5	13
No	5	26	31	No	11	18	29
Total	9	33	42	Total	19	23	42
OR 1.4, CI 0.38–5.59, <i>aP</i> = 0.77				OR 0.45, CI 0.12–1.43, <i>aP</i> = 0.21			

Abbreviations: BT, blood transfusion with packed red blood cells; CI, 95% confidence interval; NEC, necrotizing enterocolitis; OR, conditional odds ratio for discordance.

*a*Two-tailed Exact McNemar *P*-value.

Table 3

Hazard ratio of developing NEC controlling for potential confounding variables

<i>Parameter</i>	<i>HR</i>	<i>CI</i> s	<i>p</i> ^a
BT ^b	0.69	0.46–1.01	0.056
Gender (female)	0.33	0.11–0.98	0.047
Milk (formula)	1.42	0.24–8.41	0.70
Milk (formula and human milk)	2.99	0.93–9.68	0.066

Abbreviations: BT, blood transfusion with packed red blood cells; CI, confidence interval; HR, hazard ratio; NEC, necrotizing enterocolitis.

^a Cox proportional hazard model.

^b As a time-dependent covariate.