Transfusion-associated necrotising enterocolitis in neonates

Amelie I Stritzke,1 John Smyth,2 Anne Synnes,2 Shoo K Lee,3 Prakesh S Shah4

ABSTRACT

Objective To evaluate the association between blood transfusion in previous 2 days and necrotising enterocolitis (NEC) in infants admitted to neonatal intensive care units in Canada.

Patients and Methods Using the Canadian Neonatal Network database of admissions to neonatal intensive care units from 2003 to 2008, cases with NEC were matched with controls by gestational age (GA) at birth. Exposure to transfusion within 2 days of NEC (for cases) or 2 days before the median age of NEC diagnosis among cases of the same GA (for controls) was determined. After controlling for confounders, the differences in characteristics and neonatal outcomes of transfusion-associated NEC (TANEC) and NEC not associated with transfusion (non-TANEC) were compared.

Results NEC cases (n=927) were matched with 2781 controls. Transfusion in previous 2 days was significantly higher in NEC cases than in controls (15.5 vs 7.7%; adjusted OR (AOR) 2.44; 95% CI 1.87 to 3.18). TANEC cases versus non-TANEC cases had a lower mean GA (25.8 vs 29.3 weeks), a lower mean birthweight (885 vs 1373 grams), and a higher proportion of infants with SNAPPi score >20 (52.1 vs 22.9%). After adjustment for confounders, no significant differences in mortality (AOR 1.28; 95% CI 0.82 to 2.01), severe retinopathy (AOR 1.15; 95% CI 0.71 to 1.87), or severe neurological injury (AOR 0.83; 95% CI 0.43 to 1.60) were identified.

Conclusions Exposure to transfusion in previous 2 days was an independent risk factor for NEC. After controlling for confounders, no significant differences in mortality and morbidities were observed between infants who had transfusion-associated NEC and those with NEC not associated with transfusion.

INTRODUCTION

The aetiology of necrotising enterocolitis (NEC) in infants is likely multi-factorial. Despite recent improvements in the survival of extremely preterm infants, the incidence and illness burden of NEC has remained unchanged over the past 50 years.1 Effective preventive strategies remain elusive, and the prevalence fluctuates around 5% among very low birthweight (BW) infants in Canada.2 Suspected risk factors include preterm birth, small for gestational age (SGA) status, hypoxic-ischaemic events, early and rapid advancement of enteral feeds, formula feeds and bacterial overgrowth.3 Several case series have suggested a potential role for red blood cell (RBC) transfusion in cases of NEC.1–3 Some case-control studies indicated that patients with transfusion-associated NEC (TANEC) may have different characteristics and outcomes from patients with NEC that is not associated with transfusion (non-TANEC).3 6 However, the studies reported thus far have involved small numbers, and there is no population-based large cohort study to convincingly show an association of recent transfusion with NEC and outcomes of such infants.

Our primary objective was to evaluate the association between recent exposure to RBC transfusion and NEC in a Canadian population-based neonatal database. The secondary objective was to evaluate neonatal outcomes of infants who had TANEC with those infants who had NEC unassociated with recent transfusion.

METHODS AND PATIENTS

Patients and data collection

The study population was selected from 58 193 infants admitted to 26 participating neonatal intensive care units (NICUs) in the Canadian Neonatal Network (CNN) between 2003 and 2008. All NICUs are regional tertiary level referral centres, and the CNN database comprises >90% of admissions to level 3 NICUs in Canada. The NICUs ranged in size from 9 to 70 beds and had 74–1122 admissions annually. Data were collected by trained abstractors at each site until discharge from the NICU, and were entered directly from patient charts into computers using a customised data entry program with built-in error checking and a standard manual of protocols and definitions.7 Data were collected with institutional approval of

either a local Research Ethics Board or an institutional quality improvement process.

Gestational age (GA) was defined as the best estimate based on early ultrasound, or obstetric history and examination followed by paediatric estimate, in that order. Diagnosis of stage 2 or 3 NEC was made by the medical team caring for the infant according to Bell’s criteria.8 Infants with NEC had clinical and radiographical changes consistent with the diagnosis. Feeding practices, including volume and type of feed, were variable between centres and were not collected. The threshold for transfusion was variable at each unit and the practice of holding feeds during transfusion or not also varied between centres and within centres. Transfused RBCs were irradiated, cytomegalovirus-negative, leucocyte-reduced, generally not washed, type specific or type O. Range of storage for RBCs is 1–42 days, and the usual storage solution contained SAGM (saline, adenine, glucose, mannitol). Usual transfusion volumes were between 15 and 20 ml/kg as per Canadian Pediatric Society guidelines.9 All infants had an illness severity score calculated based on data from the first 24 h after birth (SNAPII – Score for Neonatal Acute Physiology).10 Diagnosis of patent ductus arteriosus (PDA) was based on clinical or echocardiographical measures or both, depending upon centre practices. Intraventricular haemorrhage (IVH) was defined according to the criteria of Papile et al from the worst findings on head ultrasound.11 Detection of periventricular echogenicity or leucomalacia (PVL) was based on ultrasound findings after 21 days of age. Severe neurological injury was defined as grade 3 or 4 IVH or PVL. Retinopathy of prematurity (ROP) was classified according to the international classification.12 Severe ROP was defined as ROP of stage 3 or higher, in either eye, or the need for surgery.

Study design
This study was a 1:3-matched case-control design. This was a secondary analyses from the CNN dataset. Each case meeting the criteria for stage 2 or 3 NEC, according to Bell’s criteria,8 was identified from the database. From the remaining neonates, for each case, three control neonates without NEC matched by GA at birth were randomly selected using SAS macro %match, a computer program designed to generate random matches. For cases and controls, infants with major congenital anomaly involving gastrointestinal tract were excluded.

Definition of exposure
Among NEC cases
Exposure was defined as the receipt of RBC transfusion within the 2 calendar days before the day of diagnosis of NEC.

Among controls without NEC
Exposure was defined as receipt of an RBC transfusion within the 2 calendar days before the median age of NEC diagnosis among cases for each of the same GA stratum in weeks. Due to the nature of the dataset that collected events for each calendar day, we were unable to break down the association to hours.

Statistical analysis
The study population was described using descriptive statistical methods. Infant characteristics, including gender, Apgar score at 5 min, SNAPII score, outborn status, prenatal steroid use and BW were compared between NEC cases and controls using χ² tests for categorical variables and t tests or non-parametric tests for continuous variables, as appropriate. A multiple conditional logistic regression model, derived using a stepwise procedure, was further employed to examine the association between recent exposure to transfusion and NEC after controlling for BW: SGA status, SNAPII score, Apgar score at 5 min, outborn status and prenatal steroid use.

For our secondary objective, the outcomes of TANEC infants were compared with outcomes for non-TANEC infants using multiple logistic regression methods. Data management and statistical analyses were performed using SAS 9.2 (SAS Institute, Cary North Carolina, USA); a significance level of less than 0.05 was used without multiple comparison adjustment.

RESULTS
Of the 58 193 infants in the CNN database between 2003 and 2008, 1026 met the criteria for the diagnosis of stage 2 or 3 NEC. After exclusion of 99 infants (97 with congenital anomalies of the digestive tract and two with missing data), 927 infants were included in the study. From the remaining 57 887 neonates who did not meet the criteria for diagnosis of stage 2 or 3 NEC, those with anomalies of the digestive tract were again excluded, then three control neonates per case (n=2781), matched by GA, were selected at random (figure 1).

Baseline comparison revealed a GA of 28.8 weeks, and higher rates of infants who were SGA or outborn among NEC cases compared with controls without NEC (table 1). The SNAPII scores were higher and the Apgar scores were lower among the cases of infants with NEC, overall transfusion rate was the same: 366 (39.5%) in NEC cases and 1091 (39.2%) in controls; 144 (15.5%) were exposed to transfusion within 2 days of diagnosis of NEC (IQ range 0–2) compared with 214 (7.7%) infants in the control group.

To understand the characteristics of the infants who had received a transfusion, we compared 355 neonates who had been exposed to transfusion, regardless of the development of NEC, to the remaining 3350 infants who had not been exposed to transfusion in the same time period. Neonates who received transfusions were more likely to have had a low 5 min Apgar score, a SNAPII score >20, and to be of lower BW. A multivariable conditional logistic regression analysis (table 2) controlling for these confounders revealed that recent exposure to transfusion remained significantly associated with NEC (adjusted OR (AOR) 2.44, 95% CI 1.87 to 3.18) (table 2).

Finally, we divided all NEC patients into TANEC (144 infants; 15.5%) and non-TANEC groups (783 infants; 84.5%), and compared their characteristics and outcomes (table 3). TANEC infants were of lower GA at birth, had lower BW, higher SNAPII scores, higher rates of PDA, and higher postnatal age at diagnosis (day 20 vs day 14) compared with non-TANEC infants. Of the total TANEC patients, 58 (42.3%) had surgical NEC. Of the non-TANEC patients 206 (26.3%) had surgical NEC. Univariate analyses revealed a significantly higher risk of mortality, severe neurological injury and severe ROP among TANEC infants compared with non-TANEC infants; however, after adjusting for confounders, no significant differences in any of these outcomes were identified (table 4).

DISCUSSION
In this large population-based study, we identified an association between RBC transfusions and NEC, after adjusting for confounding variables. In keeping with known risk factors, our NEC population was composed of more SGA and ‘sicker’ neonates, in terms of initial illness severity scores, compared with controls, despite being matched by GA. Within all our cases of NEC, comparison of TANEC infants with non-TANEC infants revealed that patients who developed TANEC were smaller and
had higher illness severity scores. In addition, the infants in whom NEC was associated with transfusion showed a higher postnatal age but lower BW and GA. Controlling for these confounders, however, there were no differences in mortality and severe morbidities during the neonatal period between infants who had TANEC compared with those who had NEC not associated with transfusion.

Our demonstration that TANEC cases were significantly more premature and more ill on the first day of life than non-TANEC is consistent with several other studies, although not all. The observation that TANEC cases were diagnosed at a higher postnatal age than non-TANEC cases is in line with several previous reports that TANEC occurs around the 23 to 37 days, compared with 11 to 16 days reported for non-TANEC. One possible explanation for this is the more severe prematurity in TANEC infants and the purported concurrence of NEC around GA 31 weeks of postmenstrual age. Although others have reported an increased length of stay, more surgery, and higher mortality and morbidities in TANEC cases, after adjusting for confounders, we found no differences in NICU mortality or severe neonatal morbidities except for an increase in surgical NEC in the TANEC group.

Several mechanisms have been presented as potential explanations for the occurrence of TANEC. In adults, immunologically mediated transfusion-associated lung injury is not uncommon. However, since 1999, all patients involved in this study have received leucocyte-depleted blood, reducing the likelihood that this is a causal mechanism of TANEC. Of interest, in Canada the introduction of universal leucocyte reduction of RBC products for transfusion to premature infants was associated with a significant decrease in NEC (OR 0.39; 95% CI 0.17 to 0.9).

Severe anaemia, with a low pretransfusion haematocrit, was thought to be a contributing factor to TANEC by triggering oxidative stress, to a hypoxic, immature gut wall during acute reperfusion. This can induce vasoconstriction in certain vascular compartments. Mally and Blau were able to show that TANEC cases had lower pretransfusion haematocrits compared with non-transfusion associated NEC patients. Singh et al reported significant differences in the haematocrits between TANEC and NEC patients (0.299 and 0.334, respectively, 95% CI 1.02 to 1.18, p=0.01). On the other hand, Josephson et al reported no difference in pretransfusion haematocrits. Unfortunately, pretransfusion haematocrit values were not recorded in our database.

### Table 1 Comparison of cases with NEC and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>NEC n=927</th>
<th>Non-NEC n=2781</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight in kg, mean (SD)</td>
<td>1.30 (0.72)</td>
<td>1.34 (0.74)</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Small for gestational age, n (%)</td>
<td>53 (5.7)</td>
<td>83 (3.0)</td>
<td>1.98 (1.39 to 2.82)</td>
<td>0.01</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>505 (54.7)</td>
<td>1549 (55.7)</td>
<td>0.96 (0.82 to 1.11)</td>
<td>0.56</td>
</tr>
<tr>
<td>Outborn, n (%)</td>
<td>297 (32.1)</td>
<td>658 (23.9)</td>
<td>1.49 (1.28 to 1.75)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5-Min Apgar score &lt;7, n (%)</td>
<td>252 (27.7)</td>
<td>602 (22.0)</td>
<td>1.4 (1.17 to 1.68)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SNAP II score &gt;20, n (%)</td>
<td>254 (27.4)</td>
<td>636 (22.9)</td>
<td>1.33 (1.11 to 1.60)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Prenatal steroids use, n (%)</td>
<td>661 (74.5)</td>
<td>1813 (70.9)</td>
<td>1.24 (1.00 to 1.53)</td>
<td>0.05</td>
</tr>
<tr>
<td>Transfusion exposure in previous 2 days, n (%)</td>
<td>144 (15.5)</td>
<td>213 (7.7)</td>
<td>2.38 (1.87 to 3.02)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*NA, not applicable; NEC, necrotising enterocolitis; SNAP, Score for Neonatal Acute Physiology.*
Another proposed mechanism is the alteration of superior mesenteric artery flow associated with RBC transfusions. In our study, the association of a haemodynamically significant PDA with TANEC supports a role for altered intestinal blood flow in the pathophysiology of TANEC. Impaired intestinal blood flow, aggravated by posttransfusion-transfusion-related reduction in superior mesenteric artery flow, is a plausible risk factors for NEC. Interestingly, El-Dib et al reported a significant decrease in NEC from 5.3% to 1.3% (p=0.047) in the 18 months following a policy change of withholding feeds during transfusion; however, a reduction in TANEC was not found. Studies to suggest the optimal time and duration for such a potential intervention are still lacking. Further investigations on feeding around the time of RBC transfusions are indicated and might provide a simple method of reducing TANEC.

The fact that our study included a large sample size, is nearly population-based and multicentre, increases confidence in the generalisability of our findings. In addition, our method of limiting our definition of exposure (within the 2 calendar days before the day of diagnosis of NEC for cases or the corresponding age for controls) strengthens the plausibility of the association. Limitations of our study include the retrospective nature of our study using a large database. In addition, data were not available regarding the exact time interval between transfusion and onset of clinical signs of NEC, as this is difficult to know with certainty. Our data collection only included days and not hours, so theoretical range of exposure time includes 23 h to 71 h preceding the onset of NEC. We elected for this gap as we wanted to rule out the receipt of transfusion in relation to disease (NEC) itself. Data about the blood, the donors and the exact indications and the degree of urgency of the need for transfusion may vary widely between centres and were also not available. Further variability included whether feeding was held or not during transfusion and practices unrelated to transfusion such as management of a PAD, advancement of feeding, and the duration of trophic feeding. Also, we did not include site in the regression model because there was no statistically significant site variation in the incidence of NEC. The heterogeneity of our sample improves the generalisability of the findings to other populations.

CONCLUSION

Exposure to transfusion within 2 calendar days before a diagnosis of NEC was an independent risk factor for NEC in infants. Infants who developed TANEC were younger, of lower BW and had higher illness severity scores. After controlling for confounders, no significant differences in mortality and neonatal morbidities were observed between infants who had TANEC versus those with NEC not associated with transfusion.

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Contributors Amelie Stritzke, initiated the concept, assembled the cohort, interpreted the data, wrote the first draft and revisions of the manuscript, and approved the final version. John Smyth, initiated the concept, revised the protocol, assisted with the data interpretation and approved the final version of the manuscript. Anne R Synnes, initiated the concept, revised the protocol, assisted with the data interpretation and approved the final version of the manuscript. Shoo K Lee, (Director, Canadian Neonatal Network); Prakesh S Shah (Associate Director, Canadian Neonatal Network and site investigator Mount Sinai Hospital, Toronto); Wayne Andrews (Janeway Children’s Health and Rehabilitation Centre, St John’s, NL); Keith Barrington (Sainte Justine Hospital, Montreal, QC); Wendy Yee (Foothills Medical Centre, Calgary, AB); Barbara Bullied (Everett Chalmers Hospital, Fredericton, NB); Roderick Canning (Moncton Hospital, Moncton, NB); Ruben Alvaro (St. Boniface General Hospital, Winnipeg, MB); Kimberly Dow (Kingston General Hospital, Kingston, ON); Michael Dunn (Sunnybrook Health Sciences Centre, Toronto, ON); Adele Harrison (Victoria General Hospital, Victoria, BC); Andrew James (The Hospital for Sick Children, Toronto, ON); Zarin Kalapesi (Regina General Hospital, Regina, SK); Lajos Kovacs (Jewish General Hospital, Montreal, QC); Orlando da Silva (St. Joseph’s Health Centre; London, ON); Douglas D. McMillan (WK Health Centre, Halifax, NS); Cecil Ojah (St. John Regional Hospital, St. John, NB); Abraham Peliowski/Khalid Aziz (Royal Alexandria Hospital, Edmonton, AB); Bruno Pietboeuf (Centre hospitalier universitaire de Québec, Sainte Foy, QC); Patricia Riley (Montreal Children’s Hospital, Montreal, QC); Daniel Faucher (Centre hospitalier universitaire de Québec, Sainte Foy, QC); Nicole Rouvinez-Bouali (Children’s Hospital of Eastern Ontario, Quebec, QC); Nicole Rouvinez-Bouali (Children’s Hospital of Eastern Ontario, Quebec, QC).

Table 2 Risk factors for necrotising enterocolitis in cases versus controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transfusion exposure in previous 2 days</td>
<td>2.44 (1.87 to 3.18)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Outborn status</td>
<td>1.89 (1.42 to 2.04)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>1.97 (1.33 to 2.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Adjusted for birthweight, SNAP, SNAP score at 5 min, outborn status and prenatal steroid use. SNAP, Score for Neonatal Acute Physiology.</td>
<td></td>
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</tr>
</tbody>
</table>

Table 3 Comparison of subjects with TANEC and subjects with NEC without association to transfusion (non-TANEC)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TANEC N=144</th>
<th>Non-TANEC N=783</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age in weeks, mean (SD)</td>
<td>25.8 (2.6)</td>
<td>29.3 (3.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Birth weight (kg), mean (SD)</td>
<td>0.885 (0.406)</td>
<td>1.373 (0.735)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5-Min Apgar score &lt;7, n (%)</td>
<td>67 (46.9)</td>
<td>195 (24.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SNAP II score &gt;20, n (%)</td>
<td>75 (52.1)</td>
<td>179 (22.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Day of life for NEC, median (IQR)</td>
<td>20 (12.5–33)</td>
<td>14 (8–27)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PDA, n (%)</td>
<td>98 (72.6)</td>
<td>249 (32.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>PDA needling surgery, n (%)</td>
<td>44 (45.8)</td>
<td>92 (38.7)</td>
<td>0.23</td>
</tr>
<tr>
<td>Mortality, n (%)</td>
<td>34 (34.0)</td>
<td>157 (20.1)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Severe ROP (stage 3 or higher), n (%)</td>
<td>28 (31.1)</td>
<td>61 (15.4)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Severe neurological injury*, n (%)</td>
<td>43 (31.9)</td>
<td>110 (17.6)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*Defined as Grade 3 or 4 intraventricular haemorrhage or periventricular leukomalacia.
NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; SNAP, Score for Neonatal Acute Physiology; TANEC, transfusion-associated necrotising enterocolitis.

Table 4 Multivariable analyses: outcomes between TANEC and non-TANEC infants

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Raw OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mortality</td>
<td>2.06 (1.40 to 3.03)</td>
<td>1.28 (0.82 to 2.01)</td>
</tr>
<tr>
<td>Severe ROP</td>
<td>2.19 (1.45 to 3.33)</td>
<td>1.15 (0.71 to 1.87)</td>
</tr>
<tr>
<td>Severe neurological injury</td>
<td>2.47 (1.47 to 4.17)</td>
<td>0.83 (0.43 to 1.60)</td>
</tr>
</tbody>
</table>

Adjusted for GA, small for gestational age, SNAP score, SNAP, Score, oxygen status, prenatal steroids use and patent ductus arteriosus. ROP, retinopathy of prematurity; TANEC, transfusion associated necrotising enterocolitis.
REFERENCES

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