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ORIGINAL ARTICLE

TITLE: Characteristics and incidence of transfusion-associated necrotizing enterocolitis in the UK

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ABSTRACT

Background and Aims: The aetiology of necrotizing enterocolitis (NEC) is unclear and postulated as being multifactorial. It has been suggested that one causative factor is the transfusion of packed red bloods cells (PRBCs) leading to the disease entity commonly referred to as transfusion-associated NEC (TANEC). TANEC has been reported in North America but its incidence has not been formally investigated in the United Kingdom (UK). Our aims were to identify the incidence of NEC and TANEC in tertiary-level UK neonatal units and to describe characteristics of TANEC cases .

Materials and Methods: Using strict case definitions for NEC and TANEC, we undertook a retrospective review to estimate the incidence of TANEC cases occurring in four UK tertiary-level centers during a 38-month period.

Results: Of 8007 consecutive neonatal admissions of all gestations to the four centers, 68 babies went on to develop NEC and all affected infants were of very low birth weight (VLBW); 34 of these had previously received a transfusion of PRBCs but did not fit the diagnostic criteria for TANEC, while 15 (22%) of the 68 babies with NEC qualified as TANEC cases. UK cases occurred at an earlier postnatal age than cases reported in multiple large North American series and were of a lower birth weight.

Conclusions: We have confirmed the presence of TANEC in the UK VLBW neonatal population. Its incidence lies within the wide range described in previous reports of this phenomenon globally, though with some local variation in characteristics. Further work is needed to clarify causation, pathophysiology, and possible mechanisms of prevention of TANEC.

INTRODUCTION

The aetiology of necrotizing enterocolitis (NEC) remains elusive. Multiple factors are considered important in its development including ischemia, reperfusion injury, gut dysbiosis, infection, mechanical injury, and immune dysfunction. Transfusion of packed red blood cells (PRBCs) has also been postulated as causative for some cases [1]. NEC occurring within 48 hours of PRBC transfusion, and usually diagnosed using Bell's criteria stage ≥2, has been termed transfusion-associated NEC (TANEC); this definition has been used in most studies. The role of PRBC transfusion in the causation of TANEC is still debated, although several plausible mechanisms are proposed [1,2].

TANEC has been reported in large retrospective North American series at widely-varying rates comprising 16-67% of NEC cases [2-9] though case definitions used for NEC and TANEC have sometimes been inconsistent. There are only isolated and recent reports of TANEC in the UK setting [10], and the proportion of TANEC among UK NEC cases is unknown. Standardized feeding practices and routine use of probiotics may reduce NEC rates,[11,12] but their impact on TANEC is unknown. It is unclear whether the characteristics of UK cases are similar to those reported from elsewhere. We therefore conducted a retrospective review of NEC cases in four UK tertiary-level neonatal intensive care units (NICUs) using strict case definitions for both NEC and TANEC. Our aims were to evaluate the incidence of TANEC over a 3-year period and to describe the characteristics of UK cases.

METHODS

This was a retrospective observational audit conducted in four of the UK's 55 tertiary-level neonatal centers (Cardiff, Imperial College London, Newport, and Norwich) using strict, pre-specified case definitions for classifying cases of NEC and TANEC. We categorised a case as being definite NEC based on a modified version of the Vermont-Oxford Network case definition[13], which diagnosed NEC by one or more of the following: i) surgically, at a proximate laparotomy; ii) pathologically, at post mortem; iii) clinico-radiologically, where there had been clinical symptoms consistent with NEC (including bilious aspirates or emesis, abdominal distension, occult or frank blood in stool) plus accompanying abdominal radiographic evidence of pneumatosis intestinalis, hepato-biliary gas, or pneumoperitoneum. Our modification excluded any case that met the clinico-radiological diagnosis where a proximate laparotomy or a post mortem found no sign of NEC. Cases of spontaneous intestinal perforation were also excluded. We defined TANEC as being a case of definite NEC where the first possible clinical symptoms of NEC (including abdominal distension, bile-stained aspirates, and apneas/bradycardias/desaturations) had begun within 48 hours of commencement of a PRBC transfusion.

Participating centers used a common data collection proforma (**Supplemental online only File S1**). Enteral feeding practices were similar in the participating units and were based on standardized guidelines (example, **Supplemental online only File S2**). Data were gathered for the 38-month period 1st October 2011 to 30th November 2014 by neonatal physicians. We reviewed clinical and radiographic records of all babies admitted in the period who had a diagnosis of suspected or definite NEC recorded in their electronic BadgerNet records (Clevermed UK Ltd). Cases identified as being definite NEC were then further evaluated to see if they had undergone any prior PRBC transfusion and to determine if they met the case definition for TANEC. Blood transfusion data were obtained from our local hospital blood transfusion departments. The medical case notes of all that met the TANEC case definition were interrogated to collect data on baseline characteristics, details of feeding, use of probiotic prophylaxis, transfusion history, nature of symptoms at the onset of NEC and their timing in relation to the PRBC transfusion, and clinical outcomes.

The study was performed as a registered multicenter audit. The study protocol had previously been reviewed by our research services manager and did not require formal ethics approval according to the contemporaneous UK National Research Ethics Service guidance.

RESULTS

Sixty eight babies across all sites had definite NEC, and all were very low birth weight (VLBW; <1500 g). There were 1608 VLBW admissions during the audit period, giving an NEC incidence among VLBW admissions of 4.2% (68/1608; 95% confidence interval: 3.3–5.3%). Fifteen (22%) of the sixty eight NEC cases met the case definition for TANEC. Overall, TANEC occurred in 0.93% of VLBW admissions (15/1608; 95% confidence interval: 0.52–1.53%). **Figure 1** shows the study flow diagram and classification of cases of NEC and TANEC among all admissions and among VLBW admissions. The 15 TANEC cases were born at a mean gestational age of 25.1 weeks and had a mean birth weight of 695 g. **Table 1** presents their baseline characteristics and details of their PRBC transfusions. Eight cases had their onset of symptoms within ≤24 hours after commencement of transfusion, and seven had their onset between 24–48 hours after commencement of transfusion. One infant had been transfused with irradiated PRBCs due to previous in utero platelet transfusion, and two infants had not received any PRBCs prior to the index transfusion. The earliest presenting NEC symptoms in these 15 babies were: abdominal distension (n=12, 80%); increased aspirates (n=9, 60%); bile-stained aspirates (n=5, 33%); and bradycardias/desaturations (n=6, 40%). Among the four NICUs, the local incidence of definite NEC ranged from 4.5 to 9.7 cases per year (an incidence of 3.7–7.8% among VLBW admissions), and the incidence of TANEC within these individual NICUs ranged from 0.50 to 1.95 cases per year (an incidence of 0.41–1.64% among VLBW admissions). The proportion of TANEC/NEC cases within individual NICUs ranged from 11%–40% and there was no correlation between comparative local NEC rates and TANEC rates of individual NICUs (Spearman's rho = 0.74, 95% CI: –0.77 to +0.99, P-value = 0.4).

Three of the four participating NICUs introduced routine probiotic supplementation during the study period (Infloran®, Desma Healthcare, Switzerland) providing a daily dose of 1 billion each of live *Lactobacillus acidophilus* and *Bifidobacterium bidfidum*. Only one NICU practiced routine cessation of enteral feeding during PRBC transfusions. Of the 15 TANEC babies, 12 (80%) were receiving enteral feeds at the first onset of NEC symptoms (6 were fully enterally fed with daily milk intakes ranging from 146-180 mL/kg; 6 were receiving lower milk volumes ranging from 10-130

mL/kg/day with concurrent parenteral nutrition). Of the 12 enterally-fed babies, 7 were exclusively on their mother's own milk, 3 were on mixed human milk and preterm formula feeds, and the milk type was unrecorded in 2 cases. Two TANEC babies were on full parenteral nutrition and were not being fed enterally at the first onset of NEC symptoms; the feeding details of a further case were unrecorded.

DISCUSSION

Our study shows that across four UK centers, NEC occurred in proximate association with PRBC transfusion at a rate within the wide range reported from many other countries (22% of overall NEC cases, 0.93% of total VLBW admissions). In 2015, a total of six TANEC cases were reported to the UK national hemovigilance scheme (Serious Hazards of Transfusion; SHOT) [10]. Yet we identified 15 cases in our four centers over the 3-year audit period, suggesting that the annual UK total is likely to be much higher. TANEC cases were identified in all our centers, although rates of both NEC and TANEC varied between centers. Reported rates of TANEC as a proportion of NEC cases, in mostly VLBW infants, have also varied markedly in the reported large North American series: 7/44 (15.9%) [9], 144/927 (16%) [7], 18/93 (19%) [3], 9/36 (25%) [4], 33/122 (27%) [5], 40/112 (36%) [2], 60/148 (41%) [8], and 116/174 (67%) [6].

NEC cases associated with transfusion have tended to affect babies born at much earlier birth gestations who developed NEC later in their neonatal course, generally at 3-5 weeks postnatal age, and following at least one previous PRBC transfusion [1–8]. Comparison of characteristics of TANEC cases in our present study with those of the North American reports reveal similarities but also some key differences: mean birth gestation of affected UK infants was slightly lower than the 26-27 weeks reported by some [2,5,8], but similarly low (mean: 25 weeks) as in others [3,4,7]. However our cohort's median birth weight (695 g) was lower than in any of the other large series which show a range between median 735 g [3] and mean 981 g [2]. UK TANEC cases occurred at earlier postnatal age (median: 16 days versus between median 20 [7] and 39 days [4]. Time of onset of NEC symptoms after transfusion was later in our series compared with that of Blau et al. [4] (median: 24 h versus 6 h), and the UK cases were transfused at a slightly higher hematocrit level (31.5% compared to 25.0% [4]–30.7% [3]). A notable feature of UK cases was the older shelf age of blood product transfused (medians: 16 days versus 7 [8]–11 days [2]). While age of blood has not been shown to differ between TANEC and non-transfusion associated NEC cases [1] or to alter the rate of NEC [14], further studies are needed to examine the potential hazards associated with transfusion of older stored blood to

premature neonates.[15] The excess of females in our series was also seen in one study [4], but was not found in others [5,8].

The existence of TANEC as a distinct subset of NEC remains controversial. No definite causal link between transfusion and NEC has been proved, and the available evidence from published studies is contradictory. Indeed three published meta-analyses have reached conflicting conclusions [16-18]. An important recent systematic analysis of the evidence behind TANEC found it to be of overall "very low" quality [19]. The odds ratio from observational/case control studies for the pooled outcome of TANEC defined as occurring within 48 h of PRBC transfusion was 1.13 (95% CI: 0.99-1.29), p=0.07, while that for the pooled outcome of NEC occurring at any time post PRBC transfusion was 1.95 (95% CI: 1.60-2.38), p<0.00001 [19].

Some consider that babies transfused for symptoms attributed to anemia may have been showing those same symptoms as their earliest feature of NEC. Nevertheless, in our present study most babies who developed TANEC were completely asymptomatic at the start of transfusions that were given routinely only to 'top up' a low hemoglobin found as an incidental finding during routine hematological monitoring. Patel and colleagues studied the association between PRBC transfusions, severe anemia (defined as hemoglobin ≤80 g/L), and NEC (Bell's stage ≥2) [9]. This large, multicenter observational study of 598 VLBW infants of whom 44 (7.4%) developed NEC found that severe anemia but not PRBC transfusion was associated with an increased risk of NEC. Interestingly, only 2 (13%) of the 15 babies in our own cohort had severe anemia prior to their index transfusion that preceded TANEC **(Table 1)**.

It is likely that the pathogenesis of TANEC, like NEC, is multifactorial. Several aetiological mechanisms have been proposed for a possible causal association between PRBC transfusion and the development of NEC [1]. Sensitization by a previous PRBC transfusion might cause an exaggerated response of 'primed' neutrophils to exogenous mediators in the subsequently-transfused blood (though two infants in the present series had not received prior PRBCs); the anemia that has often prompted the transfusion may itself adversely affect intestinal blood flow thus predisposing the immature gut to threshold ischemic injury [19, 20]. The subsequent transfusion of relatively

deoxygenated blood might precipitate reperfusion injury, perhaps especially during the periods of increased gut motility and metabolic demands associated with enteral feeding [20]; stored red cells also have reduced deformability and so are more prone to adhesion and aggregation. They are also relatively deficient in nitric oxide which impairs their ability to achieve vasodilation, thus possibly increasing the risk of hypoperfusion and ischemic injury [1].

Strengths of our study include its multicenter design, its large cohort of VLBW admissions that were screened using uniform strict definitions, and it being the first substantive report of the occurrence and estimated incidence of TANEC among NEC cases in the UK, not least within the era of use of probiotics and standardised feeding regimens. Limitations include its retrospective nature, and the lack of control data collection on the non-transfusion associated NEC cases.

CONCLUSION

We have identified a subpopulation of VLBW neonates affected by NEC, whose disease was preceded by a recent PRBC transfusion. Our data are consistent with reports of the existence of this phenomenon worldwide, and highlight that TANEC also exists in the UK neonatal population, though with some differing characteristics. In the context of the relative uncommonness of TANEC among VLBW infants and the variation in incidence between centers, the major challenge going forward is how to design a large multicenter trial that can test interventions with the potential to reduce its incidence.

ADDENDUM:

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Competing interests/ Conflict of interest statement: PC and HVN are current Steering Group members of the Serious Hazards of Transfusion UK national haemovigilance scheme (SHOT). There are no competing interests and no conflict of interests to declare in relation to this work.

Prior Presentation: These data were presented in abstract form at the 19th annual spring meeting of the Royal College of Paediatrics and Child Health, Birmingham, UK, May 2015, and at the 1st congress of joint European Neonatal Societies, Budapest, Hungary, September 16-20, 2015.

Ethics approval: This study was performed as a registered multicenter audit and did not require ethics approval according to the contemporaneous National Research Ethics Service guidance.

Author contributions:

PC conceived and designed the study and the data collection proforma. PC, SH, KDJ, KS, SG, HVN, SC, and AJ collected the data for their respective NICUs. PC analysed the data. CMF and PC wrote the first manuscript draft and PC wrote the final draft. All living authors provided intellectual input, had access to the complete dataset, contributed to manuscript revisions, and approve of the final version. PC is guarantor.

Dedication:

We dedicate this work to the memory of Dr Shobha Cherian, consultant neonatologist and our respected friend, colleague, and co-author, who sadly died in November 2015.

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Figure 1: Overall cases of NEC and TANEC among total and VLBW admissions in the four centers. Out of 1608 VLBW admissions, 68 neonates developed definite NEC; 19 (28%) had never received a PRBC transfusion, 34 (50%) received a transfusion but not in temporal relation to NEC, and 15 (22% of NEC cases) developed TANEC.

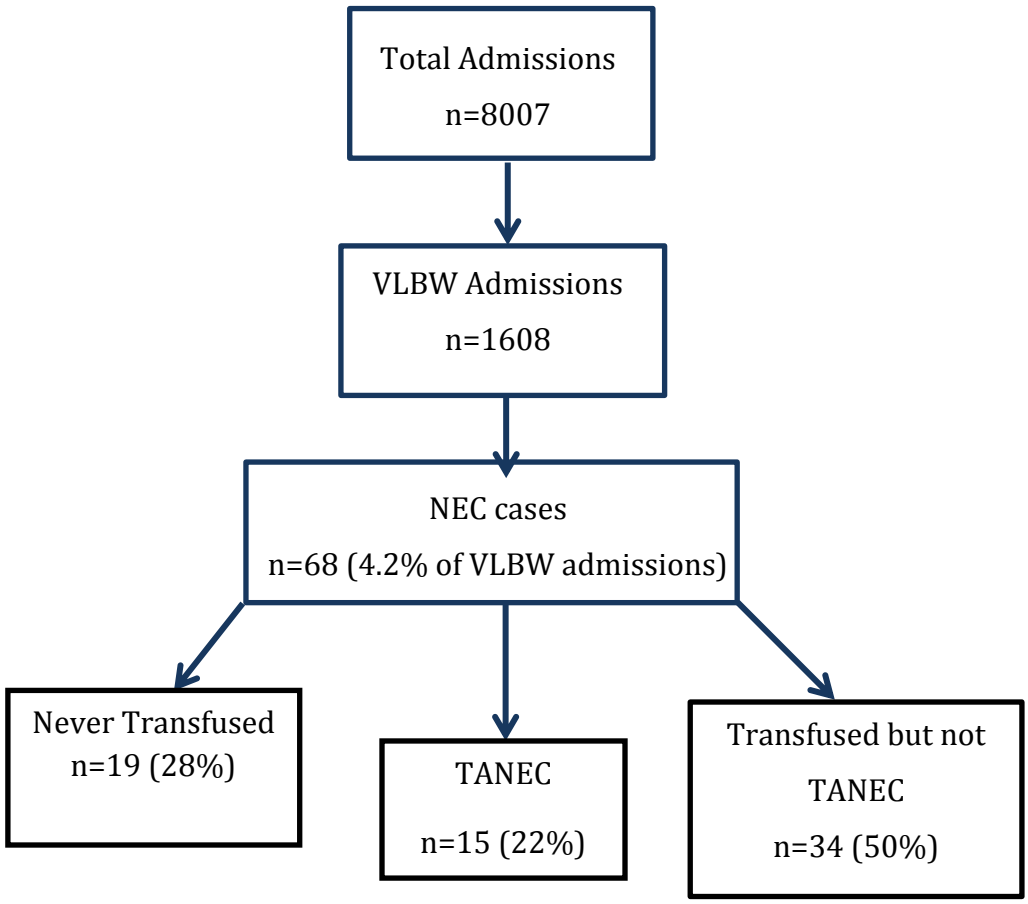

Table 1: Baseline characteristics and transfusion details of the 15 neonates with transfusion-associated necrotizing enterocolitis

Baseline characteristics	
Birth gestation, weeks	25.1 (23.3–27.0), [25.0–26.0]
Birth weight, g	695 (527–1070), [582–830]
Male gender	5 (33%)
Small for gestational age	4 (27%)
PDA on echocardiogram <2 weeks prior	7 (47%)
Ibuprofen treatment <2 weeks prior	3 (20%)
Culture-positive sepsis <1 week prior	5 (33%)
Received probiotics prior to NEC	4 (27%)
Ventilator dependant at onset of NEC	9 (60%)
Surgical intervention needed	10 (67%)
Survived to discharge	9 (60%)
Transfusion details	
Primary reason for transfusion:	
Routine 'top-up' (with no symptoms of anemia)	9 (60%)
Symptomatic anemia	5 (33%)
Other*	1 (7%)
Postnatal age at start of index transfusion, days	15 (0–69)†, [5–36]
Time between start of index transfusion and onset of first NEC symptoms, hours	24 (3–46), [15–36]
Postnatal age at diagnosis of NEC, days	16 (1–70), [6–36]
Corrected gestational age at diagnosis of NEC, weeks	27.0 (24.9–34.9), [26.0–31.7]
Hemoglobin prior to index PRBC transfusion, g/L	103 (71–147), [94–113]
Hematocrit prior to index PRBC transfusion, %	31.5 (21.5–43.0), [26.5–37.9]
Severe anemia (hemoglobin <80 g/L) prior to index PRBC transfusion	2 (13%)
Total number of previous PRBC transfusions§, n	3 (1–14), [2–6]
Volume of PRBCs transfused, mL/kg	20.0 (9.8–27.3), [19.5–20.0]
Age of transfused blood, days since donation	16 (7–35), [14–27]

Data are shown as median (range) and [interquartile range], or n (%)
NEC, necrotizing enterocolitis. PDA, patent ductus arteriosus. PRBC, packed red blood cell
*'Other' was specified for one case as 'critically unwell with moderately low Hb'
†Minimum age was 5 hours postnatal
§Including the index transfusion