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The relationship of red blood cell transfusion to intestinal mucosal injury in premature infants☆

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ABSTRACT

Objective: To determine the incidence of intestinal mucosal injury before and after transfusions in premature infants.
Study design: Urine was collected throughout the hospital stay of 62 premature infants and specimens obtained within 24 h before and after transfusion were assayed for intestinal fatty acid binding protein (iFABP). A urinary iFABP:creatinine ratio ($iFABP_u$:Cr_u) of 2.0 pg/nmol was considered elevated.
Result: Forty-nine infants were transfused. iFABPu:Cru was elevated following 71 (75.6%) of 94 transfusions for which urine was available. In 51 (71.8%) of these, iFABPu:Cru was also elevated prior to the transfusion. Among four cases of transfusion-associated NEC, $iFABP_u$ was elevated following every sentinel transfusion and prior to three of them.
Conclusion: Subclinical intestinal mucosal injury is frequent following blood transfusions in premature infants and, when present, usually precedes transfusion. This suggests that transfusion may not be a primary mediator of intestinal injury so much as anemia and its associated conditions.
Level of evidence: Prognosis study/level 3

Between 25 and 38% of cases of necrotizing enterocolitis (NEC) are associated with a red blood cell transfusion that was administered within the prior 48 h [1–6]. Recent studies have suggested that feedings should be withheld during and after a transfusion [7,8], although the status of the intestinal mucosa around the time of transfusion is unknown.

The pathogenesis of transfusion associated NEC (TANEC) is incompletely understood. Mechanisms that have been hypothesized include an exaggerated immune response to biological mediators in stored blood [2], analogous to transfusion related acute lung injury (TRALI) [9], and impaired oxygen delivery because of the characteristics of stored blood, including nitric oxide deficiency [10] and impaired erythrocyte deformability [11]. Alternatively, infants who undergo transfusions may already harbor subclinical intestinal mucosal injury because of anemia and the conditions that led to it and are at an increased risk for NEC because of it. Therefore, a transfusion may not be a primary mediator of gut injury so much as it is a marker for it [12,13].

We hypothesized that if red blood cell transfusion is a primary instigator of NEC via vasoactive or rheological effects, subclinical intestinal mucosal injury could be shown to develop following transfusions, even in the absence of progression to NEC. Conversely, if anemia is an independent risk factor for NEC, significant mucosal injury might also be detectable prior to transfusion. In order to assess the independent impact of red blood cell transfusion upon mucosal viability, we evaluated urinary levels of intestinal fatty acid binding protein ($iFABP_u$) before and after red blood cell transfusion in a population of premature infants, some of which developed NEC. $iFABP_u$ is a sensitive and specific marker for intestinal mucosal injury [14] that is elevated in infants with NEC [15], in newborns who are at the highest risk for the development of NEC [16] and in the days before NEC is diagnosed, when clinical signs are absent [17,18].

1. Methods

Approval for the study was granted by the Institutional Review Board of the Loma Linda University School of Medicine (#5,100,238).

The study was a retrospective review of a convenience sample from a prospective study that was designed to evaluate whether $iFABP_u$ might be a sensitive and specific predictor of NEC in the days prior to the first clinical signs of the disease [17]. The original cohort included 62 infants of gestational age 24–28 weeks who were admitted to the neonatal intensive care unit (NICU) of the Loma Linda University

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Table 1
NICU packed red blood cell transfusion protocol during the time period of the study.

No transfusion should be administered for an asymptomatic low hematocrit unless oxygen carrying capacity is critical to infant's condition. Transfusions should be administered when the following criteria are met:
1) Acute hemorrhagic anemia
2) Ongoing deficit replacement
3) Maintenance of effective oxygen-carrying capacity
4) Hematocrit <20% without symptoms, if reticulocyte count <100,000/ml of <2%
5) Hematocrit <25% if infant is receiving supplemental oxygen
6) Hematocrit <30% if infant is receiving CPAP or minimal mechanical ventilation
7) Hematocrit <35% if any of the following are present:
a. Significant mechanical ventilation (MAP >8, FiO_2 > 0.4)
b. Apnea/Bradycardia, despite appropriate caffeine therapy (>9 episodes or 2 events requiring bagging in 24 h)
c. Tachycardia (>180 bpm) or tachypnea (>80 bpm) persisting for >24 h
d. Poor weight gain (<10 g/kg/d for 4 days) while receiving 100 kCal/kg/d

Children's Hospital between November, 2009 and April, 2012. The 49 subjects who underwent a red blood cell transfusion during their NICU stay were the subject population of the present study.

Demographic data including gestational age and birth weight were recorded. Information pertaining to the timing and volume of red blood cell transfusions was obtained from the medical record. The transfusion criteria used by the NICU at the time of the study are provided in Table 1.

The physiological status of subjects at the time of red blood cell transfusion was assessed, including the hemoglobin level and whether the infant was enterally fed. At the time of the study, the NICU did not have a policy of holding feedings around the time of red blood cell transfusion.

Subjects who developed NEC were identified and the severity of disease was categorized according to the Bell criteria [19]. Stage 2 NEC was indicated by pneumatosis intestinalis, significant bowel wall edema, and/or a fixed loop on abdominal imaging. Operative intervention was required in cases of stage 3 NEC, although this was delayed in one case.

The temporal relationship of NEC episodes to blood transfusion was noted.

Urine was obtained from most subjects beginning at the time of hospital admission until either the occurrence of NEC or hospital discharge. In some subjects early in the study, urine samples were discontinued after full enteral feedings were achieved. At times, samples were obtained as frequently as every six hours and in other instances there were larger gaps in sample collection because of nursing compliance with the protocol. Samples were obtained by placement of a cotton ball adjacent to the urethral meatus within the diaper. This technique has proven effective for collection of urine for assessment of iFABP [18,20] and samples in which there was any concern for stool contamination were discarded. Urine was stored at 4 °C after collection then centrifuged and stored at −80 °C. Urine samples that were obtained within 24 h before the initiation of a transfusion and within 24 h after the conclusion of a transfusion were assayed for iFABP content.

The urinary concentration of iFABP was determined using an ELISA (Cell Sciences, Canton, MA). Urinary levels of iFABP were normalized to urine concentration and expressed as a ratio to urinary creatinine ($iFABP_u$:Cr_u). An $iFABP_u$:Cr_u greater than 2.0 pg/nmol was used to identify significant intestinal mucosal injury. This is the level that has been found to reliably identify infants with proven NEC among those in whom there is a suspicion for NEC [20,21]. However, this level of $iFABP_u$:Cr_u does not necessarily indicate NEC, only that there is mucosal injury, and elevations to this degree or greater are described in infants without any clinical indicators of intestinal compromise [17,18].

Ordinal data were assessed with the Student t-test and the significance of differences in categorical data was analyzed using chi-square analysis. A p-value of less than 0.05 was considered significant. The Pearson correlation coefficient was determined to assess for a linear relationship between pre-transfusion hemoglobin and $iFABP_u$:Cr_u. IBM® SPSS® Statistics v. 22 was used for statistical analysis.

Table 2
Demographic and transfusion data for subjects.

	NEC (n = 7)	No NEC (n = 42)	p
Gestational age at birth (wk)	25.4 ± 1.5	26.4 ± 2.4	0.07
Birth weight (g)	768 ± 236	956 ± 423	0.06
Gender (male, %)	4 (57%)	24 (57%)	0.92
Number of transfusions	5.3 ± 3.5	6.4 ± 5.3	0.61

2. Results

Forty-nine infants underwent 347 red blood cell transfusions. The mean gestational age of these subjects was 26.4 weeks. Other demographic features are presented in Table 2. Seven infants (12%) developed NEC. Four of these were treated for Bell stage two NEC and three for stage three disease.

For 94 of the transfusions, a urine sample was available within 24 h of the initiation of the transfusion and within 24 h after the conclusion of the transfusion. For 45% of these transfusions, feedings were being administered at the time the transfusion was ordered. In contrast, in the original study cohort of 62 subjects, feedings were administered concurrent with 35% of urine samples. A blood transfusion was administered within the 48 h prior to the diagnosis of 5 of the 7 cases of NEC and pre- and post-transfusion urine samples were available for 4 of these. In four of the transfused subjects, who received ten transfusions, no appropriately timed pre- and post-transfusion urine specimens were obtained.

The mean iFABPu:Cr_u for the overall study population was 7.04 ± 8.02 pg/nmol prior to transfusions and 7.82 ± 7.43 pg/nmol following transfusions. $iFABP_u$:Cr_u was elevated after 75.6% of transfusions. For 71.8% of these, $iFABP_u$ was also elevated prior to the transfusion (Table 3). Sixty-one (65%) transfusions were administered to babies who were not being fed at the time the transfusion was ordered and 33 were receiving ongoing feedings prior to transfusion. The pre- and post-transfusion behavior of urinary iFABP was similar in the fed and non-fed subjects (Table 4).

When the pre-transfusion $iFABP_u$ was low and became elevated following the transfusion, indicating the development of intestinal mucosal injury, the volume of blood transfused was significantly less than for transfusions in which $iFABP_u$ was low before and after transfusion (Table 3). In cases in which $iFABP_u$ was elevated prior to transfusion, the hemoglobin concentration was not significantly different than when $iFABP_u$ was not elevated. There was no linear relationship found between pre-transfusion hemoglobin and $iFABP_u$:Cr_u (correlation coefficient − 0.04). Among the subjects who were being fed at the time of a transfusion there was also no relationship of hemoglobin to $iFABP_u$:Cr_u (correlation coefficient − 0.09).

In each of the four subjects in whom a transfusion was administered within the 48 h prior to the diagnosis of NEC and urine samples were available, $iFABP_u$ was elevated following the transfusion. In three, $iFABP_u$ was also elevated prior to transfusion (Table 5).

3. Discussion

In this evaluation of intestinal mucosal viability in premature infants around the time of transfusion, we found that when subclinical injury was present following administration of blood it was also frequently detected prior to the transfusion. In the subjects in whom NEC developed after a transfusion, there was a similar, substantial incidence of pre-transfusion mucosal compromise.

The entity of TANEC is well described [2,5] and up to 56% of cases of NEC may be preceded by a blood transfusion [7], although most reports cite an incidence of 25–38% [1–6]. The pathophysiology of TANEC remains unclear, although several mechanisms by which a red blood cell transfusion might lead to NEC have been proposed. Stored blood may exert a vasoactive effect upon the mesenteric circulation or be prone to unfavorable rheological qualities that induce injury that, in some