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Anemia, blood transfusions, and necrotizing enterocolitis in premature infants

David J. Hackam [1] ✉

The persistent impact of necrotizing enterocolitis (NEC) on neonatal morbidity and mortality occurs in part from of a lack of a clear understanding of how NEC risk factors can lead to disease development. Blood transfusions have been linked to NEC development, although controversy exists regarding the relative contributions of the transfused blood versus the anemia that initiated the transfusion. In addressing this controversy, Kalteren et al. have now compared 36 anemic and non-anemic infants at various time points prior to blood transfusion. They reveal that anemia is linked to sub-clinical intestinal inflammation, as revealed by the expression of intestinal fatty acid binding protein in the urine. In seeking to understand the mechanisms involved, Kalteren et al. also show that anemia corelated with impaired intestinal oxygenation, a factor that could drive the intestinal injury. The current study thus suggests that anemia may be a risk factor for NEC development through its effects on impaired tissue oxygenation, thus switching the focus back onto the trigger for the blood transfusion as opposed to the transfused blood itself. Studies such as these enhance our understanding of the factors leading to NEC, and bring us closer to the ultimate goal of eliminating this devastating disease.

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Necrotizing enterocolitis presents clinicians with the ultimate paradox[1]. It is at first a disease with which all neonatal providers have great familiarity—a disease that attacks suddenly, can progress rapidly, and that kills many who are diagnosed with it. Yet NEC also provides neonatologists and pediatric surgeons alike with the ultimate medical mystery—a disease that lacks diagnostic biomarkers, where the pattern of intestinal involvement is unpredictable, and where the rate of disease progression is highly variable. Part of this paradox arises from our lack of a clear understanding of what the critical risk factors that lead to NEC development are, as well as an incomplete understanding of how a particular risk factor can actually lead to NEC. The roles of blood transfusion and anemia in NEC development represent relevant examples of this conundrum[2,3]. Despite years of studying the link between blood transfusions and NEC, the relative contributions of anemia as opposed to the transfusion itself in causing NEC, and the relevant mechanisms involved, remain incompletely understood.

In their recent study, Kalteren et al.[4] now provide additional information to aid in our understanding of this paradox. To do so, they compared 36 anemic and non-anemic infants at various time points prior to blood transfusion, and assessed the degree of subclinical intestinal injury, as measured by the concentration of intestinal fatty acid binding protein (I-FABP) in the urine. The major observation of this study is that urinary I-FABP levels were higher in anemic infants compared with controls prior to blood transfusion[4]. Moreover, the authors determined that the highest concentrations of I-FABP were determined within 24 h of the transfusion, suggesting the possibility that the most clinically relevant states of anemia were linked to the most severe sub-clinical intestinal injury. In seeking to understand the mechanisms by which anemia alone could lead to sub-clinical intestinal injury, the authors also determined that hemoglobin levels negatively corelated with oxygen delivery to the gut, as measured by reduced splanchnic tissue oxygen saturation and extraction (r_sSO2). Importantly, the hemoglobin (Hb) level in the blood corelated with r_sSO2 such that the more severe the anemia, the greater the reduction in r_sSO2. By contrast, the urinary I-FABP levels correlated negatively with r_sSO2, indicating that the greater the impairment in tissue oxygenation, the greater the increase in sub-clinical intestinal injury. Taken together, these findings reveal that anemia can lead to sub-clinical intestinal injury through reduced oxygenation within the intestine of the premature infant.

The current work adds to an extensive body of work that has sought to understand the link between blood transfusion practices and NEC development. On the one hand, erythrocyte transfusions have been linked to the development of NEC in both pre-clinical and clinical studies[5,6]. The mechanisms by which erythrocyte transfusion can lead to NEC remain unclear, but have been tied to the potential pro-inflammatory effects of red blood cell degradation products, that may activate inflammatory receptors including toll like receptor 4 (TLR4)[7]. As a consequence of work showing that blood transfusion practices may be linked to NEC, Kalteren et al. point out that there has been a general restriction on transfusion practices, with potential for negative consequences[8]. The current findings now reveal that one such

[1]Professor and Chief of Pediatric Surgery, Johns Hopkins University, Baltimore, MD, USA. ✉email: Dhackam1@jhmi.edu

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consequence of a more restrictive transfusion practice might include the development of sub-clinical intestinal injury attributable to the anemia itself.

Although the current study is highly clinically relevant, there are several limitations. First, the study does not actually reveal that intestinal injury has occurred, as the study was not designed to collect stool samples for evidence of sloughed epithelial cells, nor of intestinal tissue. Second, it would be interesting to know whether any of the anemic patients who showed evidence of subclinical intestinal injury also developed evidence of systemic sepsis or NEC, and whether such clinical manifestations were either worsened or improved by a blood transfusion. Finally, the study was performed at a single albeit busy European academic medical center, and was performed over a relatively short period of time, and so the degree of applicability to other centers outside of the academic centers is unclear.

Despite these limitations, the study is important. This work adds significantly to the conversation regarding the role of blood transfusions in NEC development, yet puts the focus back on the presence of anemia, and away from the transfusion itself. As a potential next step, there would be great value in conducting a follow on, multi-center study that examines the impact of anemia and tissue oxygenation before and after blood transfusion on not only NEC development, but also on inflammation in other organs that are at risk for hypoxic injury in the premature neonate, including the brain, the retina and the lungs. Moreover, given the link between an abnormal stool microbiome and NEC development[9], a careful collection of the stool microbiota in the anemic infant—before and after blood transfusion - would be a useful adjunct as part of the overall transfusion risk assessment.

It is noteworthy that the key findings fit extremely well within our general understanding of how NEC develops in premature infants. Specifically, we[10,11] and others[12] have shown that NEC develops in part through activation of the Gram-negative bacterial receptor TLR4 on the intestinal epithelium, which is expressed at higher levels in the premature than the full term intestinal epithelium[13]. The elevated expression of TLR4 in the premature gut reflects the non-immune role that TLR4 plays in gut development through the activation of the Notch signaling pathway[11]. The fetal intestine normally develops in a bacterial free environment *in utero*, where the TLR4 signaling is also inhibited by amniotic fluid[14]. Thus, after a premature birth occurs, the persistently elevated TLR4 can become activated by microbes that colonize the intestinal tract, many of which are enriched in TLR4 ligands[15,16]. The effects of persistent TLR4 signaling in the premature intestinal epithelium include epithelial death by apoptosis and necroptosis[17], impaired mucosal healing[18], and mesenteric vasoconstriction[19,20], resulting in the intestinal ischemia seen in NEC. It is noteworthy that activating mutations in pathways that result in enhancedTLR4 signaling are seen in patients with NEC[21–23], and that the NEC-protective effects of breast milk can be linked to TLR4 inhibition in the neonatal gut[24–26], providing additional human proof-of-concept for the role of TLR4 in NEC development. The current findings in which anemia and impaired intestinal oxygenation cause sub-clinical intestinal injury fit nicely into this model, as hypoxia is a major inducer of TLR4 signaling and expression[27,28]. It is therefore tempting to speculate that in the anemic infant, the reduced oxygen delivery to the gut drives persistent TLR4 expression and signaling, thus setting the stage for the proinflammatory cascade that leads to NEC when bacterial colonization occurs.

In summary, while NEC represents one of the most difficult challenges in the neonatal intensive care unit, its elimination represents one of the greatest opportunities to impact the health of premature infants. Achieving sustained NEC reduction will require a greater understanding of the risk factors that lead to disease development in the first place, including those related to blood transfusion practices. Studies such as the current work by Kalteren et al. may bring us even closer to the important goal of eliminating NEC, to the ultimate benefit of our patients.

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COMPETING INTERESTS

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ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to David J. Hackam.