Immunologic and Hematological Abnormalities in Necrotizing Enterocolitis

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KEYWORDS
- NEC • Blood counts • Inflammation • Macrophages • Signaling • Neutrophils
- Platelets • Monocytes

KEY POINTS
- Necrotizing enterocolitis (NEC) is a leading cause of morbidity and mortality in preterm infants born before 32 weeks’ gestation or with a birth weight less than 1500 g.
- Bacterial flora plays a central pathophysiological role in NEC.
- Premature intestine is at risk of NEC because of mucosal sensitivity to bacterial products and paucity of mechanisms that normally limit the interaction of luminal bacteria with mucosal cells.
- The onset of NEC is associated with elevated plasma concentrations of several inflammatory cytokines. Increased circulating interleukin-8 concentrations may provide prognostic information.
- Low circulating TGF-β concentrations on the first postnatal day may predict later occurrence of NEC.
- Hematological abnormalities such as thrombocytopenia, disseminated intravascular coagulation, increased or decreased neutrophil counts, eosinophilia, and anemia occur frequently in infants with NEC.
- In a premature infant with feeding intolerance, an acute drop in peripheral blood monocyte concentrations compared with the last presymptomatic blood counts may be an early indicator of NEC.

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INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating inflammatory condition of the gastrointestinal disease that afflicts 4% to 11% of very low birth weight infants and is a leading cause of morbidity and mortality in this population. The pathogenesis of NEC is complex and is not well understood. Clinical studies associate NEC with diverse prenatal and postnatal factors, such as placental insufficiency, prolonged/premature rupture of membranes, chorioamnionitis, gut ischemia, altered bacterial colonization, viral infections of the gastrointestinal tract, bacterial overgrowth, and red blood cell (RBC) transfusions. Although a unifying mechanism may not be readily evident in all the risk factors of NEC, some of these conditions presumably alter/disrupt the intestinal epithelial barrier to allow bacterial translocation from the lumen into the subepithelial lamina propria, where these bacteria or their products trigger an exaggerated, damaging mucosal inflammatory reaction. In severe intestinal injury, bacterial products and/or the inflammatory mediators may spill into the bloodstream, causing a systemic inflammatory response and multiorgan dysfunction. In this article, we review the immunologic aspects of the pathogenesis of NEC and its hematological manifestations. A literature search was performed using the databases PubMed, EMBASE, and Scopus. To minimize bias, keywords from PubMed’s Medical Subject Heading (MeSH) thesaurus were shortlisted before the actual search and combined with text words likely to be used in titles and abstracts.

IMMUNOLOGIC ASPECTS OF NECROTIZING ENTEROCOLITIS

Mucosal Sensitivity to Bacterial Products in the Premature Intestine

Several lines of evidence indicate that luminal bacteria play a central pathophysiological role in NEC: (1) bacterial overgrowth, and *pneumatosis intestinalis*, the accumulation of gaseous products of bacterial fermentation in the bowel wall, are prominent histopathological findings in NEC; (2) ischemic intestinal injury in the sterile in utero microenvironment may cause strictures or atresia, whereas similar insults after postnatal bacterial colonization may increase the risk of NEC; (3) enteral antibiotics can reduce the incidence of NEC and NEC-related mortality. Although specific bacterial species have not been causally associated with NEC, infants who go on to develop NEC often display a microbial imbalance (“dysbiosis”) with abnormal abundance of gammaproteobacteria (Enterobacteriaceae and Pseudomonadaceae) and *Clostridia*, but with fewer Firmicutes, the dominant Gram-positive bacterial phylum in infants who do not develop NEC. Gammaproteobacteria express lipopolysaccharides and other products that have unique microbial-associated molecular patterns, which engage with the Toll-like receptors (TLRs) on mucosal cells to activate downstream inflammatory signaling. As discussed in the following section, the developing intestine is at risk of inflammatory injury because of 2 major factors: (1) the epithelium and mucosal immune cells in the developing intestine are uniquely sensitive to bacterial products, and (2) a paucity of adaptive mechanisms that normally limit the interaction of luminal bacteria with the mucosa.

Intestinal Epithelium

Fetal intestinal epithelial cells (IECs) express a variety of innate response receptors and display a “hyperactive” TLR-activated transcriptional program, which manifests with exaggerated expression of cytokines and inflammatory mediators. This epithelial sensitivity to bacterial products correlates with high levels of expression of TLR2, TLR4, downstream adaptors such as the myeloid differentiation primary response gene 88, and tumor necrosis factor receptor-associated factor 6, and the
transcriptional regulator nuclear factor kappa B 1 (NF-κB1). In addition, fetal IECs are developmentally deficient in many negative regulators of TLR signaling, such as the single immunoglobulin interleukin-1-related receptor, interleukin-1 (IL-1) receptor-associated kinase (IRAK)-M, tumor necrosis factor-alpha–induced protein 3, and the Toll-interacting protein. Other reports implicate low levels of inhibitor of κB.17 NF-κB signaling may also be dampened after full-term birth due to posttranscriptional downregulation of IRAK-1, another key intermediate in TLR signaling.18

Goblet cells start producing mucus by week 12, but this mucus layer contains low amounts of the protective mucin 2 (muc2).19 Expression of muc2 is further compromised during NEC, possibly through bile acid–mediated mucosal injury.20 Paneth cells appear at about the same time as goblet cells and produce antibacterial proteins such as lysozyme and α-defensins. The number of Paneth cells is low in the premature intestine and increases with maturation until adulthood.21 In rodents, Paneth cell ablation may trigger NEC-like injury in the developing intestine.22 Developmental differences in secretory immunoglobulin A (sIgA) are described later in this article.

**Intestinal Macrophages and Dendritic Cells**

Macrophages first appear in the fetal intestine at 11 to 12 weeks. The resident macrophage population increases rapidly during the 12-week to 22-week period, and then a slower pace through early childhood.23–25 These cells play a critical role in host defense as the first phagocytic cells of the innate immune system to encounter luminal bacteria that breach the epithelium and gain access to the lamina propria. In the adult intestine, macrophages display avid phagocytic and bacteriocidal activity but are attenuated in their inflammatory responses,26 a unique adaptation that minimizes inflammation in the gut mucosa despite the close proximity to luminal bacteria. We have shown that macrophage precursors undergo inflammatory downregulation on recruitment to the intestinal mucosa under the influence of transforming growth factor-beta (TGF-β), particularly the TGF-β2 isoform, present in the local extracellular matrix.4 In the midgestation intestine, which is developmentally deficient in TGF-β bioactivity, macrophages are yet to acquire this inflammatory anergy and produce inflammatory cytokines on exposure to bacterial products.4,27 These inflammatory responses of the mucosal macrophages likely add to the risk of mucosal injury during NEC. Transgenic mice with defects in tissue-specific differentiation of gut macrophages due to loss of TGF-β signaling or mutations in the signal transducer and activator of transcription pathway are at increased risk of inflammatory mucosal injury.4,28

There are very limited data on fetal/neonatal intestinal dendritic cells (DCs).23 HLA-DR+ DC-like cells are detected in both lamina propria and the Peyer patches after 14 weeks, but these cells may have some overlap with lamina propria macrophages.29 In rats and nonhuman primates, DCs have been noted in the fetal lamina propria as well as in Peyer patches.30,31 The functional importance of these DCs in NEC remains unclear, although DCs were proposed as a cause of epithelial damage in mice with Cronobacter sakazakii–induced NEC-like injury.32

**Mucosa-Associated Lymphoid Tissue**

Peyer patches first appear at 11 weeks and develop during mid-late gestation (Table 1).33,34 At birth, these lymphoid aggregates are structurally complete but “naïve,” as germinal centers take a few weeks to develop.35 The number of Peyer patches in the ileum increases as a function of gestational maturation, and premature infants born before 32 weeks’ gestation may have only half as many Peyer patches as their full-term counterparts.36 Other mucosa-associated lymphoid tissue (MALT)
structures, such as the lymphoid aggregates in the vermiform appendix, develop after birth following postnatal bacterial colonization.\(^{37,38}\)

**T Lymphocytes in the Lamina Propria and Intraepithelial Compartments**

T cells are first seen in the fetal intestine at 12 to 14 weeks’ gestation.\(^{39}\) Outside the MALT, intestinal T cells are distributed in the lamina propria and the intraepithelial compartments. Lamina propria lymphocytes develop in the fetal intestine in utero and reach densities similar to the full-term intestine by 19 to 27 weeks’ gestation.\(^{39}\) In contrast, intraepithelial lymphocytes (IELs) expand mainly after birth.\(^{40,41}\) Approximately 10% to 30% of IELs express the \(\gamma\delta\) T-cell receptor\(^{34}\) and may serve specialized roles in epithelial homeostasis, cytotoxic activity, and antimicrobial immunity.\(^{42–44}\) The fetal intestine also shows some early-lineage T-cell populations, indicating that T cells may also develop locally in a mucosal, extrathymic pathway.\(^{34,39,40,45–49}\) In premature infants, the T-cell receptor shows a polyclonal repertoire that undergoes gradual restriction to a mature, oligoclonal pattern, possibly due to the emergence of a few dominant clones specific for commensal bacteria.\(^{50,51}\) Although the role of T-cell subsets in NEC remains unclear, there is an overall paucity of T cells in surgically resected bowel affected by NEC and in murine models of NEC-like injury.\(^{52–54}\) Consistent with this deficiency in T-cell development, infants who go on to develop NEC had lower circulating levels of T-lymphokines, such as IL-2, IL-18, CC-motif ligand (CCL) 4, and CCL5 in the preceding weeks than other premature infants who did not develop NEC.\(^{55}\)

**B Cells and Secretory Immunoglobulins**

The first B cells are seen in the lamina propria at 14 weeks’ gestation and display a mature B-cell phenotype similar to the thymic B cells (CD20* IgM* IgD* light

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**Table 1**

<table>
<thead>
<tr>
<th>Development of Peyer patches in the human fetus</th>
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<td>11 wk gestation</td>
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<td>16 wk gestation</td>
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<td>24 wk gestation</td>
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<td>0–4 wk postnatal</td>
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*Abbreviations: Ig, immunoglobulin; PP, Peyer patch.

\(^a\) Fetal PP T cells are predominantly of the CD4* phenotype.
chain). Some pre-B cells (IgM light chain−CD20+) also may be seen, indicating that the mucosa may serve as an alternative site for B-cell development. During the second postnatal week, some B cells in both the lamina propria and the MALT undergo IgA class-switch. The number of IgA plasma cells reaches adult levels at 2 years, although serum IgA concentrations may not reach adult levels until the second decade.

The sIgA is first detected in mucosal secretions at 1 to 8 weeks after birth. In premature infants, sIgA may first appear in secretions at a similar chronologic age as in full-term infants, although the concentrations are usually lower, as sIgA concentrations rise as a function of postmenstrual age. The IgA responses also may be functionally less robust with a predominance of monomeric (instead of polymeric) sIgA and IgA1 (instead of the sIgA2 subclass). Premature infants also show global abnormalities in their immunoglobulin responses, such as reduced antigen affinity, polyreactivity, and autoreactivity. In addition, immunoglobulin heavy chains have short complementarity-determining regions in premature neonates, which markedly lowers the potential antibody diversity available to these infants.

During the neonatal period, colostrum provides an important alternative source of sIgA. Milk antibodies may contribute approximately 0.5 to 1.0 g per day throughout lactation (comparable to the 2.5 g per day being produced by a 65-kg adult), and are directed against antigens present in the environment shared by the mother-infant dyad. Immune cells stimulated by antigens in the maternal intestine and bronchial mucosa have been shown to migrate specifically to the mammary gland. Interestingly, sIgA levels in colostrum and milk of mothers of preterm neonates may be higher than in mothers who delivered at full-term.

Platelet-Activating Factor

Platelet-Activating Factor (PAF), an endogenous phospholipid mediator produced by platelets, leukocytes, and endothelial cells, is believed to represent a final, common effector in diverse forms of intestinal injury. In rodent models, exogenous administration of PAF induces systemic hypotension, microvascular permeability, and gut mucosal necrosis, which is often most prominent in the ileum. In NEC, the role of PAF is supported by several lines of evidence: (1) compared with age-matched controls, infants with NEC have elevated plasma PAF levels and decreased plasma activity of PAF-acethylhydrolase (PAF-AH), the enzyme that normally degrades PAF; (2) human milk contains PAF-AH; (3) NEC-like injury was prevented in rat pups and piglets by PAF-receptor antagonists; (4) enteral administration and intravenous infusion of recombinant PAF-AH prevented NEC in rats; (5) PAF-AH knockout mice are more susceptible to NEC-like injury; and (6) ileum, the site of predilection of NEC, shows highest regional expression of PAF receptors in the gastrointestinal tract.

Nitric Oxide

Nitric oxide (NO) is a key regulator of vascular tone, inflammation, neurotransmission, and tissue restitution and repair. There are 3 isoforms of NO synthases (NOS): endothelial NOS (eNOS) and neuronal NOS (nNOS), which generate low (picomolar) concentrations of NO, and inducible NOS (iNOS), which produces high (micromolar) concentrations of NO. Low levels of NO play physiologic roles in epithelial homeostasis and water absorption. However, at high concentrations, NO reacts with superoxide to form peroxynitrite, a highly toxic intermediate that disrupts protein conformation, alters cellular metabolism, and can block epithelial restitution and induce apoptosis in diverse cell types. Increased iNOS expression was noted in IECs in surgically resected intestinal tissue samples of NEC. Formula-fed rat pups show increased
iNOS expression in the gut mucosa, which is further increased during NEC-like injury.\textsuperscript{97,98}

**Reactive Oxygen Species**

Reactive oxygen species (ROS) are believed to play a key proinflammatory role in NEC. The xanthine oxidase/dehydrogenase system is one of the main producers of ROS in the intestine. Xanthine dehydrogenase is constitutively expressed in IECs and normally catalyzes the transformation of xanthine into uric acid (Xanthine + H\textsubscript{2}O + NAD → Uric acid + NADH + H\textsuperscript{+}). However, during ischemia, xanthine dehydrogenase is converted into xanthine oxidase, which leads to xanthine oxidation into uric acid and superoxide (Xanthine + H\textsubscript{2}O + O\textsubscript{2} → Uric acid + 2O\textsubscript{2}− + 2H\textsuperscript{+}). ROS activates the intestinal mitochondrial apoptotic signaling pathway during oxidative stress and leads to IEC apoptosis via the p38 mitogen-activated protein kinase.\textsuperscript{99,100} Xanthine oxidase and superoxide are implicated in intestinal reperfusion injury and PAF-induced bowel necrosis, as allopurinol, a xanthine oxidase inhibitor, has been shown to be protective in these models.\textsuperscript{101,102}

**Transforming Growth Factor-β**

We have shown recently that premature infants who developed NEC had lower circulating TGF-β\textsubscript{1} levels than their non-NEC controls since birth.\textsuperscript{55} In this study, blood TGF-β\textsubscript{1} concentrations less than 1380 pg/mL on the first postnatal day predicted future onset of NEC with 64% accuracy. Although several biomarkers (such as the inter-alpha inhibitor protein, intestinal fatty acid–binding protein, hexosaminidase, proapolipoprotein CII, and des-arginine serum amyloid A) have been identified for their ability to discriminate between confirmed NEC and other causes of feeding intolerance,\textsuperscript{103–106} blood TGF-β\textsubscript{1} is the first biomarker to estimate the risk of NEC in a newly born premature infant. Even though genetic factors do not seem to be a major contributor to the risk of NEC,\textsuperscript{107} the ability of blood TGF-β\textsubscript{1} to identify infants at risk of NEC on day 1 is interesting because it indicates that the risk-stratification for NEC may start early, possibly in utero, before most hypoxic-ischemic events of the early neonatal period would have occurred or the microbial flora would have been established in the intestinal lumen. These findings are consistent with our previous reports indicating that preterm neonates may be at risk of inflammatory mucosal injury and NEC because of a developmental deficiency of TGF-β bioactivity in the intestine.\textsuperscript{4,27} The reasons for low blood TGF-β levels in infants who developed NEC were unclear, although a few interesting possibilities merit consideration. The first is increased peripheral uptake of TGF-β to compensate for low tissue expression. We have previously shown that TGF-β expression is decreased in healthy margins of tissues resected for NEC.\textsuperscript{4} A second explanation could be based on an assumption that the developing intestine is a major contributor to circulating TGF-β levels. Because TGF-β expression increases in the intestine as a function of gestational maturation,\textsuperscript{4} lower tissue expression of TGF-β in infants who developed NEC could conceivably reflect an underlying state of arrested mucosal development, where low TGF-β expression may indicate persistence of a cytokine profile corresponding to an earlier, less mature developmental epoch. In support of this possibility, we detected lower TGF-β levels in the NEC group than in controls at the same postmenstrual age. Finally, infants who developed NEC may constitutively produce less TGF-β than controls due to genetic/epigenetic factors. Premature baboons with spontaneously occurring NEC-like injury showed histone methylation in the TGF-β nucleosome, a repressive modification associated with facultative heterochromatin assembly and transcriptional silencing.\textsuperscript{27}
Cytokine Responses Associated with Necrotizing Enterocolitis

Several studies have investigated the cytokine response during NEC. Cytokines are attractive not only as potential biomarkers of the primary disease and the severity of inflammation in NEC, but also for possible pathophysiological roles in intestinal injury. At the tissue level, increased IL-1β and tumor necrosis factor expression have been detected in surgically resected tissue specimens of human NEC. In preclinical models, NEC-like intestinal injury induces chemokines such as the CXC-motif ligand 5 (CXCL5), CXCL1, and CXCL2, CCL2, CCL3, CCL5, CCL20, IL-1β, IL-6, IL-12, and IL-18. In plasma, the diagnosis of NEC in premature infants was associated with elevated IL-1β, IL-1 receptor antagonist, IL-6, IL-10, CXCL5, IL-8/CXCL8, CCL2, CCL3, neurotrophin-4, and C-reactive protein. In one study, plasma IL-8/CXCL8, epithelial-derived neutrophil chemoattractant-78/CXCL5, and IL-10 levels were higher in infants with NEC than in those with sepsis syndrome. IL-6 has a shorter half-life and may be detectable only for short periods after onset of NEC, whereas IL-8/CXCL8 levels show sustained elevation and correlate with the extent of NEC (NEC-totalis), the need for surgical resection of bowel, and higher 60-day mortality in infants with NEC.

HEMATOLOGICAL MANIFESTATIONS OF NECROTIZING ENTEROCOLITIS

Hematological abnormalities occur frequently in infants with NEC, and in many, carry important diagnostic and prognostic information. These abnormalities remain mild and require no intervention in most infants with NEC, although some with severe disease may require blood product transfusions and monitoring for complications such as hemorrhage, infections, anemia, and even death.

Platelet Counts

Thrombocytopenia, defined as a platelet count less than $150 \times 10^9/L$, is seen at clinical presentation in 50% to 95% of infants with NEC, and many have platelet counts in the range of $30 \times 10^9/L$ to $60 \times 10^9/L$. Most neonates with advanced NEC develop thrombocytopenia within 24 to 72 hours of onset of disease. The severity of thrombocytopenia correlates with the Bell clinical stage of NEC, and a rapid drop in platelet counts to less than $100 \times 10^9/L$ is a sensitive (although not specific) predictor of bowel gangrene or the need for surgical intervention. Ververidis and colleagues reviewed the clinical course of 58 neonates with advanced NEC (Bell's stages II or III, gestational age 23–41 weeks) and noted platelet counts less than $150 \times 10^9/L$ in 54 (93%) infants and counts less than $100 \times 10^9/L$ in 51 (88%). Most infants who showed a rapid drop in platelet counts to less than $100 \times 10^9/L$ were bacteremic. In patients without intestinal necrosis, platelet counts recovered to normal levels within 24 hours. These data are similar to previous reports by Hutter and colleagues, Patel, O'Neill, and Kenton and colleagues.

Platelet counts provide important predictive information for the outcome in patients with NEC. In survivors, the median time to recovery to a platelet count greater than $150 \times 10^9/L$ is approximately 7 to 10 days. Infants who die of NEC tend to have lower nadir platelet counts than survivors. In the cohort described by Ververidis and colleagues, patients who died of NEC had a lower nadir in platelet counts than the survivors. All patients with a platelet count greater than $100 \times 10^9/L$ during the course of the disease survived. Ragazzi and colleagues recorded platelet counts less than $150 \times 10^9/L$ in 86% of nonsurvivors versus 43.3% of the survivors ($P<.0001$). Severe thrombocytopenia ($<100 \times 10^9/L$) was noted more frequently in

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nonsurvivors (70%) than in survivors (33%; \( P < .0001 \)). In another study, Kenton and colleagues\(^{123} \) reported higher median platelet counts in survivors than in nonsurvivors (203 \( \times \) \( 10^9/L \) vs 33 \( \times \) \( 10^9/L; \ P < .001 \)). Severe thrombocytopenia was a predictor of mortality (adjusted odds ratio [OR] 6.39; \( P = .002 \)) and NEC-related gastrointestinal complications, such as cholestatic liver disease and short bowel syndrome (adjusted OR 5.47; \( P = .006 \)).

The primary mechanism for thrombocytopenia in NEC is widely believed to be increased platelet destruction. Indirect evidence for platelet consumption is seen in the rapid drop in platelet counts in many patients, and also in the short-lived rise in platelet concentrations after transfusions lasting less than 24 to 48 hours.\(^{126} \) The mechanism(s) of thrombocytopenia in NEC has not been investigated in clinical studies, but existing information from animal models indicates a likely role of PAF and circulating bacterial products, such as lipopolysaccharide.\(^{127} \) These mediators stimulate endothelial cells and macrophages to release inflammatory cytokines and NO, which, along with thromboplastin released from gangrenous bowel, can increase platelet activation and aggregation in the microvasculature.\(^{120} \)

Patients with NEC have higher plasma thrombopoietin (Tpo) concentrations than their healthy counterparts.\(^{128,129} \) Brown and colleagues\(^{130} \) measured plasma Tpo concentrations, circulating megakaryocyte progenitors, reticulated platelet counts, and the percentage of reticulated platelets in the circulating platelet pool (RP%) in 20 neonates with sepsis and/or NEC. They showed elevated concentrations of Tpo and a modest increase in circulating megakaryocyte progenitors and RP% in thrombocytopenic neonates. The investigators hypothesized that this dampening of thrombopoietic response may be due to increased levels of platelet factor 4, which is released from activated platelets and is a potent inhibitor of megakaryocytopoiesis.

**Coagulopathy**

Hutter and colleagues\(^{121} \) noted signs of disseminated intravascular coagulation (DIC) in 14 of their 40 infants. Many infants showed decreased plasma fibrinogen, tested positive for fibrin split products, and had elevated partial thromboplastin times. These data are similar to Patel\(^{122} \) and Sonntag and colleagues,\(^{131} \) who described a similar frequency of DIC in their respective cohorts.

**Anemia**

Patients with NEC may develop anemia due to multiple pathogenetic mechanisms. In the presence of mucosal injury, thrombocytopenia, and coagulation disturbances, occult and obvious blood loss is common. Sites of bleeding may include bloody stools, peritoneal hemorrhage, pulmonary hemorrhage, hemopericardium, myocardial hemorrhage, and intracranial hemorrhage.\(^{119} \) Iatrogenic anemia due to phlebotomy losses is an added problem. In some patients, hemolysis can cause further worsening of anemia. Thrombotic microangiopathy can cause red cell damage, which can often be seen on blood smears as tear drop cells, schistocytes, spherocytes, and acanthocytes. Hutter and colleagues\(^{121} \) reported red cell fragmentation in 25 of their 40 patients. Another possible cause is activation of the Thomsen-Friedenreich (T) cryptantigen on the RBC surface, a naturally occurring antigen that is normally concealed by a layer of N-acetylneuraminic acid but can be exposed in NEC by bacterial or other neuraminidases. T-activation can cause hemolysis and anemia in multitransfused patients who have previously received adult blood containing anti-T antibodies.\(^{132,133} \) Studies show considerable variation in the frequency of T-activation in NEC, ranging from rare occurrences to up to a third of all
patients. T-activation is associated with increased morbidity and mortality in NEC. Finally, infants with NEC may be at enhanced risk of inflammatory suppression of erythropoiesis. NEC tends to occur most frequently in extremely premature infants who show an impaired erythropoietin response for a given level of anemia and are prone to develop severe anemia of prematurity. The presence of inflammation after onset of NEC in these infants can further suppress erythropoietin and red cell production.

In some situations, anemia may be a risk factor for the development of NEC rather than its effect. Infants with severe anemia related to glucose-6-phosphate dehydrogenase deficiency, hemolytic disease of newborn, and in donor twins in twin-to-twin transfusion syndrome are at increased risk of NEC. Anemia also has been identified as a predisposing factor in patients who develop NEC after RBC transfusions. Singh and colleagues investigated the association between anemia and transfusion-associated NEC in a cohort of 111 preterm infants with confirmed NEC and 222 matched controls. In a multivariate model, lower hematocrit was associated with increased odds of NEC (OR 1.10, \( P = .01 \)) after controlling for other factors. They showed that RBC transfusions had a temporal relationship with onset of NEC, where a transfusion within the preceding 24 hours (OR 7.60, \( P = .001 \)) and 48 hours (OR 5.55, \( P = .001 \)) was associated with increased odds of developing NEC. Although the mechanisms by which anemia may increase the risk of NEC remain unclear, anemia has been shown to impair splanchnic perfusion and increase oxygen extraction as a compensatory mechanism. Anemia also can impair the normal postnatal changes in splanchnic vascular resistance, predisposing the developing intestine to hypoxemic-ischemic gut mucosal injury, and possibly, to NEC.

**Neutrophils**

Increased neutrophil counts comprise an appropriate inflammatory response in patients with mild-moderately severe disease. In contrast, neutropenia, defined as an absolute neutrophil count (ANC) less than 1500/\( \mu \)L, can be seen in severe NEC and is associated with adverse outcome. Patel noted neutropenia in 14 of their 23 patients who died of NEC, compared with 6 of 24 survivors. In another study, Ragazzi and colleagues noted that neutropenia in the initial blood counts at onset of NEC was associated with adverse outcome; there was a trend for a higher frequency of neutropenia in nonsurvivors (37%) than in survivors (25%; \( P = .136 \)), and thrombocytopenia and neutropenia occurred together more frequently in nonsurvivors (39%) than in survivors (14%; \( P = .0007 \)). In infants at greater than 34 weeks' gestation and with lower ANC, higher immature neutrophil number, and greater immature:total neutrophil ratio at onset of NEC were associated with the need for surgical intervention. Although the pathophysiology of neutropenia in NEC is not well understood, depletion of the circulating neutrophil pool due to emigration into the intestines and peritoneum, and increased margination in the microvasculature are some of the possible causes.

**Macrophages and Monocytes**

The cellular inflammatory response in NEC is characterized by the presence of a macrophage-rich infiltrate. We have previously shown that gut macrophage populations are normally maintained through continuous recruitment of circulating monocytes and in situ differentiation of these cells in the lamina propria. Because preterm infants have a limited circulating monocyte pool and lack significant reservoirs of mature monocytes in the bone marrow or elsewhere, we asked whether the migration of circulating monocytes into NEC lesions could result in an acute drop in...
peripheral blood monocyte counts that may distinguish early NEC from other causes of feeding intolerance. We compared the absolute monocyte counts (AMC) at the onset of feeding intolerance with the last available presymptomatic AMC. In patients who developed stage II NEC, the AMC fell from median 1.7 × 10^9/L (interquartile range [IQR] 0.98–2.4) to median 0.8 (IQR 0.62–2.1, P<.05), whereas those who developed stage III NEC dropped their AMCs from median 2.1 × 10^9/L (IQR 0.15–3.2) to median 0.8 (IQR 0.6–1.9, P<.05). Total white cell counts, ANC, and lymphocyte counts did not change significantly. In the control group (developed feeding intolerance for a reason other than NEC), there was no change in AMC or the white cell, ANC, or lymphocyte counts.

**Eosinophils**

Eosinophilia, defined as a blood eosinophil count exceeding the 95th percentile upper reference limit, ranges from 1180/mL during the first postnatal week to 1560/mL after 3 weeks. Christensen and colleagues noted eosinophilia in 54 (19.6%) of 275 infants who were evaluated for bloody stools. They hypothesized that premature infants with bloody stools and eosinophilia were likely to have atopic enteropathy and not develop the classic signs of NEC, and were therefore, more likely to have a benign course of disease. However, the rate of pneumatosis, bowel resection, and death in infants with bloody stools and eosinophilia was similar to those with normal blood eosinophil counts. Interestingly, infants with eosinophilia were more likely to have received an RBC transfusion in a 48-hour period before passing a bloody stool. These infants with a preceding history of a blood transfusion showed a progressive rise in eosinophil counts. In contrast, infants who developed bloody stools and eosinophilia but did not have a history of a previous transfusion showed reduction in eosinophil counts once the feedings were discontinued and had a relatively benign course of disease. Although the mechanism for transfusion-associated eosinophilia is unclear, exposure to foreign antigens on donor RBCs or to medications administered during the transfusion may play a role.

**Basophils**

Kim and colleagues showed that low basophil counts (16.5 ± 43.0/mL in the NEC group vs 60.6 ± 131.4/mL in controls, P<.001) at birth may predict the development of NEC. The investigators speculated that these differences in basophil hematopoiesis may reflect host interaction with commensal bacteria. However, additional study is needed to understand the mechanistic basis of these findings because these differences were identifiable early, possibly before the intestinal microbial flora would have been established in these infants.

**Lymphocytes**

Lambert and colleagues reported that infants who died of fulminant NEC within 48 hours of onset had low lymphocyte counts. They reviewed the medical records of 523 infants with a diagnosis of NEC, of whom 35 (6.7%) had a fulminant course. These infants were more likely to have a blood lymphocyte count less than 4000/mL (P = .018), besides having portal venous air, severe anemia, recent increase in feeding volume or introduction of human milk fortifier, and increased immature to total neutrophil ratio.

**Nucleated Red Blood Cells**

Infants who develop NEC may have a higher number of nucleated RBCs at birth than their gestation-matched controls. Mandel and colleagues compared 23 preterm
infants diagnosed with NEC with pair-matched controls who were admitted immediately after a case, had the same gestational age (±1 week), 1-minute and 5-minute Apgar scores (±1), and who did not develop NEC. Exclusion criteria included factors that may influence the absolute nucleated RBC counts at birth, such as maternal diabetes, pregnancy-induced hypertension, major congenital malformations, chromosomal abnormalities, hemolysis, and perinatal blood loss. Infants who developed NEC had higher absolute nucleated RBC counts than controls, suggesting that these neonates may have experienced intrauterine hypoxemia, which may increase the risk of NEC. These data are consistent with observations by Baschat and colleagues, who showed that nucleated RBC counts greater than 30/100 white blood cells beyond 3 days were associated with increased risk of NEC.

SUMMARY

In the premature intestine, developmental limitations in both the innate and adaptive arms of the mucosal immune system increase the risk of inflammatory injury and NEC. The systemic inflammatory response during NEC is characterized by elevated circulating cytokine levels and a consistent pattern of hematological abnormalities, which may also play a direct or indirect role in augmenting the mucosal injury. Some of these immunologic and hematological abnormalities carry important prognostic information.

Best Practices

What is the current practice?

Infants who develop necrotizing enterocolitis (NEC) frequently show immunologic and hematological abnormalities, such as elevated circulating cytokine levels, thrombocytopenia, increased or decreased neutrophil counts, low monocyte counts, and anemia. These findings show high sensitivity and in some infants, might convey important diagnostic and prognostic information. In others, the clinical utility of these tests may be limited due to low specificity and the overlap with comorbidities, such as bacterial or fungal sepsis, or cholestasis.

What changes in current practice are likely to improve outcomes?

Many hematological abnormalities associated with NEC have been identified in recent years and still need confirmation in larger studies. Cytokine measurements, although straightforward and relatively inexpensive, are not routinely available yet in clinical laboratories. Further study is needed to refine these measurements and determine whether the diagnostic accuracy of these tests could be improved if used in combination.

Is there a clinical algorithm?

No. Because of low specificity, immunologic and hematological abnormalities are likely to be less useful as diagnostic tests in infants with NEC. Instead, these findings are more likely to find utility as “early-warning” systems that indicate a need for clinical monitoring and/or imaging. Elevated plasma concentrations of interleukin (IL)-8 and IL-6, low levels of TGF-β, and the detection of worsening thrombocytopenia or an acute drop in monocyte counts are some of the more promising findings. In infants at risk of transfusion-associated NEC, a rise in blood eosinophil counts may be important.

Summary statement

NEC is characterized by elevated circulating cytokine levels and a consistent pattern of hematological abnormalities. Some of these findings may provide important diagnostic and prognostic information.
REFERENCES


