Necrotizing Enterocolitis: The Mystery Goes On

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Abstract
Necrotizing enterocolitis (NEC) has largely been present in neonatal intensive care units for the past 60 years. NEC prevalence has corresponded with the continued development and sophistication of neonatal intensive care. Despite major efforts towards its eradication, NEC has persisted and appears to be increasing in some centers. The pathophysiology of this disease remains poorly understood. Several issues have hampered our quest to develop a better understanding of this disease. These include the fact that what we have historically termed ‘NEC’ appears to be several different diseases. Animal models that are commonly used to study NEC pathophysiology and treatment do not directly reflect the most common form of the disease seen in human infants. The pathophysiology appears to be multifactorial, reflecting several different pathways to intestinal necrosis with different inciting factors. Spontaneous intestinal perforations, ischemic bowel disease secondary to cardiac anomalies as well as other entities that are clearly different from the most common form of NEC seen in preterm infants have been put into the same database. Here I describe some of the different forms of what has been called NEC and make some comments on its pathophysiology, where available studies suggest involvement of genetic factors, intestinal immaturity, hemodynamic instability, inflammation and a dysbiotic microbial ecology. Currently utilized approaches for the diagnosis of NEC are presented and innovative technologies for the development of diagnostic and predictive biomarkers are described. Predictions for future strategies are also discussed.

Introduction
Necrotizing enterocolitis (NEC) has become one of the most dreaded diseases in neonatal intensive care units. In the US and Canada, it affects approximately 7% of babies weighing between 500 and 1,500 g and approximately 20–30% of these babies die of this disease [1, 2]. Survivors may be left with significant sequelae, which include not only gastrointestinal complications, such as a short gut syndrome, but also severe neurodevelopmental delay [3, 4]. It is a very costly disease and both medical

and surgical NEC markedly increase the costs of hospitalization [5]. In addition, it is a much feared disease because its putative association with enteral feeding has caused neonatologists to refrain from using the gastrointestinal tract to feed these infants and to use prolonged intravenous nutrition. This incurs its own complications, which include prolonged hospitalization, increased use of central venous catheters and increased sepsis, as well as the possibility of increasing the likely development of intestinal inflammation and damage because of gastrointestinal mucosal complications secondary to the lack of trophic stimuli.

Although considerable effort has been made to determine the pathophysiology of NEC as well as finding optimal preventative and treatment strategies, progress in eradication of this disease has been almost zero. In fact, in some countries, the incidence of NEC has actually increased most likely due to more small babies being aggressively treated and now surviving [6]. Other reasons for the lack of progress include the fact that ‘NEC’ is actually more than one disease. Over time, the databases of what was called NEC have included infants with ischemic bowel disease secondary to cardiac anomalies, other congenital intestinal abnormalities, such as Hirschsprung’s disease, and spontaneous intestinal perforations. Table 1 lists some of these entities. Databases also have included stage I NEC [7], which is actually not a clearly defined entity. With stage I NEC, necrosis is implied by the name but is not validated with clear diagnostic criteria, which leads one to suspect that NEC is not yet present but may be developing. Another entity becoming increasingly recognized in older infants but with a poorly described pathophysiology, food protein-induced enterocolitis syndrome (FPIES), exhibits signs and symptoms similar to those in many preterm infants of NEC [8]. It will be critical to find ways to discern this entity from NEC since the treatment may be very different.

Another factor that has been problematic for developing better a understanding of the pathophysiology of NEC includes the lack of an animal model that directly represents the most common form of this disease. Most babies who develop NEC do not present shortly after birth in response to significant stressors, such as hypothermia, severe hypoxia and infection. However, the most commonly used animal model, which is a variant of that developed in the late 1970s, involves some variant of cold stress, infusing pathogenic bacteria such as Klebsiella, severe hypoxia and gavage feeding [9]. This is clearly not directly relevant to the disease seen in the preterm baby, who typically develops NEC several weeks after birth and may have no major stress indicators prior to the onset of the disease. Piglet models have been utilized for this disease, and despite the pig gastrointestinal tract having many similarities to that of the human, piglets require their mother’s colostrum or infusion of IgG to prevent death [10], whereas preterm infants do not require this for closure of the gastrointestinal tract and prevention of death. Another more recently developed model is of interest utilizing a chemical inhibitor of Paneth cells and ingestion of pathogenic Klebsiella in rodents during the later stages of pre-weaning [11]. This induces a disease similar to NEC, but fidelity of this model to the human disease remains unclear.

### Pathophysiology

For the most common form of NEC in preterm infants, the etiologic factors are multifactorial and are largely related to immaturity of the gastrointestinal tract. However, the interplay of other factors, such as feeding, type of feeding, intestinal microbial ecology and the highly susceptible intestinal mucosal surface relating to inflammatory processes, all appear to play a role. Many of these have been extensively discussed in previous reviews [12–19].

Although genetic factors have been implicated by twin studies [20], only a few have suggested actual mutations that might be related to higher risk of NEC [21–24]. The innate immune system of the newborn gastrointestinal tract appears to encompass several factors that predispose to the development of this disease [12, 25]. Furthermore, the interaction of the microbial ecosystem with this immature intestinal mucosal innate immune system appears to play a significant role [19, 26, 27]. There is a microbial ecology that differs prior to the development of NEC in these babies who subsequently develop the dis-

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**Table 1. Some of the pretenders of NEC**

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<tr>
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<th>Isolated intestinal perforations</th>
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<td>2</td>
<td>Ischemic bowel disease due to cardiac anomalies</td>
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<td>3</td>
<td>Variants of food protein-induced enterocolitis syndrome</td>
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<td>4</td>
<td>Congenital bowel anomalies (e.g. Meckel’s/Hirschsprung’s disease)</td>
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<td>Stage 1 NEC</td>
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<td>Misinterpretation of stool gas as pneumatosis intestinalis</td>
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<td>7</td>
<td>Placement of an abdominal drain prior to definitive diagnosis of NEC by surgery</td>
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ease compared to controls [28–30]. When one evaluates the population microbes in the gastrointestinal tract prior to the development of NEC, the phylum Proteobacteria appear to be more highly represented in comparison to other phyla. Certain bacteria such as the Klebsiella genus also appear to be overrepresented prior to the development of the disease [19]. The Proteobacteria also are overrepresented prior to exacerbations of symptoms of inflammatory bowel disease in older individuals [31]. This phylum contains numerous Gram-negative pathogens with high levels of lipopolysaccharides in their cell wall.

There are several predisposing conditions that occur in neonatal intensive care that may alter the microbial ecology, especially skewing toward the Proteobacteria (fig. 1). Among these is the common practice of providing little or no food to the intestine for prolonged periods of time and nourishing the infants intravenously with total parenteral nutrition. Another is the common practice of giving antibiotics shortly after birth during a ‘rule out sepsis’ period [32]. Use of acid blockers and formula instead of human milk are also common practices. Proteobacteria have been found to be the dominant phylum found in rodents fed with total parenteral nutrition rather than enteral feedings [33, 34]. Mechanisms of antibiotic induced loss of colonization resistance via loss of mucin degrading capability of commensal organisms with subsequent overgrowth of pathogens similar to those found in the Proteobacteria which thrive on sialic acid residues and nitrates have also been described [35, 36]. This phylum is responsive to the acid microclimate of the intestine with Proteobacteria growing very poorly in an acid (pH 5.5) microclimate [37] and therefore may partially be related to the finding that treatment of the preterm infants with H-2 blockers is associated with greater risk of NEC [38]. Human milk feeding is also associated with a lower intestinal pH, but whether this is enough to affect growth of various taxa of microbiota is unclear.

Proteobacteria, which are Gram negative, have a cell wall rich in the pathogen-associated molecular pattern lipopolysaccharide, which is recognized by Toll-like receptor (TLR)4, which is developmentally regulated and is thought to play a role in the pathogenesis of the inflammation associated with the pathogenesis of NEC. These microbes may also play a role in the stimulation of certain TLR, such as TLR4, which has been implicated in the pathogenesis of NEC. The modulating effects of the adaptive immune response where T regulatory cells stimulate production of IL-10 and TGF-β may also not yet be fully developed, hence allowing the proinflammatory cascade to dominate.

Secretory IgA is not released by the immature intestine in large quantities, but is found in human milk, and this may actually stimulate the development of subsequent intestinal mucosal IgA production as well as induce epithelial tight junction integrity and a decrease in intestinal permeability [39].

In addition to numerous other bioactive components that are conducive to human premature gastrointestinal tract development [40], it has been found recently that microbes present in human milk may act as commensal gastrointestinal microorganisms [41, 42] and potentially have beneficial effects for the developing gastrointestinal tract. The taxa from individual mother’s samples over time remains similar, but each mother’s samples differ from other mothers’ milk microbiota [41], suggesting a specific microbial ecology for each mother-infant dyad. The site of origin of these microbes remains uncertain, but it has been speculated that because of increased permeability of the maternal intestine during pregnancy, this would be a strong possibility [43], especially since many of the taxa are shared between the milk and the maternal and infant intestine [42]. Whether these microbes play a role in the protection against NEC has not been determined, and this will be an exciting area for future research.

The concept of the entero-mammary system is one that dates back to the late 1970s where studies in animals suggested that exposure of the mother to the infant can lead
to an ingestion of the microbes that the infant is colonized with, which can lead to the development of specific humoral and cellular immune responses to those specific microbes, which can then be transmitted to the infant via breast milk [44]. This system has been somewhat understudied in human infants, but the recent finding that some (or term?) neonates that have certain infections may actually stimulate a specific immune response in the mother that modifies the breast milk immunoglobulins and immune cells is of major interest [45, 46]. This finding supports the commonly used technique in neonatal intensive care units called kangaroo mother care [47] or skin-to-skin care, where there is close contact between mother and infant, and this could potentially have a salutary effect on the mucosal immune system of the preterm infant. All these factors may be involved in the protective effect provided by human milk to the preterm infant.

Over the past several years, the contribution of hypoxic ischemic injury to the pathogenesis of NEC has been modified [48]. It is no longer thought to be as significant as a predisposing factor as once thought. However, it is very likely that the microvasculature and blood flow in the small vessels of the intestine may be mitigated as a final component in the inflammatory cascade which results in intestinal injury [49]. Studies evaluating blood flow to the gastrointestinal tract using infrared techniques have not been very helpful in the evaluation of predisposition to development of NEC [50].

Thus, although we are beginning to put together some pieces of the pathophysiologic puzzle of NEC, there remains considerable work to be done before we have the knowledge base to confidently provide strong evidence-based strategies for NEC prevention and treatment.

**Diagnostic Considerations**

Several clinical factors lead to the diagnosis of NEC, including distended abdomen, peri-umbilical erythema, bloody stools, feeding intolerance and overall instability of the infant. However, these are not specific criteria. Diagnosis is usually made with abdominal radiographs with findings of pneumatosis intestinalis and/or portal venous gas [51]. Bowel wall thickening, persistent bowel loops that are filled with gas, and overall gaseous distention are suspicious signs but are not specific. Pneumoperitoneum is a sign that the intestine has perforated, but this may be due to either spontaneous intestinal perforation or NEC. The diagnosis of the pneumatosis intestinalis is sometimes very difficult with various radiologists having different opinions on the same radiograph [52]. Occasionally, a neonate with a very distended abdomen and complex ascites lacks bowel gas and radiographs suggest considerable fluid in the abdominal cavity without free air. This should be highly suspicious of NEC [53]. Such a finding unfortunately often delays surgery or does not lead to operation because of lack of free air in the abdominal cavity. Approximately 50% of babies who have surgical NEC do not have free air on abdominal radiographs, but if there is fluid on ultrasonography, this should be taken very seriously as a potential surgical emergency.

At present, there are no biochemical biomarkers for NEC in clinical use that are highly specific and sensitive. C-reactive protein, white blood cell and platelet counts have been used quite commonly, but these do not specifically represent NEC if they are abnormal [54]. Biomarkers for diagnosis under study include intestinal fatty acid-binding protein, claudin 3, calprotectin and IL-8 [54–57]. Use of these appears to be promising, but exactly how they will be utilized in the clinical setting remains to be seen.

**Preventative Measures**

As previously mentioned, the use of human milk is of major importance in the prevention of NEC. Baby’s own mother’s milk appears to confer major benefits in terms of prevention of NEC, sepsis and feeding intolerance [58]. However, neither baby’s own mother’s milk nor donor milk have all the nutrients required for optimal growth and development, and fortification is suggested for many of the smallest infants [59]. The provision of donor milk has been highly recommended for use in all preterm infants by the American Academy of Pediatrics [60]. However, the evidence to support widespread use of donor milk in this population remains debated.

Fear of NEC has been a major factor in neonatologists not feeding babies by the enteral route for significant periods of time and relying on total parenteral nutrition. However, over the past 2 decades several studies have suggested that using minimal enteral nutrition, which involves providing small quantities of feeding, initially usually less than 20 ml/kg/day for the first couple of days and then advancing to 20–35 ml/kg/day, appears to be relatively safe [61, 62]. Several neonatal intensive care units have instituted nutritional and feeding guidelines [63, 64], and these have been successfully utilized and actually have been shown to be efficacious in the prevention of NEC [65].

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Other modalities which have been recommended include oral antibiotics, IgA, steroids, growth factors, anti-inflammatory agents and amino acids such as arginine and glutamine [17, 66, 67]. Although some studies in animals and humans have suggested efficacy, these have not yet been sufficiently evaluated to recommend their routine use in preterm infants.

Significant controversy exists over the use of probiotics, which are defined by the World Health Organization as live microorganisms, which when administered in adequate amounts confer health benefits on the host. There are well over 100 types of probiotics with over 50 Lactobacillus species alone. There has been considerable debate on whether these should be routinely instituted for the prevention of NEC in preterm infants largely based on meta-analyses of trials done over the past 10 years [68]. Although they appear to be promising, there are still numerous safety and efficacy issues that should be addressed, and an adequately powered trial of efficacy should be completed [69–72]. Regulatory issues need to be addressed in terms of safety and efficacy if a specific probiotic is to be used to prevent diseases such as NEC. For the prevention of NEC, a probiotic would be considered by definition a pharmacologic agent and would need to undergo rigorous safety evaluation as a drug, which would provide for more reasonable quality and safety standards than if used as a food. Furthermore, use of live agents suggests the possibility of microbial mutation and possible long-term unforeseen outcomes since no long-term studies are available. Use of non-live microbial components is being studied and may represent a suitable alternative [73].

### Predictive Biomarkers

If one is to use routine interventional strategies for the prevention of NEC, it would be reasonable to target babies at highest risk for development of the disease using predictive biomarkers [57, 74]. Several studies have begun to address this and the ideal biomarker would include a bedside tool that includes a noninvasive assessment, such as urine, buccal swab, cord blood or noninvasive hemodynamic technique, such as evaluation of heart rate activity and or abdominal electrical activity or intestinal blood flow using noninvasive near-infrared techniques. Such studies are underway, but none has yet proven effective in the clinical setting for the prediction of NEC.

### Conclusion

Despite considerable frustration in the progress in our understanding of NEC including its treatment and prevention, it appears that with improved definitions of the disease, improved technologies to evaluate the microbial ecology as well as the immune responses of the gastrointestinal tract and other ‘omic’ techniques, it is likely that new strategies will be developed that will help predict those at highest risk for this disease and additional strategies will be developed to prevent NEC in most preterm infants.

### Disclosure Statement

The author discloses that he is a consultant to Infant Microbial Therapeutics and is on the Scientific Advisory Board of Medela.

### References


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Neonatology 2014;106:289–295

293


23 Moonen RM, Paulussen AD, Souren NY, Kes-...


