

New Frontiers of Necrotizing Enterocolitis: From Pathophysiology to Treatment

Umut Zubarioglu, Sinan Uslu*, Ali Bulbul

Division of Neonatology, Department of Pediatrics, Sisli Hamidiye Etfal Educational and Research Hospital, Istanbul, Turkey
Email: uzubari@hotmail.com, *sinanuslumd@hotmail.com, drbulbul@yahoo.com

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Abstract

Necrotizing enterocolitis [NEC] is an inflammatory disease of intestine largely occurring in preterm infants with a wide range of damage from minimal injury limited to mucosa to extensive necrosis of bowel wall and perforation. Despite advancements in neonatal care, mortality remains high [30% - 50%] and controversy still persists with regards to the most appropriate management of neonates with necrotizing enterocolitis. The main factors thought to be involved in the pathogenesis of NEC are: relatively hyper-reactive state of premature intestine, enteral feeding and bacterial colonization. In this review, we discuss current knowledge about the epidemiology, pathophysiology, imaging, medical and surgical management of necrotizing enterocolitis and describe novel strategies for prevention and treatment.

Keywords

Preterm Infants, Necrosis, Necrotizing Enterocolitis

1. Introduction

Necrotizing enterocolitis [NEC] is an inflammatory disease of intestine largely occur in preterm infants with a wide range of damage from minimal injury limited to mucosa to extensive necrosis of bowel wall and perforation. Necrotizing enterocolitis is the most common reason of death originated from intestinal tract in preterm babies especially for very low birth weight [VLBW] infants [1]. Although early recognition and aggressive treatment of this disorder has improved clinical outcomes, NEC accounts for substantial long-term morbidity in survivors of neonatal intensive care, particularly in VLBW infants.

Here, we will discuss current knowledge about epidemiology, pathophysiology, management strategies and describe new strategies about prevention and treatment.

2. Epidemiology

Worldwide, large multicentre studies coordinated by neonatal research networks have determined the incidence of NEC to be up to 13% among infants born ≤ 33 weeks of gestation or whose birth weight is ≤ 2500 g [1]-[9]. Although the majority of cases of NEC occur among premature infants, a small subset of babies born at term or ≥ 35 weeks of gestation develop NEC-like gastrointestinal signs and symptoms, frequently in association with other conditions such as congenital heart disease, perinatal asphyxia, polycythemia, sepsis, and respiratory disease [10].

3. Pathogenesis

The main factors thought to be involved in the pathogenesis of NEC are: relatively hyper-reactive state of premature intestine, enteral feeding and intestinal microflora.

3.1. Hyper-Reactive State of Premature Intestine

To understand the reasons for why preterm babies are at a particularly high risk of developing NEC compared with term babies, researches have focused on identifying the differences between the premature and the full-term intestinal tract. These studies have shown significant differences in bacterial colonization, microcirculatory perfusion and the maturity of the innate immune system of gut [11] [12]. These differences can provide and explain us the multi-factorial pathogenesis of NEC. Major difference found in studies was expression of Toll-like receptor 4 [TLR4] at higher levels in the premature than the full-term intestine in mice, humans and other species [13]. The elevated expression of TLR4 in the premature gut is reflective to function of TLR4 exhibits in the regulation of normal gut development [13]. In the premature birth situation, intestinal TLR4 levels remain elevated and activation of TLR4 on the lining of the premature intestine by the Gram negative bacteria that colonize the premature gut leads to a number of deleterious effects, including increased enterocyte apoptosis, impaired mucosal healing and enhanced proinflammatory cytokine release, which in aggregate lead to the development of NEC [14] [15]. Also, the translocation of Gram-negative bacteria through the gut mucosa leads to activation of TLR4 on the lining of the endothelium of the premature bowel mesentery, resulting in a reduction in blood flow and the development of intestinal ischaemia and necrosis [16]. This explanation for the pathogenesis of NEC termed as “the cross-switching hypothesis” partially explains the reasons for which the premature infant is at risk of NEC development and why the disease occurs upon bacterial colonization.

Also additional factors are known to differ between the premature and full-term host that might contribute to this disease.

- high baseline level of cellular endoplasmic reticulum stress within the premature intestine which increases the likelihood of apoptosis in the epithelial lining [17].

- the decreased number of mucus-producing goblet cells in the premature intestine results in deficient mechanical protection [13] [18].
- the impaired clearance of luminal contents, owing to decreased motility [19] [20] [21] [22].
- decreased digestion and absorption as a result of enterocyte immaturity [23] [24].
- increased microvascular tone within the preterm intestinal mesentery [16] [25].
- presence of immature tight junctions [26] [27].

All of these factors can render the bowel at risk of proinflammatory signalling, bacterial translocation and NEC development [19] [25] [28]. Notably, some of these important factors are linked to TLR4 signalling. Furthermore, T lymphocytes have been shown to participate in the adaptation of the premature intestinal mucosa to bacterial colonization and contribute to NEC development [29] [30]. NEC is associated with lymphocyte imbalance within the intestinal mucosa, as TLR4 signalling in the intestinal epithelium leads to an upregulation of proinflammatory T helper 17 cells and a reduction in protective T regulatory cells [30]. Specifically, various investigators have identified roles for the increased expression and function of platelet-activating factor in the mucosal injury and barrier dysfunction associated with NEC [28] [31] [32]. Infants with NEC have high circulating levels of platelet-activating factor associated with the increased expression of this protein as well as with deficient activity of platelet-activating factor acetylhydrolase, the enzyme involved in its degradation [31] [33] [34]. Additionally, platelet-activating factor has been demonstrated to induce TLR4 expression and signalling [28] [35].

3.2. Enteral Feeding

There have been 2 conditions known about relationship between enteral feeding and NEC; which are predominance of enterally fed premature babies in NEC epidemiology and protective effects of human milk against NEC [36]. A wide range of protective ingredients present in breast milk has been appointed and some part of them have been found as promising agents in NEC prevention and treatment [37]. Recent researches suggested the epigenetic effects of diet type on intestinal genomic structure. Human milk and other enterally given products change gene expression especially with methylation [38]. In studies performed in preterm infants and pig models, it was shown that proinflammatory genes became upregulated by enteral feeding [39] [40]. Also important risk factors in NEC pathogenesis such as intestinal microflora and intestinal splanchnic perfusion has mutual interaction with enteral feeding type [41] [42].

3.3. Intestinal Microflora

How bacterial pathogens involve in NEC pathophysiology still unclear but studies showed that they got interaction with several ways [36]. The most important evidence about bacterial involvement is occurrence of NEC as out-

breaks occasionally with the grown of same organisms from babies' cultures and such clusters of cases got controlled with the start of infection control measures [43] [44]. However, different centers reported separate microorganisms are grown in their outbreaks so someone can not claimed NEC development dedicated to a distinct bacterial agent. Also researchers found that endotoxemia, blood culture grows by bacteria and 30% hydrogen content of pneumatosis [an unique gas produced only by bacteria] in their studies which were demonstrate association of bacteria with NEC pathogenesis [45] [46] [47].

Earlier studies about NEC pathogenesis suggested the "dysbiosis" hypothesis [48]. Even so, recent studies claimed that bacterial diversity in microflora of gut disappeared just before beginning of NEC with following domination of pathogenic bacteria [49] [50].

4. Diagnosis

The cornerstone of effective NEC treatment relies on accurately diagnosing the disease, which can usually be established on the basis of readily available clinical, radiographic and laboratory data. The typical neonate with NEC is a premature infant who is thriving, yet suddenly presents with feeding intolerance, abdominal distension, bloody stools and signs of sepsis [11] [51]. For descriptive purposes and for disease stratification, the Bell scoring system has been widely utilized, which assesses the degree of NEC severity as mild [Bell stage I], moderate [Bell stage II] or severe [Bell stage III], as shown in **Table 1**.

Biomarkers and Noninvasive Testing for the Diagnosis of NEC

The relative nonspecificity of the readily available clinical and radiographic tests suggest the need for additional molecular markers to improve early diagnosis of NEC in premature infants. In this regard, the presence of several molecules that are detected in the blood have been assessed for their value in establishing the diagnosis of NEC and a number of them have shown considerable promise, including acute-phase reactants [such as C-reactive protein] and proinflammatory cytokines [for example, TNF α , IL-6 and IL-8] which were found as nonspecific [55] [56] [57].

In addition, organ-specific biomarkers, such as those that would indicate enterocyte injury or intestinal barrier impairment, include intestinal fatty acid-binding protein, liver fatty acid-binding protein, faecal calprotectin studied for early identification of NEC [58] [59]. Among these circulating molecules, one of the most promising might be intestinal fatty acid-binding protein, a cytoplasmic protein involved in enterocyte lipid metabolism that is released into circulation and secreted into the urine after enterocyte damage, which has been suggested to be useful in the prediction of NEC development and to correlate with the extent of intestinal necrosis [53] [60]. Nevertheless, I-FABP has handicaps in detection of NEC in early stages. Plasma half life is short and normal values in healthy preterm babies are variable that limits the use of I-FABP [36]. Also some NEC cases with extensive necrosis with ongoing damage can come against us with low

Table 1. Modified Bell staging criteria for necrotizing enterocolitis [52] [53] [54].

Stage	1	2A	2B	3A	3B
Description	Suspected	Mild	Moderate	Severe	Severe
Systemic signs	Temperature instability, apnea, bradycardia	Similar to stage 1	Mild acidosis, thrombocytopenia	Respiratory and metabolic acidosis, need for mechanical ventilation, hypotension, oliguria, disseminated intravascular coagulopathy	Further deterioration and shock
Intestinal signs	Increased gastric residuals, mild abdominal distention, occult blood in the stool	Marked abdominal distension ± tenderness, absent bowel sounds, grossly bloody stools	Abdominal wall edema and tenderness ± palpable mass	Worsening wall edema with erythema and induration	Evidence of perforation
Radiographic signs	Normal or mild ileus	Ileus, dilated bowel loops, focal pneumatosis	Extensive pneumatosis, early ascites ± portal venous gas	Prominent ascites, fixed bowel loop, no free air	pneumoperitoneum

I-FABP levels paradoxically because of this short half-life [53] [61]. In recent researches about biomarkers for NEC discovered novel urinary peptides and proteins which are linked to poor prognosis [62] [63].

Although plain radiology still accepted as the primary imaging procedure for diagnosis and staging the infants with NEC some new modalities especially ultrasound [US] has attracted research interest [64] [65]. Abdominal US can detect both pneumatosis intestinalis and portal venous gas [PVG] which is earlier than plain radiography especially for PVG [66]. Also US gives more details about perfusion and thickness of gut wall and motility from plain radiographs which may detect babies with more advanced disease and those who may benefit from surgery [67] [68].

Further information that can be obtained from ultrasound also includes the presence of free intraabdominal gas and the presence and nature of any free intraabdominal fluid that may be indicative of intestinal perforation [36].

Doppler ultrasonography can be used for evaluating coeliac trunk and superior mesenteric artery blood flow velocity, which can demonstrate cases at risk of necrotizing enterocolitis with poor perfusion, also for assessment of the intestinal wall viability in patients with NEC [54] [67] [68] [69] [70].

Near infra-red spectroscopy [NIRS] has got research interest recently which

may be useful for risk prediction of NEC, separation of cases with NEC from those without and detection of advanced NEC [71] [72]. Important limitation for NIRS is placement of probes on the skin and tissue penetration has short depth. So, measures of NIRS may give only oxygenation of underlying intestine of probes rather than entire intestine. On the other hand, NIRS measures can be used to detect differences in splanchnic tissue oxygenation in preterm babies who subsequently developed NEC and those who did not [71] [72] [73].

5. Prevention

Given that NEC occurs in a well-defined population of patients who are premature, there might be benefit in identifying specific preventive strategies that, if administered successfully to the appropriate patients, could reduce the incidence of NEC. In this regard, there has been tremendous interest in developing specific nutritional and pharmacological strategies to reduce the incidence of NEC.

5.1. The Use of Breast Milk

Multiple randomized clinical trials have now validated the empirical observation that breast milk statistically significantly reduces the incidence of NEC [74] [75]. Human milk contains a variety of beneficial bioactive factors, among which several have been shown to reduce NEC incidence and progression [74] [76].

In **Table 2**, a list of human milk components which had protective effects against NEC presented.

Considerable research efforts have been deployed to identify these critical factors in the hope that new preventive strategies can be developed [75]. Although the precise mechanisms by which breast milk protects against NEC are not yet fully understood, emerging experimental evidence suggests that breast milk inhibits TLR4 signalling by preventing glycogen synthase kinase 3 β activity [77]. Consequently, breast milk-mediated downregulation of TLR4 signalling can reverse the inhibition of intestinal stem cell proliferation and mucosal healing, which are themselves inhibited by TLR4 [77] [78]. Moreover, these effects were shown to be partially dependent upon activation of epidermal growth factor

Table 2. Protective factors of human milk against NEC.

Laktoferrin
Oligosaccharides and prebiotics
Secretory IgA
L-arginine
Nitrate/Nitrite
Platelet-activating factor acetylhydrolase
Antioxidant factors
Growth factors [Epidermal GF, Heparin-binding EGF-like GF, Transforming GF B ₂]
Erythropoietin

receptor signalling [77].

5.2. Donor Milk

The lack of availability of human breast milk [which can arise for a number of reasons, such as insufficient production by the mother of an infant remains a major challenge in neonatal care and has led to the use of donor breast milk as a potential substitute or supplement to formula-feeding [74] [79]. Multiple reports support the use of donor human milk as a potentially effective strategy for reducing the incidence of NEC [80] [81].

5.3. Probiotics in the Prevention of NEC

Probiotics are defined as live microorganisms that provide a health benefit to the host. These agents have been shown to protect against NEC and reduce disease severity and overall mortality in premature infants [82] [83]. The finding that a degree of perturbation in the normal gut microbial flora exists in patients with NEC supports a rationale of using probiotics to treat and prevent this disease [84] [85] [86]. Considering the vulnerability of premature infants, routine administration of probiotic agents has elicited substantial controversy regarding the type of agent to be used, dosing and timing [83] [87]. A systematic review evaluated the efficacy and safety of probiotics for preventing NEC and suggested that oral administration of probiotics decreases all-cause mortality and incidence of severe NEC in preterm infants; however, the precise probiotic agent, timing and length of therapy still remains to be established [83] [88]. Emerging consensus is that the use of probiotics in NEC could be effective in reducing the incidence of the disease without increasing rates of sepsis or other adverse events [88] [89] [90] [91].

Administration of the probiotic bacteria *Lactobacillus rhamnosus* was shown to increase enterocyte proliferation and differentiation of Paneth cells in enteroids grown in a 3D bioscaffold [92]. Furthermore, treatment with CpG-containing bacterial DNA, which bypasses the potential adverse effects of live bacteria, is effective against experimental NEC in mice and piglets, and acts by activating Toll-like receptor 9 and inhibiting TLR4, providing a potential alternative to the use of live probiotics [93].

6. Treatment

6.1. Medical Supportive Management

Most infants with Bell's stage I or stage II NEC are managed with appropriate supportive therapies which includes cessation of enteral nutrition, support of ventilation, stabilization of fluid-electrolyte and acid-base balances, correction of ongoing coagulopathy and/or thrombocytopenia, bowel rest, and antibiotics [54]. About which antibiotics should be used for NEC, there is still no sufficient evidence and concensus in literature and this confusion was demonstrated by both an international survey and a Cochrane review [94] [95]. So, antibiotics may be ordered according to protocol of center and regulated according to

grows in cultures and sensitivity results of the case. Similarly, the optimal duration time for withholding enteral feeds and bowel rest is not on evidence-based treatment and based on institutional approach [94].

6.2. Surgical Management

Surgical intervention is required in up to 50% of the NEC cases in large, population-based and hospital-based multicentre studies coordinated by neonatal research networks and typically includes the removal of necrotic intestine [5] [8] [9] [96] [97]. In rare cases, the placement of a peritoneal drain and abdominal irrigation might be sufficient. Although several studies have reported that patients undergoing peritoneal drainage and laparotomy could have similar outcomes [87] [94] [95]. Several surgical guidelines have been published [89] [98] [99] [100]. Given that up to 74% of infants initially managed with peritoneal drainage will require a subsequent laparotomy [96] [99] a commonly accepted approach has been to reserve primary peritoneal drainage for those patients with substantially elevated intra-abdominal pressure that impairs ventilation, or for extremely small infants under 750 g.

6.3. New Medical Treatments in Research

In consequence of the role of hyper-reactive and immature immune system in NEC etiopathogenesis; research studies focused on new treatment agents that regulate the immune response. Pentoxifylline is one of these agents studied but a very weak evidence found for usage as combination therapy to antibiotics in neonatal sepsis in a Cochrane review with a relative risk of NEC of 0.62 [101] [102]. In another study which intra-peritoneal pentoxifylline used in neonatal rat model, it was found that NEC incidence and severity reduced [103].

6.4. Promising New Agents

- Stem cells; Recent evidences came from research groups suggested that amniotic fluid stem cells [AFS], mesenchymal stem cells and enteric neural stem system cells have potential to change the trend of the NEC in experimental models [104] [105] [106] [107] [108].
- Amniotic fluid; In experimental animal models, *in vitro* proliferation and migration of gut epithelial cells was provided by both porcine and human amniotic fluid [109]. In another study which amniotic fluid used in postnatal minimal enteral feeding of preterm piglets; NEC incidence, severity and inflammation decreased [110]. In a mouse model, it was shown that NEC severity also decreased with amniotic fluid given enterally which contains epidermal growth factor [EGF] and its receptor as major factor in this effect [111].
- Growth Factors; Heparin-binding EGF-like growth factor has been identified as a biologic agent capable of preventing NEC in various animal models, and of reversing the effects of established NEC, via positive effects on mucosal healing, intestinal stem cell function and vascular perfusion [112]

[113] [114].

- TLR4 inhibitor; nontoxic oligosaccharide that inhibits TLR4 was shown to prevent NEC in mice and piglets and to reduce intestinal inflammation in *ex vivo* human intestine obtained during the treatment of NEC [32].
- Human milk oligosaccharides; established an important role for in NEC prevention and treatment [115] [116].
- Lactoferrin; Emerging evidence also suggests a prophylactic benefit against the development of NEC by oral administration of lactoferrin with or without probiotics to preterm infants at risk of NEC [gestational age <32 weeks or birth weight <1500 g] [75].

7. Outcome

Despite advancement in medical and surgical treatment over the last decades, the average mortality from NEC is 20% - 30%, with mortality as high as 50% in those infants requiring surgical management [117]. Necrotizing enterocolitis has also severe morbidities affects survived infants which are originated from intestinal or systemic insults [54]. These complications include;

- Relapsing of NEC; About 10% of babies who had undergone surgery for NEC develop a relapsing episode and this causes long-term parenteral nutrition dependency [54].
- Intestinal strictures; About 25% of patients who had NEC and especially treated surgically, can develop one or more intestinal strictures [54].
- Gut failure; Infants who had undergone surgery for NEC will develop intestinal failure with high proportion and it depends on many factors (such as low birth weight, antibiotic use, ventilator use, and greater extent of bowel resection) also associated with NEC development [54].
- Parenteral nutrition associated complications
- Neurodevelopmental disturbances; seen in the nearly half of neonates, but by which mechanisms this develops is stil not identified clearly [118] [119]. It is found that infants with NEC had white matter anomalies on magnetic resonance imaging at term which increases the risk for motor impairment [120].

8. Conclusion

NEC is the most common and lethal gastrointestinal pathology that affects premature infants. Characterized by high morbidity and mortality, complex pathogenesis and devastating short-term and long-term sequelae. Only within the past decade have substantial strides been made in the understanding of the molecular mechanisms that determine NEC pathogenesis. These advances undoubtedly hold the promise to improve the development of effective preventive and diagnostic strategies to curtail the devastating consequences of the disease.

Conflict of Interests

The authors have indicated they have no financial relationships and conflict of

interest relevant to this article to disclose.

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