33 Necrotizing Enterocolitis

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Despite nearly two centuries of recognition and five decades of increasingly intensive study, necrotizing enterocolitis (NEC) remains an unsolved problem. Reports from France as early as the 1820s describing "gangrenous necrosis" of the intestine were echoed in Vienna 30 years later.[1] Not until the mid-20th century was the disease process systematically observed in Switzerland in 1944 and in New York in the 1960s.[2] A condition of premature neonates, the magnitude of the problem has dramatically expanded with our ability to resuscitate infants at very early gestational ages. Research interest has exploded as the number of publications on NEC increased from 1–2 per year in the early 1970s to more than 400 in 2015.[3] Many individuals have devoted their entire careers to understanding, preventing, or treating NEC. In spite of tremendous effort, the mortality associated with NEC has remained essentially unchanged over the past three decades.[4–9] Furthermore, the precise risk factors, prevention strategies, and optimal medical and surgical interventions also remain unclear.

Epidemiology

Though the overall incidence of NEC is around 1 per 1000 live births, it is primarily a disease of premature neonates. NEC affects about 10% of very low birth weight infants (VLBW, birth weight <1500 g), and the incidence is inversely proportional to birth weight.[4,10–14] The incidence for a given gestational age has not changed significantly over the last three decades. Despite a number of studies aimed at identifying risk factors for NEC, prematurity (either gestational age or low birth weight) appears to be the only consistently demonstrated association. Some regions have higher reported rates of the disease than others, and urban areas may be more significantly affected than rural ones.[15–17]

The overall mortality of NEC probably approaches 30%.[5,10,12,16,18,19] Lower birth weight and younger gestational age correlate with higher risk of death.[4,5,20] Approximately 20–40% of affected neonates undergo an operation for NEC worldwide. Among VLBW infants in the United States, about half undergo operative intervention.[5] The mortality associated with surgical NEC is significantly higher than with medical NEC, with some sources citing 50% fatality.[20] Moreover, the apparent protective effect of increasing birth weight is substantially blunted in surgical NEC.[5] Almost all of the long-term morbidity from NEC occurs in the surgical group. Babies with surgical NEC suffer higher rates of neurodevelopmental delay and intestinal failure than their medically managed NEC counterparts.[21]

NEC in full-term infants is rare (0.5 per 1000 live births) and may reflect a different pathophysiology.[22] Although the clinical and pathologic findings are similar, reduced mesenteric perfusion stemming from congenital heart disease, sepsis, respiratory disease, or global hypoxic events probably drives bowel necrosis. Once NEC develops in term babies, however, the mortality rates are similar to those for preterm infants.[23]

Though NEC is a relatively rare disease, its economic burden is substantial. The median length of hospital stay (LOS) for extremely low birth weight (ELBW, birth weight <1000 g) neonates is between 2 and 3 months.[24] The addition of a diagnosis of successfully treated medical NEC increases that by 20 days, and treatment with surgery increases LOS by 60 days. Medically treated infants incur >$70,000 hospital costs over baseline and surgical NEC adds another $330,000.[25–27] The long-term morbidity associated with NEC is likely even more costly than the initial hospital admission. A child who develops short bowel syndrome, for example, may require multiple operations and incur a mean cost over a 5-year period in excess of $1.6 million.[28,29]

Pathophysiology

Our understanding of the molecular and cellular basis for NEC is becoming increasingly sophisticated. Traditionally NEC has been defined by clinical and radiographic criteria. These findings are discussed in detail later. Radiographically, pneumatosis intestinalis, or air within the bowel wall, is thought to be related to gas produced by the overgrowth of enteric bacteria in concert with failure or breakdown of mucosal barriers (Fig. 33.1).[30] Progressively produced air escapes into the mesenteric veins or lymphatics and may appear on plain films as branching bands of air projecting over the liver (Fig. 33.2). Pneumoperitoneum indicates perforation with complete disruption of the intestinal wall with leakage of intraluminal gas (Fig. 33.3). The histologic findings in NEC typically reflect inflammatory changes, bacterial overgrowth, and coagulation necrosis (Fig. 33.4).[31]

Although this clinical, radiographic, and histologic constellation is typically referred to as NEC, it is likely that this condition can result from a number of different inciting events or contributing factors. Poor cardiac output, hyperviscosity, a variety of food-protein–related enteropathies, and possibly "spontaneous intestinal perforation" (SIP) may represent distinct pathophysiologic processes that are often clinically grouped together under the heading of NEC.[32]

This review of pathophysiology will focus on "classic" NEC—the type seen in premature neonates and associated

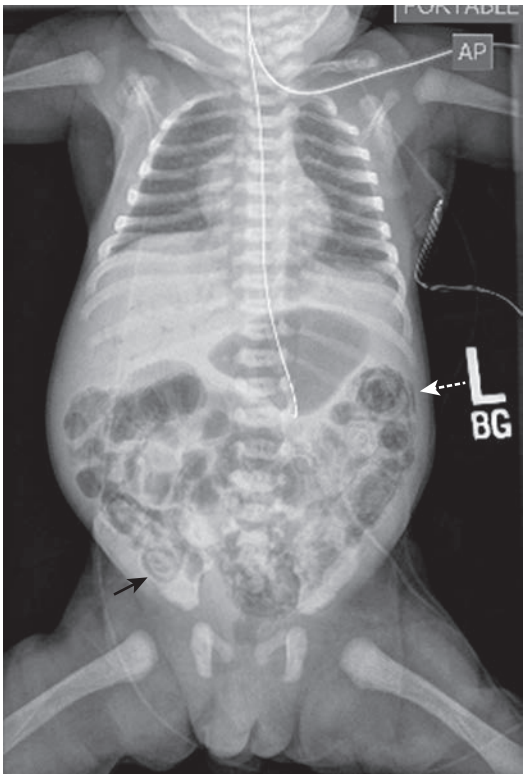

Fig. 33.1 Pneumatosis intestinalis is the classic radiographic finding in NEC. The air within the bowel wall may be cystic (solid arrow) or linear (dotted arrow) on the abdominal film, and may be seen in a focal intestinal segment or diffusely throughout the bowel as is visualized on this abdominal film.

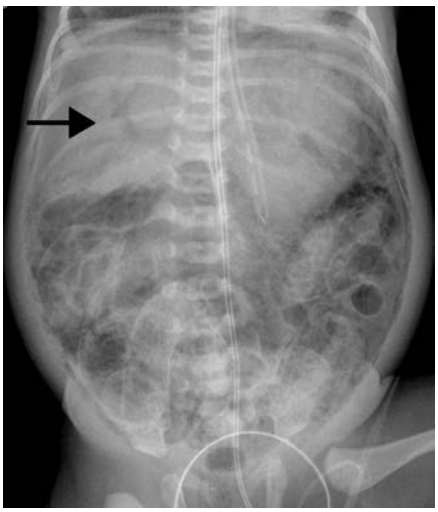

Fig. 33.2 Portal venous gas (arrow) is demonstrated on this abdominal radiograph. This finding is considered a poor prognostic sign. This baby also has widespread pneumatosis intestinalis.

with inflammation.[33] The essence of the current prevailing theory is that the premature intestinal tract paired with some type of insult triggers an exaggerated immune response in the setting of failure of protective factors. The insult may be microbial dysbiosis, disturbed nutrient metabolism, genetic predisposition, or something else. The insult results in a stress that alters the intestinal metabolome and releases cytokines. This subsequently results in

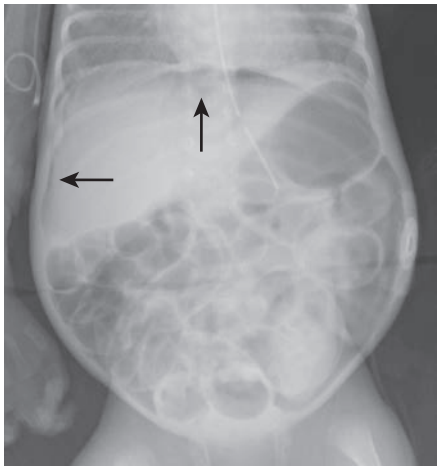

Fig. 33.3 Free air (arrows) is seen on this radiograph. This finding is an indication of perforation and is considered an absolute indication for intervention, whether drainage or exploration.

increased intestinal epithelial permeability, which allows translocation of bacteria and foreign proteins that in turn further activate the immune response, and ultimately result in necrosis and a global inflammatory state.[33,34]

THE INTESTINAL BARRIER

Contemporary evidence suggests that an essential element in the development of NEC is not only a bacterial breach of the physical intestinal barriers, but also a failure and/or inappropriate response by the innate and adaptive immune systems in the bowel. The physical barriers that protect the gastrointestinal (GI) tract include gastric acid secretion, intestinal motility, the mucus layer, the epithelial barrier, and antimicrobial peptides.[34] Nonmechanical factors include innate and adaptive immunologic defenses, cellular homeostasis, and regeneration.

Gastric Acid

The low pH of the stomach is one of the GI tract's first defenses against pathogens. The process of gastric acid secretion does not appear to mature until about 24 weeks of gestation.[35] The absence of this acidic environment, as seen in neonates on H_2 blockers, has been associated with both NEC and late-onset sepsis.[36,37]

Intestinal Motility and Digestion

Intestinal motility develops during the third trimester of pregnancy but may not be fully mature until the eighth month of gestation.[30,38–40] In premature infants, immature motility leads to increased epithelial exposure to potentially noxious substances, and poor clearance of bacteria with subsequent overgrowth. Additionally, the immature intestine has decreased nutrient digestion and absorption, which may lead to direct epithelial injury.[41–43] The failure of chemical digestion that results from the decreased gastric and pancreatic exocrine function in newborns contributes to bacterial proliferation.[44]

Increased ileal bile acid levels may play a role in the pathogenesis of NEC. Bile acids are known to be cytotoxic, resulting in the development of mucosal injury.[45] In premature infants, levels of ileal bile acid-binding proteins are

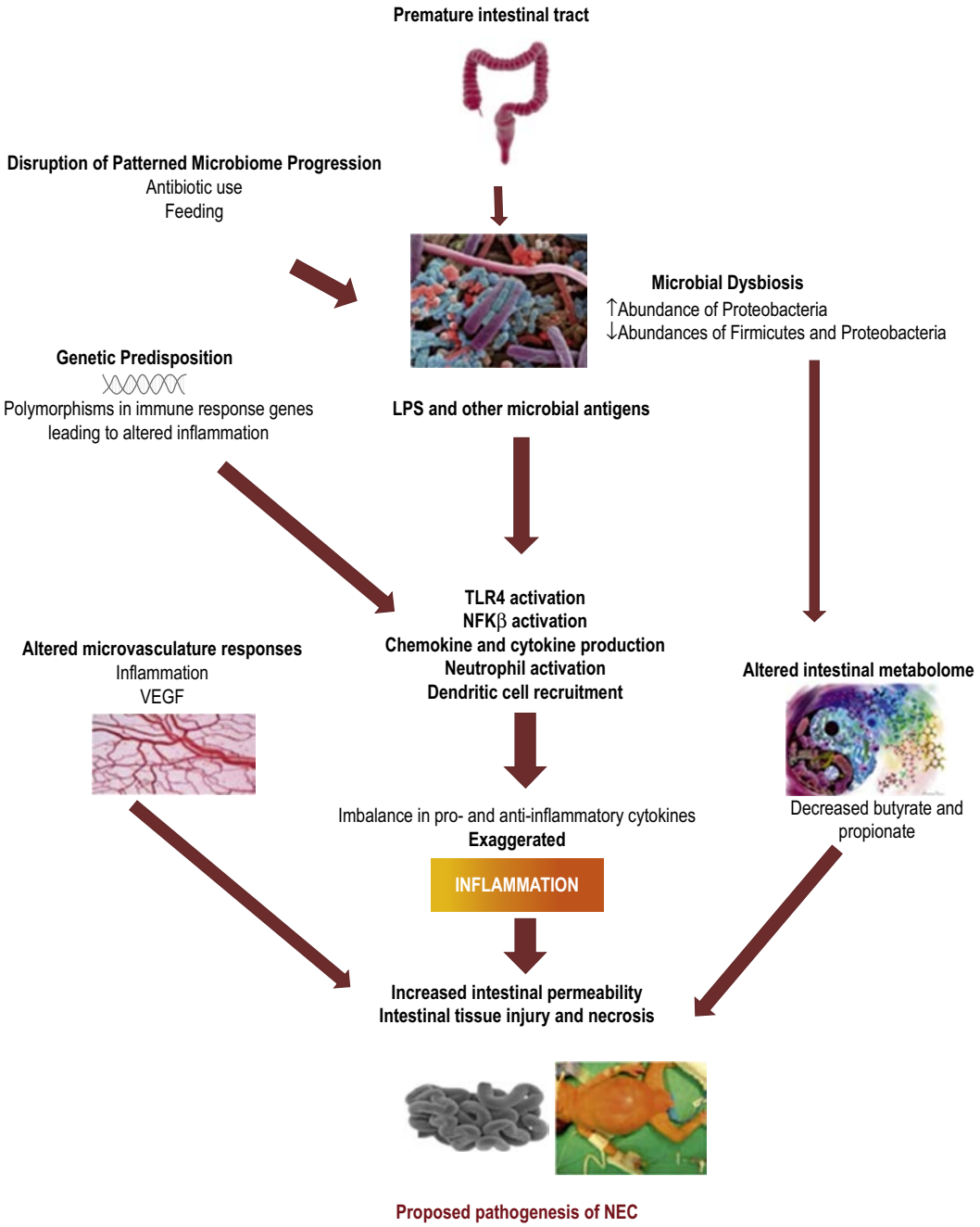

Fig. 33.4 This diagram summarizes the pathophysiology of NEC.

lower, leading to increased levels of bile acids in the intestinal lumen and in enterocytes.[46] Interestingly, formula feeding, which is clearly more closely associated with NEC then breast milk, elicits more secretion of bile acids.[47]

Mucous Layer

The mucous coat overlying the intestinal epithelium plays a key role in the barrier function. One essential element of mucous is mucin, a highly glycosylated protein secreted by goblet cells in the epithelial layer that concentrates enzymes near the intestinal surface.[48,49] Mucin aids in lubrication, provides a mechanical barrier to the approach of bacteria and damage from gastric acids,[18] and assists in the fixation of pathogens.[50] Mature mucins have higher viscosity, better pH buffering, and resistance to bacterial breakdown, and are thus more effective.[50–52] Mucin production and composition changes with gestational age, bacterial challenges, and colonization by commensal organisms.[53–55] Low numbers of goblet cells have been noted in both experimental rodent models and in human neonates with NEC.[49,56] Deficiencies in the production or composition of mucin may contribute to the ability of bacteria to invade the intestinal epithelium and thus contribute to the pathogenesis of NEC.[44,49,53–55,57–60]

Tight Junctions

The intestinal epithelial cells create a complex and highly regulated physical barrier. In addition to adherens

junctions, tight junctions link the mucosal poles of epithelial cells and form a semipermeable membrane. Mature tight junctions are composed of the transmembrane proteins occludin, claudin, and junctional adhesion protein, which normally present a barrier to diffusion of large molecules.[61] Tight junctions are not static, but may be altered by disease processes.[62] A significant portion of mucosal maturation occurs between 26 weeks of gestation and term.[42] Immaturity in the composition of tight junctions likely plays a role in the increased permeability of the epithelium of the newborn intestine, [63] and weakening of the barrier function related to cytokines and tight junctions has been implicated in the pathogenesis of NEC.[64,65] Further, intestinal fatty acid binding protein (I-FABP) and claudin-3 are markers of gut barrier disruption that may find clinical utility.[66] Maintenance and regeneration of the intestinal barrier is an important focus of considerable recent study into the pathophysiology of NEC.

IMMUNOLOGIC DEFENSES OF THE GASTROINTESTINAL TRACT

Passive Immunity

Immunoglobulin G (IgG) antibodies transferred via the placenta may offer one of the neonate's first passive defenses. While antibody transfer starts at 13 weeks, the majority of delivery occurs during the last 4 weeks of gestation. Neonates born at 22 weeks have <10% of maternal levels, whereas those delivered at term have up to 130%.[67] Despite this finding, clinical trials attempting to replace IgG and IgA orally in preterm infants have not altered the risk of NEC.[68]

Breast milk contains a number of factors that may contribute to its protective effects against infection and inflammation. In addition to the homeostasis promoted by the delivery of fats, proteins, and sugars, some specific nutritional elements, such as caseins, may both prevent attachment of bacteria to the epithelium and stimulate the production of protective mucin.[69,70] Breast milk also contains bioactive proteins, such as lactoferrin and lysozyme, that are involved in a number of antimicrobial processes.[71,72] Additionally, it contains interleukin (IL)-10 and transforming growth factor-beta (TGF-β), important anti-inflammatory cytokines that promote intestinal homeostasis, prevent enterocolitis, and induce production of gut IgA.[73–76] Lastly, breast milk contains growth factors such as epidermal growth factor (EGF) and insulin-like growth factors (IGF-1, IGF-2). The IGF family reduces apoptosis of epithelial cells while promoting their proliferation and reducing NEC in animal models.[77–79] EGF is further discussed later in this chapter.

Innate and Adaptive Immunity

A number of cell lines play a role in the host's defense against bacterial invasion and are involved in the normal and pathologic inflammatory response that occurs in NEC.

Intraepithelial lymphocytes reside between bowel epithelial cells and play an important role in innate immunity. Specifically, the T-cells identified by γσ receptors are among the first immune cells present in the developing gut.[80] Mice without γσ-intraepithelial lymphocytes had a greater severity of injury in an experimental NEC study.[81] Also, in another study, reduced numbers of these cells were found in ileal specimens from infants with NEC compared with controls.[81] These cells also secrete epithelial growth factor and other signaling molecules that support the epithelial barrier and promote regeneration.[82]

Emerging data suggest that natural killer (NK) cells have a role in supporting the intestinal barrier and suppressing inflammation. The experimental absence of NK cells results in higher levels of inflammatory cytokines.[83] Low levels of NK cells have also been noted in NEC.[84]

Neutrophils appear be involved in both protective and harmful processes that occur in NEC. They contribute to intestinal protection against microbes through phagocytosis and proinflammatory processes, such as the production of reactive oxygen species, aimed at destroying microbes.[85] Neutrophil-dependent release of IL-22 may result in proliferation of epithelial cells and thus support regrowth of damaged intestinal tissue.[34] Neutropenia is associated with an increased severity of NEC,[86] and impaired neutrophil function is also associated with more severe disease.[87] However, high concentrations of neutrophils have been noted in specimens resected for NEC.[88] It is possible that neutrophil invasion and the cascade that follows may contribute to the destruction of healthy tissues.[34]

Intestinal macrophages are constitutively present in the bowel wall and play a role in tolerance of bacteria related to their hyporesponsiveness to exotoxins including lipopolysaccharide (LPS).[89] Blood macrophages, on the other hand, infiltrate into tissues in response to injury and differentiate into activated M1 macrophages. These elaborate a variety of proinflammatory cytokines including IL-6, IL-8, IL-12, and TNF-α, and are associated with epithelial cell apoptosis.[90–92] Dendritic cells (DCs) are another group of antigen-presenting cells present in the gut wall. In adults, DCs have a role in mediating tolerance to microbes.[93] In premature neonates, DC activation may contribute to pathologic inflammation and has been associated with NEC in an animal model.[94]

MOLECULAR MECHANISMS OF INFLAMMATION AND INJURY

The pathologic findings of NEC arise not only from alterations in the integrity of the intestinal barrier but also from an impaired ability to regenerate.[95] Premature infants have a reduced capacity for intestinal repair, likely contributing to the pathogenesis of NEC.

Lipopolysaccharide

LPS is the endotoxin portion of the Gram-negative bacterial cell wall, and is one of the most abundant proinflammatory stimuli. LPS is seen in high levels in NEC.[96] LPS impairs intestinal barrier function by inhibiting repair and promoting the release of signaling molecules and proinflammatory cytokines such as nitric oxide (NO), interferon-gamma (IFN-γ), cyclooxygenase-2 (COX-2), and RhoA from enterocytes.[62,95,97,98] These mediators promote intestinal injury. LPS causes increased expression and function of integrins on the cell surface, resulting in increased cell adhesion to the basement membrane,[99] and compounds the effects of platelet-activating factor (PAF).[100,101]

Nitric Oxide

Though constitutive production of low levels of NO promotes vascular and mucosal homeostasis, high concentrations produced in the setting of inflammation drive destruction of the intestinal barrier.

Two enzymes primarily mediate the production of NO from arginine. Metabolism via endothelial NO synthase (eNOS) is associated with the constitutive low levels of NO, whereas inducible NO synthase (iNOS) is upregulated during inflammation.[102] In normal concentrations produced by eNOS, NO has multiple beneficial effects including regulating vascular smooth muscle tone, maintenance of mucosal capillaries, and the scavenging of free radicals, which protects against oxidative stress.[103,104]

Inducible NOS produces concentrations of NO up to a million-fold higher.[103] These levels produce the highly toxic peroxynitrite, which induces enterocyte apoptosis and inhibits enterocyte proliferation and migration. High NO also results in impaired mitochondrial function and decreased endothelial leukocyte recruitment.[105,106] NO-related damage to the gut barrier is also associated with bacterial translocation.[107,108] These effects may be compounded in the presence of high levels of LPS, which leads to increased iNOS expression and function within the intestine.[109,110] Ford and colleagues directly linked these processes and NEC by demonstrating the increased expression of iNOS in affected tissues.[111]

The pathway that leads to activation of iNOS in humans with NEC has not been fully elucidated. One cellular/signaling cascade has been described in a specific mouse strain that links certain pathologic bacteria or molecules such as LPS with iNOS upregulation, but this finding has not been reliably produced in other models.[104] Further understanding of the interaction of the microbiome and intestinal immunity may lead to an improved grasp of this pathway and ultimately allow therapeutic intervention.

Platelet-Activating Factor

PAF is a potent phospholipid inflammatory mediator that is produced by most cells and tissues.[112] The cytotoxic effects of PAF are due to initiation of the inflammatory cascade. PAF-induced bowel injury is associated with the production of oxygen-derived free radicals and leukocyte migration, activation, and capillary leakage, resulting in apoptosis in affected enterocytes.[113]

Various studies have shown the importance of PAF in the pathogenesis of NEC. Higher concentrations of PAF have been found in NEC patients compared with controls.[113–115] Activity of the PAF-degrading enzyme PAF acetyl hydrolase (PAF-AH) has been shown to be deficient in sick infants with NEC, and the administration of PAF-AH or of a PAF receptor antagonist in animal models of NEC reduces the degree of intestinal injury.[114,116,117] PAF-AH is present in maternal breast milk, which may contribute to its protective effect.[117]

Epidermal Growth Factor

EGF, a peptide secreted into the intestinal lumen, plays a key role in both development and maturation of gut tissue as well as intestinal repair and adaptation. It is an important element of the mechanism that keeps the gut barrier healthy, preventing bacterial translocation.[73,118–124] In addition to support of the barrier, it may also downregulate inflammatory cytokines.[119,120]

EGF binds to the EGF-receptor (EGFR), a member of the ErbB family of cell surface growth factor receptors that includes ErbB2/HER2, ErbB3, and ErbB4.[125] Decreased levels of EGF have been demonstrated in the saliva and serum of premature infants with NEC.[126] Furthermore, in preterm infants, low salivary levels of EGF in the first 2 weeks of life are associated with the subsequent occurrence of NEC.[127] A small randomized controlled trial (8 infants) suggested increased rates of early intestinal repair in the group treated with recombinant EGF.[128]

Another member of the EGF family, heparin binding EGF (HB-EGF), found in amniotic fluid and breast milk, is protective against the development of NEC.[129] Animals with overexpression of HB-EGF have decreased susceptibility to NEC,[130] while animals with deletion of the HB-EGF gene have increased susceptibility.[119,130] These effects seem to be at least in part due to cytoprotective effects of HB-EGF on intestinal stem cells, and the promotion of enterocyte proliferation and migration.[119,131] HB-EGF also leads to improvement in microvascular blood flow.[132] These effects appear to be mediated through the ErbB4 and EGFR receptors.[125] In animal models of NEC, administration of HB-EGF has been shown to reduce the incidence of bowel injury by half and more than double survival,[130–133] as well as preserve the integrity of the intestinal barrier.[134]

Experimentally, the coadministration of stem cells and HB-EGF results in additive protective effects.[135] Similarly, administration of stem cells modified to overexpress HB-EGF have a more beneficial effect than unmodified stem cells or HB-EGF given alone.[136]

While this growth factor offers a promising protective effect against NEC, one significant challenge to the widespread use of this factor in infants is that HB-EGF is associated with tumor formation. HB-EGF is upregulated by certain tumors, and its expression is a key component of tumor resistance to therapy. In some animal studies, it has even led to development of new tumors.[137]

Neuregulin-4 (NRG4) is a selective ErbB4 ligand that appears to have a protective effect against apoptosis in Paneth cells. Experimentally, exogenous NRG4 delivery has been shown to halt intestinal necrosis is a rat model of NEC.[138] Highly specific ligands may offer novel treatment options in NEC that may avoid some of the issues with more broadly binding compounds.[125,138,139]

NEONATAL VASCULATURE AND THE PATHOGENESIS OF NEC

Newborn intestinal circulation is characterized by a low resting vascular resistance,[140,141] and is controlled both extrinsically by the autonomic nervous system and intrinsically via local signaling pathways.[142] The intrinsic regulation is mediated by two vascular effector mechanisms produced and released within the intestine—one vasoconstrictive and one vasodilatory.[143] Endothelin (ET)-1 is the primary vasoconstrictor stimulus in the newborn intestine and is produced by the endothelium.[140,144] Although constitutively produced, it can also be stimulated by decreased flow, hypoxia, and various inflammatory cytokines.[145,146]

NO is the primary vasodilator stimulus and is produced by both eNOS and iNOS as described earlier.[147,141] In the neonate, the balance of ET-1 and NO favors vasodilation generating the characteristic low vascular resistance. In pathologic states, endothelial dysfunction leads to ET-1–mediated vasoconstriction, causing compromised blood flow, intestinal ischemia, and injury.[148] Increased expression of ET-1 has been identified in surgical specimens from infants with NEC.[142] Furthermore, the concentration of ET-1 was proportional to the degree of histologic injury in that study.

THE MICROBIOME AND NEC

Though bacteria have long been implicated in the pathogenesis of NEC, the concept of intestinal dysbiosis was first detailed in 2001.[149] In this paradigm, the secondary inflammation that occurs as a result of the host–microbe interaction, rather than a specific infectious microorganism, is at the heart of NEC pathophysiology. Neutrophil activation in response to bacteria results in the release of inflammatory cytokines, vasoconstriction, and disruption of the intestinal barrier. Changes in the intestinal microbiome and an associated exaggerated immune response have been further implicated in NEC pathogenesis.[150,151]

To summarize simplistically, the concept of the "microbiome" emerged after the development of non-culture–based techniques for identifying microorganisms such as genomics and metabolomics. The ability to isolate and sequence RNA and DNA rapidly and accurately has allowed for the accrual of huge amounts of data that can be statistically analyzed, which yields a broader picture of all the organisms identified in a sample.[152,153] Other "omics" fields, including metabolomics and proteomics, employ similar accrual techniques using massive data from biologic samples processed through bioinformatics. A number of review articles are available on this subject.[154,155]

Studies using molecular techniques have implicated specific changes in the microbial pattern in infants with NEC.[156] Further, characterizing the microbiomata and metabolic milieu may allow for determining which neonate is likely to develop NEC.[157,158] A recent prospective trial that evaluated the microbiota of VLBW infants before any of them developed NEC concluded that relatively higher levels of Gram-negative facultative bacilli (Gammaproteobacteria) and lower levels of strict anaerobic bacteria, such as Negativicutes, were present in the neonates who developed clinical NEC.[158]

Clinically, the few factors that affect NEC development have direct effects on the microbiome that may mediate their influence on the pathophysiology. Exposure to antibiotics has a significant effect on the microbiome and carries a duration-related effect on the risk of NEC.[159,160] Additionally, acid suppression is linked both to specific changes in GI bacterial content and the development of NEC.[37,161] H_2 blockers are associated with a larger percentage of Proteobacteria over Firmicutes, a change that has been identified in infants who develop NEC.[158]

Based on these observations, Neu and Pammi have proposed an updated theory of NEC pathophysiology (see Fig. 33.4).[33] In some infants, genetics may predispose a higher risk of NEC.[162,163] The specific stage of intestinal development, or lack thereof, dictated by postconceptual age combines with specific microbiota to set up the conditions for

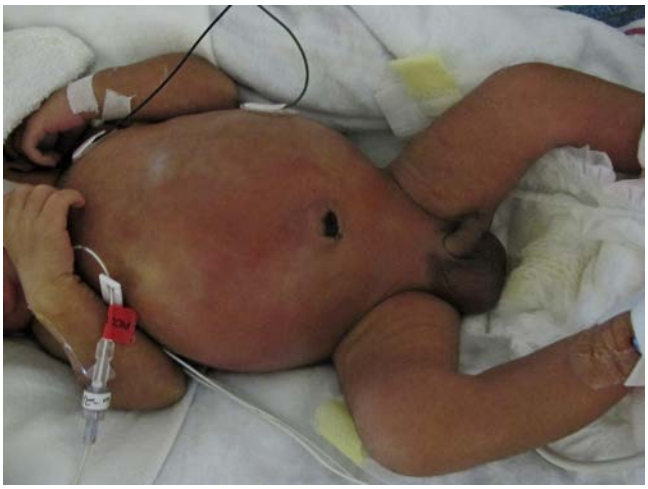

Fig. 33.5 This infant has NEC. Note the abdominal distension and abdominal wall erythema.

NEC. They postulate that rather than a primary hypoxic-ischemic event, NEC is triggered via changes in the microvasculature in response to endothelial growth factor in response to inflammatory mediators. Cell receptors (Toll-like receptors) respond to these microbial elements and trigger cytokine elaboration (via NFKβ) that leads to tissue damage caused by an exaggerated immune response.[33]

Clinical Diagnosis

NEC is typically diagnosed when characteristic radiologic findings are noted in the appropriate clinical setting. Classically, NEC presents clinically with feeding intolerance manifested as vomiting or high gastric residuals and abdominal distention. Early signs may be even more nonspecific and include apnea, bradycardia, lethargy, and temperature instability. Hematochezia or occult fecal blood may also occur. The surgeon may also elicit a history of sudden increase ventilatory requirements at the onset of the NEC, suggestive of increased metabolic requirements combined with increased intra-abdominal pressure.[164]

Abdominal distention is the most common finding on exam. Visual inspection may reveal bowel loops projecting through the skin. Skin color changes should be noted. Duskiness of the abdominal wall may reflect underlying discoloration of bowel or stool through thin soft tissue. Erythema may suggest peritonitis with inflammation transmitted through the wall (Fig. 33.5). Palpable loops of bowel typically raise concern. When present, the findings of a fixed abdominal mass and erythema of the abdominal wall are strongly predictive of NEC. However, these findings are present in only 10% of patients with NEC.[165]

Confirmation of the diagnosis of NEC combines signs and symptoms with radiologic findings. These findings have been combined into the clinical staging system proposed by Bell that aids in describing the severity of disease (Table 33.1).[166]

LABORATORY STUDIES

The diagnosis of NEC is not made with laboratory tests, but they may aid in establishing the degree of the infant's

Table 33.1 Modified Bell Classification for NEC

	Clinical Findings	Radiographic Findings	Gastrointestinal Findings
Stage I	Apnea, bradycardia, and temperature instability	Normal gas pattern or mild ileus	Mild abdominal distention, stool occult blood, gastric residuals
Stage IIA	Apnea, bradycardia, and temperature instability	Ileus with dilated bowel loops and focal pneumatosis	Moderate abdominal distention, hematochezia, absent bowel sounds
Stage IIB	Metabolic acidosis and thrombocytopenia	Widespread pneumatosis, portal venous gas, ascites	Abdominal tenderness and edema
Stage IIIA	Mixed acidosis, coagulopathy, hypotension, oliguria	Moderate to severely dilated bowel loops, ascites, no free air	Abdominal wall edema, erythema, and induration
Stage IIIB	Shock, worsening vital signs and laboratory values	Pneumoperitoneum	Bowel perforation

systemic illness. The degree of metabolic acidosis may reflect bowel and/or whole body perfusion. Leukocytosis with bandemia or leukopenia may be present. Worsening thrombocytopenia, especially a precipitous drop, may be an ominous sign.[167]

Early identification and grading of NEC would allow for earlier medical intervention and could guide the role and timing of operative intervention. An accurate diagnostic tool could also more effectively rule out NEC and thus preserve resources. A number of putative biomarkers have been evaluated.[168]

Certain serum acute phase proteins and cytokines are elevated in NEC. Increased levels of IL-6, IL-10, and C-reactive protein (CRP) have been documented in premature infants with NEC, with the highest levels of IL-10 being found in those patients who did not survive.[169] Rapid elevation in CRP may accompany the clinical onset, and some prospective data suggest this change may discriminate NEC from other GI disorders.[170] More importantly, a number of complications, including abscesses, strictures, and sepsis, are associated with failure of CRP to normalize.[171] In one study, the negative predictive value of a normal CRP level for stricture formation was 100%.[172] In two related studies, IL-8 levels were higher in neonates with surgical versus medical NEC, and were statistically different in those with NEC totalis versus multifocal versus unifocal disease.[173,174]

Fecal calprotectin is a marker of intestinal inflammation that has been shown to differentiate limited NEC from NEC with system illness (Bell III) with 76% sensitivity and 92% specificity.[175] Higher levels of a similar fecal protein were seen in infants with suspected NEC who developed perforation over those who did not.[176] The wide variability in levels of these proteins and difficulty in reliably collecting stool in low birth weight neonates significantly limit the clinical utility of these markers.[176–178] I-FABP is located in the enterocytes in small bowel villi. On cell lysis, this protein is released into the blood and subsequently cleared in the urine.[179] High levels in infants with NEC have been found in those neonates who developed surgical NEC.[180,181]

Lastly, genomics, proteomics, and metabolomics methodologies are currently helping to find additional marker arrays that may be clinically useful in NEC. The use of such technologies represents a paradigm shift in the search for biomarkers. Rather than testing one putative biochemical at a time, thousands of chemicals can be analyzed and candidate markers identified prospectively. One such study found a panel of seven urinary proteins that can identify patients who later developed surgical NEC.[182] A variety of biomarkers and metabolic arrays are being evaluated for diagnosis and prognosis.[183]

The majority of these advanced biomarkers have yet to reach the surgeon at the bedside. International survey data published in 2015 showed that pediatric surgeons report following platelet count (99%), CRP (90%), white blood cell count (83%), and lactate levels (43%) most frequently with only 10% using fecal calprotectin and IL-6 or -8 levels.[184]

IMAGING

Radiography

Pneumatosis intestinalis seen on plain film is the hallmark radiologic finding in NEC (see Fig. 33.1). Given the lack of specificity of the typical signs, symptoms, and laboratory results, pneumatosis is often the critical discovery that makes the diagnosis. The early stage of NEC (Bell I) may present with dilated loops of bowel or a paucity of bowel gas. Pneumatosis can be followed by portal venous gas, which is generally considered a poor prognostic sign (see Fig. 33.2). This finding tends to be fleeting; it may be seen on one film and not the next without truly indicating a change in clinical status. A "fixed loop" of bowel, or multiple plain films showing a dilated loop in the same place, may represent a nonfunctioning segment concerning for necrosis. Some surgeons consider a fixed loop a clear operative indication.

Ultrasound

Abdominal ultrasound (US) to evaluate NEC was first described in 2005.[185] US allows observation of bowel peristalsis, wall thickening, vascularity, and echogenicity in addition to pneumatosis intestinalis, free fluid, and pneumoperitoneum. Based on a number of studies, US is more sensitive than plain films for the diagnosis of NEC given its greater ability to identify smaller air or fluid collections, and to more completely characterize the bowel wall.[186–188] US does appear to aid in defining a prognosis. One study showed that even beyond free air and fluid, having any three of nine additional US findings was predictive of poor outcome.[188] Clinically, US may be a useful tool in at least three clinical settings: (1) concern for NEC without pneumatosis on plain film; (2) questionable plain film findings in an otherwise well infant; and (3) in making a decision to operate upon an infant with clear well-demonstrated pneumatosis/medical NEC with a poor clinical course and an otherwise equivocal set of laboratory findings. However, no large prospective studies have been performed, and US is still not widely used as a primary tool to either diagnose NEC or to delineate treatment.

Other Imaging Modalities

Computed tomography and contrasted fluoroscopy have no clear role in evaluating infants with acute NEC.[189–191] There is limited literature regarding the use of magnetic resonance imaging.[192]

Near-infrared spectroscopy (NIRS) is an emerging modality that may allow an improved assessment of bowel perfusion in neonates. Noninvasively NIRS measures tissue hemoglobin oxygen saturation. It has been used clinically to monitor cerebral oxygenation and to evaluate shock in critically ill adults.[193,194] In a porcine model of NEC, NIRS saturation measures have been able to distinguish poor splanchnic perfusion and predict the animals in which NEC developed.[195]A recent study using continuous NIRS paired with I-FABP in piglets suggested that these combined modalities may identify NEC earlier than conventional tests.[196] Though this tool has yet to demonstrate clear utility in neonates with NEC, its feasibility and safety in the premature neonate have been established[197] as have normal values for tissue oxygenation in this population.[198] Specific differences in proprietary systems may represent a barrier to the widespread use of NIRS.[177] Additional studies in neonates are ongoing.[199]

Differential Diagnosis

Septic ileus may present with near-identical findings to early NEC and is the most clinically relevant diagnosis in the differential. Clinical and biochemical indices of systemic illness coupled with abdominal distention and abnormal appearing bowel on plain films are common to both diagnoses. Until hallmark signs such as pneumatosis appear, radiographs typically do not distinguish NEC. Other causes of neonatal bowel obstruction should be considered such as Hirschsprung disease, ileal atresia, volvulus, meconium ileus, and intussusception.

SPONTANEOUS INTESTINAL PERFORATION

SIP or focal intestinal perforation (FIP) is either a variant of NEC or a distinct clinical entity that presents similarly in premature neonates as pneumoperitoneum.[200,201] SIP is defined by findings of a small "punched out" hole in the intestinal wall without surrounding necrosis at operation or on postmortem.[202] Clinically, SIP and NEC both primarily affect the smallest premature infants. Those with SIP tend to have lower birth weights and may be less likely to develop severe physiologic derangements consistent with shock (hypotension, acidosis) as well as hyponatremia, neutropenia, and thrombocytopenia. SIP has been linked with indomethacin exposure and early postnatal steroids.[203–205] Both surgical NEC and SIP typically are diagnosed with the finding of pneumoperitoneum, but SIP is not associated with bowel injury beyond the focal perforated area. When confirmed at laparotomy, SIP carries half the mortality of surgical NEC.[206]

Despite important implications of this entity on researching outcomes and interventions in neonates and its potentially differing pathophysiology, the clinical management of the two is typically similar. Some surgeons may be more likely to treat a suspected SIP with a peritoneal drain over laparotomy.[207] However, while some contend that SIP can clearly be differentiated from NEC preoperatively by the clinical team, a large prospective multicenter study found the ability to distinguish the entities preoperatively was only moderate.[20] For the surgeon approaching a small neonate with intestinal perforation, the distinction is largely academic. However, in the future, a better understanding of the disease states may provide opportunities for a more tailored surgical approach.

Grading System

In 1978, Bell described a classification system[166] for NEC that was later modified slightly (see Table 33.1).[208] These criteria have mostly been used in studying NEC interventions and outcomes to grade severity of an infant's disease. Critics contend that there are two significant problems with the use of this classification.[209] First, Bell I (temperature instability, apnea, bradycardia, gastric residuals, mild abdominal distention, normal motility or perhaps mild ileus, with occult positive stools) is quite nonspecific and may reflect any septic illness in a preterm neonate, particularly a VLBW infant. For this reason, many contemporary studies do not include Bell I as NEC.

The other criticism of Bell's classification is that it groups all diagnoses that appear as NEC together. As these are typically treated in the same way, this may be reasonable from a clinical standpoint. Given the differences in outcomes of varying underlying diseases (NEC vs SIP and others), the Vermont Oxford Network (and others) has abandoned using Bell's classification as it is likely an incomplete description. The alternative classification is based on treatment rendered (medical vs surgical NEC) and differentiates SIP when it is found on opening the abdomen.[202] Though the decision for surgery is often made by an individual surgeon and may not reflect an identical disease state across infants, this distinction does appear to discriminate outcomes.[5,210,211] In this schema, Bell I is essentially "NEC watch."

The severity of NEC found at laparotomy is highly variable. Any segment of the GI tract can be involved with both colonic and small bowel involvement present in the majority of patients with isolated small bowel areas being the next most common.[31,212,213] The spectrum of involvement ranges from the focal perforation seen in SIP to massive necrosis of the entire intestine, termed "NEC totalis," which is considered uniformly fatal.[212] Identifying which neonates are at risk for NEC totalis has proven difficult.[214]

Medical Management

The primary management of medical NEC is supportive. The suspicion of NEC typically prompts treatment with bowel rest, gastric decompression, intravenous fluid, and parenteral nutrition. Clinically, the appearance of pneumatosis intestinalis on plain film is often the key finding. Most clinicians will add broad-spectrum antibiotics with anaerobic and Gram-negative coverage early in the process. Though a number of combinations are currently in use, none is clearly superior.[215] As in any septic patient, cardiopulmonary support focuses on delivering oxygen via appropriate

resuscitation with fluids and blood products, adequate oxygenation and ventilation, and vasopressor support when necessary. No supportive specific strategy has yet emerged in NEC therapy. Close clinical and radiographic observation by the neonatology and surgical services with ongoing reevaluation for operative indications is important in these neonates.

Surgical Management

INDICATIONS

Among VLBW neonates with NEC, up to 52% undergo an operation.[5] Although some infants followed for medical NEC develop surgical indications under observation, many required an operation at presentation. The only absolute indication for operative intervention is pneumoperitoneum on an abdominal radiograph. Paracentesis has been used in the diagnostic algorithm for NEC. A tap that is positive for enteric contents within the abdomen is also considered a clear reason for operation.[8] Deciding which neonates with severe NEC without obvious evidence of perforation should undergo an operation remains difficult. Some surgeons elect not to operate in the absence of pneumoperitoneum, though many will intervene in the face of worsening clinical status or a "fixed loop" of bowel seen on serial radiographs. Many attempts have been made to identify which of the myriad clinical data can be combined to predict those neonates with NEC who will benefit from exploration. A prospective multicenter study found that clinical factors alone could not predict which infants will need surgical therapy.[216] A number of studies have evaluated markers for intestinal necrosis, such as severe thrombocytopenia, that may identify neonates without perforation who may benefit from an operation, but none has yet to be widely adopted.[217,218] Currently, in practice, the decision for surgery is highly personal based on the overall assessment by the surgeon at the bedside.

Early identification of infants who are likely to require operation may allow for intervention before perforation occurs. In a series of studies, Tepas and colleagues identified seven clinical and laboratory findings that were indicative of significant metabolic derangement in neonates with NEC (positive blood culture, pH < 7.25, bandemia with I/T >0.2, sodium <130, platelets <50,000, mean arterial pressure less than gestational age or on vasopressors, absolute neutrophil count <2000/mm^3).[219] Treated as binary variables, they proposed that the presence of 3 of the 7 is a relative surgical indication. They then compared two similar neonatal ICUs and found that the awareness of the "MD7" and integration of the concept into clinical practice, without a hard mandate for surgery based on the criteria, significantly decreased the number of neonates who died or required long-term PN.[220] These findings underline the concept that clinical predictors of surgical NEC prior to perforation may help to improve outcomes. Further study to better define the role of the MD7 is needed.

OPERATIVE APPROACH

Exploratory Laparotomy

Laparotomy with resection of necrotic bowel and creation of stomas is the traditional operation of choice. When limited disease is identified with healthy surrounding bowel, intraoperative decision making is straightforward. A finding of NEC totalis should prompt abdominal closure followed by a frank discussion with the family regarding goals of care and expectant management for the moribund infant. Diffuse or patchy intestinal involvement may pose an operative dilemma (Fig. 33.6) as a large resection may leave the infant with short bowel syndrome (SBS) but failure to resect injured areas may result in worsening illness or recurrent perforation. In this situation, the use of a "second-look" laparotomy[221] has been widely adopted. Similar in concept to the damage-control approach for abdominal trauma, clearly nonviable intestine is resected using a "clip and drop" technique and questionable areas are left in place.[222] The GI tract is left in discontinuity and the abdomen left open with a temporary silo or negative pressure dressing with plans to reexplore at 24–72 hours, at which time further resection and/or closure typically with stoma creation is performed. Multiple reexplorations may be required prior to final closure. Large areas of necrosis have also been treated by proximal diversion alone with some favorable results.[223]

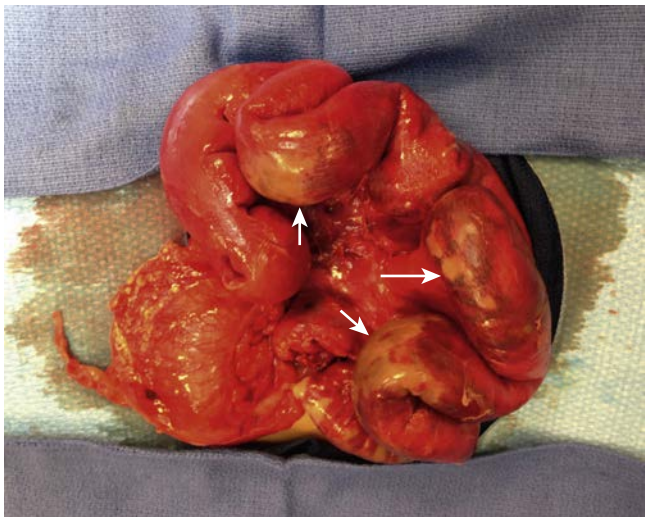

Fig. 33.6 This infant was explored for pneumoperitoneum. As is evident, there is diffuse involvement of the bowel and pneumatosis (arrows) is readily visualized. However, much of this bowel looks uninvolved, so the bowel was returned to the abdominal cavity and a second look operation performed 48 hours later, at which time segmental resections were performed.

As an alternative to universal enterostomy creation in surgical NEC, some surgeons advocate for primary anastomosis citing stoma complications and the need for a second operation as disadvantages. Limited data are available that compare the two approaches, but one study suggests that mortality is nearly doubled in neonates who undergo a primary anastomosis compared with stomas.[224]

Primary Peritoneal Drainage

Peritoneal drainage was initially developed as a temporizing measure in the smallest and sickest of premature neonates and intended as a bridge to laparotomy. Pediatric surgeons in Toronto noticed that some of the patients did quite well after drain placement and never required further operative intervention and published their experience in 1977.[225] Over the following two decades, the concept of primary peritoneal drainage (PPD) entered into mainstream practice.

Table 33.2 Comparison of Study Design Between NET and NECSTEPS Trials

	NET Trial (Europe)[a]	NECSTEPS (North America)[b]
Number of patients	69	117
Centers represented	18 centers in 8 countries	15 centers in USA and Canada
Birth weight criteria	<1000 g	<1500 g
Gestational age criteria	None	<34 weeks
Confirmation of intestinal perforation (indication for surgery)	Radiologic evidence of pneumoperitoneum required	Radiologic evidence of pneumoperitoneum, paracentesis results, or clinical decision accepted
Randomization	Assigned by weighted minimization techniques accounting for weight at enrollment, gestational age, platelet count, mechanical ventilation, inotropic support, facilities for onsite laparotomy, and geographic location	Permuted blocks of four and stratified by birth weight (<1000 g vs 1000–1500 g)
Primary peritoneal drainage instruction	One-fourth inch soft drain inserted in the right or left lower quadrant and irrigations via the drain were not recommended	One-fourth inch, right lower quadrant incision with manual expression of stool and pus and irrigation until clear followed by Penrose drain placement. Additional drain placement as per operating surgeon
Postoperative care	Per operating surgeon and treating neonatologists	Uniform care pathway
Primary outcome measure	Mortality at 1 and 6 months	Mortality at 90 days
Secondary outcome measures	Total hospital length of stay, ventilator dependence, dependence on parenteral nutrition and time to full enteral feeding	Parenteral nutrition dependence at 90 days and length of stay for patients surviving 90 days postoperatively

[a]Rees CM, Eaton S, Kiely EM, et al. Peritoneal drainage or laparotomy for neonatal bowel perforation? A randomized controlled trial. Ann Surg 2008;248:44–51.
[b]Moss RL, Dimmitt RA, Barnhart DC, et al. Laparotomy versus peritoneal drainage for necrotizing enterocolitis and perforation. N Engl J Med 2006;354:2225–2234.
Adapted from Raval MV, Hall NJ, Pierro A, Moss RL. Evidence-based prevention and surgical treatment of necrotizing enterocolitis: a review of randomized controlled trials. Semin Pediatr Surg 2013;22:117–121.

Though a number of studies were published on the relative outcomes of PPD versus laparotomy, a meta-analysis in 2001 concluded that there was insufficient evidence to recommend one approach over the other.[226]

Three prospective studies have evaluated outcomes after laparotomy versus PPD in NEC. The NICHD Neonatal Research Network conducted a cohort study at 16 centers.[227] In this purely observational study, 156 infants with either NEC or SIP underwent either laparotomy or PPD as determined by their treating surgeons. Overall 50% (n = 78) of the patients died and 72% (n = 112) either died or had some element of neurologic impairment at 18–22 months. Extensive prospective data were collected, allowing for risk-adjusted multivariable regression analyses. The odds ratio (OR) for death after adjusting for differences in the two treatment groups was 0.97 for laparotomy compared with peritoneal drainage. Despite the lack of statistical significance, there was a trend toward better overall outcomes at 18–22 months of age in the laparotomy group. The OR for the combined outcome of death or neurodevelopmental impairment (NDI) at 18–22 months was 0.44 for laparotomy compared with drainage.

Two multicenter randomized controlled trials comparing PPD to laparotomy began recruiting patients in the early 2000s, one in the United States and the other in Europe.[228,229] The North American trial, known as NECSTEPS, enrolled 117 VLBW infants at 15 tertiary care centers and randomized them to PPD or laparotomy. There was no difference in mortality at 90 days (the primary outcome variable), and the LOS was similar between groups. The study concluded that, at least in the short term, the choice of surgical intervention does not affect mortality.[228]

The NET trial was performed across 31 centers in 13 European countries.[229] Sixty-nine ELBW neonates were randomized. The primary outcomes were mortality at 1 and 6 months. There was a trend toward better survival in the laparotomy group (65%) compared with the PPD group (51%), with a nonsignificant relative risk of mortality of 0.5. The authors concluded that there was no evidence from the trial to support the use of PPD in ELBW infants with intestinal perforation.

There are some important differences in how these studies were performed that inform their collective interpretation (Table 33.2). Among these differences was the approach to postoperative management. In attempt to minimize confounding variables, NECSTEPS included a prescribed postoperative protocol that was used across centers, while the NET trial allowed for variability in postoperative care as dictated by the treating physician. Both the "real-world" and more rigidly scientific approach yielded similar findings. Also, the NET authors allowed crossover from drain to laparotomy if the infant's condition did not improve within 12 hours, whereas in NECSTEPS, clinicians were encouraged to keep patients in the assigned group for the duration of care whenever possible. Taken together, these studies suggest that the choice of initial operative intervention in VLBW infants with perforated NEC does not affect mortality. The similar outcomes despite differences in the trials serve to underscore the lack of a significant difference in surgical approach on short-term outcomes. It is important to note that long-term data are unavailable.

In the United States, more than two thirds of VLBW neonates with surgical NEC undergo laparotomy first. In a recent prospective cohort study, among those who underwent drainage first, nearly half also received a laparotomy.[5]

The Necrotizing Enterocolitis Surgical Trial (NEST) completed enrollment in late 2016.[230] NEST is a multicenter study that randomized ELBW infants with perforated NEC or SIP to PPD versus laparotomy. It was designed to compare long-term outcomes with a primary outcome of death or NDI at 18–22 months corrected gestational age. The forthcoming results from this trial should further inform the surgeon's decision at the bedside and may allow better prognostication of developmental outcomes.

Outcomes

RECURRENCE

The reported incidence of recurrence in NEC varies in the literature but may be as high as 10%.[231] A second episode may be more likely in lower birth weight infants, those with persistent cardiac issues, and neonates with other major congenital anomalies.[232–235] While one study found a low rate of surgical NEC among those who recurred,[235] another found that >80% of recurrences require an operation.[231] The mortality and stricture rate after recurrent NEC appear to be similar to that following a single episode.[231]

MORTALITY

The mortality for NEC has remained in the range of 30% over the last four decades. [5,10,12,16,18,19] The most well documented risk factor for death is prematurity. Mortality is inversely proportional to birth weight and gestational age.[4,5,20] Medical NEC carries a mortality of about 20%, whereas surgical NEC mortality is probably in excess of 35% and may be as high as 50%.[5,10,20,236,237] Further, higher birth weight is less protective against mortality in surgical NEC compared with medical NEC.[5] The presence of other congenital comorbidities, particularly severe congenital heart disease, also significantly increases mortality.[238] The degree of bowel involvement also correlates with risk of death.[239,240]

Given the likelihood that a surgeon will intervene in the setting of a florid illness from NEC, the concept of mortality related to medical NEC may be problematic. A number of studies cite mortality for medical NEC in a range from 5–20%.[5,10,236,237] However, in most of these papers, there is no comparison between "medical NEC mortality" and the expected rate for corresponding gestational age.[10,236,237] The Vermont Oxford Network (VON) data suggest that infants with medical NEC do have mortality significantly higher than their birth weight-matched peers without NEC.[4,5] No clear explanation for this is evident from the data, but it is important to note that VON entries for NEC diagnosis are not timed[201] so it is possible that their deaths may be related to other causes. Still, it is also possible that a history of medical NEC decreases survival even if death is not caused by NEC itself. An alternative hypothesis is that "medical NEC mortality" reflects a group of moribund infants for whom an operation was deemed futile. To the authors' knowledge, no study has directly assessed these questions.

REGIONALIZATION OF CARE AND NEC MORTALITY

Evidence to support the relationship between patient volume and outcomes is mounting both in the surgical[241,242] and neonatal literature.[243–245] Recognizing that neonates with complex medical needs fare better at large referral centers, the American Academy of Pediatrics (AAP) has recommended regionalization of care since the 1970s.[246] Available data suggest better outcomes in neonates who undergo surgery in specialized centers, a trend reflected in management of other pediatric surgical conditions.[247,248] In recent years, the pediatric surgical community has increasingly recognized the importance of matching the needs of the patient to the resources of the treating hospital.[249] To that end, The American College of Surgeons has created the Children's Surgery Verification Program, the pilot phase of which concluded in 2016.[250] NEC is a helpful marker for studying these concepts given its relative frequency and the medical and surgical complexity that often accompanies the disease.

Administrative database studies by Kastenberg[251] and Jensen[252] support a relationship between level of care and mortality in NEC specifically. Further, Jensen demonstrated that lower neonatal intensive care unit (NICU) patient volume is an independent risk factor for mortality in neonates with NEC.[252] The question that follows is: if mortality for NEC is lower at high level of care centers with a large volume of patients, do infants who are transferred there have better outcomes? One reason sometimes given for not transferring is that the transport itself of a critically ill preterm infant between centers may entail substantial risk. However, a study of administrative data in California by Kelley-Quon et al. compared mortality between infants transferred for urgent operations for NEC (surgery <2 days from transfer) to those with NEC who were not transferred and found no statistical difference in mortality.[253] Fullerton et al. reviewed VON data on VLBW infants with surgical NEC and found that neonates who were transferred prior to their operation had lower mortality (32%) than those who remained in the same center (45%).[254] Each of these datasets has limitations, but taken together, these results indicate that transfer itself is not a risk factor for mortality, and transfer to a higher level of care is likely to yield better outcomes.

Despite data to support regionalization for neonatal care and emerging data for NEC specifically, some areas of the United States are becoming increasingly deregionalized. The substantial charges associated with the long NICU stay provide a strong financial incentive for smaller hospitals not to transfer infants with NEC. Increased accuracy and transparency in neonatal outcomes may result in realigning monetary incentives with the improved outcomes that follow regionalization.[255,256]

INTESTINAL FAILURE

NEC is the leading cause of pediatric intestinal failure (IF) resulting in more than a third of IF patients.[257] IF can be defined as inadequate functional bowel to satisfy the nutrient and fluid homeostasis via digestion and absorption. One quantitative definition is the requirement of PN for >90 days.

SBS is a large subset of IF in which the lack of functional intestine results from loss of a substantial length of intestine. Although the majority of infants with IF from NEC have SBS, even those who have not undergone resection can develop IF. A multicenter cohort study found that 42% of infants with surgical NEC and 2% of those with medical NEC developed IF.[258] Risk factors for IF in this population included parenteral antibiotics on the day of NEC diagnosis, birth weight <750 g, mechanical ventilation on the day of diagnosis, and exposure to enteral feeds prior to diagnosis.

The remaining length of intestine is also an important prognostic factor. Fifty percent of infants with >35 cm of small bowel will wean from PN,[259] although some children with as little as 10 cm have become enterally autonomous. With current hepatoprotective strategies and multidisciplinary intestinal rehabilitation programs, survival for infants with <10 cm of small bowel is excellent, despite a requirement for long-term PN or intestinal transplantation.[260]

The anatomic location of the segment of bowel resected and remaining also affects outcomes. The mortality in patients with small bowel disease is higher than infants with colonic NEC.[240,261–263] Furthermore, neonates with large jejunal resections appear to fare better than those with large ileal resections. This is likely secondary to the ileum's greater capacity for adaptation.[264,265]

Interestingly, among children with IF, those with a history of NEC are both more likely to wean from PN and become enterally autonomous earlier than their peers with IF from other causes.[266]

STOMA COMPLICATIONS

Enterostomal complications can lead to significant morbidity with complication rates exceeding 50% in some series.[187,206–209] The most serious of these include prolapse, stricture, and retraction, all of which may require surgical intervention. Proximal jejunostomies can cause significant electrolyte and water losses that can lead to problems with fluid balance and weight gain[194,210] in addition to skin breakdown if not managed appropriately.[194,211]

A variety of approaches to stoma placement and techniques for creation have been advocated. Small studies comparing complication rates between these various strategies have not found differences in complications, including retraction, prolapse, hernias, or wound infections.[203–205] Some surgeons do not mature the stoma citing concern for further compromise of the tenuous blood supply.

The timing of enterostomy closure remains controversial. Recommendations vary from as early as 1 month to as late as 4 months after stoma creation.[212–215] Most suggest waiting 1–2 months after the initial operation, and/or until a weight of 2000 g is reached, as long as adequate feeding and growth is being maintained.[194,210,213] Earlier closure may be necessary with very proximal stomas due to fluid and electrolyte losses and an inability to gain weight. The additional medical comorbidities must also be considered in determining the optimal time of closure.

INTESTINAL STRICTURES

Strictures occur in 12–35% of infants with medical and surgical NEC.[267–272] Resection and primary anastomosis at the time of the original operation does not increase the stricture rate when compared with initial enterostomy and subsequent stoma closure.[9,267–271,273–276] The colon is the most common site for stricture formation, the descending colon in particular.[233,270,271] The standard approach to stricture is laparotomy with resection and reanastomosis, though spontaneous resolution has been reported.[190,272,277] Balloon dilation may be an option for focal lesions in select patients.[278]

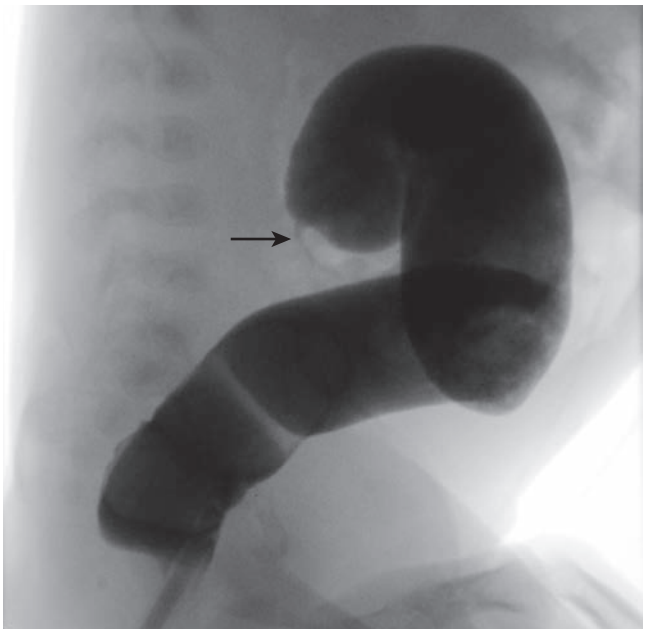

Fig. 33.7 This infant was born at 34 weeks of gestation and developed NEC at 3 weeks of age. He did not require an operation and was managed medically. About a month later, he began to develop abdominal distention and feeding intolerance. A barium enema was performed and revealed this stricture (arrow) in the left colon. At operation, a 3-cm segment of the left colon, including the stricture, was resected, and a primary anastomosis performed. He recovered uneventfully.

Patients treated by laparotomy and stoma creation for NEC should undergo routine imaging of the distal intestine before enterostomy closure to evaluate for a possible stricture. Patients managed medically, by peritoneal drainage, or with primary anastomosis can all develop strictures (Fig. 33.7). Some patients remain asymptomatic while others present acutely with partial or intermittent bowel obstruction. Occasionally infants with stricture will present in distress due to perforation.[267] Due to this occurrence, some surgeons advocate contrast studies in all NEC patients prior to feeding,[190,267,268,270] although this is not widely practiced.

Any long-term implications of stricture formation are likely related to the need for additional operations, including the risk of general anesthesia on neurodevelopmental outcome and the potential for SBS after multiple intestinal resections.

NEURODEVELOPMENTAL OUTCOMES

The majority of studies evaluating treatment strategies for NEC have focused on mortality as the primary outcome. Among survivors, however, there has been increasing concern regarding neurologic and developmental impairment. In 1980, a groundbreaking study reported that less than half of children surviving NEC were neurodevelopmentally normal at 3-year follow-up.[279] Subsequently, multiple observational studies have cited "intellectual delays,"[280] "moderate-to-severe developmental delay with speech and motor impairment,"[281] "developmental delay requiring special educational classes,"[282] and delays in "locomotor," "hearing and speech," "intellectual performance," and "personal and social" skills.[283]

Strong data have since confirmed that in ELBW neonates, the diagnosis of NEC is an independent risk factor for NDI.[284–286] Two systematic reviews, one of which included more than 4000 VLBW neonates born between 1977 and 2002, [287] not only redemonstrated the association of NEC with NDI, but moreover showed that surgical NEC survivors were at especially high risk of NDI.[287,288]

The risk for those treated surgically was twice the risk for those treated medically. Most infants with NEC who are successfully treated medically develop similar to age-matched premature infants without NEC, whereas those with more severe disease requiring operative intervention have a significantly increased risk of poor neurodevelopmental outcomes.

The underlying reason for higher rates of NDI among neonates with surgical NEC is unclear. As discussed previously in this chapter, requiring or undergoing surgery for NEC is considered a marker for severity of disease. The systemic illness that accompanies the intestinal disease, along with the corresponding hemodynamic instability and release of cytokines (such as TNF-α, IL-6, PAF), are associated with white matter injury.[113,289,290]

It is also unclear if the mode of surgical intervention affects the risk of NDI. The impact of anesthesia for laparotomy in this patient group is also unknown. Alternatively, PPD may result in a longer duration of exposure to inflammatory cellular mediators and thus more brain injury. A multicenter prospective cohort study published in 2006 (despite a lack of statistical significance in adjusted ORs) suggested that infants undergoing laparotomy may have a lower risk of NDI at 18 months than those who undergo PPD.[227] The forthcoming NEST trial results should improve our understanding of neurodevelopmental differences in survivors of these two operative strategies.[230]

It is important to note that the measures used to track neurodevelopmental outcomes, such as the Bayley Scales of Infant Development, in premature neonates may be imperfect tools in assessing this population.[291] Functional MRI, while expensive and time consuming, may be helpful. Parent-completed surveys could be a more practical alternative.[292] Further study into the best methods for tracking NDI will be essential to a better understanding of this outcome.[176]

Prevention

Our inability to uncover the best treatment for NEC and its persistently high mortality underscore the need for effective strategies for prevention. Although a wide range of measures have been studied, feeding neonates with human breast milk remains the most effective intervention in avoiding NEC in premature neonates. Probiotics have offered the most recent compelling data regarding prevention.[293–303]

PROBIOTICS

The use of probiotics has become routine in many parts of the world. The term *probiotics* refers to an enterally delivered supplement or medication containing live organisms aimed at improving health. They are generally given in attempt to change or control the composition of the intestinal microbiome. The most commonly delivered species include *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Escherichia*, *Enterococcus*, *Bacillus*, and *Saccharomyces.* Bifidobacteria and lactobacilli have been most studied in NEC. A large number of studies, including many randomized controlled trials, have demonstrated a protective effect of probiotics against both acquiring NEC and NEC-related mortality.[293] The ability of these microbes to reach and thrive in the intestine depends on their resilience to stomach acid and bile, and their interaction and competition with existing gut bacteria.

Recent data have shown that infants are exposed to bacteria even in utero as the amniotic fluid is not sterile.[294] The neonate's microbiome is further altered after exposure to maternal vaginal bacteria. Healthy, breast-fed infants have intestinal flora that are markedly different from those of preterm neonatal ICU patients who are exposed to antibiotics, often a prolonged absence of enteral feeding, the hospital environment, and gastric acid blockade. All of these factors significantly alter the microbiome by increasing the proportion of Gram-negative Proteobacteria and Gram-positive Firmicutes, both of which have been linked with sepsis and NEC.[295,296]

Thirty-five randomized placebo-controlled clinical trials in premature neonates with NEC, death, and/or sepsis as the primary outcome have been performed.[293] When combined, a total of 5559 patients received probiotics and 5513 received placebo or nontreatment. When Bell II NEC or greater was the outcome, the incidence was 3.3% in the probiotic groups and 6.1% in the control groups. For the studies reporting death, mortality was 5.1% in the probiotic arms and 7.2% in the control groups. Eleven cohort studies had similar findings with even larger differences.[293] Interestingly, probiotic organisms given to some neonates in the NICU show up in the stool of others who did not receive them.[297] Further, one large trial found that 49% of neonates in the control group were colonized with the study probiotic.[298] This phenomenon may significantly decrease differences seen between treatment and nontreatment groups, and could imply that the protective effects of probiotics are even greater than they appear in the quoted trials.[293]

The commonly studied probiotics have been shown to have broad effects on the microbiome itself as well as the inflammatory cascades occurring in the bowel wall. Both bifidobacteria and lactobacilli species inhibit growth of harmful bacteria by producing bacteriocins and secrete factors with anti-inflammatory effects.[293] In rats, *B. infantis* both decreased NEC and reduced expression of IL-6, IL-8, TNF-α, IL-23, and iNOS.[299] Factors released by *L. acidophilus* inhibit induction of NFκB and IL-8 by PAF.[300]

Some potential limitations in the use of probiotics include sepsis from the delivered bacteria, contamination of the product with other pathogens, and lack of standardization of "probiotic" products.[293] The actual risk of infection in neonates from lactobacilli and bifidobacteria is unknown. Sepsis from these in adults or neonates is rare, though it has been seen in immunocompromised populations.[301,302]

Currently available probiotic products used in some of the studies include FloraBaby, Infloran, Natren Life Start powder, and Biogaia ProTectis. A number of laboratories have focused on mechanisms to more reliably deliver these

bacterial species to the intestine. One such product, a formulation of *Lactobacillus reuteri* made by Infant Bacterial Therapeutics (Stockholm, Sweden), has recently completed enrollment in a phase 2 FDA trial.[303]

HUMAN MILK

Sufficient evidence is available for the protective effects of breast milk against a variety of poor outcomes, including NEC, that it has become the standard of care diet for premature neonates.[304–306] Human milk provides a variety of factors that support passive immunity (IgA) and help to mature the infant's adaptive immunity (described earlier in this chapter). Human milk prevents colonization by pathologic bacteria and microbial invasion by lowering the gastric pH, decreasing intestinal permeability, as well as providing beneficial intestinal flora (bifidobacteria and lactobacilli) and oligosaccharides.[307] It is also better tolerated than formula in premature neonates.[308]

It is important to distinguish the source of the human milk, however. The benefits seen with mother's own milk (MOM) are not clearly demonstrated when infants are given donor human milk (DHM). DHM is pasteurized in order to decrease pathogens. This also results in the destruction of many of the protective factors such as IgA, growth factors, protective bacteria, and lactoferrin.[309,310] Donors are often mothers of older infants and may have decreased levels of the various protective elements.[311] Pasteurization also destroys lipase, which leads to less stimulation of bile salts, and thus decreases fat absorption.[310,312] For this and other reasons, DHM is associated with decreased growth in neonates when compared with formula or MOM-fed infants.[308,312,313] While some studies suggest a protective effect of DHM over formula,[314] the first randomized controlled trial comparing DHM to formula saw no difference in the combined rate of sepsis/NEC.[308]

The protective effects of MOM appear to be dose dependent with a threshold of 50% of total calories providing optimal protection.[308,315,316] Additionally, the first 2–4 weeks of life may be a critical time period during which MOM is most helpful.[315,317]

FEEDING STRATEGIES

Timing of Initiation and Trophic Feeds

The optimal volume and postnatal age to start enteral feeds in premature neonates remains controversial. Despite strong historic clinician concerns, early feeding does not seem to increase the incidence of NEC,[318] and when MOM is used, it appears to be protective.[319] One cohort study compared VLBW neonates fed within 48 hours of birth to those started after 72 hours.[320] Both arms started at 1–2 mL/kg every 4–6 hours and advanced 1–2 mL/kg/day. The early feeding group had decreased duration of PN, decreased time to weight gain, and a shorter LOS.

In one study, infants started and maintained on a low volume of feeds without advancement (i.e., trophic feeding) were less likely to acquire bacterial sepsis than fasting infants.[321] However, a Cochrane review found insufficient evidence to support trophic feeding over fasting to prevent NEC.[322]

Feeding Advancement

After starting feeds, the rate at which to advance feeds is another concern with regard to NEC. For infants to quickly regain their birth weight and achieve full feeds, rapid advancement is advocated.[323] Concerns have been raised about the safety of this strategy with regard to increasing the incidence of NEC. In one randomized trial, the study was terminated early due to a higher incidence of NEC in the group that had their feeds rapidly advanced.[324] Results from this study were confounded by a questionable randomization model, an unusually high incidence of NEC, early termination of the study, and exclusion of 4 patients who died or developed intestinal perforation. A Cochrane review found five studies that evaluated slow versus fast advancement (15–20 mL/kg/day or 30–35 mL/kg/day, respectively) in VLBW infants and concluded that there was no significant difference in the risk of NEC or death.[325] Though the current literature suggests that the rate of advancement does not change the risk for NEC, large prospectively collected data are needed to definitively answer this question.

OTHER PREVENTATIVE STRATEGIES

Though small studies of amino acid supplementation were promising,[326] Cochrane reviews of both arginine and glutamine demonstrated no significant benefit.[327,328] Another Cochrane meta-analysis reviewed five studies of prophylactic enteral antibiotics. While there was a significant NEC risk reduction, concern regarding the safety of widespread use of antibiotics and the risk of bacterial resistance prevented the authors from advocating their use.[329]

Lactoferrin is a glycoprotein found in high concentrations in colostrum that has broad-spectrum antimicrobial activity via sequestration of iron and/or microbial cell membrane lysis.[72] Further, the metabolite lactoferricins produced on exposure to gastric acid also has antimicrobial properties.[330]A Cochrane review evaluated four randomized controlled trials for the use of lactoferrin supplementation in the prevention of NEC and late-onset sepsis.[331] It found a reduced risk of NEC (Bell II or greater) with a risk ratio (RR) of 0.30 and a reduction in all-cause mortality (RR 0.30). The effect of lactoferrin appeared to be greatest if started in the first three days of life.[332] The optimal duration of treatment is unknown as studied lengths included 28–45 days. Additional prospective studies are forthcoming.

A wide variety of experimental methods for reducing NEC risk are currently being assessed. Among these are the ErbB receptor ligands such as HB-EGF and NRG4 that are discussed in detail earlier this chapter. Innovative methods of packaging probiotics, such as using biofilms, may also allow for more effective delivery and better NEC prophylaxis.[333]

Conclusion

NEC is a frustrating disease that continues to plague NICUs, resulting in death in nearly a third of affected premature neonates. Despite a tremendous, growing, research effort that continues to elucidate the pathophysiologic mechanisms of the disease, outcomes have not significantly improved over the last four decades. Recent understanding of the microbiome

and the bacterial interaction with the immune system and microvascular homeostasis of the intestinal wall have fueled a paradigm shift in the central theory of NEC pathogenesis. Using proteomics and genomics, subtle changes in an infant's microbiome indicative of impending NEC may allow us to intervene earlier. Even before such advanced testing makes it to the bedside, we may be able to tabulate readily available clinical and laboratory data to identify infants who need operations before perforation occurs.

The operative strategy chosen by the surgeon does not appear to significantly affect mortality. We now recognize that the majority of survivors have some degree of neurodevelopmental impairment. New data regarding intermediate and long-term neurodevelopmental outcomes in surgical NEC are forthcoming and may guide the choice of operation. Given our shortcomings in treating this disease and its devastating effects, efforts should be focused on prevention. A diet of MOM is the most important prophylactic intervention, while DHM may be less helpful. Commensal bacteria delivered in the form of probiotic supplements may offer substantial protection against NEC if they can be properly formulated and delivered. With rapid expansion in the understanding of this area of the disease, we may be on the verge of a new era in which we can finally reduce the morbidity and mortality from NEC.

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