Infectious Causes of Necrotizing Enterocolitis

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KEYWORDS

- Necrotizing enterocolitis • Neonate • Bacteria • Virus • Fungi

KEY POINTS

- Necrotizing enterocolitis (NEC) is the most common cause of gastrointestinal morbidity and mortality in premature infants.
- The exact role of microbes in the pathogenesis of NEC is still incompletely understood.
- The presence of specific bacteria, viruses, and fungi has been associated with NEC predominantly in relatively rare outbreak situations.
- Aberrant bacterial colonization seems necessary for NEC development but is unlikely to cause disease by itself.
- Future studies are needed to determine how therapeutic interventions on microbial communities may prevent the development of NEC.

INTRODUCTION

Necrotizing enterocolitis (NEC) is the most common surgical emergency in premature infants, affecting approximately 7% of infants with less than 1500 g birth weights.[1] Universally described risk factors include prematurity, aberrant microbial colonization, and lack of human milk feeding.[2] NEC's clinical presentation is nonspecific and can range from signs limited to the gastrointestinal (GI) tract (eg, feeding intolerance, ileus, abdominal distention, hematochezia) to catastrophic illness with multiorgan failure (eg, lethargy, apnea, metabolic acidosis, shock, disseminated intravascular coagulopathy) and death.[3] Since its first mention in the medical

literature more than 150 years ago, NEC has stimulated intensive research in its cause; despite seminal discoveries of epidemiologic and molecular risk factors and pathways, the pathogenesis remains unclear.[4] One reason for the lack in progress is inclusion of diseases closely resembling classic NEC as a complication of preterm birth, such as spontaneous intestinal perforation (SIP), NEC in term infants, cow-milk intolerance, and viral enteritis.[5]

The role of bacteria as significant contributors to NEC has been identified since the first systematic descriptions of this disease.[6,7] Pneumatosis intestinalis and portal venous gas are pathognomonic radiographic signs of NEC[8] and thought to be caused by anaerobic bacteria, specifically clostridia.[9] Gram-negative bacteria have been most frequently associated with NEC, and the epithelial receptor and innate immune sensor Toll-like receptor (TLR) 4 is elevated in the premature intestine and required for the development of experimental NEC.[10,11] NEC can occur in clusters, and seasonal outbreaks of virus-associated NEC cases have been reported.[12–16] Here the authors attempted to summarize the main published data on the role of microbes in NEC.

BACTERIA

Bacteria are clearly involved in the pathogenesis of NEC (**Table 1**); despite the paucity of randomized control trials to determine the optimal antimicrobial regimen in premature infants, treatment with intravenous broad-spectrum antibiotics remains a mainstay of the clinical management.[17,18] However, many open questions remain, including the role of specific bacterial overgrowth as the cause or the consequence of NEC, timing of bacterial colonization during fetal/neonatal development, and type of molecular interactions between different microbes and their host.[19] Despite the abundance of bacteria in the premature intestine early in life[20] and the clinical appearance of gram-negative sepsis, a positive blood culture is uncommon in infants with NEC.[21,22] This finding is surprising given the frequent growth of bacteria in peritoneal fluid.[23] In 80 cases of NEC with intestinal perforation, *Enterobacteriaceae* were present in the peritoneal fluid in 75% of cases, coagulase-negative *Staphylococci*

Table 1
Infectious causes of NEC

Bacterial	Viral	Fungal
Clostridium spp	Astrovirus[15,184,185]	*Candida* spp[194,197–199]
Butyricum[83–89]	Cytomegalovirus[163–165]	
Difficile[76,79,80]	Coronavirus[167,168]	
Perfringens[61–68]	Coxsackievirus B2[176,177]	
Cronobacter (Enterobacter)	Echovirus[180]	
sakazakii[91,101–103,105]	Human immunodeficiency virus	
Enterococcus (VRE)[205]	(maternal exposure)[186–188]	
Escherichia coli[22,114,116–119]	Norovirus[16,147,149,150]	
Klebsiella spp[22,112–116]	Rotavirus[14,133–135]	
Pseudomonas aeruginosa[123–125]	Torovirus[170,171]	
Salmonella[206,207]		
Staphylococcus aureus (MRSA)[208]		
Staphylococcus epidermidis[48,212]		
Ureaplasma urealyticum[25,128,129]		

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci.

(CoNS) in 14%, and anaerobes in 6%.[23] Despite similar age at the time of intestinal perforation and similar mortality, the distribution of predominant organisms cultured from peritoneal fluid differed significantly between patients with NEC and SIP. *Candida* species (44%) and CoNS (50%) dominated samples from 36 patients with SIP.[23] Specific bacteria have been suggested as important contributing factors in NEC,[24,25] and NEC occurs typically after the first week post partum after the intestine has been colonized. In contrast, one study on human NEC samples using laser capture microdissection and subsequent sequencing combined with fluorescent in situ hybridization and bacterial rRNA-targeting oligonucleotide probes did not detect dominating potential pathogenic bacteria and suggested that NEC is a "non-infectious syndrome."[9]

Bacteria shape normal immune development including the development of T regulatory cells (Treg), which are critical for reducing inflammation-mediated injury.[26–29] Another example is recruitment of intestinal intraepithelial lymphocytes (IEL) after microbial colonization of germ-free mice.[30] IEL are reduced in human NEC suggesting that paucity of normal commensals in the newborn gut may alter intestinal immune development.[31] Infectious complications of pregnancy, such as chorioamnionitis, increase the risk for NEC either by direct bacterial colonization or through the anatomic and immunologic changes following the inflammatory challenge of the developing intestine.[25,32–36] Independent epidemiologic association between chorioamnionitis and NEC is difficult to prove, as chorioamnionitis is also the most important risk factor for prematurity and most severe NEC cases occur in extremely premature infants. However, after adjustment for antenatal steroid prophylaxis, gestational age, and surfactant treatment, the presence of intrauterine infection and the fetal inflammatory response syndrome (FIRS) remained independent predictors for NEC in several studies.[32,33] Increased gastric neutrophil counts have been demonstrated in chorioamnionitis-exposed preterm infants, reflecting a proinflammatory state of the gut shortly after birth.[37] Moreover, presence of microbes and inflammatory markers in the gut mirror that of the amniotic fluid when chorioamnionitis is present.[38] Preterm labor and chorioamnionitis are also linked with abnormal intestinal development and fetal proliferation of activated T cells in the immature intestinal mucosa.[35] At the same time, ileum Treg cell proportions are reduced in chorioamnionitis, whereas activated T effector cells are increased.[39,40] Reduced Treg proportions in the small intestinal lamina propria characterize NEC in human disease and in animal models, suggesting the possibility of bacteria-induced fetal immune priming as a risk factor for NEC.[41–43]

Gram-Positive Bacteria

The C-type lectin RegIIIγ and its human counterpart, hepatocarcinoma-intestine-pancreas/pancreatic-associated protein (HIP/PAP), are antimicrobial proteins that bind peptidoglycan, a molecule that is exposed on the surface of gram-positive bacteria. RegIIIγ expression is developmentally regulated and dependent on normal microbial ecology.[44] Although the exact role and developmental regulation of HIP/PAP is unknown in human infants, lower levels, especially in preterm infants, could lead to aberrant intestinal colonization with gram-positive bacteria.

Staphylococcus epidermidis

Colonization of the maternal genital tract with *Staphylococcus* sp has been associated with a significantly increased risk for chorioamnionitis (odds ratio 18.4).[33] The small intestine is colonized with staphylococci shortly after birth and in patients with or without NEC, specifically in infants delivered via cesarean section.[20,45] CoNS were found to

preferentially translocate through the intestinal wall after ischemia-reperfusion injury in mice.[46] Importantly, a lack of enteral nutrition and exposure to total parenteral nutrition alone reduce intestinal barrier function.[47] CoNS are frequently cultured from postnatal stool samples and seem to increase the risk for NEC development.[48]

Clostridia species

Clostridia are spore-forming anaerobic motile gram-positive rods. They can be found in soil and the human GI tract and can be considered part of the normal intestinal flora in newborns, especially premature infants exposed to the neonatal intensive care unit (NICU) environment and infants fed formula.[49,50] Therefore, when isolated during disease, it is difficult to establish if they are pathogens or normal flora.[51] However, patients with NEC with positive cultures for Clostridia spp have more extensive pneumatosis intestinalis, a higher incidence of portal venous gas, faster progression to more severe necrosis, and intestinal perforation with higher mortality.[52,53] Clostridium spp were significantly more prevalent among samples from a preterm piglet model of NEC.[54] Clostridia spp have been implicated in NEC for many years because the clinical presentation of diseases caused by these toxin-producing strains often resemble NEC. For example, pseudomembranous colitis as a result of overgrowth of toxin-producing Clostridium difficile in the colon can present with hematochezia and multiorgan failure.[55] Enteritis necroticans, known as pigbel in Papua New Guinea, is a segmental necrotizing infection of the jejunum and ileum caused by Clostridium perfringens, type C.[56,57]

Clostridium perfringens

Clostridium perfringens frequently colonizes the intestine of preterm infants within the first 2 weeks post partum.[58] Clostridium perfringens types A to E form 12 different toxins: major toxins (eg, α-toxin = phospholipase C), collagenase, protease, hyaluronidase, deoxyribonuclease, enterotoxin, and neuraminidase.[59] Clostridium perfringens α-toxin is produced by all 5 types of bacteria (A–E); increases capillary permeability; induces platelet aggregation, hemolysis, and myonecrosis; decreases cardiac contractility; and is lethal.[60] Clostridium perfringens was identified as a causative agent of NEC in 22% of cases in one study.[61] Compared with the control group (n = 32), the onset of disease was earlier in life, portal venous gas was more common (77%), the clinical course was more severe, and the mortality rate was more than twice as high (44%).[61] Another study isolated Clostridium perfringens in patients with fatal outcomes and suggested it has the potential to trigger a fulminant and often lethal course.[62] Clostridium perfringens has been declared as a possible risk factor for NEC as it was recognized by molecular techniques in the first 2 weeks post partum in 3 infants who later developed the disease.[63] In one study, Clostridium perfringens was isolated from intestinal flora in 40% of infants with NEC compared with 13% of controls ($P = .03$)[64] and has been associated with an NICU outbreak of NEC in another.[65] Clostridium perfringens has also been associated with NEC in several animal models.[66–68]

Clostridium difficile

Clostridium difficile is part of the commensal intestinal flora in humans but has recently attracted the attention of researchers because of its role as the most common cause of severe and refractory health care–associated diarrhea.[69] After intestinal overgrowth following antimicrobial use, toxigenic strains can cause pseudomembranous colitis, ranging from mild diarrhea to fulminant colitis. Clostridium difficile's 2 major toxins, Clostridium difficile toxin A (TcdA) and Clostridium difficile toxin B (TcdB), disrupt host cell function by inactivating small GTPases that regulate the actin cytoskeleton.[70]

Both toxins can manifest disease on their own.[71] During infancy, asymptomatic colonization with toxin-producing *Clostridium difficile* is common and has been associated with changes in the intestinal microbiome composition.[58,72–75] Delivery or exposure to human flora has no effect on colonization, and *Clostridium difficile* originates from the NICU environment rather than maternal transmission.[76,77] The involvement of *Clostridium difficile* in NEC is controversial because toxin-producing *Clostridium difficile* strains are not more frequently recovered in NEC.[78] However, *Clostridium difficile–associated* NEC cases have been described during a *Clostridium difficile* outbreak.[79,80]

Clostridium butyricum

Clostridium butyricum produces butyric acid through fermentation and a specific strain (MIYAIRI 588 strain of *Clostridium butyricum*) is widely used as a probiotic in Asia.[81] It can be isolated from soil, feces of healthy children and adults, as well as soured milk and cheeses. Type E can produce a neurotoxin and has been implicated in cases of botulism.[82] Several reports state isolation of toxin-producing *Clostridium butyricum* from peritoneal fluid, blood, and cerebrospinal fluid of patients with NEC.[83] *Clostridium butyricum* has been suggested as the primary cause of NEC in outbreak situations; but because of a lack of adequate controls, its primary role has been questioned.[84,85] Isolation of *Clostridium butyricum* in blood samples of infants with NEC may have resulted from mucosal breakdown and transmigration of these bacteria into the bloodstream.[86] In a community analysis of bacteria found in tissue specimens from infants with NEC, the presence of *Clostridium butyricum* and *Clostridium parputrificum* highly correlated with histologic pneumatosis intestinalis.[21] *Clostridium butyricum* strains isolated from NEC cases can cause cecal lesions in animals with gas cysts, hemorrhagic ulceration, and necrosis.[87–89] Lactose fermentation and production of butyric acid seem to be a prerequisite, and colonization with bifidobacterium was protective.[67,90] Attachment of *Clostridium butyricum* to the ileal mucosa has been associated with NEC in preterm, cesarean-derived, and formula-fed piglets.[54]

Gram-Negative Bacteria

Cronobacter sakazakii

With a reported incidence of one infection per 10,660 very low birth weight (VLBW, <1500 g) infants,[91] *Cronobacter sakazakii* (formerly *Enterobacter sakazakii*)[92,93] infection is rare. *Cronobacter sakazakii* has been isolated from powdered infant formula worldwide,[94,95] and NICU outbreaks of invasive disease have been reported.[96–100] Meningitis is the most prominent clinical manifestation,[101] but outbreaks of NEC occurred in NICUs with isolation of *Cronobacter sakazakii* from multiple patients' body fluids and cans with powdered infant formula.[102,103] *Cronobacter sakazakii* is commonly found in soil, food items, and other environmental sources.[104] Therefore, inappropriate hygiene practices including storage, temperature control, and hand, nipple, and bottle cleaning after powdered formula reconstitution may contribute to infection. Powdered formula is not a sterile product, and the World Health Organization recommends formula reconstitution with hot water (>70°C) (http://www.who.int/foodsafety/publications/micro/pif2007/en/).

Cronobacter sakazakii binds to villi in the distal small intestine and can induce NEC from a direct toxic effect to gut epithelium in the rat pup model.[105] *Cronobacter sakazakii*'s best-characterized virulence factor, outer membrane protein A (*ompA*), binds and invades human epithelial cells[103] and brain endothelial cells,[106–108] whereas its enterotoxin functions similarly to lipopolysaccharide (LPS) and modulates the activation of TLR 4.[109] *OmpA* also mediates recruitment of dendritic cells at the expense of

neutrophils and macrophages leading to epithelial injury through transforming growth factor-β production and iNOS activation.[110,111]

Klebsiella species

Klebsiella sp have been described in NEC outbreaks with nosocomial origin.[112,113] It is also one of the most common organisms responsible for bacteremia in NEC.[22,114,115] A 1998 outbreak in Johannesburg was significant for isolation of a single clone of an extended-spectrum beta-lactamase–producing *Klebsiella* in blood cultures of patients with NEC, notable for sudden decompensation leading to shock and severe thrombocytopenia in all cases and for the absence of diarrhea or hematochezia.[112]

Escherichia coli

E coli is a similarly common organism found in normal gut flora; among infants with NEC, it has been isolated in blood in up to one-third of cases.[22,114] Both *E coli* and *Klebsiella* were isolated in feces at markedly higher rates in infants with NEC than those without.[116] Several outbreaks of NEC associated with *E coli* have been described.[117,118] In one report, 15 of 16 infants with suspected or confirmed NEC had either enterotoxigenic *E coli* or its heat-labile enterotoxin recovered in stool.[118] A report of NEC associated with *E coli* O157:H7 in a term infant resulted in death secondary to widespread intestinal necrosis.[119]

Pseudomonas

Pseudomonas is well known for its role in nosocomial and immunocompromised infections. It forms biofilms and can colonize hard surfaces and respiratory equipment, with mechanical ventilation as a risk factor for infection. However, *Pseudomonas* also colonizes the GI tracts of 10% to 42% of newborns[120,121] and 25% to 35% of normal adults.[122] Among VLBW infants, it is primarily responsible for late-onset disease (sepsis, pneumonia, NEC). There are several reports of *Pseudomonas*-associated NEC. A Taiwanese study reports 45 infants with *Pseudomonas* in the stool, of whom one had NEC, 4 had colonic perforations, and 2 infants died of sepsis.[123] Other studies noted an increased rate of NEC in infants with *Pseudomonas* bacteremia compared with nonbacteremic infants (36% vs 7%, respectively) and with it a much higher mortality rate (up to 50%), especially when signs of septic shock were present.[124,125]

Atypical Bacteria

Unique in their lack of a cell wall, *Ureaplasma* are obligate intracellular mycoplasma that colonize human adult genital tracts. They may be vertically transmitted intrapartum, with nasopharyngeal colonization reported among 22% of NICU patients.[126] Colonization is associated with chorioamnionitis,[127] a known risk factor for NEC. However, the existence of a direct relationship between colonization with *Ureaplasma* and development of NEC is controversial. One study found a 2-fold increase in incidence of stage 2 or greater NEC associated with elevated interleukin (IL)-6 and IL-1 beta among infants colonized with *Ureaplasma* (12.3% vs 5.5%).[25] Two other groups disagreed and found no increased incidence of NEC associated with *Ureaplasma* colonization.[128,129]

VIRUSES
Rotavirus

A double-stranded DNA member of the Reovirus family, rotavirus causes GI disease by invading enterocytes and disrupting their absorptive and digestive activities.[130] Fecal excretion of rotavirus can be found in up to half of infants in the newborn

nursery.[131] Although most infants shed the virus asymptomatically, 8% to 30% of infants present with vomiting and diarrhea.[131,132] Rotavirus infection tends to peak in the late winter/early spring, though introduction of a vaccine in young children has interrupted this seasonality.[130] Several outbreaks of NEC have been associated with rotavirus infection with virus isolated from stool or serologic diagnosis and concomitant evidence of infection among a significant portion of NICU staff members.[133,134] Risk factors for the development of serious GI disease included low birth weight and younger age.[133] Notably, rotavirus-associated NEC has been found to cause less severe disease compared with NEC without rotavirus.[14] Anatomic distinctions were also noted: left-sided, more distal colonic pneumatosis intestinalis in rotavirus NEC compared with right-sided, ileal pneumatosis in nonrotavirus NEC.[14,135]

Norovirus

Norovirus (Norwalk virus), a nonenveloped positive-sense single-stranded RNA virus,[136] is the most important cause of foodborne outbreaks of gastroenteritis[137,138] and the second leading cause (after rotavirus) of gastroenteritis in young children.[139] Both individual infections and outbreaks most commonly occur during winter.[98] Seventeen percent of 75 premature infants less than 32 weeks' gestation shed the virus in their stool over the first 4 weeks after admission in a NICU in Sydney.[140] Norovirus prevalence was 1.9%, representing roughly half of all infants in that cohort who shed the virus.[140] Controversy exists regarding the best methods for viral identification, with one report noting several norovirus-positive cases by enzyme-linked immunosorbent assay that were not corroborated by reverse transcription polymerase chain reaction or electron microscopy.[141] The specificity of each aforementioned method is reportedly greater than 90%, and positivity in 2 of the 3 tests confirms norovirus infection.[142] Norovirus has been thought to primarily affect the small intestine based on pathologic findings of villus blunting, crypt hypertrophy, and edema among adults infected with norovirus[143–145] and mononuclear infiltrate and apoptosis in the jejunum and ileum of pediatric small bowel transplant recipients.[146] However, a recent report described 3 premature infants with norovirus infections with radiographic evidence of extensive colonic pneumatosis and pathologic insult (fibrosis and hyperplastic vessels) limited to the colon.[147] Apnea was noted as the primary presentation of norovirus infection in a preterm infant who subsequently developed watery diarrhea and positive stool cultures.[148] Several small outbreaks associated with NEC have been described. The largest involved 8 cases of NEC with a 25% mortality rate and noted that, in comparison with nonoutbreak NEC cases, those associated with norovirus had significantly lower levels of neutrophil band forms.[149] An outbreak of 8 cases of norovirus among premature infants was marked by abdominal distention, apnea, and increased gastric residuals. Vomiting and acute diarrhea were not predominant clinical features (27% and 0%, respectively), but one infant with proven norovirus developed NEC.[150] A case-control study noted an increased prevalence of norovirus in stools of infants with NEC compared with non-NEC controls (40% vs 9%, respectively) and suggested an etiologic role of norovirus in the pathogenesis of NEC.[16]

Cytomegalovirus

Cytomegalovirus (CMV), a double-stranded DNA herpesvirus, is well known to cause serious neonatal disease in its congenital form but has also been implicated in NEC. CMV transmission may occur via transplacental, intrapartum, or postpartum routes.

Rates of perinatal CMV infection in premature infants have been reported to be as high as 15% to 20%.[151,152] Given their immunocompromised status, premature infants are at particular risk for postnatal infection from breast milk (transmission rates 5%–37%)[153–155] or via transfused blood products.[156–159] Among immunocompromised patients, CMV enteritis is common and marked by diarrhea, hematochezia, and toxic megacolon.[160] In infants, however, CMV enteritis is unusual; the virus is a disputed player in the development of NEC. Patients may present with diarrhea or with disease resembling NEC but without distinguishing features, such as intestinal pneumatosis.[161] However, several case reports linking confirmed cases of NEC to CMV infections have been reported, with clinical manifestations including abdominal compartment syndrome,[162] viremia and sepsis,[163] and colonic strictures.[164] In one particularly severe case, fulminant NEC leading to death was associated with stool culture positive for CMV but with a notable reduction in diversity of bacterial flora, prompting speculation that intestinal CMV infection may predispose infants to NEC by altering intestinal immune responses and promoting secondary bacterial infection.[165]

Coronavirus (Torovirus)

Coronaviruses are enveloped viruses with positive-sense RNA genomes that are known to cause respiratory[166] and serious GI disease among infants.[167,168] A coronavirus outbreak was associated with hemorrhagic NEC, and viral particles were visualized both in intestinal and fecal specimens.[168] In another outbreak of NEC, coronavirus was detected in stool in 23 of 32 (72%) infants. Sixty percent of bedside nurses also shed the virus in stool, prompting speculation of nosocomial transmission.[167] As members of the coronavirus family, toroviruses are a known agent of diarrhea in cattle and horses and have been associated with GI disease in children. Torovirus infections are known to occur year-round, with a substantial portion thought to be acquired nosocomially.[169] Its association in neonatal disease was first described in 1982, when "virus-like particles" similar to coronavirus were detected in the stool of 80% of infants in an outbreak of bloody diarrhea, bilious gastric aspirates, and abdominal distention.[170] Transmission was thought to be vertical because all but one mother had flulike or GI symptoms within 2 weeks of delivery, and viral particles were detected in meconium of several infants.[170] One study reported the detection of torovirus in the stools of 48% of its patients with NEC and in 60% of those with stage III disease, although the presence or absence of torovirus did not affect mortality.[171]

Enteroviruses

Enteroviruses are positive-sense, single-stranded RNA viruses encompassing multiple serotypes, including 2 specific viruses that have been associated with NEC: coxsackievirus and echovirus. These infections are seasonal, with most cases occurring during summer and fall.[172–175] Among 27 infants with enterovirus, 3 had NEC marked by fevers, abdominal distention, and bloody diarrhea and one had coxsackievirus B and died following exploratory surgery revealing dusky jejunum.[176] Another fatal case of NEC associated with widespread coxsackievirus B infection demonstrated ischemic ileum with subserosal hemorrhage; the child's parents were both febrile at the time of birth.[177] The clinical presentation of echovirus infection among premature infants ranges from asymptomatic to diarrheal illness[178] to upper and lower respiratory tract infections.[179] An outbreak of echovirus type 22 in a NICU resulted in a diarrheal illness among 12 premature infants, 6 (50%) of whom developed stage I NEC and one had pneumatosis intestinalis, but all survived.

Identification of echovirus was via stool culture in most infants, though a few only had increased serum antibody titers.[180]

Astrovirus

As single-stranded RNA viruses, astroviruses were first described in infants during an outbreak of gastroenteritis in a newborn nursery.[181] Few reports on the pathology of human astrovirus infection are available, but one study in a child following bone marrow transplant revealed villous blunting and inflammatory cell infiltrate in the duodenum and jejunum (not consistent with graft-versus-host disease).[182] Alternatively, intestinal astrovirus infection in a turkey model leads to rearrangement of the actin cytoskeleton on ultrastructural examination and evidence of sodium malabsorption secondary to redistribution of sodium-hydrogen exchangers.[183] There are multiple reports associating astrovirus with NEC.[15,184,185] One report detected astrovirus in the stools of 6% of infants with either gastroenteritis or NEC. Infants with astrovirus more frequently acquired NEC (9 of 14) than those with norovirus (1 of 8) or rotavirus (2 of 12).[184] Compared with uninfected infants, those with astrovirus had increased hematochezia (54% vs 15%) and Bell stage II and III NEC (21% vs 4%).[185]

Human Immunodeficiency Virus

One case-control study suggests that maternal human immunodeficiency virus (HIV) infection places premature infants at higher risk for the development of NEC (at a rate of 8.8% vs 1.2% in children of HIV-positive and HIV-negative mothers, respectively).[186] Additional case reports describe development of NEC in 2 infants born to HIV-positive mothers; one infant also had trisomy 21.[187,188] All HIV-positive mothers received antiretroviral drugs during pregnancy and/or labor, and all infants received antiretrovirals after birth; no infant was HIV positive. The investigators speculate that the reduced production of IL-12 and/or use of zidovudine may have predisposed infants of HIV-positive mothers to NEC.[186]

FUNGI
Candida

Candida is a classically dimorphic organism that produces both yeast and hyphal forms, though certain species differ slightly (Candida glabrata does not form hyphae, and Candida parapsilosis forms pseudohyphae). In VLBW infants, fungal colonization occurs in the first week of life at an estimated rate of 27%, with Candida spp making up most of the organisms.[189] Candida albicans is isolated in more than 60% of cases of candidemia.[190] Among the NICU population, the risk factors for both colonization and invasive disease include the use of central venous lines, intravenous lipids, and histamine H_2 receptor antagonists.[191–193] It is unclear whether intestinal colonization with Candida spp is protective or a risk factor for NEC. In one study, none of 7 infants with NEC had viable fungal organisms detected in stool.[194] Further complicating the picture is the frequent association of Candida with SIP.[195,196] When Candida is linked to NEC, however, the results can be severe[197]; 27% of fatal cases of surgically treated NEC were associated with Candida sepsis, an outcome complicated by late diagnosis occurring either within 48 hours of death or at autopsy.[198] On pathologic examination of 84 patients with NEC, yeast and pseudohyphae were detected in both the intestinal lumen and wall.[199] Antifungal prophylaxis has been shown in a randomized controlled trial[200] to have benefit in reducing both colonization and invasive disease[201,202] but not NEC[201] or overall mortality.[203]

SUMMARY

NEC is a common and devastating problem for premature infants. Bacterial colonization seems to be a necessary but not sufficient contributor to NEC. Although intestinal pathogens may cause NEC-like illness in animal models or occasional clinical outbreaks, they are not detected in most cases of classic NEC.[204] NEC outbreaks have been associated with clusters of viral GI infections, but the clinical presentation may vary and often affect the large intestine. Future studies are needed to determine the impact of host-specific GI tract microbial communities on the development of NEC.

Best practices

What is the current practice?

NEC

The guidelines of the Surgical Infection Society and the Infectious Diseases Society of America recommend fluid resuscitation, bowel decompression, antimicrobial therapy, and surgical intervention (laparotomy or drainage) if needed.[18] Recommended antibiotics for complicated intra-abdominal infections in infants include combinations of ampicillin, gentamicin, and cefotaxime with or without anaerobic coverage with metronidazole, piperacillin-tazobactam, or meropenem.[17,18] In spite of their frequent use, the safety and efficacy of various antimicrobial combination treatment strategies for NEC has not been established in randomized controlled trials. The American Pediatric Surgical Association Outcomes and Clinical Trials Committee advises probiotics to decrease the incidence of NEC, and human milk should be used when possible.[209] They conclude that there is a lack of evidence-based data to support definitive recommendations for the type of surgical treatment or length of antimicrobial therapy.

What changes in current practice are likely to improve outcomes?

Prevention of NEC is the most effective strategy because once the disease becomes clinically evident, a mucosal and systemic inflammatory cascade has already been activated and multiorgan injury is likely. For the same reasons, earlier diagnosis of NEC before clinical onset is an important goal.[210]

Major recommendations

Medical management consists of bowel decompression, discontinuation of enteral feedings and medications, maintaining intravascular volume and electrolyte balance, and initiating broad-spectrum antibiotics based on known sensitivities of prevalent pathogens in the individual NICU. Typical regimens include ampicillin plus gentamicin to cover for common intestinal bacteria. Often the addition of a third antibiotic that provides more targeted anaerobic coverage (eg, clindamycin or metronidazole) is indicated when there is evidence of pneumatosis or bowel perforation. As an alternative, piperacillin-tazobactam may offer the advantage of broad-spectrum antimicrobial coverage including typical anaerobes of the intestinal flora. However, downsides are variable penetration into the cerebrospinal fluid and concerns for the emergence of drug resistance. Second-line therapy for severely ill infants can include meropenem and vancomycin in cases of positive cultures with resistant organisms, possible central nervous system infection, perforated bowel, and/or failed first-line or alternative therapy. Almost all of the drugs mentioned do not have a Food and Drug Administration label for use in this population because safety and efficacy data are lacking. Supportive management may require respiratory and blood pressure support and correcting anemia and thrombocytopenia and/or other coagulation defects. Serial abdominal radiographs are often recommended to monitor for intestinal pneumatosis, portal venous gas, and pneumoperitoneum. Early consultation with a pediatric surgeon is advised. Intestinal perforation or evidence of bowel necrosis is a common indication for operative management. As a preventive measure, a more consistent practice style including the implementation of early

breast milk feedings, standardized feeding regimens, and reduction of unnecessary antibiotics is recommended.

Clinical algorithms

The management of patients with NEC (medical and/or surgical) can be guided by Bell staging criteria as reported recently by Sharma and Hudak[211] (**Fig. 1**).

Rating for the strength of the evidence

C (Recommendation based on consensus, usual practice, expert opinion, disease-oriented evidence, and case series for studies of diagnosis, treatment, prevention, or screening)

Summary statement

NEC is a multifactorial disease, but bacteria and other microorganisms have been uniformly implicated somewhere along the pathogenic process. Because no specific microorganism can be considered causative in most cases of NEC, broad-spectrum antimicrobial therapy remains a mainstay in NEC treatment. More research is needed to determine the optimum therapy and to develop effective strategies for NEC prevention.

Data from Refs.[17,18,209–211]
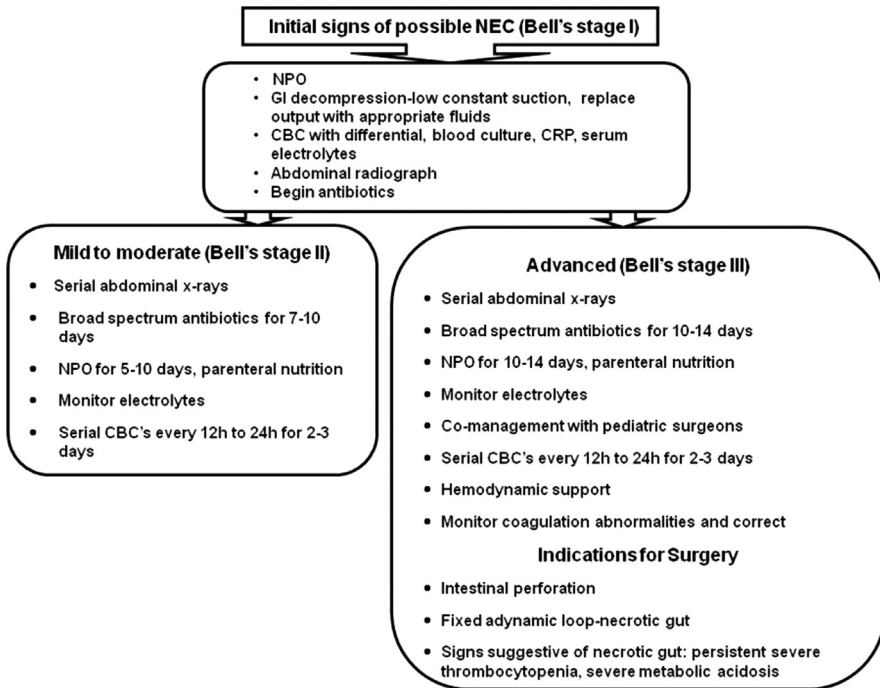
Fig. 1. Clinical decision algorithms. CBC, complete blood cell count; CRP, C-reactive protein; NPO, nil per os (nothing by mouth). (*Adapted from* Sharma R, Hudak ML. A clinical perspective of necrotizing enterocolitis: past, present, and future. Clin Perinatol 2013;40(1):27–51.)

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