What next in necrotizing enterocolitis?

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Necrotizing enterocolitis (NEC) is a devastating disease of premature infants, with a mortality rate of 10–50%. It is uncommon in term infants and in premature infants who have not yet been fed. Most commonly NEC develops suddenly in a preterm infant who was otherwise well, with initial symptoms of abdominal distention, bilious or bloody emesis or gastric aspirates, hematochezia, and pneumatosis intestinalis, and sometimes progresses quickly to include bowel perforation, acidosis, shock, and death. Trigger factors (i.e. perinatal hypoxia, mild infection or formula feeding) cause focal mild intestinal mucosal injury. In the presence of proliferation of commensal bacteria, local breakdown of mucosal barrier may cause entry of bacterial products (e.g. lipopolysaccharides, platelet-activating factor). Endothelial platelet-activating factor and/or tumor necrotizing factor and/or direct stimulating effects of polymorphonuclear leukocytes cause proinflammatory cascade and focal necrosis, which increase the entry of large amounts of bacterial toxins, and then severe NEC, sepsis, and shock develop. Therapies for the prevention of NEC that appear to have some benefit are breastfeeding and antenatal steroids, and probably probiotics. Enteral immunoglobulin, polyunsaturated fatty acids, and arginine or glutamine supplementation are therapies for the prevention of NEC that do not appear to be of benefit. Enteral erythropoietin and enteral granulocyte colony-stimulating factor are promising novel therapies. Treatment options are limited to gut rest, parenteral nutrition, broad-spectrum antibiotics, and surgical interventions for enteral perforation. Two commonly used methods for NEC with intestinal perforation are laparotomy or primary peritoneal drainage ("patch, drain and wait"); however, the preferred method is controversial.

***Key words**: preterm infants, necrotizing enterocolitis, pathogenesis, prophylaxis, management.*

Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in newborn infants. Despite improvement in other areas of neonatal care, there have been no significant advances in the prevention, incidence, or mortality from NEC over the last several decades. In fact, incidence of death from NEC has actually increased, since advances in pre- and postnatal care have resulted in a survival of a greater number of very-low birth weight (VLBW) infants[1].

Necrotizing enterocolitis remains a leading cause of mortality, ranging from 10-50% and nearly 100% in patients with the most severe of the disease (pan-necrosis). Long-term morbidity is also high, e.g. intestinal adhesions, strictures and short gut syndrome, and poorer neurodevelopmental outcome arising from a combination of factors including NEC-associated infection, inflammation, delayed nutrition, and commonly, complicating hypoperfusion.

Incidence is 0.9-2.4 infants per 1000 births, with 1-5% of admissions to neonatal intensive care units (NICUs). The disease involves mostly premature infants, and is uncommon in term infants (only 10% of the cases). Five to 15 percent of infants with a birth weight less than 1500 g at birth develop NEC. Incidence increases with decreasing gestational age, and risk for NEC remains high until the postconceptual age of 35-36 weeks[1].

Clinical Findings

A change in feeding tolerance with gastric retention is a frequent early sign. Vomiting, feeding intolerance, abdominal distension

and periumbilical and flank erythema on the abdominal wall, blood in the stools, lethargy, apnea, and temperature instability; and in severe cases, progressive systemic shock with metabolic acidosis, oliguria, hypotension and disseminated intravascular coagulation (DIC) may develop.

When NEC is suspected, serial abdominal X-ray films are recommended to check for the presence of pneumatosis intestinalis and pneumoperitoneum and for assessing disease progression. Occult blood in stool and sepsis are evaluated in suspected cases. The presence of abdominal distention, blood in stool, and pneumatosis intestinals confirm the clinical diagnosis of NEC. However, presence of occult blood is not specific for NEC. At least one positive occult blood is found in 58% of infants <1800 g over a six-week period[2].

Pneumatosis intestinalis (gas bubbles within the bowel walls) is thought to be produced by bacterial fermentation of substrates and diagnostic of NEC (present in 85% of cases). In severe cases, portal air can be seen and is associated with severe bowel necrosis in about 40% of the cases. However, radiological signs may vary with gestational age; pneumatosis intestinalis is present in 100% of full-term infants and in 29% of infants whose gestational ages are ≤26 weeks, while portal venous gas is present in 47% and 10%, respectively[3]. Pneumoperitoneum is present in severe cases. "Football sign" for free gas in the peritoneal cavity is a large hypolucent area in the central abdomen with markings from the falciparum ligament. Pneumatosis coli (pneumatosis in the colon without small intestinal involvement) is a benign form of NEC. Sonographic findings are also useful in predicting outcome and therefore might help guide management[4].

The severity of the disease was categorized in stages by Bell et al.[5] in 1978, later modified by Walsh and Kliegman[6] in 1986. Briefly, abdominal distention in stage I (mild), pneumatosis intestinalis in stage II (moderate), and pneumoperitoneum in stage III (severe) are the diagnostic parameters. In 25% of cases, NEC is suspected but not confirmed (stage I). The symptoms resolve gradually in these infants. In 25-40% of cases, the progression of NEC is fulminant with sepsis, DIC, and shock (stage III).

Predominant pathological lesion is coagulative or ischemic necrosis and most commonly involves the ileocecal region (insufficient blood supply?). In about half of the cases, the necrosis involves both the small and large intestines, either continuous or segmental. In severe cases, gas bubbles, which may be grossly visible in the intestinal wall, involve the entire colon more commonly in the term infant than in the premature.

Pathogenesis

Risk factors associated with NEC have been suggested as small prematurity (infectious, pathogenic bacteria/viral colonization of lumen, sepsis), oxygen delivery - consumption imbalance (perinatal hypoxia and ischemia, congenital heart disease, anemia, abnormal hemoglobins, polycythemia), and iatrogenic (umbilical arterial or venous catheterization; drugs - indomethacin, methylxanthines, H2 blockers; feeding regimens - advancing too fast, high osmolality; feeding additives - calcium, vitamin E; and formula feeding).

Pathogenesis of NEC is poorly understood. It may be a multifactorial disorder: prematurity, enteral feeding and uncontrolled inflammation in the bowel are three important factors for development of NEC[7].

Prematurity and ischemia-reperfusion injury

Highest incidence occurs during the first few days of postnatal life in term babies, at the end of the first week of life for neonates greater than 33 weeks gestational age, during the first two-and-a-half weeks in neonates 28-32 weeks and after more than four weeks in neonates below 28 weeks gestational age. In recent years, "late-onset" or "new" NEC is proposed to define late-onset NEC developed in VLBW infants[8].

Late-onset cases often affect "stable" growing neonates who are often breathing without assistance and are tolerating full enteral feeding volumes, and who at diagnosis are completely without any of the traditional risk factors other than a history of extreme prematurity. These cases probably arise from abnormal combination of developmentally immature digestive tract (reduced gastric acidity, reduced intestinal peristalsis, thinner goblet cell secretions, looser tight junctions between enterocytes,

lesser amounts of secreted antimicrobial factors such as lactoferrin, secretory IgA, defensin, intestinal trefoil factor and lysozyme, and fewer and less active Paneth cells)[9], pathogenic gut colonization, and dysregulation of the gut-associated lymphoid system, causing exaggerated and aberrant local and/or systemic immune response[10,11].

In older infants, any intestinal cellular destruction may lead to diarrhea, followed by epithelial regeneration from proliferating intestinal crypt cells. However, in high-risk or premature neonates whose regenerative capacity may be compromised, epithelial cell necrosis may not be counterbalanced by sufficient cellular regeneration, and as a result, systemic bacterial invasion or intestinal perforation may ensue.

Bowel ischemia is a suspected contributing factor in NEC. Perinatal asphyxia, presence of umbilical lines, polycythemia, hypotension, and congenital heart disease such as hypoplastic left heart and truncus arteriosus are risk factors for developing intestinal ischemia. However, epidemiologic studies have failed to confirm an association between NEC and most of these risk factors[12]. Whether the implicated role of ischemia is the cause or the end result of NEC remains unknown, but according to the results of an in vitro study with submucosal arterioles harvested from human intestine for NEC, it is unlikely that vascular events are the primary or initiating factors in NEC pathogenesis[13]. Ischemia-reperfusion injury following perinatal asphyxia and the prolonged state of low flow perfusion in growth-retarded fetuses are important risk factors to be proven.

Feeding and abnormal intestinal bacterial flora

Although the fetus ingests as much as 500 ml daily by term, NEC does not occur in utero. Ninety percent of cases occur after infants have been fed. Human milk reduces the incidence but does not prevent it entirely. Infants often develop symptoms following recent volume advancement or after reinitiating feeds. Osmolality in damaging the intestine have failed to support this. However, enteral feeding, especially formula feeding, may cause abnormal intestinal bacterial flora.

Healthy breast-milk fed neonates are colonized with normal bacterial flora with a predominance of the probiotic *Bifidobacteria* and *Lactobacilli*, whereas coliforms, enterococci and bacteroides predominate in formula-fed infants. Intestinal microbiota of the premature infant differs greatly from that of the term infant due to decreased contact with maternal flora and increased exposure to broad-spectrum antibiotics and nosocomial pathogens. Broad spectrum antibiotics and delayed initiation of enteral feeds contribute to abnormal colonization. Intestinal bacterial colonization of babies with NEC is abnormal[1].

Necrotizing enterocolitis usually does not occur before bacterial colonization of the intestine.

The endemic cases of NEC are not consistently associated with a single infectious agent. Gram-negative bacteria are the most common, followed by Gram-positive bacteria (*Escherichia coli*, Klebsiella, Enterobacter, Pseudomonas, Salmonella, *Clostridium perfringens*, *Clostridium difficile*, *Clostridium butyricum*, coagulase-negative staphylococci), but yeast and even viruses (coronavirus, rotavirus, and enteroviruses) have been implicated. Cases of NEC are usually sporadic, but the many reports of clusters suggest colonization with particularly virulent strains may be important. Only 20-30% of infants will have a positive blood culture, but bacteremia is seen more often with advanced disease.

In the anaerobic environment of the colon, bacteria rapidly ferment carbohydrates to gases (hydrogen, carbon dioxide, and in some cases, methane) and SCFAs (short chain fatty acids - mainly acetic acid, propionic acid and butyric acid). Pneumatosis intestinalis most likely results from these gases. Bacterial production of P-galactosidase, which reduces pH by fermentation of lactose, has been suggested to contribute to the development of intestinal pneumatosis. However, the ability of colonizing bacteria to ferment lactose is not correlated with the production of NEC. Intraluminal administration of lactic acid, the fermentation product of lactic acid-producing probiotics, does not induce intestinal mucosal injury.

In the premature infant who has a relative lactase deficiency, lactose ingested in the form of milk may be fermented into SCFAs and subsequently absorbed. SCFA overproduction may arise during periods of significant carbohydrate malabsorption and/or bacterial overgrowth. Overproduction or accumulation

of SCFAs, but not lactic acid, in the proximal colon and/or distal ileum may play a key role in the pathogenesis of NEC[14].

Uncontrolled inflammation

Patients with NEC often have elevated inflammatory mediators. Intestinal epithelial cells produce many of the cytokines that are implicated as mediators of intestinal inflammation and injury. Intraepithelial lymphocytes are also responsible for cytokine response. In the neonate, the functions of intraepithelial lymphocytes may be relatively depressed until "adequate" antigenic exposure has occurred (minimum of two weeks even in full-term babies). During that period, especially in hypoxia or other perinatal insults, dysregulated transfer of antigen (including bacteria) across the intestinal epithelium may occur, resulting in widespread activation of the mucosal immune system.

Activated T cells can cause injury in the gut by producing proinflammatory cytokines, by direct epithelial damage, by stimulating local release of inflammatory mediators that leads to further tissue injury (enterocyte apoptosis) and inhibition of tissue repair mechanisms (enterocyte proliferation and migration), and by recruiting additional blood-borne inflammatory cells, which in turn become activated in the inflammatory cytokine milieu. All these lead to uncontrolled inflammatory response with release of other mediators. The net effect is further tissue destruction, intestinal perforation, and sepsis. In small preterms, dysregulation of the gut- associated lymphoid system, causing exaggerated and aberrant local and/or systemic immune response, may cause NEC.

Cytokine gene polymorphisms are characterized by the overproduction of inflammatory mediators or diminished expression of antiinflammatory cytokines. Infants with NEC may have a "pro-inflammatory" genotype, and polymorphism in the cytokine gene may account for variation of disease. Although mutant variants of interleukin-4ra (IL-4ra) are less frequent in NEC, no differences were found in tumor necrosis factor (TNF), IL-1β, IL-6, and IL-10 and TAP (transferring antigen peptide) gene polymorphism between NEC and controls[15-17]. However, further studies with larger sample sizes are needed.

Platelet activating factor (PAF) is one of the mediators most intensely studied. PAF is an endogenous mediator of inflammation that is released during inflammatory states. It also seems to be the endogenous mediator for hypoxia-induced bowel injury. PAF is produced by inflammatory cells, endothelial cells, platelets and bacteria. The ileum is sensitive to PAF, since the greatest receptor expression is found in the ileum - the most common site of involvement in NEC. Conversion by PAF-acetylhydrolase renders it inactive; human neonates have low or absent circulating PAF-acetylhydrolase, and human milk contains significant quantities. Stool PAF levels in infants with NEC are 3-4 times higher[18,19].

It is probably the most potent agent to induce intestinal injury. Activation of the PAF receptor induces the production of additional molecules such as TNF-α, IL-6, and IL-8. PAF activates pathways triggering apoptosis in intestinal epithelial cells, increases gut mucosal permeability, and may facilitate the entry of bacterial products including lipopolysaccharides (LPS) from the gut lumen into the tissues, triggering the inflammatory cascade. PAF also causes capillary leak, myocardial dysfunction, renal dysfunction, neutropenia, thrombocytopenia, and hypotension[18,19].

Toll-like receptors (TLRs) on the cell surface act as sensors of microbial infection and play a role in the initiation of the inflammatory and immune defense response. LPS are a potent "priming" agent for PAF secretion, and PAF may be the endogenous mediator for LPS-induced intestinal injury, since LPS-induced intestinal injury is blocked by pretreatment with PAF antagonists. Activation of TLRs does result in cytokine activation and, potentially, a considerable inflammatory response. Abnormal TLR activation, perhaps via the influence of PAF, in the developing neonate increases the likelihood of developing NEC[19].

Tumor necrosis factor-α (TNF) has many proinflammatory actions, such as inducing leukocyte and endothelial adhesion molecules, activating polymorphonuclear leukocytes (PMNs) and endothelial cells, and causing production of other cytokines, including TNF itself, eicosanoids, and PAF. Both LPS and PAF stimulate TNF gene expression. LPS may induce TNF production via both PAF-dependent and –independent pathways. PAF and LPS (partly mediated via PAF and TNF) activate nuclear factor κB (NF-κB), a central

transcription factor in the regulation of many proinflammatory cytokines[20]. Proinflammatory cytokines cause PMN activation and tissue inflammation. Neutrophils adhere to the mesenteric endothelium, release further inflammatory mediators, and cause further intestinal inflammation and necrosis. The complement system, especially C5, may also participate in producing NEC.

The final step of intestinal injury is most likely *free oxygen radicals*. These radicals can be released by activated PMN, but the major source of free oxygen radicals in the intestine is probably the xanthine dehydrogenase/xanthine oxidase complex (XD/XO). XD is the precursor of XO. During ischemia/reperfusion, XD is converted to XO. XO generates superoxide, which, in the presence of iron, forms the potent tissue damaging hydroxyl radicals. Pretreatment with allopurinol, a XO inhibitor, largely prevents PAF-induced bowel necrosis. Infusion of superoxide dismutase plus catalase and antioxidant enzymes also alleviates the injury[21].

Nitric oxide (NO) increases intestinal blood flow. Inadequate NO leads to vasoconstriction of the intestinal vessels, which may lead to ischemia and a predisposition to NEC. It also inhibits leukocyte adherence, modulates the inflammatory responses in the intestine, and acts as a neurotransmitter for enteric non-adrenergic non-cholinergic neurons that regulate peristalsis (lack or inadequacy of NO can alter intestinal motility). It has been shown that NO donors reduce PAF-induced bowel injury[22]. NO also protects against hypoxia-induced intestinal injury[23].

Nitric oxide synthase (NOS) is essential in NO synthesis. Degree of intestinal injury is inversely related to the neuronal NOS (nNOS) activity, and PAF rapidly decreases intestinal nNOS. Tetrahydrobiopterin (BH_4), a nNOS cofactor essential for its action, protects rats from PAF-induced intestinal ischemia and necrosis[24].

Local release of inflammatory mediators such as interferon-γ and TNF by neighboring cells leads to sustained upregulation of inducible NOS (iNOS) and overproduction of NO, which reacts with superoxide to produce peroxynitrite radical ($ONOO^-$). NO or $ONOO^-$ leads to further tissue injury (enterocyte apoptosis) and inhibition of tissue repair mechanisms (enterocyte proliferation and migration)[25].

In summary, trigger factors (i.e. perinatal hypoxia, mild infection or formula feeding) cause focal mild intestinal mucosal injury. In the presence of proliferation of commensal bacteria, local breakdown of mucosal barrier may cause entry of bacterial products (e.g. LPS, PAF?). Endothelial PAF and/or TNF and/or direct stimulating effects of PMN cause proinflammatory cascade and focal necrosis, which increase the entry of large amounts of bacterial LPS, and then severe NEC, sepsis, and shock develop.

Prophylaxis

Therapies for the prevention of NEC that appear to have some benefit are breastfeeding, antenatal steroids, fluid restriction and enteral antibiotics. Although enteral antibiotics have some protective effect in prevention of progression of NEC, they should not be used because of the colonization of resistant bacteria[26].

Antenatal steroids

Antenatal steroids have some protective effects on fetal and neonatal intestine (e.g. increase cardiovascular stability, decrease the incidence of patent ductus arteriosus [PDA], have anti-inflammatory effects, promote intestinal maturation, increase the activity of PAF-acetylhydrolase that breaks PAF, and decrease the activity of PAF-acetyltransferase, the key enzyme in PAF biosynthesis).

Although it has been claimed in previous reports that prenatal steroids reduce NEC in approximately 70%, a recent meta-analysis showed only a "non-significant" trend of benefit[27].

Enteral feeding

Breast milk reduces the incidence of NEC 6-10 times compared to formula-feeding, although it does not prevent it entirely. There are no evidence-based feeding strategies for the prevention of NEC or for optimal nutrition during active and recovering NEC. Prospective randomized controlled trials are needed to evaluate safety and efficacy of age of initiation of feeding (early versus delayed) and rates of advancement (slow versus rapid) of feedings.

Most authors agree that 20 ml/kg/day is a safe advancement rate[28]. Many NICUs have a policy of attempting "minimal enteral nutrition" or "trophic feedings" to not increase the risk[29,30].

According to "*experience-based practice*", late-onset, slow enteral feeding protocol may be valuable in the prevention of NEC[31].

After the diagnosis of NEC, infants who are re-fed sooner (median 4 days) reach full enteral feedings sooner compared to infants who are re-fed 10 days after diagnosis. In cases with portal air, enteral feedings should be initiated in infants when portal gas is absent for three consecutive days on abdominal radiographs[32].

Probiotics

Probiotics are living organisms, anaerobic bacteria and yeast that promote maturation of intestinal functions, reduce growth and adherence of potentially pathogenic organisms, stimulate the immune system to develop a regulated immune response, and induce dendritic cells to enhance the production of anti-inflammatory cytokines and secretory IgA[33].

A meta-analysis of seven randomized placebo-controlled trials in VLBW infants to evaluate probiotics in the prevention of NEC showed that probiotics reduce the risk of NEC, shorten the time (mean −2.7 days) to full feeding, and reduce overall mortality without changing the mortality due to NEC and sepsis[34]. The inconsistency of all measured outcomes may raise concerns regarding the stage of NEC and different probiotics[35]. Although no side effects are reported, *Lactobacillus* GG sepsis[36] and fungemia due to *Saccharomyces boulardii*[37] in premature infants have been reported. Trials with heat-killed probiotics may solve the problem in the near future.

Therapies for the prevention of NEC that do not appear to be of benefit are enteral immunoglobulin[38] and polyunsaturated fatty acids[39]. There are also some reported novel therapies as summarized below.

Arginine supplementation

Local NO affects the intestinal blood flow and potentially predisposes to NEC. Adequate NO concentration may be achieved by supplementing substrates such as arginine for its precursor. The intestine is an important source of arginine, and enteral glutamine is catabolized by the small intestine and serves as a major precursor for intestinal synthesis of arginine. Arginine synthesis is low in preterm infants[40]. Lower plasma arginine levels were reported in NEC[41,42]. Although prophylactic arginine (15 mmol/kg per day) reduces NEC[43], multicenter trials are required before arginine supplementation[44]. However, NO may also play a role in the generation of peroxynitrites[45], and it has been reported that arginine supplementation increases mortality in sepsis in adults[46].

Nitric oxide production is regulated by the DDAH/ADMA/NOS pathway. Normally, ADMA (asymmetric dimethylarginine) is a NOS inhibitor and decreases NO production and is itself catabolized with DDAH (dimethylarginine dimethyl aminohydrolase). Sepsis causes increased ADMA levels in adults[47]. However, reduced ADMA levels and arginine: ADMA ratios in NEC may cause increase in NO[48]. Therefore, overall nutrition covering arginine and ADMA is important in the prevention of catabolism-induced production of ADMA[49].

Glutamine supplementation

Glutamine is the most abundant amino acid in the body and is a non-essential amino acid, but during times of stress (e.g. sepsis), the body may not be able to produce adequate quantities of glutamine to meet increased demands. Glutamine is approved by the Food and Drug Administration (FDA) as a protein supplement and is available in health food stores. It is mainly used by body builders for anabolic purposes. Glutamine is the principal metabolic fuel for the small intestine and major precursor for intestinal synthesis of arginine. It stimulates crypt cell proliferation (a mitogenic signal), increases the effects of growth factors (i.e. epidermal, insulin-like, transforming - EGF, IGF-1, TGF), and stimulates intestinal salt and water absorption. Glutamine also has some immunological functions: as nutrient for immune cells, in improving gut barrier function, as precursor of glutathione, which plays a role in reducing oxidative stress by scavenging free radicals, and as inhibitor of the inflammatory response by preventing action of NF-kB[50]. Glutamine deprivation induces apoptosis in intestinal cells[51]. Although low glutamine levels have been reported before NEC[52], neither enteral (max. 0.3 g/kg/day) nor parenteral glutamine supplementation makes a difference in the rate of systemic infection or of NEC in VLBW infants[53,54].

Growth factors

Many growth factors, including EGF, IGF-1, TGF-α, erythropoietin, and granulocyte colony-stimulating factor (G-CSF), are present in relatively high concentrations in the liquids swallowed by the fetus and neonate, namely, amniotic fluid, colostrum, and human milk, and are relatively protected from digestion. Enterally administered growth factors to neonates are not absorbed. The receptors for growth factors are also expressed on enterocytes of the fetus and neonate, and induce growth and development of the gastrointestinal tract[55].

The majority of *epidermal growth factor* (EGF) is produced in the submaxillary salivary glands, and lesser amounts in Brunner glands of the duodenum and in the exocrine pancreas, and it plays an important role in the function of intestinal epithelial barrier function (i.e. matures the intestinal mucosal barrier) by enhancing the migration and proliferation of enterocytes in response to mucosal injury; it also decreases intestinal apoptosis and down-regulates the proinflammatory response[56]. EGF, which is the major trophic factor for the developing intestine, is found in many endogenous fluids bathing the developing intestine (amniotic fluid, fetal urine, breast milk, bile, saliva). In amniotic fluid, the EGF levels increase as gestation progresses[55]. Urinary EGF levels increase as gestation progresses[57]. In rabbits and Rhesus monkeys, in utero EGF infusion accelerates the maturation of intestinal enzymes and stimulates intestinal growth[58]. EGF-receptor knockout mice die in utero or early in the neonatal period with a hemorrhagic enteritis that is similar to human NEC[59]. Single nucleotide polymorphisms in the human EGF gene may account for variation of disease[60].

Human milk feeding is the only currently accepted modality for NEC prevention. This finding may be related to the presence of EGF in human milk[61]. Saliva also contains EGF, which increases with gestational age as well as with postnatal days, and small for gestational age (SGA) infants and formula-fed infants have lower salivary EGF levels. Infants who developed NEC have lower salivary and serum EGF levels in the first week, with a greater increase in subsequent weeks; a two to three times increase in sEGF levels may be due to intestinal injury[62,63]. Supplementation of formula with EGF reduces the incidence and severity of NEC in rats and mice[64,65]. Therefore, it may have a therapeutic value in newborn infants with NEC[60].

In VLBW infants, *erythropoietin* (in a daily dose of 200 U/kg intravenously as a continuous infusion in the hyperalimentation solution or as 400 U/kg subcutaneously, 3 days/week) reduces the incidence of NEC (4.6% vs 10.8%, p=0.028)[66]. However, a FDA warning (May 10, 2007) cautioned that erythropoietin used to treat anemia caused by chemotherapy has the potential for tumor promotion and thromboembolic events[67].

Recombinant human granulocyte colony-stimulating factor (rhG-CSF) increases the absolute neutrophil count and neutrophil functions. In VLBW septic-neutropenic and even in preeclampsia-associated neonatal neutropenia, rhG-CSF causes a significant increase in neutrophil cell number, although the function of those cells remains sub-optimal. Intravenous rhG-CSF given at the time of diagnosis decreases NEC mortality[68,69].

Enterally administered rhG-CSF likely has local actions, and may reduce the severity of intestinal damage or may lead to an acceleration in the reparation of intestinal tissue or may control local inflammation[70]. In a preliminary study, it has been shown that enteral rhG-CSF in stage I NEC limits progression to more severe stages, which is also supported by an animal study[71,72].

Pneumatosis typically occurs 12-48 hours after presenting signs of NEC and 1-4 days prior to perforation. Therefore, the presence of pneumatosis intestinalis may be an objective criterion for the initiation of rhG-CSF therapy. Although responses to rhG-CSF begin as early as a few hours, a peak response is seen in neonates at 10-14 days following the initiation of a three-day course of treatment. Therefore, the patient must be able to survive long enough using conventional support until any putative rhG-CSF effects have sufficient time to occur[73].

Drugs

Some drugs are used with caution for the prevention of NEC. Hyperosmolar formulas and drugs (e.g. multivitamins, phenobarbital, theophylline) may cause predisposition to NEC. Use of histamine type 2 receptor antagonists (e.g. cimetidine, ranitidine, famotidine), which eliminate the gastric barrier by reducing the gastric pH in premature infants, increases

the NEC risk by 1.7 times[74]. Vitamin E may increase risk for NEC because of a reduction of antimicrobial defenses by excessive scavenging of oxygen free radicals[75].

Patent ductus arteriosus is an independent risk factor for the development of NEC in VLBW infants. Therapy with indomethacin has no significant effect on the risk for NEC[76]. However, if it is given with steroids, NEC risk is 9.6 times higher[77]. In addition, prolonged use of indomethacin is associated with an increased risk of NEC by 1.9 times[78]. Enteral feedings do not need to be interrupted when on a course of indomethacin[79].

Management

Management is determined by the specific stage of the infant's disease. In stage I NEC, intravenous antibiotics (ampicillin + amikacin for sepsis, and metronidazole to reduce abnormally colonized bacteria for 10-14 days, although blood culture is positive in one-third of the patients), no enteral feeding and nasogastric decompression (2-3 days in suspected cases) are sufficient, and serial abdominal radiographs are performed to evaluate those that demonstrate radiographic progression. Management in stage II includes no enteral feeding and prolonged nasogastric decompression (7-14 days). Surgical intervention is generally recommended in stage III.

Portal venous gas had been thought to be a predictor of poor outcome and an indication for surgical intervention. However, there is no difference in survival rates between those with portal venous gas and those without (17% vs. 20%). Of the infants with portal venous gas, those who are treated medically have a higher survival rate than those treated surgically (91% vs. 74%). Therefore, portal venous gas and extensive pneumatosis are not accepted as a surgical indications[80].

Multiple retrospective analyses have been unable to answer the question as to why some babies with NEC recover uneventfully, while others develop fulminant disease. Patients with NEC who will not respond to medical therapy are unpredictable. Nevertheless, intestinal perforation is an absolute indication for operation. Intestinal perforation (which is often multiple), occurs in about 20% of those babies who develop NEC, and mortality rate is 30-50% in babies with intestinal perforation due to NEC. Unfortunately, abdominal radiographs are specific but not very sensitive in the diagnosis of perforation.

Localized intestinal perforation (LIP) without NEC also occurs in premature babies. It is only about a third as common as in those affected by NEC. Affected neonates appear to remain clinically relatively well despite LIP until sudden onset of abdominal distension, which coincides with the onset of perforation. LIP is usually in the terminal ileum and unlike in NEC the remaining intestine appears normal. NEC and LIP are different ends of a spectrum of the same intestinal pathological disorder; LIP is a more benign condition that responds well to treatment and carries a good prognosis. LIP is the isolated nature of the perforation. Other aspects of these diseases (pathogenesis, pathology, clinical presentation, morbidity, mortality) are less obviously different[81].

Two commonly used methods for NEC with intestinal perforation are laparotomy or primary peritoneal drainage ("patch, drain and wait"). The preferred method is controversial. Laparotomy with surgical resection and enterostomy formation has traditionally been considered the safest method. Resection of gangrenous bowel reduces bacterial translocation. Formation of enterostomies allows for resolution of peritonitis and further disease before reestablishing continuity of the intestine[82].

According to the findings of a multicenter randomized control trial, the type of intervention (primary peritoneal drainage vs. laparotomy) for perforated NEC does not influence survival, dependence on parenteral nutrition, or length of hospital stay in preterm infants. However, long-term neurodevelopmental impairment is not known in infants treated with primary peritoneal drainage[83]. The critical questions regarding surgical care remain unanswered: what is the optimal time for operative intervention; what is the optimal strategy for intervention; and what are the specific techniques that are appropriate during that intervention?

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