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Immunonutrition for Preterm Infants

Verena Walsh William McGuire

Centre for Reviews and Dissemination, University of York, York, UK

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Abstract
Care and outcomes for very preterm infants continue to improve, but important causes of mortality and acute and long-term morbidity associated with prolonged hospitalisation remain. Necrotising enterocolitis (NEC) and late-onset infection have emerged as the major causes of death beyond the early neonatal period and of neurodisability in very preterm infants. Although the pathogenesis of these conditions is incompletely understood, it appears to be related to the content and mode of delivery of the enteral diet, particularly the impact of immunonutrients from human breast milk on the microbial and metabolic balance within the immature intestine. Evidence exists to support investment in measures to help mothers to express breast milk as the primary source of nutrition for their very preterm infants. In the absence of maternal milk, pasteurised donor breast milk provides protection against NEC, but its nutritive adequacy is not clear and its cost-effectiveness is uncertain. Supplementation with individual immunonutrients, including immunoglobulins and lactoferrin, has not been shown to be effective in preventing NEC or infection in randomised controlled trials. The evidence base for prebiotics and probiotics is stronger, but concerns exist about the choice, safety and availability of formulations. Other strategies – including avoidance of drugs such as gastric acid suppressants that compromise innate immunity, as well as evidence-based progressive feeding strategies that reduce exposure to invasive interventions – are emerging as key components of care packages to reduce the burden of NEC, infection and associated growth and developmental faltering for very preterm infants.

Background

Over the past 50 years, major advances in key areas of care have dramatically improved outcomes for very preterm infants. While mortality and morbidity associated with respiratory distress syndrome have been reduced substantially by widespread use of antenatal corticosteroids and exogenous surfactant, other major morbidities associated with the need for prolonged invasive or intensive care remain. These include necrotising enterocolitis (NEC), late-onset (hospital-acquired) infection, and associated slow growth and developmental faltering secondary to nutritional deficiency [1]. Late-onset infection affects about 20% and NEC occurs in about 5% of all very preterm infants [2, 3]. The attributable mortality can be in excess of 20%, especially for severe NEC and gram-negative bacterial, enterococcal or fungal infections. These are now the commonest causes of acute morbidity and death beyond the early neonatal period for very preterm infants [1]. Infants who develop severe NEC or infection have longer durations of hospital stay than age-equivalent infants without these conditions, and they are more likely to develop bronchopulmonary dysplasia or be

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E-Mail karger@karger.com
www.karger.com/neo

Prof. William McGuire
Centre for Reviews and Dissemination
University of York
York YO10 5DD (UK)
E-Mail william.mcguire@york.ac.uk

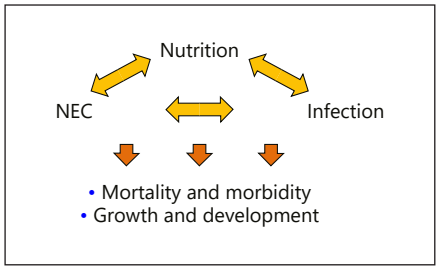

Fig. 1. Necrotising enterocolitis (NEC), infection and associated nutritional compromise, and growth and developmental faltering are associated with mortality and long-term morbidity.

diagnosed with neurological impairment or disability with consequent higher (life-long) health service needs and costs [4, 5] (Fig. 1).

Risk Factors and Pathogenesis

Early-stage translational research to determine the underlying aetiology and pathogenic pathways of NEC and invasive infection in very preterm infants is the key to developing strategies to better prevent and treat these conditions [6]. Frustratingly, the pathogenesis of NEC remains incompletely defined. The most commonly accepted "unifying model" invokes contributions from intestinal vascular insufficiency, enteric immaturity, and intraluminal and mucosal intestinal dysbiosis, infection and inflammation [7, 8]. Risk factors for invasive infection are similarly related to organ immaturity, including skin and gut immaturity [2]. While exposure to intensive care practices and invasive procedures, principally intravascular access devices to deliver parenteral nutrition or drugs, is a major risk factor for coagulase-negative staphylococcal infection, emerging evidence suggests that more serious infections with gram-negative bacilli, enterococci and fungi are associated with intestinal dysbiosis [9].

Immunonutrition

Given these putative mechanisms of disease, interventions or care practices that enhance innate immunity, particularly enteric immunity, or modify risk factors that impair intestinal integrity or immunity ("immunonutrition") have the potential to reduce the risk of infection and NEC in very preterm infants. These include harnessing the immunological benefits of maternal breast milk or its beneficial components; minimising exposure to invasive procedures such as intravascular access devices by optimising early enteral feeding strategies; exploring the role of particular micronutrients such as conditionally essential amino acids; and avoiding interventions that compromise innate immunity, such as use of gastric acid suppressants [10].

Table 1. Immuno-active components of breast milk [11]

– Immunoglobulins
– Immuno-regulatory cytokines and growth factors
– Medium- and long-chain polyunsaturated free fatty acids
– Milk oligosaccharides – prebiotics
– Probiotic bacteria
– Lactoferrin
– Lysozyme

Human Milk: The Ultimate Immunonutrient

Expressed maternal breast milk is the recommended source of nutrition for very preterm infants primarily because of its immunoprotective properties. As well as supplying macro- and micronutrients optimised by evolution for newborn infants, human milk, and especially human colostrum, is rich in a range of bioactive components and cells (Table 1). These work in synergy to optimise the intestinal microbiological and metabolic milieu and to protect infants from infection and inflammation [11].

There are no randomised controlled trials of expressed maternal breast milk versus formula for very preterm infants because of lack of equipoise and associated ethical concerns. Large observational studies, however, have consistently demonstrated strong, dose-dependent associations with the level of maternal breast milk intake and the risk of infection and NEC [12, 13]. Further evidence for the immunoprotective benefits of human milk comes from meta-analyses of randomised controlled trials that assessed the effect of donor human breast milk versus formula for feeding preterm infants [14]. Despite the potential for donor milk processing, including freeze-thaw cycles and pasteurisation, to reduce levels of its bioactive components, recent high-quality trial data have confirmed that feeding very preterm infants with donor breast milk versus formula reduces the risk of NEC [15]. Some concerns remain about the nutritional sufficiency of donor breast milk, but this can be compensated by addition of multi-nutrient fortifiers extracted from cow's

Table 2. Evidence-based practices to ensure mothers are optimally supported to express their own breast milk [19]

– Kangaroo care and skin-to-skin contact
– Simultaneous expression from both breasts (using an electric pump)
– Peer support in the hospital and community
– Multi-disciplinary, skilled, professional support
– UNICEF "Baby Friendly" accreditation

milk [16]. Currently, investigators are exploring whether using multi-nutrient fortifiers extracted from human milk rather than cow's milk might be associated with reductions in the risk of infection and NEC [17].

Further trials are ongoing to determine the effectiveness of this strategy and to assess its cost-effectiveness, particularly in the context of overall high levels of feeding with expressed maternal human milk [18]. The key uncertainty is whether the opportunity and capital costs of maintaining a service to provide donor breast milk would be better invested in, for example, peer or professional support for mothers who wish to express breast milk for their babies – particularly vulnerable and socially disadvantaged women, who are less likely to provide expressed breast milk [19] (Table 2).

Oropharyngeal Colostrum

Colostrum, produced in the first few days after birth, contains a higher concentration of secretory immunoglobulin A, lysozyme, lactoferrin, cytokines and growth factors than mature breast milk [20]. This immunonutrient-rich mixture is evolutionarily adapted to provide early postnatal protection from infection, stimulate development of the gastrointestinal tract and modulate development of the infant immune system [21]. Although it is feasible to deliver maternal colostrum to very preterm infants via an intragastric tube, mothers may be able to provide only very small volumes during the first few days after birth. Another option is instilling these small amounts (typically <0.5 mL) into the infant's oropharyngeal cavity [22]. The rationale for this approach is that the oral epithelium contains receptors for the immunological and trophic factors in colostrum and that oropharynx-associated lymphoid tissue is a key interface between the infant's immune system and the enteric microbial flora [10]. Trial evidence of benefits is currently limited, but large, high-quality randomised controlled trials to evaluate more precisely and reliably the effects of oropharyngeal colostrum on outcomes for preterm infants are planned or in progress [22, 23].

Human Milk Components

The possibility that the immunonutritive benefits of human breast milk in preterm infants are mediated via factors such as secretory immunoglobulin A and lactoferrin has generated interest and research efforts to assess whether enteral supplementation with these individual components might protect very preterm infants against NEC and late-onset infection [10].

Immunoglobulins

Immunoglobulins are transferred to the fetus through the placenta from the second trimester. Because most transfer occurs in the last few weeks of a term pregnancy, preterm infants have lower levels of immunoglobulins than term infants, making them more susceptible to invasive infection. Despite the plausibility that enteral supplementation of immunoglobulins may enhance preterm infants' intestinal immunity and reduce the risk of infection and NEC, large randomised controlled trials have not found any evidence of beneficial effects [23]. Most trials, however, have used immunoglobulin G, whereas enteral immunoglobulin A prophylaxis (which is more expensive) is likely to be a more biologically appropriate intervention [24].

Lactoferrin

Lactoferrin, an antimicrobial glycoprotein present in colostrum and breast milk, is a key component of the mammalian innate response to infection [25]. Lactoferrin has broad microbicidal activity against gram-positive cocci, gram-negative bacilli and fungi. Very preterm infants have low lactoferrin levels, and this deficiency is exacerbated by delay in establishing enteral feeding. The current Cochrane review of enteral lactoferrin supplementation for preterm infants (6 randomised controlled trials with 1,071 participants in total) suggests that lactoferrin supplementation reduces the incidence of late-onset invasive infection by about 40% and the risk of NEC by about 60% [26]. However, the trials included were small and contained various methodological weaknesses, and a recently completed, large, high-quality randomised controlled trial has contradicted this evidence [27] (Box 1).

Box 1. ELFIN – a pragmatic placebo-controlled randomised controlled trial [27]

Participants	2,203 very preterm infants (<72 h old at randomisation)
Setting	134 UK neonatal units
Intervention	Enteral bovine lactoferrin (150 mg/kg/day until 34 weeks' postmenstrual age)
Comparison	Sucrose placebo
Outcomes	Microbiologically confirmed or clinically suspected late-onset infection, NEC, mortality, ROP, and BPD
Results	No significant differences: – Late-onset infection: RR 0.95 (95% CI 0.86–1.04) – NEC: RR 1.13 (99% CI 0.68–1.89) – Mortality: RR 1.05 (99% CI 0.66–1.68) – ROP: RR 0.89 (99% CI 0.62–1.28) – BPD: RR 1.01 (99% CI 0.90–1.13) – Composite of infection, mortality, NEC, ROP, BPD: RR 1.01 (99% CI 0.94–1.08)
Conclusion	Enteral supplementation with bovine lactoferrin does not reduce the risk of late-onset infection, other morbidity or mortality in very preterm infants

NEC, necrotising enterocolitis; ROP, retinopathy of prematurity; BPD, bronchopulmonary dysplasia; RR, risk ratio; CI, confidence interval.

Prebiotics and Probiotics

Breast milk contains numerous "prebiotic" substances such as human milk oligosaccharides and lactoferrin that promote the growth of non-pathogenic "probiotic" microorganisms, predominantly lactobacilli and bifidobacteria, at the expense of potentially pathogenic bacteria [28]. Probiotic microorganisms predominate in the intestinal microbiome of breastfed term infants. Very preterm infants, however, tend to harbour fewer probiotic microorganisms and more potential pathogens, which might be due to dysbiotic effects of enteral fasting and antibiotic exposure [10]. Furthermore, the intestinal microbiome in preterm infants who develop NEC or late-onset infection is less diverse and contains fewer bifidobacteria than that in healthy preterm infants. Pathogens such as enterococci and Enterobacteriaceae causing late-onset bloodstream infection are in a dominant or near-dominant proportion within the intestinal microbiome at diagnosis [29]. Although the emerging evidence suggests that NEC is also strongly related to the intestinal microbiomic structure, there is insufficient evidence at present to determine whether any specific bacteria or fungi are causally associated with development of NEC [30].

Probiotic Supplementation

Given the plausibility that intestinal dysbiosis increases the risk of NEC and late-onset infection in very preterm infants, numerous randomised controlled trials have assessed the effects of enteral supplementation with probiotic organisms for very preterm infants. Meta-analyses of these trials which have assessed a range of different species and multi-organism combinations suggest that prophylactic probiotics halve the risk of NEC and reduce all-cause mortality by one-third [31, 32]. Most trials and meta-analyses of their data do not show statistically significant effects of probiotics on the risk of late-onset infection. Based on this accumulated evidence, some commentators have advised that probiotic supplementation could be considered as a standard care practice for very preterm infants at risk of developing NEC [33]. Although this policy has been adopted in some centres, persistent concerns about methodological weaknesses in the primary trials, the optimal choice of probiotic organisms and doses, and licensed product availability, quality and safety have limited its widespread use internationally [34].

Prebiotic Supplementation

Administering enteral prebiotics to support the growth of intestinal probiotic microorganisms may be a potentially simpler and safer alternative to direct probiotic supplementation for very preterm infants [28]. Prebiotics in breast milk are resistant to gastric acid digestion, and feeding very preterm infants with formula supplemented with prebiotics, typically a mixture of galacto- and fructo-oligosaccharides, stimulates the growth of an intestinal microflora that is similar to that found in infants fed with maternal breast milk [35]. Randomised controlled trials have indicated that prebiotic supplements are safe and well tolerated by preterm infants. Evidence of their effectiveness in very preterm infants, however, is limited by concerns about the quality of the existing trial data [36]. There remains a need to conduct large, high-quality randomised controlled trials to assess the effect of prebiotic supplementation or of supplementation with prebiotic-probiotic combinations ("synbiotics") on NEC, late-onset infection and other morbidity, and mortality amongst very preterm infants [37].

Conditionally Essential Amino Acids

Glutamine Supplementation

Glutamine, a "conditionally essential" amino acid, is the principal respiratory fuel for rapidly dividing cells such as enterocytes. It is abundant in breast milk but present at much lower levels in formula. Low plasma levels of glutamine are associated with a higher risk of NEC in preterm infants [38]. In animal models of NEC, glutamine supplementation reduces mucosal damage and lowers the risk of invasive infection and death. However, despite the biological plausibility that glutamine supplementation might reduce the risk of adverse outcomes including NEC for very preterm infants, high-quality randomised controlled trials, in which almost 3,000 infants in total have participated, have not shown any evidence of benefit [39]. Research efforts have shifted to assessing the effect of glutamine supplementation as an adjuvant therapy for infants recovering from established NEC.

Arginine Supplementation

Arginine is involved in the generation of nitric oxide, a key mediator of intestinal vasomotor tone. Observational studies have shown that plasma arginine levels are lower in preterm infants who develop NEC than in control infants [38]. Enteral arginine supplementation may enhance endothelial nitric oxide generation and thereby improve intestinal perfusion. Three small randomised controlled trials have tested this hypothesis, but although their meta-analysis indicates some protective effects (a statistically significant reduction in the risk of NEC development), the current evidence is insufficient to support a practice recommendation. A large, high-quality randomised controlled study to assess whether arginine supplementation affects the risk of NEC or late-onset infection is needed to resolve this uncertainty [40].

Early Enteral Feeding Strategies

As well as seeking to develop interventions that promote intestinal integrity and immunity, research into the epidemiology and aetiology of NEC and late-onset infection has examined effects of different early enteral feeding strategies for very preterm infants [41]. Historically, clinicians have favoured a conservative approach to the introduction and advancement of enteral feed volumes for very preterm infants because of concerns that early introduction or rapid advancement might increase the risk of NEC. However, because enteral milk feeds stimulate gastrointestinal hormone secretion and motility, delayed introduction or slow advancement of enteral feeds might diminish the functional adaptation of the preterm gastrointestinal tract [42]. Prolonged use of parenteral nutrition might increase the risk of infectious and metabolic complications that increase mortality and morbidity, prolong hospital stay, and adversely affect growth and development. It has been argued that the "fear of NEC" should not be considered in isolation from other potential clinical outcomes when early enteral feeding strategies for very preterm infants are determined [43].

Recent large randomised controlled trials have provided more robust evidence to guide policy and practice in this key area [44] (Box 2). Systematic reviews and meta-analyses of data from these trials do not provide evidence that delayed introduction or slow advancement of feed volumes reduce the risk of NEC or mortality in very preterm infants, but they do raise concerns that conservative

Box 2. SIFT – a pragmatic randomised controlled trial [44]

Participants	2,804 very preterm or very-low-birth-weight infants
Setting	176 UK neonatal units
Intervention	Enteral feeds advancement 30 mL/kg/day
Comparison	Enteral feeds advancement 18 mL/kg/day
Outcomes	Microbiologically confirmed or clinically suspected late-onset infection, NEC, mortality, growth and duration of parenteral feeding
Results	*No significant differences* – Late-onset infection: RR 0.94 (95% CI 0.84–1.06) – NEC: RR 0.89 (95% CI 0.65–1.22) – Mortality: RR 0.92 (99% CI 0.59–1.44) – Weight SDS at discharge home: MD –0.01 (99% CI –0.11 to 0.09) – Head circumference SDS at discharge home: MD –0.06 (99% CI –0.23 to 0.11)
	Significant differences – Days of parenteral feeding: MD –2.14 (99% CI –2.74 to –1.57)
Conclusion	Advancing enteral feed volumes at daily increments of 18 mL/kg (compared with 30 mL/kg) does not affect the risk of NEC, infection or death in very preterm or very-low-birth-weight infants

NEC, necrotising enterocolitis; RR, risk ratio; CI, confidence interval; SDS, standard deviation score; MD, mean difference.

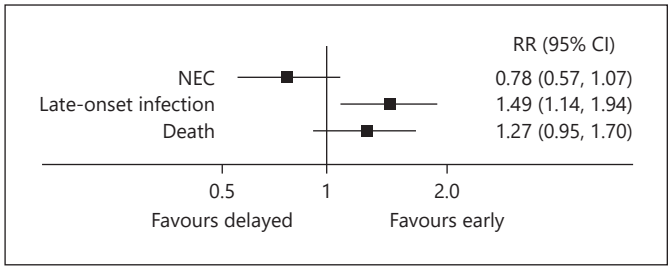

Fig. 2. Summary meta-analysis of delayed versus early (<4 days) introduction of progressive enteral feeds [45]. NEC, necrotising enterocolitis.

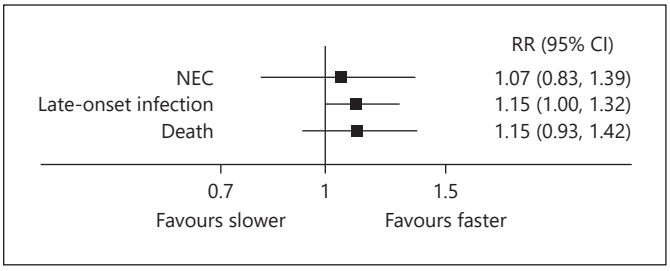

Fig. 3. Summary meta-analysis of slower (≤24 mL/kg/day) versus faster rates of advancement of enteral feed volumes [46]. NEC, necrotising enterocolitis.

enteral feeding strategies in the early neonatal period prolong exposure to parenteral nutrition and might be associated with an increased risk of late-onset infection (Fig. 2, 3) [45, 46].

Gastric Acidity Suppression

The key role that gastric acidity plays in innate gastrointestinal immunity for preterm infants has been recognised partly because of the adverse effects of drugs that suppress gastric acid production (histamine receptor type 2 blockers or proton pump inhibitors). Large observational studies show that exposure to these drugs is associated with a higher risk of NEC and late-onset infection in very preterm infants [47]. Given lack of evidence that gastro-oesophageal reflux is a cause of apnoea in preterm infants, use of gastric acid suppressants should be restricted until robust evidence that benefits outweigh harms is obtained [48].

Conclusion

Immunonutritive factors and practices make important contributions towards averting the pathogenesis of NEC and late-onset infection in very preterm infants. Feeding with human breast milk rather than formula is the simplest family-centred, valued and effective immunoprotective strategy [49, 50]. Donor breast milk may be beneficial for infants whose mothers cannot provide expressed breast milk, but the cost-effectiveness of this policy remains to be established in some contexts and settings. Individual breast milk immunonutrients such as immunoglobulins or lactoferrin or micronutrients such as conditionally essential amino acids that have appeared to be plausible interventions in preclinical studies or small randomised controlled trials have generally not been shown to be effective when assessed in large, high-quality trials. Similarly, prebiotics, probiotics and synbiotics all appear to be promising immunonutritive interventions, but concerns remain about the quality of the evidence base and the safety and availability of formulations. Avoidance of iatrogenic harm, for example, from gastric acid suppressants, as well as using early enteral feeding strategies that reduce exposure to invasive interventions including intravascular access devices, has emerged as an evidence-based component of quality improvement initiatives to reduce the risk of NEC, late-onset infection and associated morbidities, and mortality in very preterm infants.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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