PROBIOTICS: POSSIBLE STRATEGIES TO PREVENT NECROTIZING ENTEROCOLITIS IN NEONATES

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Abstract: *Necrotizing enterocolitis (NEC) is an inflammatory bowel disease that mainly affects low birth weight preterm infants. Because the etiology and pathogenesis of NEC are still incompletely understood, therapeutic options, morbidity and mortality were not significantly improved in the last decade of time. Taking into account the catastrophic development of this disease, it is necessary to focus research on prevention strategies and identify predictive risk factors for its occurrence. Many preventive measures have been tried and accepted in clinical practice, one of these is represented by probiotics supplementation. This article is a review of the literature regarding the efficacy of probiotics as a preventive strategy for NEC in premature infants with low birth weight.*

Cuvinte cheie: *enterocolita ulceronecrotică, prematur, strategii preventive, probiotice*

Rezumat: *Enterocolita ulcero-necrotică (EUN) este o afecțiune inflamatorie a intestinului care afectează în special prematurul cu greutate mică la naştere. Pentru că etiologia și patogeneza EUN sunt încă incomplet înțelese, opțiunile terapeutice, mortalitatea și morbiditatea nu s-au îmbunătățit în mod semnificativ în ultima decadă de timp. Luând în considerare evoluția dezastruoasă a acestei boli, este necesară focalizarea cercetărilor pe strategii de prevenție și pe identificarea factorilor de risc predictivi pentru apariția acesteia. Multe măsuri preventive au fost încercate și acceptate în practica clinică, una dintre acestea fiind reprezentată de suplimentarea cu probiotice. Acest articol este o revizuire a literaturii de specialitate privind eficacitatea utilizării probioticelor pentru prevenţia EUN la prematurii cu greutate mică la naştere.*

NEC is an acute inflammatory bowel disease of the newborn, the most common gastrointestinal emergency in this age(1), with a high mortality rate between 10-30%, surgical cases exceeding 50%. EUN incidence is inversely proportional to gestational age (GA), over 90% of affected infants being preterm. With an incidence of 1-5% of all newborns admitted in the neonatal intensive care, a prevalence of 7-14% of very low birth weight infants (VLBW, <1500g), NEC remains a significant clinical problem.(1,2) NEC incidence differs by country and centers, generally varying between 1-3 cases per 1000 live births.(3)

Etiology and pathogenesis. Despite extensive research focused on understanding the disease, the etiology and pathogenesis of NEC remain incompletely understood. NEC is a multifactorial disease that occurs in a susceptible newborn. The most important risk factors are: prematurity, infant milk formula feeding, enteral feeding, intestinal hypoxia-ischemia, antibiotic use, and intestinal colonization with pathogenic bacteria.(4) The main pathogenic link is represented by intestinal ischemia and reperfusion injury with an inadequate inflammatory response.(5,6) Epidemiological studies have shown a significant association between prematurity and NEC due to structural and functional intestinal immaturity at this age.

Preventive strategies: Because NEC incidence and mortality associated with NEC remained unchanged in recent years, the optimal strategy remains prevention and reducing exposure to risk factors. Several preventive strategies have been tried, and these can be divided into two categories: strategies with proven and unproven efficacy (limited evidence).(7)

Table no. 1. NEC prevention - methods(2)

Strategies with proven scientific efficacy	**Strategies supported by limited data**
Breast-milk feeding	Slow increase of the milk intake
Trophic nutrition (nonaggressive enteral feeding)	Fluid restriction
	Oral immunoglobulin
Antenatal steroids	L-Arginine supplementation
Prophylactic enteral administration of antibiotics	Administration of polyunsaturated fatty acids
	Acidification of milk
	Probiotics, prebiotics, and synbiotics
	Growth factor and erythropoietin

Strategies with the highest impact in preventing or reducing the severity of NEC are: natural nutrition, diet strategies and supplementation with probiotics and prebiotics.

Search strategies and selection criteria: We searched in the Cochrane database, PubMed, Medline, Embase and scientific papers presented at the conferences in the field. We selected from the articles we have found meta-analyzes and randomized controlled clinical trials that studied the efficacy of probiotics in preventing NEC at VLBW.

Probiotics

Definition. Probiotics are live microbial nonpathogenic preparations that colonize the gut and provide benefits for the host when they are administered in adequate amounts.(8,9)

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The intestinal tract is host to a variety of microbes necessary to ensure his health, but which may have the potential to contribute to the development of some disease through various mechanisms. This potential is a topic of ongoing research. Part of this research involves voluntary manipulating of the intestinal microflora for therapeutic purposes. There are generally three types of intervention: antibiotics, probiotics (beneficial bacteria), and prebiotics (food ingredients promoting growth and metabolic activity of beneficial bacteria).(10)

The mechanisms of action are incompletely understood. Several mechanisms are described:

- suppression of the growth or epithelial binding/invasion by pathogenic bacteria;
- improvement of intestinal barrier function, decreasing mucosal permeability;
- immune system modulation by controlling the production of cytokines pro/anti-inflammatory;(11,12)
- pain perception modulation (effect seen with some lactobacilli strains).(13)

Not all probiotics act the same way, so that the results given for a particular species or combination of species are not necessarily identical to the results given by the other species.

There are several questions about the use of probiotics in reducing the risk of NEC, which researchers tried to answer to through all the studies and clinical trials that have been completed:

1. Are there probiotics effective in preventing NEC in preterm infants? The intestinal microbial community of the term newborn is obtained from the passage through the birth canal and from parental contact after birth. In contrast, the preterm infants acquire colonizing bacteria rather from the intensive care unit. These preterm infants have a delayed colonization with beneficial bacteria such as Bifidobacteria and Lactobactilli. In addition to this is added empirical treatment with antibiotics with the role of preventing a possible sepsis, but which is delaying the colonization of the digestive tract too.(14,15,16) Administration of probiotics to a vulnerable population seems, theoretically, to change intestinal colonization with so-called "good" bacteria. It has been suggested that introduction of probiotics in preterm infants may prevent the growth of pathogenic organisms, can improve gastrointestinal tolerance, may decrease the number of days to full enteral feeding administration and can prevent nosocomial infections.(17) A recent meta-analysis of 11 randomized controlled trials (N=2176) conducted by Deshpande et al, found that the use of probiotics reduced the NEC risk with about 65%, and estimates that to prevent one case of NEC 25 children must be treated.(18) It was not been possible to establish yet which are the probiotic bacteria species and the doses that will provide optimal safety. Alfaleh and colleagues published in 2010 a meta-analysis of 9 trials that randomized 1425 eligible children. They compared the efficacy of enteral probiotics versus placebo or no treatment in the prevention of NEC in preterm infants. The included trials varied significantly in terms of study design. The results were as follows: probiotic group versus the control group had a lower incidence of NEC [RR 0.32,(95% CI 0.17-0.60)], and a lower mortality rate [(RR 0.43,(95% Cl 0.25,0.75)]. It has not been shown a significant decrease in neonatal sepsis and of the number of days of total parenteral nutrition in the probiotic group versus the control group. Trials did not report any case of systemic infection due to probiotics supplementation. Data regarding the safety of probiotics administration in VLBW could not be extracted.(19) Other examples of randomized controlled trials using different preparations of probiotics for NEC prevention in preterm infants are illustrated in the following table:

Table no. 2. Randomized controlled trials using probiotics

Source	Probiotic Agent/s	Dosage and Duration	Primary outcome
Dani,2002	LB-GG (Dicloflor)	6×10^9 CFU/d, from first feed until discharge	UTI, sepsis, m**NEC** 1,4% v 2,7%
Bin Nun,2005	BI, ST, BBB	0.35×10^9 CFU/d, (BI, ST, BBB) from first feed to 36 wk corrected age	**NEC:** 1% versus 14%, p 0,013
Lin, 2005	LB-A, BI	LB-A 1004356 and BI 1015697 organisms, 2x/d from day 7 until discharge	**NEC** or death 1,1% v 5,3%, p<0,5
Manzoni,2006	LB-C (Dicloflor)	6×10^9 CFU/d, from day 3 of life to 6 wk or discharge	Gut colonization by Candia species
Stratiki, 2007	BB-L	Preterm formula 1×10^7 CFU/g started within 48 h to 30 d	Intestinal permeability
Lin,2008	BBB, LB-A	2×10^9 CFU/d for 6 wk	**NEC** or death 1,8% v 6,5%, p 0,2
Samanta, 2009	BBB, BB-L, BI,	2.5×10^9 CFU/d until discharge	**NEC**, death, sepsis 5,5 v15,8% p=0,4

BB, Bifidobacterium breve; LB GG, Lactobacillus GG; SB, Saccharomyces boulardii; BI, Bifidobacteria infantis; ST, Streptococcus thermophilus; BBB, Bifidobacterium bifidus; LB-A, Lactobacillus acidophilus; LB-C, Lactobacillus casei; BB-L, Bifidobacterium lactis; BB-LG, Bifidobacterium longum; CFU, colony-forming units; UTI=urinary tract infection

2. What probiotic should be used? A probiotic or a combination of probiotics? The most commonly used are strains of Lactobacilli, Bifidobacteria, Streptococcus salivarius and Saccharomyces boulardii. Bifidobacteria and Lactobacilli have been shown to be the most promising in preterm infants. Note that based on the clinical benefits of probiotics are different mechanisms species-specific. Bifidobacteria is the dominant strain in childhood. There is no clear evidence to show if a preparation comprising several probiotic strains is more efficient than a preparation consisting of a single strain.(20) Since trials conducted varied trough study design, the optimal strains and dosses remains uncertain. Because there are differences in composition, doses and biological activity of the various commercial preparations, the results vary depending on the product.

3. What is the optimal dose that can be administered? A probiotic strain in optimal dose colonize suitable the intestinal tract in order to provide benefit to the host. The records indicate that, to have the desired effect, a probiotic must to be viable and in a suitable dose of 10^6-10^7CFU/g of the product.(20) Being based on data from several clinical trials, it was suggested that a daily dose of $3x10^9$ CFU/day is appropriate for preterm infants with GA <32 weeks. There are no available data regarding the safety dose for VLBW. Therefore, it is recommended that the starting dose to be $1.5x10^9$ CFU/day for VLBW.(20)

4. When should we start and when should we stop probiotics administration? Studies are recommending early

probiotic supplementation to prevent intestinal colonization by pathogenic bacteria and to destroy beneficial bacteria, usually when enteral feeding is started. An important condition is that the infant is clinically stable and has an optimal intestinal function. It was not been yet established a specific probiotic formula for preterm infants.(21,22) Based on the results of the published studies (table no. 2) it seems to be appropriate to stop the supplementation after reaching the corrected GA at 36-37 weeks, when the risk of prematurity complications is minimal. Most studies provide limited data regarding the potential adverse effects of probiotics. Cases of sepsis due to Lactobacillus GG in preterm infants, and cases with fungal infection have been reported in the study by Dani et al.(23) There are studies showing increased number of deaths from sepsis in adult patients from intensive care unit who received probiotics.(24)

The etiology and pathophysiology of NEC is multifactorial and has many unknowns. Treatment options are insufficient and without particularly success in decreasing morbidity and mortality, so that prevention remains the only option. Although data from studies have shown that probiotic therapy appears to reduce the risk of NEC, their use is not yet safe in preterm prevention strategies, especially if they are compared to proven preventive therapies. Further studies are needed to confirm the efficacy, safety and optimal dose (type, time of introduction, duration, and dosage) before routinely recommending this preventive measure.

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