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Bovine lactoferrin supplementation for prevention of necrotizing enterocolitis in very-low-birth-weight neonates: a randomized clinical trial

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ABSTRACT

Importance: NEC is a common and severe complication in premature neonates, particularly those with very-low-birth-weight (VLBW, <1500 g at birth). Probiotics including lactobacillus rhamnosus GG (LGG) proved effective in preventing NEC in preterm infants in several RCTs.
Objective: Lactoferrin, a mammalian milk glycoprotein involved in innate immune host defences, can reduce the incidence of NEC in animal models, and its action is enhanced by LGG. We tried to assess whether bovine lactoferrin (BLF), alone or with the probiotic LGG, has a similar effect in human infants, something that has not yet been studied.
Design: An international, multicenter, randomized, double-blind, placebo-controlled trial conducted from October 1st, 2007 through July 31st, 2010.
Setting: Thirteen Italian and New Zealand tertiary neonatal intensive care units.
Participants: 743 VLBW neonates were assessed until discharge for development of NEC.
Intervention: Infants were randomly assigned to receive orally either BLF (100 mg/day) alone (group LF; $n=247$) or with LGG (at 6×10^9 CFU/day; group BLF+LGG; $n=238$), or placebo (Control group; $n=258$) from birth until day 30 of life (45 for neonates <1000 g at birth).
Main outcome measures: ≥ stage 2 NEC; death-and/or-≥ stage 2 NEC prior to discharge.
Results: Demographics, clinical and management characteristics of the 3 groups were similar, including type of feeding and maternal milk intakes. NEC incidence was significantly lower in groups BLF and BLF+LGG [5/247 (2.0%)] and 0/238 (0%), respectively] than in controls [14/258 (5.4%)] (RR=0.37; 95% CI: 0.136–1.005; $p=0.055$ for BLF vs. control; RR=0.00; $p<0.001$ for BLF+LGG vs. control). The incidence of death-and/or-NEC was significantly lower in both treatment groups (4.0% and 3.8% in BLF and BLF+LGG vs. 10.1% in control; RR=0.39; 95% CI: 0.19–0.80; $p=0.008$. RR=0.37; 95% CI: 0.18–0.77; $p=0.006$, respectively). No adverse effects or intolerances to treatment occurred.
Conclusions and relevance: Compared with placebo, BLF supplementation alone or in combination with LGG reduced the incidence of ≥ stage 2 NEC and of death-and/or ≥ stage 2 NEC in VLBW neonates. BLF might be a

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promising strategy to prevent NEC in NICU settings. Further data on larger sample sizes are warranted before BLF can be widespreadly used in clinical settings.

Trial registration: ISRCTN53107700—http://www.controlled-_trials.com/ISRCTN53107700.

1. Introduction

Necrotizing enterocolitis (NEC) is a devastating bowel disease affecting approximately 7% of very-low-birth-weight (<1500 g, VLBW) infants.

NEC is associated with substantial morbidity and mortality, prolonged stay in neonatal intensive care units (NICUs), high costs and late neurodevelopmental impairment as well as decreased quality of life in the survivors [1]. NEC-associated mortality exceeds 20%, and survivors often have failure-to-thrive and impaired enteric absorption [1]. NEC is multifactorial and its pathogenesis involves immaturity of the gut, infections, enteric colonization by pathogens, local vascular alterations as well as gut damage by pro-oxydative and toxic factors [2].

In recent years, a number of RCTs suggested that prophylactic administration of probiotics—including *Lactobacillus rhamnosus* GG (LGG)—can prevent the most severe stages of NEC [3]. However, this strategy is not yet adopted in all Centers due to a number of unaddressed concerns.

Lactoferrin (LF) is the major whey glycoprotein in mammalian milk, is highly represented in colostrums and plays an important role in innate immune host defences. In addition, it promotes maturation and differentiation of nascent gut, as well as immunomodulation in the Gut Lymphoid Associated Tissue (GALT) [4,5]. LF can be found also on mucosal surfaces, with LF receptors being present on several immunitary cells and also in epithelial cells and enterocytes.

LF reduces the incidence of experimental NEC in animal models, with its action enhanced by LGG [1], but no studies have been done in humans. Of note, bovine and human lactoferrin share a high structural homology, and the same antimicrobial and immunomodulatory properties [4,5].

In a trial in VLBW neonates, bovine lactoferrin (BLF), ±LGG, proved effective in preventing sepsis [6] which is often associated with NEC. However, the study was underpowered to determine the effect on NEC of BLF, alone or with LGG.

This study assesses whether oral supplementation with BLF alone or with LGG can prevent the occurrence of NEC in preterm VLBW neonates.

2. Methods

This is a continuation of a large, multicenter, double-blind RCT of BLF for prevention of late-onset-sepsis in VLBW infants [6], that was not powered to properly assess the effects of BLF on prevention of NEC.

When that study ended, 7/11 centers [6 in Italy, 1 in New Zealand] agreed to continue recruitments for 18 additional months (January 2009–July 2010), targeting to enroll some 800 patients, to address whether BLF (±LGG) prevents NEC.

The design, enrollment criteria, randomization, blinding, BLF/LGG dosages/schedules were the same as the original study, and have been already described elsewhere [4]. Ethics approval and written, informed consent from parents/guardians were obtained.

Briefly, all VLBW neonates (inborn and outborn) admitted to a participant NICU prior to 48 hrs of life were eligible, provided that administration of any Probiotic product had not been already instituted, and that informed parental consent had been released.

Infants were randomly allocated to 1 of 3 groups in a 1:1:1 ratio. Randomization was stratified by center, and randomly permuted blocks of size 9, 12, and 15 were used. The random allocation sequence was generated using ralloc.ado version 3.2.5 in Stata 9.2 (Stata-Corp, College Station, Texas). The pharmacy at each center used these computer-generated randomization lists to form the 3 groups and prepared the drug doses. Clinical and research staff remained unaware of study group assignments during the study.

Infants were randomized to receive orally either BLF (100 mg/day) ±LGG (6×10^9 CFU/day), or placebo from birth until day 30 (45 for neonates <1000 g at birth).

Drug and placebo administration began on the third day of life with 1 daily dose; all doses including placebo were diluted in prepared milk so as to maintain blinding. Neonates not feeding in the first 48 hours received the drug(s) or placebo by orogastric tube.

The 100 mg/day dose of BLF was based on the mean human lactoferrin (HLF) intake that preterm neonates should ingest with mother's fresh milk in the first two weeks of life (30–150 mg/day) [7].

The BLF dose yielded osmolarity of 386 mOsm/l (Meyer, Alexander et al, unpublished data)

The 6×10^9 CFU/day dose of LGG was taken from published data [8].

Nutritional strategies were consistent within all Centers and followed a common protocol [6]. Fresh, expressed maternal milk, whenever possible, the earliest possible, was the first nutritional choice for all Centers. When needed, feeding supplementations in all groups were made with a Preterm Formula not supplemented neither with Bovine Lactoferrin nor with Probiotics.

Systematic surveillance of adverse events (e.g., vomiting, feeding intolerance, skin rashes) was performed through daily infant examination until 2 days after end of treatment.

The primary endpoints were NEC $\geq$ 2nd Bell's stage, defined as clinical signs with the presence of pneumatosis intestinalis on abdominal X-rays, and NEC-and/or-death [9]. The primary outcome was thus individual components (NEC > 2nd Bell's stage) and composite (NEC-and/or-death); this composite outcome was included since "NEC" and "death" are mutually competing.

Mortality not attributable to NEC was defined as "all deaths occurring prior to discharge, for any cause but NEC". Mortality associated with NEC was defined as "all deaths occurring within three days from the diagnosis of NEC > 2nd Bell's stage, in absence of any other major cause".

The statistical analysis was intention-to-treat and performed as already described [6]. BLF and BLF+LGG groups were compared separately to controls. Proportions and continuous variables were compared using Fisher exact 2-tailed and *t*-tests, respectively. Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated to compare cumulative between-group incidences using Stata (9.2.version). A multilevel (random-intercept) logistic regression model was used. All tests were 2-tailed; $p < 0.05$ was considered statistically significant.

The pre-trial incidence rate of NEC was 7.0%. Based on that assumption, the sample size analysis predicted that 319 patients would have been needed for each group (total 957), based on 2-sided type I error rates of 0.05 or less and 80% power to detect a relative difference between treated and not-treated infants of at least 66% (decrease from 7% to 2.33%), and that 238 patients would have been needed for each group (total 816), to detect a relative difference between treated and not-treated infants of at least 75% (decrease from 7% to 1.75%) for this outcome. The planned recruitments were based on this last scenario and all Centers were