Is Lactoferrin More Effective in Reducing Late-Onset Sepsis in Preterm Neonates Fed Formula Than in Those Receiving Mother's Own Milk? Secondary Analyses of Two Multicenter Randomized Controlled Trials

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Abstract

Background Lactoferrin is the major antimicrobial protein in human milk. In our randomized controlled trial (RCT) of bovine lactoferrin (BLF) supplementation in preterm neonates, BLF reduced late-onset sepsis (LOS). Mother's own milk (MM) contains higher concentrations of lactoferrin than donor milk or formula, but whether BLF is more effective in infants who receive formula or donor milk is uncertain.

Aim To evaluate the incidence of LOS in preterm infants fed MM and in those fed formula and/or donor milk.

Study Design This is a (A) post hoc subgroup analysis, in our RCT of BLF, of its effects in preterm infants fed MM, with or without formula, versus those fed formula and/or donor milk (no-MM) and (B) post hoc meta-analysis, in our RCT of BLF and in the ELFIN

(Enteral Lactoferrin in Neonates) RCT, of the effect of BLF in subgroups not exclusively fed MM.

Results (A) Of 472 infants in our RCT, 168 were randomized to placebo and 304 were randomized to BLF. Among MM infants, LOS occurred in 22/133 (16.5%) infants randomized to placebo and in 14/250 (5.6%) randomized to BLF (relative risk or risk ratio (RR): 0.34; relative risk reduction (RRR): 0.66; 95% confidence interval (95% CI) for RR: 0.18–0.64; $p < 0.0008$). Among no-MM infants, LOS occurred in 7/35 (20.0%) randomized to placebo and in 2/54 (3.7%) randomized to BLF (RR: 0.19; RRR: 0.81; 95% CI for RR: 0.16–0.96; $p = 0.026$). In multivariable logistic regression analysis, there was no interaction between BLF treatment effect and type of feeding ($p = 0.628$). (B) In 1,891 infants not exclusively fed MM in our RCT of BLF and in the ELFIN RCT, BLF reduced the RR of LOS by 18% (RR: 0.82; 95% CI: 0.71–0.96; $p = 0.01$).

Conclusion Adequately powered studies should address the hypothesis that BLF is more effective in infants fed formula or donor milk than those fed MM. Such studies should evaluate whether a specific threshold of total lactoferrin intake can be identified to protect such patients from LOS.

Lactoferrin (LF) is a bioactive milk protein, with major immunological, antimicrobial, and gut maturational effects. Bovine LF (BLF) and human LF have high biochemical homology and share the same N-terminal, 11-aminoacidic peptide, which has antimicrobial antibioticlike properties. Several randomized controlled trials (RCTs) suggest that BLF supplementation can reduce late-onset sepsis (LOS) in preterm neonates in NICU.[1–6] However, these data are rated as low-to-moderate quality in a Cochrane review[7] and seem to be inconsistent with a recent RCT in 2,203 preterm infants that found no effect of BLF supplementation on LOS.[8]

Human colostrum is rich in LF, with concentrations five- to sixfold higher than that in mature milk.[9] Infants fed since birth with fresh milk from their mother might therefore receive adequate quantities of LF. In a case–control study in 97 preterm infants, those who developed LOS had consumed significantly less breast milk and lower doses of LF and other milk antimicrobial proteins than the controls.[10]

In our original RCT of BLF supplementation in preterm infants,[1] we collected nutritional data and reported days of exposure to, as well as intakes of, human milk, donor milk, and formula milk for all patients. There were no differences in the relative proportions of mother's own milk (MM) fed and formula/donor milk fed infants between the three randomization groups (BLF alone, BLF + the probiotic *Lactobacillus rhamnosus GG* [LGG], placebo). We showed a significant protective effect of BLF that remained independent of the type of feeding after multiple logistic regression analysis.

In this exploratory analysis, we evaluated the hypothesis that lactoferrin may be more effective in reducing LOS in preterm infants who receive a lower proportion of their own mother's milk.

Materials and Methods

(A) This was a secondary analysis of data obtained during a multicenter RCT performed in Italy and New Zealand from 2006 to 2008; its original protocol is published previously.[1,11] Preterm very low birthweight (VLBW) neonates from 11 tertiary NICUs were enrolled before 72 hours of life and were randomly assigned to receive BLF alone (LF100, Dicofarm SpA, Rome, Italy; 100 mg/day, group A1) or in combination with LGG (Dicoflor60, Dicofarm SpA, Rome, Italy; 10^6 colony-forming units per day, group A2) or placebo (group B) from birth to DOL 30 (DOL 45 for those <1,000 g at birth). The drugs and placebo were administered orally once a day. Neonates not feeding in the first 48 hours received the drug(s)/placebo by orogastric tube. Results from this RCT showed that BLF supplementation, alone or in combination with LGG, reduces the risk of LOS and necrotizing enterocolitis[1] in VLBW infants compared with placebo.

Per protocol, clinical and management data were collected prospectively for all enrolled infants until death or discharge. Systematic clinical surveillance for adverse events was performed through daily infant examination until 2 days after the end of treatment.

Nutritional and feeding policies were stable during the study and consistent among centers, following common guidelines and adherence to the study protocol. In particular, the use of fresh MM was encouraged; when it was not available, the neonates were fed either a standard preterm milk formula, not supplemented with LF,[1] and/or with donor milk obtained through processing (including 62.5°C Holder pasteurization, followed by refrigeration) of pooled milk.

Clinical surveillance for the detection of sepsis was performed in all enrolled infants, with complete laboratory and microbiology evaluation in case of suspected LOS.

In this secondary analysis, the primary aim was to test the hypothesis that BLF has a lower impact in reducing LOS in infants who receive MM than in those who receive formula or donor milk.

Standard laboratory methods were used to identify bacteria from cultures.[12] For *Candida* species, specimens were

incubated on chromogen culture plates (Albicans ID, bioMérieux, Marcy l'Etoile, France) to identify *Candida* albicans blue staining colonies after 48 hours of incubation at 37°C. Colonies were speciated biochemically (Vitec Yeast, bioMérieux).[13]

The criteria for hospital discharge were birthweight of 1,800 g, full oral feeding, and resolution of acute medical conditions. Sepsis episodes were treated with antibiotics/antifungal agents in accordance with the existing literature, guidelines from international consensus documents, and the Italian Neonatology Society's Fungal Infections Task Force recommendations.[14] Blinding was not broken to guide therapy.

Infants who received fresh MM, exclusively or with formula or donor milk, were compared with those not exposed to MM (no-MM, i.e. formula milk and/or donor milk) with regard to the incidence of the first episode of LOS in patients receiving or not receiving BLF.

(B) We combined subgroup data in a meta-analysis of data from our RCT of BLF[1] and the ELFIN (Enteral Lactoferrin in Neonates) RCT[8] using RevMan 5.3 software package (Cochrane).[1,15]

Statistical Analysis

(A) Randomly allocated groups A1 (BLF) and A2 (BLF + LGG) were combined to increase the size of the BLF comparator group. We created a binary variable with a value of 1 if the infant received MM, exclusively or with formula, and with a value of 0 if the infant did not receive MM.

In univariable analyses, relative risks (RRs, i.e., risk ratios) and 95% confidence intervals (CIs) were calculated to compare the cumulative between-group incidence of all infections, in subgroups according to infants who received MM and those who did not.

We also undertook multivariable logistic regression analysis in the whole cohort to explore whether MM modifies the effect of BLF treatment (and hence if stratification by type of feeding is needed). We derived odds ratios and 95% CIs after fitting a multilevel (random-intercept) logistic regression model similar to that presented in ►Table 1 of our original paper. We replaced the original variable representing the type of feeding with the new dichotomous one (MM versus no-MM) and calculated a likelihood ratio statistic to detect any interaction between treatment and type of feeding.

(B) We calculated risk ratios (i.e., RRs) and 95% CIs for the effect of BLF on LOS in a post hoc, subgroup meta-analysis in the two RCTs using fixed effects models in RevMan 5.3.[16]

Results

(A) Complete data for analysis were available for 472 infants. Among them, 383 were fed MM, either exclusively or with formula, and 89 were fed formula or donor milk (72 formula, 17 donor milk). A Consort flowchart of enrolled and analyzed patients is shown in ►Fig. 1.

The main results are summarized in ►Tables 1 to 6>. As expected, the incidence of LOS was less frequent in MM infants than in no-MM infants. LOS occurred in 22 infants who were fed MM, 7 infants who were not fed MM and who received placebo, and 16 of those who received BLF (14 MM and 2 no MM).

The point estimate for the RR of LOS in infants receiving MM alone (in the absence of treatment with LF) is close to 1.0 (OR = 0.91), with large CIs (0.21–3.96). (►Table 6).

When we stratified the analysis for type of feeding in univariable analyses, the point estimate for the RR of LOS after BLF treatment in infants receiving MM (RR: 0.34; 95% CI: 0.18–0.64) was almost double than that of those infants not receiving MM (RR: 0.19; 95% CI: 0.04–0.84) (►Tables 1–4).

In the multivariable logistic regression model, the point estimate for the odds of LOS associated with BLF treatment was more than doubled in infants who received MM (OR: 0.38 for BLF and 0.22 for BLF + LGG vs. placebo) than those who did not (OR: 0.12 for BLF and 0.15 for BLF + LGG vs. placebo). However, the introduction of an interaction term between type of feeding and treatment did not improve the model fit significantly (likelihood ratio test *p*-value: 0.628), indicating no significant interaction (►Tables 5 and 6).

(B) In 1,891 infants not exclusively fed MM in our RCT[1] of BLF and in the ELFIN RCT[8], BLF reduced the RR of LOS by 18% (RR: 0.82; 95% CI: 0.71–0.96; *p* = 0.01) (►Fig. 2). When cases of fungal sepsis were excluded from the analysis, the RR for LOS after BLF treatment was 0.85 (95% CI: 0.73–0.99; *p* = 0.04).

Discussion

Our hypothesis was that receiving MM may reduce the effect of BLF on LOS. In analysis A, univariable analyses are consistent with this hypothesis, but multivariable logistic regression analysis does not confirm it as there was no significant interaction between feeding type and BLF treatment. In analysis B, there was evidence that BLF may reduce LOS in infants not exclusively receiving MM.

Our results are therefore consistent with data showing that human milk has a protective effect against infection[15]

Table 1 Univariable analysis of BLF and LOS in infants receiving mother's own milk: BLF treatments combined versus placebo

	Groups (total *n* = 383)		A1 + A2 vs. B		
	BLF + LGG (A1 + A2), *n* = 250	Placebo (B), *n* = 133	RR	95% CI	*p*-Value
Total LOS (*n* = 36)	14/250 (5.6%)	22/133 (16.5%)	0.34	0.18–0.64	0.0008

Abbreviations: BLF, bovine lactoferrin; CI, confidence interval; LGG, *Lactobacillus rhamnosus GG*; LOS, late-onset sepsis; RR, relative risk.
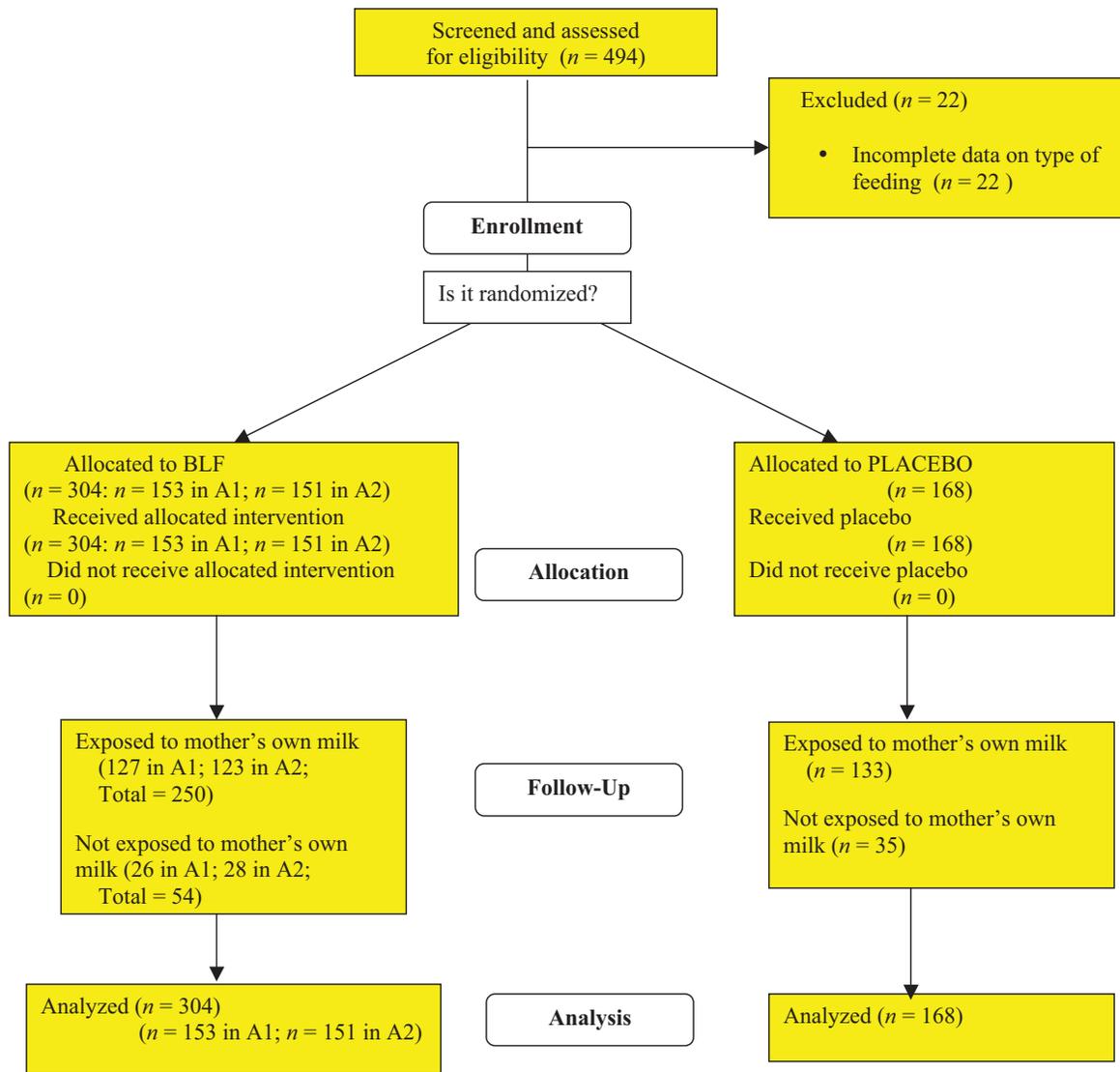
Fig. 1 CONSORT (Consolidated Standards of Reporting Trials) e-flowchart.

Table 2 Univariable analysis of BLF and LOS in infants fed MM

	Groups (total n = 383)			A1 vs. B			A2 vs. B		
	BLF (A1), n = 127	BLF + LGG (A2), n = 123	Placebo (B), n = 133	RR	95% CI	*p*-Value	RR	95% CI	*p*-Value
Total LOS (n = 36)	8/127 (6.3%)	6/123 (4.9%)	22/133 (16.5%)	0.38	0.17–0.82	0.011	0.29	0.12–0.70	0.004

Abbreviations: BLF, bovine lactoferrin; CI, confidence interval; LOS, late-onset sepsis; MM, mother's own milk; RR, relative risk.

Table 3 Univariable analysis of BLF and LOS in infants *not* fed MM

	Groups (total n = 89)			A1 vs. B			A2 vs. B		
	BLF (A1), n = 26	BLF + LGG (A2), n = 28	Placebo (B), n = 35	RR	95% CI	*p*-Value	RR	95% CI	*p*-Value
Total LOS (n = 9)	1/26 (3.8%)	1/28 (3.6%)	7/35 (20.0%)	0.19	0.03–1.47	0.122	0.18	0.02–1.37	0.066

Abbreviations: BLF, bovine lactoferrin; CI, confidence interval; LGG, *Lactobacillus rhamnosus GG*; LOS, late-onset sepsis; MM, mother's own milk; RR, relative risk.

Table 4 Univariable analysis of BLF and LOS in infants *not* receiving MM: BLF treatments combined versus placebo

	Groups (total n = 89)		A1/A2 vs. B		
	BLF + LGG (A1 + A2), n = 54	Placebo (B), n = 35	RR	95% CI	p-Value
Total LOS (n = 9)	2/54 (3.7%)	7/35 (20.0%)	0.19	0.04–0.84	0.026

Abbreviations: BLF, bovine lactoferrin; CI, confidence interval; LGG, *Lactobacillus rhamnosus GG*; LOS, late-onset sepsis; MM, mother's own milk; RR, relative risk.

Table 5 Multivariable logistic regression analysis controlling for the most important risk factors possibly associated with LOS

	OR	95% CI		p-Value
BLF and MM (referent: placebo and formula or donor milk (no-MM)	0.38	0.15	0.96	0.008
BLF + LGG and MM (referent: placebo and formula or donor milk (no-MM)	0.22	0.08	0.64	
Sex (referent: male)	1.85	0.87	3.96	0.112
Gestational age (weeks)[a]	0.70	0.57	0.88	0.002
Birthweight (grams)[a]	1.00	1.00	1.00	0.331
Use of H2 blockers (total days)[a]	1.03	0.99	1.08	0.154
Use of postnatal steroids (total days)[a]	1.10	0.54	2.22	0.796
Daily average amounts of MM intake (mL/kg)	1.00	0.98	1.01	0.600

Abbreviations: BLF, bovine lactoferrin; CI, confidence interval; LGG, *Lactobacillus rhamnosus GG*; LOS, late-onset sepsis; MM, mother's own milk; OR, odds ratio.
[a]OR for a one-unit increase.

Table 6 Interaction between type of milk feeding and BLF treatment

	OR	95% CI		p-Value
Interaction milk and treatment				
A1 (referent: placebo) in infants fed no MM	0.12	0.01	1.23	0.094
A2 (referent: placebo) in infants fed no MM	0.15	0.02	1.59	
Milk type: maternal (referent: nonmaternal) in infants treated with placebo	0.91	0.21	3.96	0.897

Abbreviations: BLF, bovine lactoferrin; CI, confidence interval; OR, odds ratio.
Note: p-Value of likelihood ratio statistic to detect any interaction between treatment and type of feeding: 0.628. Within-center correlation: 4.2%; p-value: 0.18.
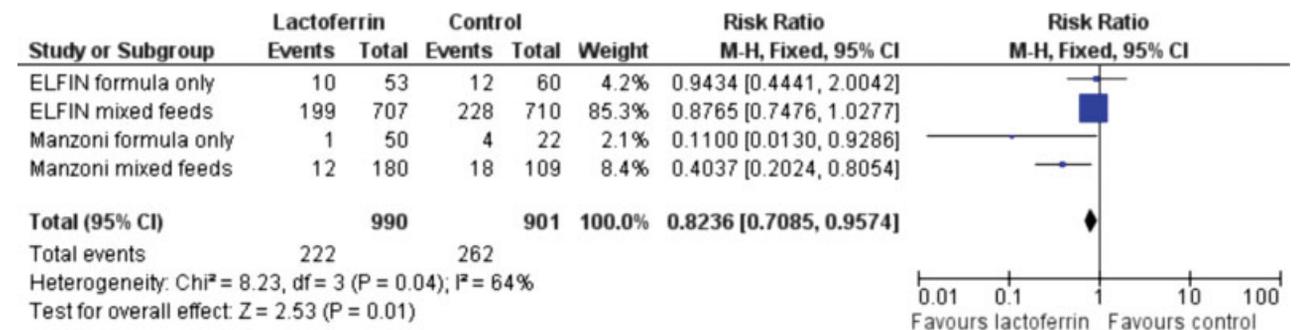

Fig. 2 Meta-analysis of the effect of bovine lactoferrin on late-onset sepsis in 1,891 preterm infants who were not exclusively fed mother's own milk in two RCTs of lactoferrin supplementation. In Fig. 2 of the original ELFIN trial report, the subtotals 10/ 53 and 12/60 in the subgroup analysis for infants fed formula only were incorrectly transposed between the groups randomized to lactoferrin and control, producing a risk ratio of 1.06 instead of 0.94. This error is corrected in the diagram shown here. CI, confidence interval; ELFIN, Enteral Lactoferrin in Neonates. (Adapted from ELFIN Trial Investigators Group.[8])

and that this beneficial effect may be related to cumulative intake, with intake thresholds that confer significant protection compared with lower intakes.[17] In a retrospective case–control study, Trend et al showed that infants who experi-enced LOS were exposed to lower cumulative intakes of lactoferrin through human milk than controls.[10] That study demonstrated that the average consumption of LF by infants without LOS was 300 to 800 mg/kg/day, which is much

higher (three- to fourfold) than the LF consumed by infants who had sepsis. In the same study, fresh breast milk samples and formula were also assessed for their antimicrobial properties in in vitro experiments. Specific threshold LF levels consistent with the intakes coming from breast fresh milk were needed to limit pathogen growth and hence achieve clinical protection from sepsis.

It is known that colostrum and intermediate milk are rich in LF compared with mature milk and that this may be even more true with human milk from mothers of premature infants.[18,19] Our group has calculated that exposure to LF through human milk in a well premature infant could rapidly increase to some 100 to 150 mg/day soon after the first week of life if the baby tolerates full feeds with MM from soon after birth.[11]

These findings suggest that intakes of LF in excess to those already delivered by MM might not confer an additional advantage, being in line with the assumption that concentrations in colostrum and MM should be naturally tailored for the needs of the infant. In contrast, LF supplementation replacing the gap in intakes in those babies not receiving the correct amounts of MM (as in infants fed formula or fed donor processed milk) could improve protection from infections.

These data may help reconcile the inconsistencies between LF studies in the past 12 years and the UK ELFIN trial including 2,203 preterm infants, which reported no reduction in LOS after BLF treatment.[8] One reason for the difference between our earlier trial in 2009[1] and the ELFIN trial[8] may be a reduction in the use of formula only from 15% in our trial to 5% in ELFIN. The hypothesis that BLF is more effective in infants not receiving MM could be evaluated further in an individual participant data meta-analysis of published studies.

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Conflict of Interest
None declared.

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