Lactoferrin: A Key Nutrient to Support Gut Health in Developing Infants

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Disclosures

Consultant

- Abbvie – antivirals
- Astellas – antifungals
- Janssen – antivirals
- Mead Johnson Nutrition – infant nutrition

Speakers Bureau

- AbbVie – antivirals
- AstraZeneca – antivirals

Learning Objectives

- Describe the components of human milk that support brain and gut development
- Explain how GI processes and functions are impacted by lactoferrin in the developing infant
- Use evidence-based research to develop a supplementation plan that best aligns with nutrients provided in human milk

Major Bioactive Factors in Human Milk

Component	Function
Cells	
Macrophages	Protection against infection, T-cell activation
Stem cells	Regeneration and repair
Immunoglobluins	
IgA/sIgA	Pathogen binding inhibition
IgG	Anti-microbial, activation of phagocytosis (IgG1, IgG2, IgG3); anti-inflammatory, response to allergens (IgG4
IgM	Agglutination, complement activation
Cytokines	
IL-6	Stimulation of the acute phase response, B cell activation, proinflammatory
IL-7	Increased thymic size and output
IL-8	Recruitment of neutrophils, proinflammatory
IL-10	Repressing Th1-type inflammation, induction of antibody production, facilitation of tolerance
IFNγ	Proinflammatory, stimulates Th1 response
TGFβ	Antiinflammatory, stimulation of T cell phenotype switch
TNFα	Stimulates inflammatory immune activation
Chemokines	
G-CSF	Tophic factor in intestines
MIF	Macrophage Migratory Inhibitory Factor: prevents macrophage movement, increases anti-pathogen activity of macrophages
Cytokine Inhibitors	
TNFRI and II	Inhibition of TNFα, antiinflammatory

Major Bioactive Factors in Human Milk (cont.)

Component	Function
Growth factors	
EGF	Stimulation of cell proliferation and maturation
HB-EGF	Protective against damage from hypoxia and ischemia
VEGF	Promotion of angiogenesis and tissue repair
NGF	Promotion of neuron growth and maturation
IGF	Stimulation of growth and development, increased RBCs and hemoglobin
Erythropoietin	Erythropoiesis, intestinal development
Hormones	
Calcitonin	Development of enteric neurons
Somatostatin	Regulation of gastric epithelial growth
Antimicrobial	
Lactoferrin	Acute phase protein, chelates iron, antibacterial, antioxidant
Lactadherin/ MFG E8	Antiviral, prevents inflammation by enhancing phagocytosis of apoptotic cells
Metabolic hormones	
Adiponectin	Reduction of infant BMI and weight, antiinflammatory
Leptin	Regulation of energy conversion and infant BMI, appetite regulation
Ghrelin	Regulation of energy conversion and infant BMI
Oligosaccharides & glycans	
HMOS	Prebiotic, stimulating beneficial colonization and reducing colonization with pathogens; reduced inflammation
Gangliosides	Brain development; anti-infectious
Glycosaminoglycans	Anti-infectious
Mucins	
MUC1	Block infection by viruses and bacteria
MUC4	Block infection by viruses and bacteria

Human Milk

its content in substances with putative anti-infective and anti-NEC actions

1. Lymphocites
2. Macrophages
3. Neutrophils
4. **Lactoferrin**
5. Lactoperoxidase
6. Lysozyme
7. IgA–G-M
8. Cytokines
9. Interferon
10. Oligosaccharides
11. Bifidogenic Factors
12. PAF AH
13. Vitamin E
14. Beta Carotene
15. Ascorbic Acid
16. Bifidobacteria

NEC, necrotizing enterocolitis; Ig, immunoglobulin; PAF AH, platelet-activating factor acetylhydrolase

Neonatal and Infant Nutrition as a Preventative, Anti-infective Strategy: the Benefits of Human Milk

Human Fresh Milk Prevents:

- BPD/CLD *(Furman 2003)*
- ROP *(Hylander 2001, Manzoni 2014)*
- NEC *(Lucas 1990, Schanler 2005)*
- Infections *(Hylander 1998, Schanler 2005)*

BPD, bronchopulmonary disease; CLD, chronic lung disease; ROP, retinopathy of prematurity.

Furman L, Taylor G, Minich N, Hack M. *Arch Pediatr Adolesc Med*. 2003;157(1):66-71; Hylander MA, et al. *Journal of Perinatology*. 2001;21:356-362; Manzoni P, et al. *Early Hum Dev*. 2014;90 Suppl 2:S29-33; Lucas A. *Lancet*. 1990;336:1519-1523; Schanler RJ, Lau C, Hurst NM, Smith EO. *Pediatrics*. 2005;116:400-406.

Human fresh milk feeding prevents infections in neonates.

TABLE 5. Reduced Logistic Regression Model for Infection in Relation to Confounding Variables*

Variable	Odds Ratio	95% CI	*P* Value
Gestational age (wk)	0.80	(0.68–0.95)	.009
Apgar score at 5 minutes	0.93	(0.77–1.14)	.494
Days without enteral feedings (NPO)	1.03	(0.99–1.07)	.153
Mechanical ventilator days	1.01	(0.99–1.03)	.184
Human milk-fed	0.43	(0.23–0.81)	.010

* The total for the regression model is 212 cases. The number of cases with imputed values on any single variable is 11 (5.0%).

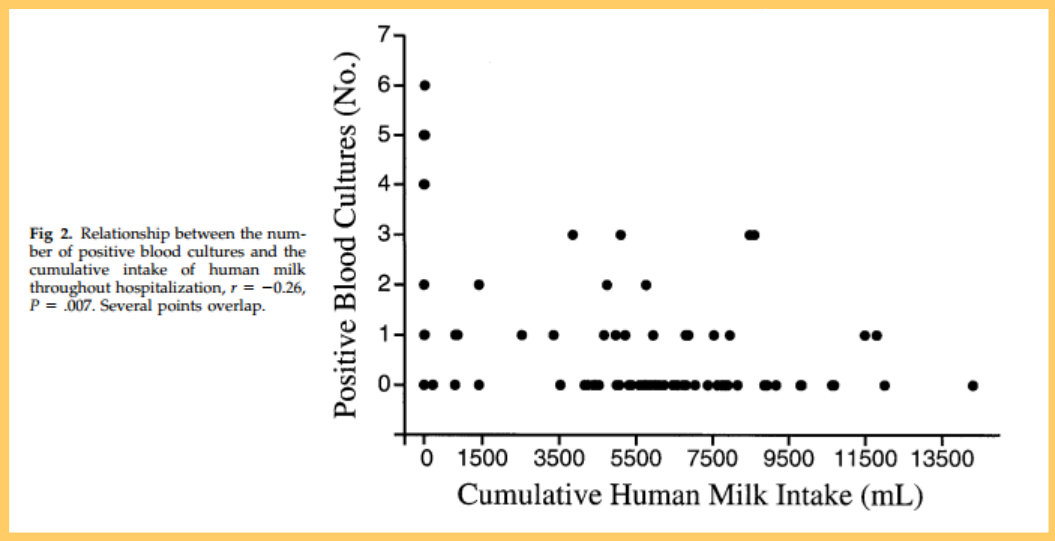


Fig 2. Relationship between the number of positive blood cultures and the cumulative intake of human milk throughout hospitalization, $r = -0.26$, $P = .007$. Several points overlap.

The beneficial effects of human fresh milk are linearly associated with the intake volumes.

Only human fresh milk daily mean intakes higher than a certain amount might deliver the preventative effect on infections

			BW (mean gms)	Sepsis	NEC	Any ROP	CLD	LOS (mean day)
>50	ml / kg / die	n=32	1163	6% *	0%	44%	19%	61
< 50	ml / kg / die	n=87	1016	37% *	8%	54%	39%	80

Please note:

a) Human fresh/donor milk needs to be fortified in protein content to meet the correct nutritional requirements for preterm infants

b) When HM/DM is fortified, the anti-infective benefits might be (partially or entirely) lost[1]

BW, birthweight; LOS, late-onset sepsis; HM, human milk; DM, donor milk.

1. Schanler RJ, Lau C, Hurst NM, Smith EO. *Pediatrics.* 2005;116:400-406.

What About Lactoferrin?

Lactoferrin is a key nutrient in supporting gut and infant health in preterm and term neonates, as well as in developing infants.

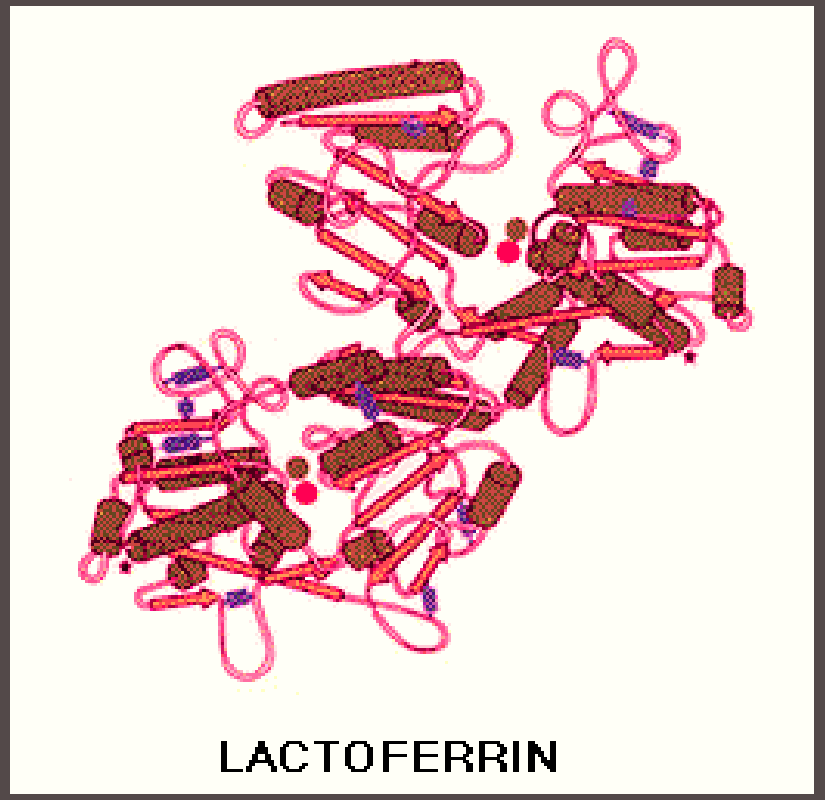


Image courtesy of www.raw-milk-facts.com

Overview of Lactoferrin's Biological Functions

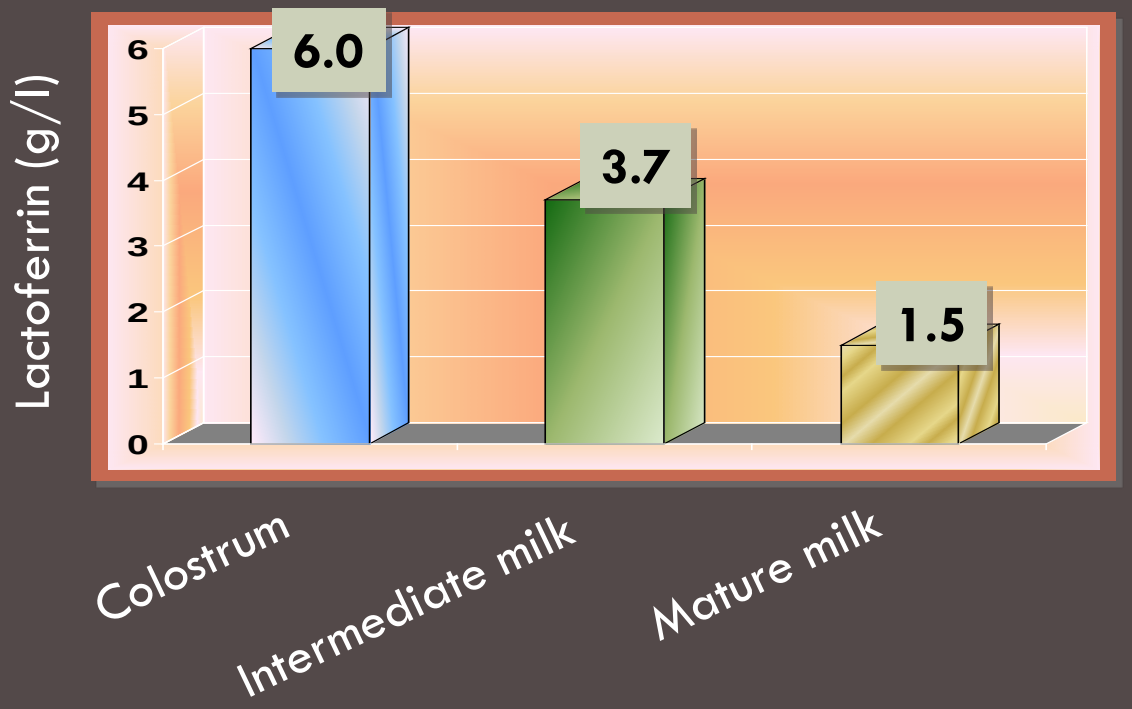
Is lactoferrin the "magic bullet" in human fresh milk responsible for its anti-infective actions?

- LF is the major whey protein in mammalian milk, in all mammals
- High [77%] structural homology between:
  - Bovine LF → extracted and purified by cow's milk
  - Human LF → recombinant engineering: *talactoferrin*
- In the stomach, pepsin digests and releases a potent peptide antibiotic called lactoferricin from native LF
- Human and bovine LF share the same:
  - LACTOFERRICIN (N-terminal, 11-aminoacidic peptide with antimicrobial activity) *(Lupetti 2004)*
  - High rate of survival from stomach passage after oral administration
  - High intestinal uptake and gut actions *(Lönnerdal 2011)*
  - Poor (10%) intestinal absorption
- Bovine LF is added to commercial formula milk in many countries and has been granted a GRAS status by the FDA

GRAS, Generally Recognized As Safe; LF, lactoferrin.

Lupetti A, et al. *J Antimicrob Chemother*. 2004;43:603-608.
Lönnerdal B, Jiang R, Du X. *J Pediatr Gastroenterol Nutr*. 2011;53:606-614.

Concentrations of Lactoferrin Decrease in Mature Human Milk vs Colostrum



This decrease typically occurs in all mammals

Milk	*Concentrations of lactoferrin*
Woman	2 (mature milk) – 6 (colostrum) mg/mL
Cow	0.2-0.5 mg/mL
Rat	<50 mcg/mL
Rabbit	<50 mcg/mL
Dog	<50 mcg/mL
Goat	0.2 mg/mL
Pig	0.2 mg/mL

Courtesy of Paolo Manzoni, MD, PhD

Gut Permeability and Human Milk: a Specific Role of Lactoferrin on the Nascent Gut

- Intestinal permeability changes as a function of age and type of feeding
- Gut permeability and mucosal trophic effect of human milk are key factors for prevention of infections and NEC
  - The feeding of human milk may modulate the trophism of the gastrointestinal tract of preterms, with more rapid maturation of intestinal epithelium (Goldman AS, *J Nutr. 2000*)
  - The feeding of human milk (versus formula) is associated with decreased permeability at 28 days of age (Shulman RJ et al, *Pediatr Res. 1998*)

Is this related to lactoferrin? Probably YES, based on at least 4 recent studies:

- Buccigrossi et al, *Ped Res.* 2007 (*in vitro* study)
- Lönnerdal et al, *JPGN.* 2011
- Jiang et al, *JPGN.* 2014
- Reznikov et al, *J Nutr.* 2014 (piglet study)

Goldman AS. *J Nutr*. 2000;130(2S Suppl):426S-431S; Shulman RJ, et al. *Pediatr Res*. 1998;44(4):519-523; Buccigrossi V, et al. *Pediatr Res*. 2007;61(4):410-414; Lönnerdal B, et al. *J Pediatr Gastroenterol Nutr*. 2011;53(6):606-614; Jiang R, et al. *J Pediatr Gastroenterol Nutr*. 2014;59(5):642-652; Reznikov EA, et al. *J Nutr*. 2014;144(9):1401-1408.

Lactoferrin and Its Trophic Effect on The Enterocytes and Gut Function

Lactoferrin Induces Concentration-Dependent Functional Modulation of Intestinal Proliferation and Differentiation

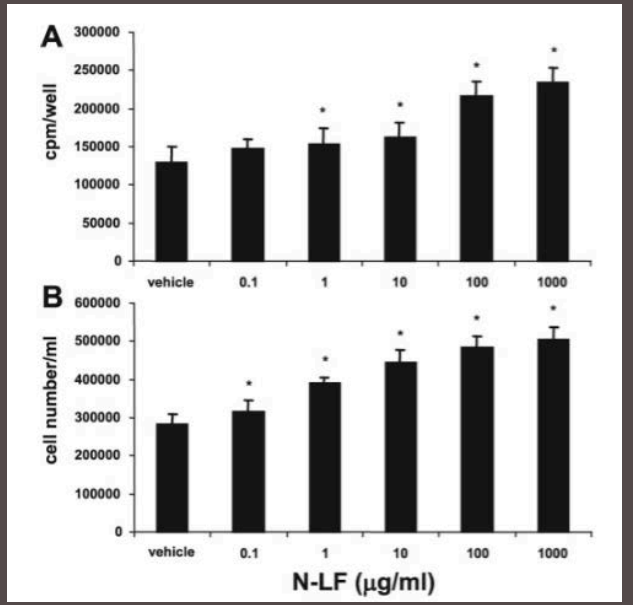
This study assesses the *in vitro* effects of a wide range of bovine and human lactoferrin concentrations on:

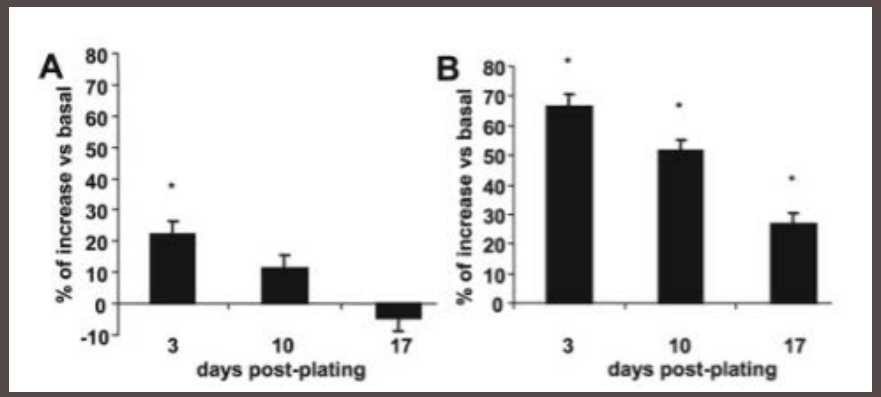
1. Proliferation of rapidly growing enteric Caco-2 cells (as number of enterocytes)
2. Differentiation of enteric Caco-2 cells (as sucrase and lactase activities)
   - Bovine LF was compared with human LF
   - Bovine LF was used in concentrations equimolar to human LF

Buccigrossi V, et al. *Pediatr Res.* 2007;61(4):410-414.

1 - Lactoferrin has a trophic effect on the enterocytes related to its concentrations

→ the higher the LF concentrations, the faster the enterocytes proliferate





2 - Lactoferrin promotes gut function related to its concentrations

→ the lower the LF concentrations, the faster the enterocytes differentiate

These actions occurred with both bovine and human LF

Conclusions

1. **Lactoferrin is a key modulator of the intestinal epithelium development**

   Speculation → less permeability, less colonizing pathogens that can disseminate to bloodstream, less infections

2. **Bovine and human lactoferrin have similar actions on the nascent gut**

   → Commercial bLF is biologically active as well as purified bLF and hLF

   → Commercial bLF exerts several of the bioactivities of hLF if added to infant formula *(Lönnerdal, JPGN 2011; Jiang, JPGN 2014)*

bLF, bovine lactoferrin; hLF, human lactoferrin.

Lönnerdal B, et al. *J Pediatr Gastroenterol Nutr*. 2011;53(6):606-614. Jiang R, et al. *J Pediatr Gastroenterol Nutr*. 2014;59(5):642-652.

Lactoferrin and the Mechanisms Accounting for Its Anti-Infective Activity

DIRECT MECHANISMS - Antibiotic-like action

- Anti-LPS (vs Gram-negatives)
- Anti-LTA (vs Gram-positives)
- Anti-Candida cell wall components

INDIRECT MECHANISMS

- Iron-sequestring (→bacteriostasis)
- Functional modulation of intestinal proliferation and differentiation (→enhancement of gut barrier)
- Bifidogenic action on gut microflora

IMMUNOMODULATORY ACTIONS in the GUT lymphoid tissues (GALT)

- IL-18 production, NK cell activity
- Maturation and differentiation of T- lymphocytes - Th1/Th2 balance
- $CD8^+/4^-$ DCs maturation
- Recruitment and activation of APCs

ANTIFLOGISTIC MECHANISMS

- Inhibition of formation of reactive oxygen species (ROS) by suppressing free radical activity
- Decrease the levels of oxidative products when medicinal iron is present in a formula

Fischer R, et al. *Biochem Cell Biol.* 2006;84(3):303-311; Kuhara T, et al. *J Interferon Cytokine Res.* 2006;26(7):489-499; de la Rosa G, et al. *J Immunol.* 2008;180(10):6868-6876; Ishii K, et al. *Hepatol Res.* 2003;25(3):226-233; Sherman MP, et al. *Neonatology.* 2011;100(4):409-411; van der Does AM, et al. *Biometals.* 2010;23(3):493-505; González-Chávez SA, et al. *Int J Antimicrob Agents.* 2009;33(4):301.e1-308; Lönnerdal B, et al. *J Pediatr.* 2010;156(2 Suppl):S26-S30; Lönnerdal B, et al. *J Pediatr Gastroenterol Nutr.* 2011;53(6):606-614; Legrand D, et al. *Biometals.* 2010;23(3):365-376; Raghuveer TS, et al. *Pediatr Res.* 2002;52(6):964-972; Sherman MP, et al. *Biometals.* 2004;17(3):285-289.

Why Lactoferrin Might Also Prevent NEC?
The Rationale (1)

- LF prevents late-onset sepsis in VLBWs *(Manzoni et al, JAMA. 2009)*
- Lactoferrin and lysozyme in breast milk are synergistic and kill bacteria.
- The antimicrobial characteristics of LF may facilitate a healthy intestinal microbiome ⟶ LF is bifidogenic, promoting Bifidobacteria and Lactobacilli microflora in the gut *(Mastromarino et al, Biometals. 2014)* ⟶ these probiotics prevent NEC *(Cochrane 2011; Deshpande et al, Lancet. 2007)*
- LF has trophic and pro-proliferative activity on the nascent enterocytes, regulating gut permeability *(Buccigrossi et al, Ped Res. 2007)*
- LF enhances anoikis of infected enterocytes in the gut *(Sherman et al, Med Hypoth. 2005)*

VLBW, very low birth weight.

Manzoni P, et al. *JAMA*. 2009;302:1421-1428; Mastromarino P, et al. *Biometals*. 2014;27:1077-1086; *Cochrane Database Syst. Rev*. 2011;Mar 16: CD005496; Deshpande G, Rao S, Patole S. *Lancet*. 2007;369:1614-1620; Buccigrossi V, et al. *Pediatr Res*. 2007;61:410-414; Sherman MP, Petrak K. *Med Hypotheses*. 2005;65:478-482.

Why Lactoferrin Might Also Prevent NEC?
The Rationale (2)

- The immuno-modulatory activates of LF activate dendritic cells (DC) and DCs then induce a Th1 helper cell population that resists neonatal infection.
- Lactoferrin has anti-inflammatory actions that may mitigate the proinflammatory state that is present in the gut before the onset of necrotizing enterocolitis.
  - LF attenuates oxidation by suppressing free radical activity, and decreasing levels of oxidative products (Raghuveer et al, *Ped Res. 2002)*
  - LF downregulates pro-inflammatory cytokines upexpressed in intestinal epithelial cells (Berlutti et al, *Biochem Cell Biol. 2006)*
- A trend towards a protective effect had been seen in the JAMA study, but it was not powered enough for the NEC outcome (Manzoni et al, *JAMA. 2009)*

Th1, type 1 helper.

Raghuveer TS, et al. *Pediatr Res.* 2002;52:964-72; Berlutti F, et al. *Biochem Cell Biol.* 2006;84:351-357; Manzoni P, et al. *JAMA.* 2009;302:1421-1428.

The First RCT on Lactoferrin Feeding in Neonates

Bovine Lactoferrin Supplementation for Prevention of Late-Onset Sepsis in Very Low-Birth-Weight Neonates: A Randomized Trial

on behalf of the GSIN - Italian Task Force for the Study and Prevention of Neonatal Infections, affiliated with the Italian Society of Neonatology

Manzoni P, et al. *JAMA*. 2009;302:1421-1428.

Design of the Study

- Multicenter RCT in VLBW (<1500 g) neonates
- 10-month period
- 11 tertiary NICUs in Italy
- Enrollment within 48 hours of birth
- Randomization 1:1:1 by center to 3 groups by means of computer-generated randomization lists

Randomization

- Group A1 – Lactoferrin 100 mg (LF100®, *Dicofarm spa*, Rome, Italy)
- Group A2 – Lactoferrin 100 mg (LF100®), + Lactobacillus GG, 6 x 10^9 CFU/day (Dicoflor 60®, both *Dicofarm spa*, Rome, Italy)
- Group C – Placebo - 2 mL of 5% glucose solution added to milk feeding, daily for 4-to-6 weeks

* the 100 mg dosing was calculated based on the theoretical mean intakes of 1,000 g-weighing preterms fed fresh maternal milk during their first 2 weeks of life

CFU, colony-forming units.

Manzoni P, et al. *JAMA*. 2009;302:1421-1428.

Objectives

- Primary → to evaluate the effectiveness of lactoferrin (alone, or in combination with LGG) compared with placebo for prevention of late-onset sepsis (LOS) by any pathogen
- Secondary → Invasive fungal Infections (IFI), NEC >2nd stage, threshold ROP, overall and sepsis-attributable mortality

Definitions

LOS = presence of clinical and laboratory signs consistent with infection, together with a positive culture from:

- blood (drawn from peripheral sites)
- cerebrospinal fluid
- peritoneal fluid

LGG, probiotic *lactobacillus rhamnosus* GG.

Manzoni P, et al. *JAMA*. 2009;302:1421-1428.

Demographics, Clinical, Nutritional Characteristics: No Differences Between Groups

	Group A1 LF	Group A2 LF+LGG	Group B Placebo	*P* values
Patients (total = 472)	153	151	168	
Birth weight (grams), M (± sd) (range)	1095 (±247) (630-1435)	1100 (±272) (550-1495)	1070 (±280) (495-1500)	ns
Gestational age (weeks), M (± sd) (range)	29.3 (±2.5) (24-35)	29.6 (±2.8)(23-35)	29.1 (±3.0) (23-34)	ns
Apgar score at 5 min	7.5	7.4	7.5	ns
Daily average amounts of human fresh milk intake (mL/kg)	71.1	70.0	71.8	ns
Total days of human fresh milk feeding	20.8	21.6	21.9	ns
TPN duration (total days)	18.0	15.7	17.4	ns

Results: LF Combined (alone or with LGG) vs Placebo

	LF combined n = *(153+151)* = 304	**PLACEBO n = 168**	**RR**	**95% CI**	***P* value**
Late-onset sepsis (all agents)	**5.3%**	**17.3%**	**0.28**	**0.16-0.50**	**<0.001**
LOS by Gram-Positive	1.2%	5.4%	0.21	0.07-0.82	0.02
LOS by Gram-Negative	3.4%	6.5%	0.48	0.35-0.98	0.05
LOS by Candida spp (IFI)	0.7%	5.4%	0.24	0.09-0.77	0.009
NEC	**0.8%**	**6.0%**	**0.16**	**0.09-0.58**	**0.001**
Mortality (all causes prior to discharge)	**3.3%**	**7.1%**	**0.39**	**0.15-0.92**	**0.04**
Death or NEC	**4.1%**	**13.1%**	**0.30**	**0.16-0.70**	**0.007**

Power calculations: 0.95 for LOS, 0.74 for NEC, 0.65 for IFI, 0.45 for mortality

RR, risk ratio; CI, confidence intervals.

Lactoferrin Trial for Prevention of NEC

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

- Design, study protocol, enrollment criteria and timing, randomization 1:1:1, LF and LGG dosages were the same as the *JAMA* study
- Administration of any probiotic product was an exclusion criterion
- The BLF dose yielded osmolarity of 386 mOsm/l *(Meyer et al, unpublished data)*
- The LGG dose was taken from published data *(Dani et al, 2002)*
- Demographics, clinical characteristics, presence of major risk factors for NEC were similar in all 3 groups
- 743 patients were analyzed:
  - Mean BW 1085, 1095 and 1065 g in the 3 groups
  - Mean GA 29.2, 29.5 and 29.1 weeks in the 3 groups

mOsm/l, milliosmoles/liter; GA, gestational age.

Manzoni P, et al. *Early Hum Dev*. 2014;90 Suppl 1:S60-65; Dani C, et al. *Biol Neonate*. 2002;82(2):103-108.

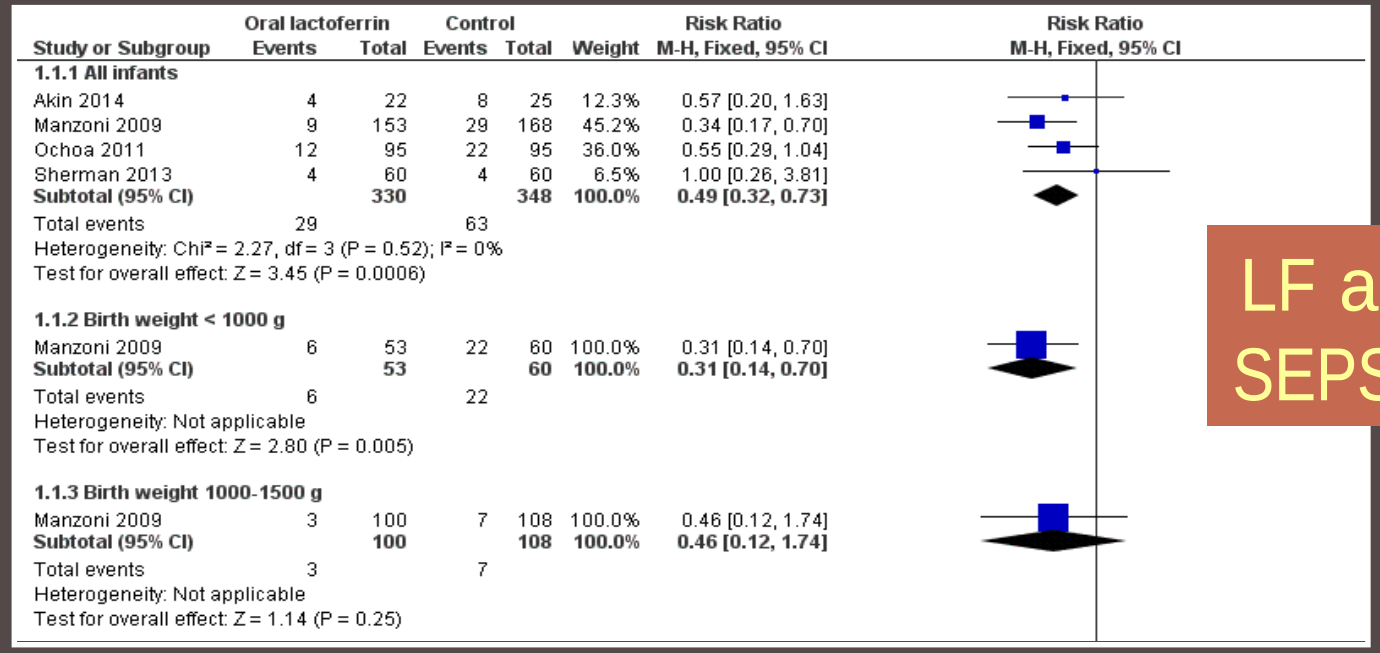
Lactoferrin Trial for Prevention of NEC Results: LF With or Without LGG

	LF N=251	*PLACEBO N=259*	*RR*	*95% CI*	*P value*
Severe NEC (>2nd stage)	2.0%	5.4%	0.37	0.14-1.00	0.05
Overall Mortality	2.0%	6.9%	0.28	0.11-0.76	0.007
NEC and/or Death	4.0%	10.1%	0.39	0.19-0.80	0.008

	LF + LGG N=242	*PLACEBO N=259*	*RR*	*95% CI*	*P value*
Severe NEC (>2nd stage)	0%	5.4%	0.00	---	<0.001
Overall Mortality	3.8%	6.9%	0.53	0.24-1.16	0.11
NEC and/or Death	3.8%	10.1%	0.37	0.18-0.77	0.006

Oral Lactoferrin for Prevention of Sepsis and Necrotizing Enterocolitis in Preterm Infants (Review)

Figure 1. Forest plot of comparison: 1 Lactoferrin alone vs placebo, outcome: 1.1 Any late-onset sepsis.



LF and SEPSIS

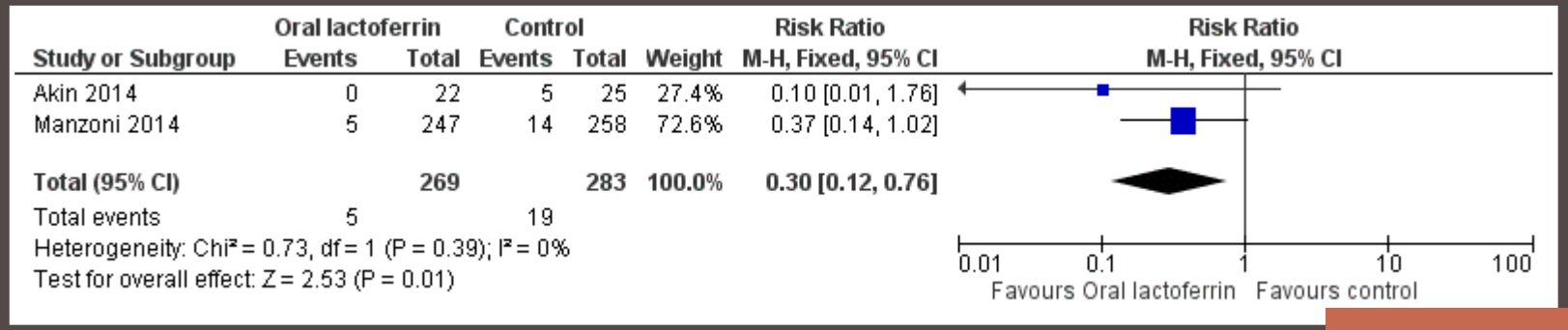
- 4 RCTs retrieved (3 BLF, 1 rHLF)
- 648 VLBW infants analysed. No heterogeneity
- RR 0.49
- NNT 11
- Current available evidence graded as "moderate quality"

rHLF, recombinant human lactoferrin; NNT, number needed to treat.

Pammi M, Abrams SA. *Cochrane Database Syst Rev*. 2015 Feb 20;2:CD007137. ©John Wiley & Sons, Inc.

Oral Lactoferrin for Prevention of Sepsis and Necrotizing Enterocolitis in Preterm Infants (Review)

Figure 2. Forest plot of comparison: 1 Lactoferrin alone vs placebo, outcome: 1.2 NEC ≥ stage II.



LF and NEC

- 2 RCTs retrieved (all with BLF)
- 552 VLBW infants analyzed. Moderate heterogeneity
- RR 0.30
- NNT 20
- Current available evidence graded as "low-to-moderate quality"

Pammi M, Abrams SA. *Cochrane Database Syst Rev*. 2015 Feb 20;2:CD007137.

Oral Lactoferrin for Prevention of Sepsis and Necrotizing Enterocolitis in Preterm Infants: The 2017 July Update

Prevention of Late-Onset Sepsis:

- 6 RCTs retrieved (5 BLF, 1 rHLF)
- 886 VLBW infants analyzed. No heterogeneity
- RR 0.59
- NNT 17
- Current available evidence graded as “low-moderate quality”

Prevention of NEC:

- 4 RCTs retrieved (3 BLF, 1 rHLF)
- 740 VLBW infants analyzed. No heterogeneity
- RR 0.40
- NNT 25
- Current available evidence graded as “low quality”

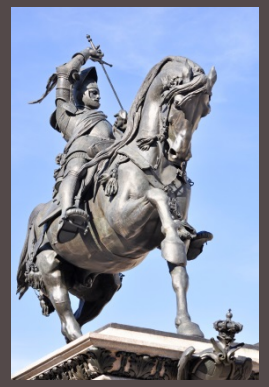
rHLF, recombinant human lactoferrin; NNT, number needed to treat.

Pammi M, Suresh G. *Cochrane Database Syst Rev*. 2017 Jun 28;6:CD007137.

…and now?

Ongoing Studies and Research Projects for Use of Lactoferrin in the Nursery

Italy GSIN Network: secondary analysis of the data from the *JAMA* trial



1. LF and IFI *(→ Pediatrics 2012)*
2. LF and NEC *(→ Early Hum Dev 2014)*
3. LF and H2 Blockers *(PAS-SPR platform presentation 2015; submitted for publication)*
4. Relationships of LF exposure with human fresh milk *(unpublished data)*
5. LF and neuro-developmental outcomes *(unpublished data)*

Manzoni P, Stolfi I, Messner H, et al. *Pediatrics*. 2012 Jan;129(1):116-23.
Manzoni P, et al. *Early Hum Dev*. 2014 Mar;90 Suppl 1:S60-65.

Main Ongoing Studies – Overview

Study	Population	Intervention Group	Control Group	Primary Outcome	Data Out
Enteral Lactoferrin In Neonates (ELFIN); ISRCTN88261002	UK Neonates <32 wks g.a. first 72 h of age (n = 2,200)	Bovine LF 150mg/kg/day (max: 300 mg) until discharge	Milk with placebo	1. Culture-proven or clinically suspected LOS from trial entry until discharge	2018
Trial of lactoferrin for prevention of infections in very premature babies (LACUNA); ISRCTN66482337	CANADA Neonates >23 <30.6 wks g.a. first 48 h of age (n = 79)	Bovine LF 100 mg/day, 2 doses per day until 36 weeks g.a. or discharge	Milk without LF	1. Death or at least 1 health care- associated infection before discharge 2. Tolerance of LF	2018
Lactoferrin Infant Feeding Trial (LIFT) to prevent sepsis and death in preterm infants; ACTRN12611000247976	AUSTRALIA, INDIA, CANADA, ITALY Neonates with BW<1,500 g and g.a. 22–28 wks first 7 days of age (n = 1,100)	Bovine LF 200 mg/kg/day until 34 weeks g.a. corrected or discharge	Breast milk or formula without BLF	1. Incidence of sepsis or brain injury or CLD or NEC or severe ROP	2018

Courtesy of Paolo Manzoni, MD, PhD

Intermezzo

What does this mean for doctors, nurse practitioners, nurses and dieticians who care for preterms, newborns and infants in the NICU and hospital?

How does this affect your practice?

What do I do in my practice?

Lessons Derived From the Existing Clinical Data of LF in Neonates

- Bovine LF has a protective effect against sepsis and NEC in preterm VLBW infants
- Similar efficacy has not yet been proven for human recombinant LF (talactoferrin)
- Concomitant administration of the probiotic LGG enhances the NEC preventative action, suggesting that LF-induced promotion of bifidogenic microflora is a key step, in addition to the other properties of lactoferrin, in protecting premature infants against NEC

Practice Actions

- Start LF as soon as possible *(Manzoni 2009 vs Ochoa 2015)*
- Do not expect efficacy in preventing sepsis/NEC in larger neonates *(Ochoa 2015)*
- Use higher than 100 mg/day dosage *(Manzoni 2009)*, possibly weight-tailored
- If you want to tackle NEC, better combine BLF with a probiotic (lower NNT) *(Manzoni 2014)*
- BLF is much more effective in prematures than term neonates *(Ochoa 2015)*
- Effective in preventing infections, not effective in preventing enteric colonization *(Manzoni 2012)*
- Efficacy on Gram-positive sepsis is still questionable

Manzoni P et al. *JAMA*. 2009;302:1421-1428; Ochoa TJ et al. *Pediatr Infect Dis J*. 2015;34(6):571-576;
Manzoni P et al. *Early Hum Dev*. 2014;90 Suppl 1:S60-65; Manzoni P et al. *Pediatrics*. 2012;129(1):116-123.

Lactoferrin Supplementation to Prevent Nosocomial Infections in Preterm Infants

"…Because colostrum contains the highest lactoferrin content (6 g/L, vs 1.5 g/L in mature human milk), nature is providing 'added protection' for a short period to full-term infants—but for preterm infants this added protection is needed for weeks or months."

"…Very low-birth-weight infants receiving human milk have inadequate protection because it can take 2 to 3 weeks until they receive full-volume enteral feedings and the full amounts of the protective components in human milk. Even this may not be enough until infants are close to full-term corrected age…"

Kaufman D. *JAMA*. 2009;302(13):1467-1468.

How Much Lactoferrin Do We Need?

Day of life	Human milk intakes (mL/feed) and no. of feeds	Concentration of LF in colostrums and early human milk (mg/mL)[1]	Presumed weight in grams	Estimates of mean daily amounts of human lactoferrin ingested with feeds (mg/kg)
1	0.5 x 6	7	1,000	21
2	0.5 x 8	7	950	26.6
3	1 x 8	6.5	900	46.8
4	1 x 12	6.5	850	66.3
5	2 x 12	6	850	122.4
6	2 x 12	6	850	122.4
7	3 x 12	5.5	870	172.6
8	3 x 12	5.5	870	172.6
9	4 x 12	5.5	870	229.6
10	4 x 12	5	890	213.6
11	5 x 12	5	890	267
12	5 x 12	5	910	273
13	6 x 12	4.5	910	294.8
12	6 x 12	4.5	930	301.3
13	7 x 12	4.5	930	351.4
14	7 x 12	4	950	319.2
15	8 x 12	4	950	364.8
16	8 x 12	4	950	364.8

[1]Patterns of mean daily human lactoferrin amounts for a 1,000 g birth weight preterm infant in the first 2 weeks of life

Antimicrobial Protein and Peptide Concentrations and Activity in Human Breast Milk Consumed by Preterm Infants at Risk of Late-Onset Neonatal Sepsis

This study assessed the levels and antimicrobial activity of antimicrobial proteins and peptides, including lactoferrin, in breast milk consumed by preterm (<32 wks) infants, and whether deficiencies of these factors were associated with late-onset neonatal sepsis

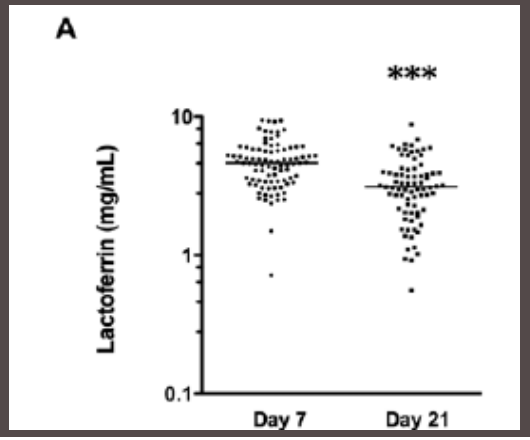
- Breast milk from mothers of preterm infants (32 wks g.a.) was collected on days 7 (n = 88) and 21 (n = 77) postpartum
- Concentrations of lactoferrin, LL-37, beta-defensins 1 and 2, and alpha-defensin 5 were measured by Elisa
- The antimicrobial activity of breast milk samples against *Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli*, and *Streptococcus agalactiae* was compared to the activity of infant formula, alone or supplemented with physiological levels of AMPs
- Samples of breast milk fed to infants with and without subsequent LOS were compared for levels of AMPs and inhibition of bacterial growth

AMPs, antimicrobial proteins.

Trend S, et al. *PLoS One*. 2015;10(2):e0117038.

Results

- Levels of most AMPs, including LF, and antibacterial activity in preterm breast milk were higher at day 7 than at day 21.
- The range of total daily LF consumed by infants ranged from 0–794 mg/kg on days 7 and 21 postpartum
- Lactoferrin was the only AMP that limited pathogen growth >50% when added to formula at a concentration equivalent to that present in breast milk.
- Levels of AMPs were similar in the breast milk fed to infants with and without LOS, however, infants who developed LOS consumed significantly less breast milk and lower doses of milk AMPs than those who were free from LOS.

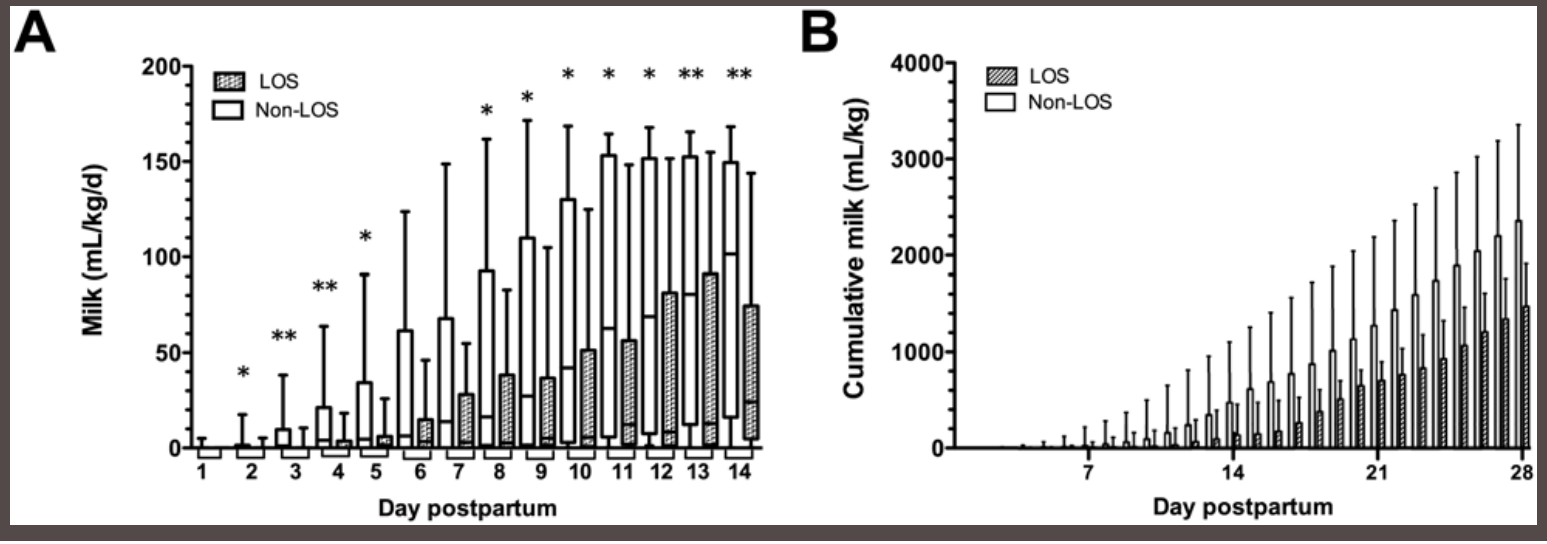


Levels of LF in breast milk are higher at 7 days than at 21 days ($P<0.001$)

Trend S, et al. *PLoS One*. 2015;10(2):e0117038.

Late-Onset Sepsis and Levels/Intake of LF

- The concentration of LF in breast milk showed negative correlation with the colony forming units of *E. coli* and *S. aureus* after incubation with breast milk
- The median doses of LF consumed by LOS cases were lower on day 7 (14 mg/kg LF in LOS cases and 52 mg/kg in controls, respectively; $P=0.03$) and day 21 (131 mg/kg LF in LOS cases and 298 mg/kg LF in controls, respectively; $P=0.04$).



*$P<0.05$; **$P<0.01$. Consumption of breast milk by preterm infants in the case-control study.
Trend S, et al. *PLoS One*. 2015;10(2):e0117038.

Antimicrobial Activity of Lactoferrin When Added to Infant Formula

- In a secondary experiment, it was determined that physiological milk levels of individual AMPs, including LF, were independently capable of inhibiting bacterial growth in LBWF.
- The addition of LF to LBWF at doses equivalent to the median concentration measured in preterm breast milk samples (3.8 mg/mL) had >50% bacteriostatic effect against all bacterial species, with >97% inhibition of growth for *S. epidermidis, S. aureus* and *E. coli*, and 67% for *S. agalactiae (Fig. 3, Trend, 2015)*.
- The effect was dose-dependent, with inhibition of all species >97% when 9.5 mg/mL LF (equivalent to the highest concentration detected in preterm breast milk) was used.
- No significant effect on growth inhibition was seen when 0.5 mg/mL LF (the lowest concentration detected in preterm breast milk) was added to LBWF.
- The other AMPs that were tested did not show similar efficacy in inhibiting pathogens.

LBWF, low birth weight formula.

Trend S, et al. *PLoS One*. 2015;10(2):e0117038.

Gaps in the Current Knowledge (1)

- Dosages → likely higher than 100 mg/kg *(Manzoni, 2011)*, but how high? Fixed or pro-kg dosage?
- Dosing/Schedule → once a day? Or many times a day (mimicking human milk)?
- Duration → in preterms, how long? And in infants, how long and since when?
- Interactions with human milk → better effects when added to HM or to formula?
- Interactions with probiotics → better effects when added to BB or LB strains?

BB, *Bifidobacterium;* LB, *Lactobacillus.*

Manzoni P, Mostert M, Stronati M. *Curr Opin Infect Dis.* 2011;24(3):177-182.

Gaps in the Current Knowledge (2)

- Short-term and long-term safety
- Effect on other outcomes of prematurity (eg, ROP, BPD)
- Generalizability of the bovine LF findings
- Generalizability also to Human Recombinant Lactoferrin (*Talactoferrin*) → the clinical preliminary data of THLF are somewhat disappointing (?)
- Is there a possible protective effect of LF extended also to toddler's age when BLF is administered in the early ages of life such as in the NICU?

Manzoni P, Mostert M, Stronati M. *Curr Opin Infect Dis.* 2011;24(3):177-182.

Conclusions

- Lactoferrin is a multifunctional protein with anti-infective activity
- Lactoferrin is a natural, key player in the gut and systemic health of preterm and term neonates, and young infants
- In clinical trials, bovine lactoferrin supplementation was demonstrated to prevent NEC and sepsis in neonates
- Lactoferrin appears to be safe and well tolerated, although long-term studies are needed
- Very promising findings already available, but many unanswered questions and much ongoing research needed