Clinical Studies of Lactoferrin in Neonates and Infants: An Update

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Disclosure

Speaker fee from:
Prolacta, MJN, Nestlè
Audience Poll

Which of the following HM factors is/are likely associated with the anti-infective benefits of human milk in a time-dependent, dose-dependent and concentration-dependent manner?

1)Lysozime
2)Lactoferrin
3)LC-PUFA
4)HMOS

Outline

- Human milk and its benefits
- Lactoferrin- a major bioactive protein in human and mammalian milk
- Preclinical data on Lactoferrin
- Clinical data on Lactoferrin in neonates and infants
- Unmet needs and current gaps
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.


Human fresh milk feeding prevents infections in neonates

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wks)</td>
<td>0.80</td>
<td>0.68-0.95</td>
<td>.009</td>
</tr>
<tr>
<td>Apgar score at 5 minutes</td>
<td>0.93</td>
<td>0.77-1.14</td>
<td>.494</td>
</tr>
<tr>
<td>Days without enteral feedings</td>
<td>1.03</td>
<td>0.99-1.07</td>
<td>.153</td>
</tr>
<tr>
<td>(NPO)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical ventilator days</td>
<td>1.01</td>
<td>0.99-1.03</td>
<td>.184</td>
</tr>
<tr>
<td>Human milk-fed</td>
<td>0.43</td>
<td>0.23-0.81</td>
<td>.100</td>
</tr>
</tbody>
</table>

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

# Major Bioactive Factors in Human Milk

<table>
<thead>
<tr>
<th>Component</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cells</strong></td>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
<td>Protection against infection, T-cell activation</td>
</tr>
<tr>
<td>Stem cells</td>
<td>Regeneration and repair</td>
</tr>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td></td>
</tr>
<tr>
<td>IgA/IgG1</td>
<td>Pathogen binding inhibition</td>
</tr>
<tr>
<td>IgG</td>
<td>Anti-microbial, activation of phagocytosis (IgG2, IgG3); anti-inflammatory; response to allergens (IgG4)</td>
</tr>
<tr>
<td>IgM</td>
<td>Agglutination, complement activation</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>Stimulation of the acute phase response, B cell activation, proinflammatory</td>
</tr>
<tr>
<td>IL-7</td>
<td>Increased thymic size and output</td>
</tr>
<tr>
<td>IL-8</td>
<td>Recruitment of neutrophils, proinflammatory</td>
</tr>
<tr>
<td>IL-10</td>
<td>Repressing Th1-type inflammation, induction of antibody production, facilitation of tolerance</td>
</tr>
<tr>
<td>IFNg</td>
<td>Proinflammatory, stimulates Th1 response</td>
</tr>
<tr>
<td>TGFb</td>
<td>Anti-inflammatory, stimulation of T cell phenotype switch</td>
</tr>
<tr>
<td>TNFa</td>
<td>Stimulates inflammatory immune activation</td>
</tr>
<tr>
<td><strong>Chemokines</strong></td>
<td></td>
</tr>
<tr>
<td>G-CSF</td>
<td>Tropic factor in intestines</td>
</tr>
<tr>
<td>MIF</td>
<td>Macrophage Migratory Inhibitory Factor: prevents macrophage movement, increases anti-pathogen activity of macrophages</td>
</tr>
<tr>
<td><strong>Cytokine Inhibitors</strong></td>
<td></td>
</tr>
<tr>
<td>TNFR1 and 2</td>
<td>Inhibition of TNFα, antiinflammatory</td>
</tr>
</tbody>
</table>

Lactoferrin: A Multifunctional Milk Protein

- Glycoprotein (80kDa) with iron binding property
- Major whey protein in human milk
- Also found in other mucosal secretions, such as tears and saliva, as well as in plasma, neutrophils and epithelial cells
- Resistant to degradation in the newborn digestive tract
- LF historically low in infant formulas due to low levels in bovine milk; newer technology allows LF to be concentrated for addition to infant formula
- Bovine and human lactoferrin share strong (77%) sequence homology and the same antimicrobial peptide (n-Lactoferricin)
- Bovine LF is resistant to proteolytic digestion
- Of note, both piglet and human intestinal epithelial cells express lactoferrin receptors
Concentrations of Lactoferrin Decrease In Mature Human Milk vs Colostrum

This decrease typically occurs in all mammals

<table>
<thead>
<tr>
<th>Milk</th>
<th>Concentrations of lactoferrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woman</td>
<td>2 (mature milk) – 6 (colostrum) mg/ml</td>
</tr>
<tr>
<td>Cow</td>
<td>0.2-0.5 mg/ml</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt;50 mcg/ml</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;50 mcg/ml</td>
</tr>
<tr>
<td>Dog</td>
<td>&lt;50 mcg/ml</td>
</tr>
<tr>
<td>Goat</td>
<td>0.2 mg/ml</td>
</tr>
<tr>
<td>Pig</td>
<td>0.2 mg/ml</td>
</tr>
</tbody>
</table>

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-sequestrating (→ 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
Functions of Lactoferrin in Gut Development and Immune Defense

Intestinal development
- Improves intestinal mucosal structure, increased villus height and crypt proliferation, less diarrhea\(^1,2\)

Antimicrobial effects
- Inhibits growth, adhesion, translocation, and virulence of pathogens\(^3,4\)
- Sequesters iron

Immune modulation
- Stimulates cells involved in innate and acquired immunity\(^5\)

Lactoferrin and Intestinal Mucosa Development: Preclinical Data

Newborn piglets fed cow milk with low vs. high LF content

Feeding with high LF milk for 30 days improved intestinal mucosal structure compared to control milk, with greater villus height and reduced crypt depth in ileum.

OM=ordinary cows milk. LFM=cows milk enhanced with recombinant human lactoferrin.

Supplementation of bovine milk for 7 days with lactoferrin significantly decreased bacterial translocation through the mucosal epithelium.

Lactoferrin and Immune Defense: Preclinical Data

**Mice** fed bovine milk with or without LF supplementation

Supplementation of bovine milk for 7 days with lactoferrin significantly decreased bacterial translocation through the mucosal epithelium.

- PHA-stimulated peripheral lymphocyte proliferation increased 36% ($P<$0.01)
- Spleen lymphocyte proliferation increased 258% ($P<$0.01)
- Enhanced serum immune indices:
  - IgG, +20% ($P<$0.05)
  - IgA, +13% ($P<$0.05)
  - IgM, +15% ($P<$0.05)
  - IL-2, +12% ($P<$0.01)
  - Decreased diarrhea ($P<$0.05)

Results support possible use of lactoferrin to improve immune function

Lactoferrin and Immune Modulation: Preclinical Data

Weaning piglets received basal diet with or without dietary supplementation with bovine lactoferrin for 15 days

- PHA-stimulated peripheral lymphocyte proliferation increased 36% ($P<$0.01)
- Spleen lymphocyte proliferation increased 258% ($P<$0.01)
- Enhanced serum immune indices:
  - IgG, +20% ($P<$0.05)
  - IgA, +13% ($P<$0.05)
  - IgM, +15% ($P<$0.05)
  - IL-2, +12% ($P<$0.01)
  - Decreased diarrhea ($P<$0.05)

Results support possible use of lactoferrin to improve immune function
BACKGROUND:
Bovine lactoferrin (bLf) reduces Staphylococcus aureus infection in premature infants and promotes the growth of Bifidobacterium infantis, a predominant infant gut species. We hypothesized that bLf in combination with B. infantis would reduce the severity of systemic S. aureus infection.

OBJECTIVE:
The aim was to determine the effects of oral administration of bLf and B. infantis on the course of systemic S. aureus infection.

METHODS:
Colostrum-deprived piglets were fed formulas containing 4 g whey/L (CON group) or bLf (LF group). One-half of the piglets in each group were gavaged with B. infantis (10⁹ colony-forming units/d), resulting in 2 additional groups (BI or COMB, respectively). On day 7, piglets were intravenously injected with S. aureus. Blood samples were collected preinfection and every 12 h postinfection for immune analyses. Tissue samples were collected on day 12 for analysis of bacterial abundance and gene expression.

RESULTS:
After infection, dietary bLf increased piglet weight gain, reduced staphylococcal counts in the kidneys, and tended to lower staphylococcal counts in the lungs and heart. Dietary bLf also decreased kidney IL-10 and increased lung interferon γ (IFN-γ) mRNA.
CONCLUSIONS:
Dietary bLf and B. infantis produced independent and tissue-specific effects. 
Piglets fed bLf alone or in combination with B. infantis mounted a more effective immune response and exhibited lower bacterial abundance. 
This study provides biological underpinnings to the clinical benefits of bLf observed in preterm infants, but does not support B. infantis administration during S. aureus infection.

Lactoferrin and Its Trophic Effect on The Enterocytes and Gut Function in human infants

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


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


humanmilkscience.org
This study measured the content of LF and the microbiota of breast milk and feces of infants of 48 mother–infant pairs (34 full-term and 14 pre-term infants) at birth and 30 days after delivery.

**LF content of breast milk:**
- In the term group, a significant decrease of mean LF concentration between colostrum (7.0 ± 5.1 mg/ml) and mature milk (2.3 ± 0.4 mg/ml) was observed.
- In pre-term group, breast milk LF levels were similar to those observed in full-term group.

**LF content of feces:**
- Fecal LF concentration of healthy infants was extremely high both in term and pre-term infants, higher than the amount reported in healthy children and adults.
- In term infants mean fecal LF levels significantly increased from birth (994 ± 1,828 lg/ml) to 1 month of age (3,052 ± 4,323 lg/ml).
- The amount of LF in the feces of 30 day-old term infants was significantly associated with maternal mature milk LF concentration (p = 0.030), confirming that breast milk represents the main source of LF found in the gut of infants.
- In pre-term infants higher mean concentrations of fecal LF at birth (1,631 ± 2,206 lg/ml) and 30 days after delivery (7,633 ± 9,960 lg/ml) were observed in comparison to full-term infants.
Microbiota composition and relationships with LF intakes:

- The amount of fecal bifidobacteria and lactobacilli resulted associated with the concentration of fecal LF at measurements at 3 days after delivery ($p = 0.01$ and $p = 0.02$, respectively).

Conclusions and speculations:

- These results suggest that Lactoferrin promotes a bifidogenic microflora in the gut in neonates and preterms.
- High levels of fecal LF in neonates, particularly in the first days of life, could represent an important factor in the initiation, development and/or composition of the neonatal gut microbiota.
- Since early host–microbe interaction is a crucial component of healthy immune and metabolic programming, high levels of fecal LF in neonates may beneficially contribute to the immunologic maturation and well-being of the newborn, especially in pre-term infants.


Plasma Lactoferrin levels in newborn preterm infants with sepsis

Journal of Materna-Fetal & Neonatal Medicine, 2017

- 15 VLBW neonates with confirmed or suspected sepsis.
- LF serum concentration was significantly lowest in 4 neonates with confirmed sepsis than in 11 with clinical sepsis.
  - The AUC was 0.90 (95%CI: 0.63-0.99)
  - The optimal cut-off for LF was <1.2 mg/ml with a sensibility of 100% and a specificity of 81.8%.
- LF serum concentration was positively correlated with WBC or neutrophil

These data underline the role of plasma LF (in addition to that of milk LF) in neonatal sepsis, supporting the need of oral LF supplementation in human newborns to prevent infection or decrease the severity of an existing infection.
A review of the Clinical Evidence In Support of Lactoferrin in neonates and infants

Benefits to health and immune function

Multiple studies have suggested a number of potentially favorable biologic effects associated with lactoferrin (LF)

- King et al JPGN 2007
- Egashira et al Acta Paed 2007
- Ochoa et al CID 2008
- Zuccotti et al JBRHA 2009
- Manzoni et al JAMA 2009
- Manzoni et al Pediatrics 2012
- Ochoa et al J Peds 2013
- Manzoni et al EHD 2014
- Mungan et al AJOP 2014
- Ochoa et al PIDJ 2015
- Kaur et al J Trop Pediatr 2015
- LIFT ANZ Study, in press, 2018
Healthy, formula-fed infants, >34 weeks’ gestation and 4 weeks of age, were randomized to receive for 12 months:

A- formula supplemented with lactoferrin (850 mg/L), or
B- commercial cow milk–based formula (102 mg/L)

Hematologic and illness data was recorded during the first year of life.

Primary Outcomes: Growth parameters; gastrointestinal, respiratory, colic illnesses in the first year.
Effect of Lactoferrin supplementation on Respiratory Morbidity in Term or Near-Term Infants: Results

The Lactoferrin-enhanced formula was well tolerated.

Lactoferrin supplementation was associated with significantly fewer lower respiratory tract illnesses (0.15 episodes/year vs 0.5 episodes/year), primarily wheezing, in the first year.


Egashira Acta Paed 2007
Lactoferrin and Rotavirus Infection and Symptoms in Children

Children <5 years were given a LF supplement (100 mg/day) daily for 12 weeks

Oral lactoferrin supplementation was associated with reduced clinical severity, but not incidence, of rotavirus infection.

Ochoa CID 2008
**Design** → RCT; patients were randomly assigned to receive lactoferrin (0.5 g/day; Tatua Nutritionals) or maltodextrin (0.5 g/day) for 9 months.

**Assessments** → Community HCPs visited the children twice daily, 6 days per week for 9 months to give the supplement. Children were evaluated monthly by a physician at the outpatient clinic.

**Definitions and Outcomes** →

- **Diarrhea** = 3 loose or watery stools in a 24-h period or 1 loose stool containing blood.
- **Colonization** = identification of a pathogen (viral, bacterial, or parasitic) from a child without diarrhea for at least 7 days before and after the sample was collected.

**Hypothesis** → lactoferrin given daily will decrease the prevalence of pathogen colonization and/or diarrheal illness in previously weaned children aged 12–36 months.

**Ochoa TJ et al. CID 2008**

- The overall incidence of diarrhea in the study groups was similar (1.3 vs. 1.2 episodes/child-year (p = NS).  
- The mean (SD) duration of diarrhea episodes was not significantly different in the lactoferrin and placebo groups (1.91 vs. 2.61 days).

**✓ However →** there was significantly lower incidence of colonization with *Giardia spp* in the LF group.
Immune modulation by lactoferrin and curcumin in children with recurrent respiratory infections.

Zuccotti GV, Trabattoni D, Morelli M, et al

The clinical and immunologic effects of lactoferrin and curcumin (LC) oral supplementation were examined in healthy children with recurrent respiratory tract infections (RTI).

LC supplementation resulted in all of the following immunological changes:
- Significant skewing of CD8+T lymphocytes maturation.
- Significant increase of CD14+, toll like receptor (TLR) 2-expressing cells
- Significant decrease of CD14+/TLR4+
- Significant reduction of IL10 production by CD14+ cells

Conclusions → LC supplementation results in immune modulation and could be clinically beneficial.
Manzoni et al JAMA 2009

The First RCT on Lactoferrin Feeding in Neonates

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

Paolo Manzoni; Matteo Rinaldi; Silvia Cattani; et al.


on behalf of the GSIN - Italian Collaborative Group for the study and prevention of Neonatal Infections, affiliated to the Italian Society of Neonatology


humanmilkscience.org
Design of the Study

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

Randomization

- Group A1 — Lactoferrin 100 mg (LF100®, Dico farm spa, Rome, Italy)
- Group A2 — Lactoferrin 100 mg (LF100®), + Lactobacillus GG, 6 x 10⁹ CFU/day (Dicoflor 60®, both Dico farm 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 1,000g-weighing preterms fed fresh maternal milk during their first two weeks of life

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

CFU = colony-forming units.

### Demographics, Clinical, Nutritional Characteristics:
No Differences Between Groups

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


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

<table>
<thead>
<tr>
<th></th>
<th>LF combined n = (153+151) = 304</th>
<th>PLACEBO n = 168</th>
<th>R.R.</th>
<th>95% C.I.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late-Onset sepsis (all agents)</td>
<td>5.3%</td>
<td>17.3%</td>
<td>0.28</td>
<td>0.16-0.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LOS by Gram-Positive</td>
<td>1.2%</td>
<td>5.4%</td>
<td>0.21</td>
<td>0.07-0.82</td>
<td>0.02</td>
</tr>
<tr>
<td>LOS by Gram-Negative</td>
<td>3.4%</td>
<td>6.5%</td>
<td>0.48</td>
<td>0.35-0.98</td>
<td>0.05</td>
</tr>
<tr>
<td>LOS by Candida spp (IFI)</td>
<td>0.7%</td>
<td>5.4%</td>
<td>0.24</td>
<td>0.09-0.77</td>
<td>0.009</td>
</tr>
<tr>
<td>NEC</td>
<td>0.8%</td>
<td>6.0%</td>
<td>0.16</td>
<td>0.09-0.58</td>
<td>0.001</td>
</tr>
<tr>
<td>Mortality (all causes prior to discharge)</td>
<td>3.3%</td>
<td>7.1%</td>
<td>0.39</td>
<td>0.15-0.92</td>
<td>0.04</td>
</tr>
<tr>
<td>Death or NEC</td>
<td>4.1%</td>
<td>13.1%</td>
<td>0.30</td>
<td>0.16-0.70</td>
<td>0.007</td>
</tr>
</tbody>
</table>

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

Lactoferrin and Sepsis Risk in Preterm Infants

Very low birth weight infants (<1500 g) were supplemented daily with LF, LF+Lactobacillus GG, or placebo to 30 days of age. Supplemental lactoferrin reduced incidence of late-onset sepsis in VLBW infants.

Manzoni et al Pediatrics 2013
**LACTOFERRIN prevents also Candida infections**


<table>
<thead>
<tr>
<th></th>
<th>LF (± LGG)</th>
<th>Placebo</th>
<th>O.R.</th>
<th>95% C.I.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida Enteric Colonization</td>
<td>17.1%</td>
<td>13.9%</td>
<td></td>
<td>0.24</td>
<td>0.43</td>
</tr>
<tr>
<td>Candida Systemic Infection</td>
<td>0.8%</td>
<td>5.4%</td>
<td></td>
<td>0.09-0.77</td>
<td>0.009</td>
</tr>
<tr>
<td>Rate of Progression from Candida Colonization to Infection</td>
<td>7.8%</td>
<td>41.9%</td>
<td>0.29</td>
<td>0.09-0.89</td>
<td>0.02</td>
</tr>
<tr>
<td>Mortality attributable to Candida spp</td>
<td>0%</td>
<td>1.2%</td>
<td></td>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

➢ Please note: LF decreased infections but not colonization rates in the gut

→ ....does it mean that Bovine Lactoferrin prevent pathogens’ translocation through enhancement of gut maturation, just like experimental data on cell lines had shown?

**Manzoni et al EHD 2014**
Lactoferrin and NEC in Preterm Infants

VLBW infants (<1500 g) were supplemented daily with LF, LF+ Lactobacillus GG, or placebo to 30 days of age

Supplemental lactoferrin decreased the incidence of necrotizing enterocolitis and/or mortality in VLBW infants

Ochoa J Peds 2013
Theresa J. Ochoa, Elsa Chea-Woo, Nelly Baiocchi et al
Randomized double-blind controlled trial of bovine lactoferrin for prevention of diarrhea in children.

Objective

✓ To determine the effect of bovine lactoferrin on prevention of diarrhea in children.

Study design

✓ Community-based double-blind placebo controlled RCT comparing supplementation with bovine lactoferrin versus placebo.
✓ Enrolments occurred at 12–18 months
✓ Children received 0.5g twice a day of bLF or placebo (diluted in 25 mL of water).
✓ The dose of lactoferrin was chosen based on the estimated amount consumed by a breastfeeding 12 month old previously weaned child
✓ Children were followed-up for 6 months with daily home visits for data collection and supplement administration. Anthropometric measures were done monthly.

Results

✓ 277 children were randomized to lactoferrin and 278 to placebo; 65 dropped out; 147,894 doses were administered (92% compliance).
✓ Overall there were 1,235 diarrhea episodes lasting 6,219 mean days.
✓ The diarrhea incidence was not different between groups: 5.4 vs. 5.2 episodes/child/year for lactoferrin and placebo, respectively (p=0.375).
✓ However, the diarrhea longitudinal prevalence was lower in the lactoferrin group (6.6% vs. 7.0%, p=0.017) as well as the median duration of episodes (4.8 vs. 5.3 days, p=0.046), proportion of episodes with moderate or severe dehydration (1.0% vs. 2.6%, p=0.045) and liquid stools load (95.0 vs. 98.6) liquid stools/child/year, p<0.001).
✓ There were no adverse events related to the intervention.

Conclusions

✓ Although there was no decrease in diarrhea incidence, longitudinal prevalence and severity of diarrhea were decreased with lactoferrin.
Mungan AJOP 2014

Single-Center, RCT of ORAL BOVINE LACTOFERRIN PROPHYLAXIS (200 mg/kg/die) for prevention of SEPSIS and NEC in VLBW NEONATES in NICU, and EFFECT ON T-REGULATORY CELLS

I. Mungan Akin1, B. Atasay1, F. Dogu2, E. Okulu1, S. Arsan1, H.D. Karatas2, S. Alan1, A. Kiliç1, A. Ikinciogullari2, T. Turmen1

ClinicalTrials.gov Identifier: NCT01287507

1 Ankara University Faculty of Medicine Department of Neonatology. Turkey
2 Ankara University Faculty of Medicine Department of Pediatric Allergy and Immunology. Turkey

<table>
<thead>
<tr>
<th></th>
<th>Lactoferrin (n=25)</th>
<th>Placebo (n=25)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>29.5 ±1.6</td>
<td>30.3 ±2.5</td>
<td>0.19</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1290 ±346</td>
<td>1307 ±262</td>
<td>0.84</td>
</tr>
<tr>
<td>SNAPPE-II</td>
<td>7 (0-43)</td>
<td>5 (0-47)</td>
<td>0.54</td>
</tr>
<tr>
<td>Culture proven sepsis (total number of episodes)</td>
<td>4</td>
<td>14</td>
<td>0.03</td>
</tr>
<tr>
<td>Stage 1 NEC (n) (%)</td>
<td>1 (4%)</td>
<td>1 (4%)</td>
<td>1</td>
</tr>
<tr>
<td>Stage 2 and 3 NEC (n) (%)</td>
<td>0</td>
<td>5 (20%)</td>
<td>0.05</td>
</tr>
<tr>
<td>Mortality (n) (%)</td>
<td>0</td>
<td>1 (4%)</td>
<td>1</td>
</tr>
<tr>
<td>Duration of hospitalisation</td>
<td>36.4 ±14</td>
<td>32.4 ±13</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Am J Perinatol 2014
Design and Methods

- Peru – NEOLACTO study
- Infants 500-2500 g at birth
- Randomization <72 hrs, block-stratified (4 blocks). ITT analysis
- **Intervention**: oral bovine LF (200 mg/kg/day) or placebo (maltodextrin - 200 mg/kg/day), both given in three divided doses each day.

- Initiation: as soon as the patient started receiving any amount of oral or tube feedings
- Duration: four weeks since the day of enrolment
- **Primary outcome**: risk of first episode of late-onset clinically-defined sepsis (culture-proven sepsis and culture-negative clinical infection) within 4 weeks (28 days) from enrollment.
- **Secondary outcomes**: frequency of culture-proven sepsis, pathogen-specific late-onset sepsis; necrotizing enterocolitis (NEC), duration of hospitalization, overall mortality rate, infection-related mortality, frequency of adverse events, and treatment intolerance
**RESULTS**

- Finally enrolled 190 neonates; 80 (42.1%) had a birth weight <1500g
- The intervention was administered completely per protocol in 82% of 3,244 child-days of observation.
- It was started on average at 4.0 ± 1.4 dol
- Of interest, after day 10 of intervention, there was 1 sepsis episode in the LF group versus 6 in the placebo group

<table>
<thead>
<tr>
<th></th>
<th>Lactoferrin (n=95)</th>
<th>Placebo (n=95)</th>
<th>R.R. and 95% C.I</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age (wk)</td>
<td>32.2 ± 2.6 [26-38]</td>
<td>32.0 ± 2.6 [26-37]</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1,582.5 ± 421.8 [770-2,482]</td>
<td>1,600.3 ± 395.2 [710-2,470]</td>
<td>ns</td>
<td></td>
</tr>
<tr>
<td>Complete supplement administration in days, mean ± SD</td>
<td>12.5 ± 8.1</td>
<td>13.9 ± 8.0</td>
<td>0.23</td>
<td></td>
</tr>
</tbody>
</table>

**MAIN RESULTS**

<table>
<thead>
<tr>
<th></th>
<th>Lactoferrin (n=95)</th>
<th>Placebo (n=95)</th>
<th>R.R. and 95% C.I</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LOS episodes</td>
<td>12/95 (12.6%)</td>
<td>21/95 (22.1%)</td>
<td>0.57 (0.30-1.09)</td>
<td>0.085</td>
</tr>
<tr>
<td>LOS in &lt;1500g</td>
<td>8/40 (20 %)</td>
<td>15/40 (37.5 %)</td>
<td>0.53 (0.26-1.12)</td>
<td>0.047</td>
</tr>
<tr>
<td>LOS in 1501-2500g</td>
<td>4/55 (7.3%)</td>
<td>6/55 (10.9%)</td>
<td>0.67 (0.20-2.23)</td>
<td></td>
</tr>
<tr>
<td>Age at onset of first LOS episode, mean days</td>
<td>6.3 (3-12)</td>
<td>9.25 (4-25)</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>NEC (surgical stage)</td>
<td>3/93 (3.2%)</td>
<td>2/95 (2.1%)</td>
<td></td>
<td>0.68</td>
</tr>
<tr>
<td>Overall Mortality (all causes)</td>
<td>7/95 (7.4%)</td>
<td>3/95 (4.1%)</td>
<td></td>
<td>0.31</td>
</tr>
<tr>
<td>Sepsis-attributable mortality</td>
<td>4/95 (4.2%)</td>
<td>2/95 (2.1%)</td>
<td></td>
<td>0.68</td>
</tr>
</tbody>
</table>
India

3rd level NICU; single-center study

Double-blind, placebo controlled, RCT

LBW infants (BW less than 2,000g)

BLF in sachets to be diluted in feeds

BLF dosing: 100 to 200 mg/day, according to the increasing BW 1,000 to 2,000g

First 30 days of life, starting <48hrs of life

Main outcome: first episode of proven Late-Onset Sepsis (LOS)

Secondary outcome: probable (clinically suspected) LOS

Main results

Table 2. Primary and secondary outcomes among the BLF and placebo groups

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Lactoferrin (n = 63)</th>
<th>Placebo (n = 67)</th>
<th>p value</th>
<th>RR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Culture proven sepsis [n (%)]</td>
<td>2 (3.2)</td>
<td>9 (13.4)</td>
<td>0.036</td>
<td>0.211</td>
<td>0.044–1.019</td>
</tr>
<tr>
<td>Bacterial sepsis [n (%)]</td>
<td>2 (3.2)</td>
<td>8 (11.9)</td>
<td>0.061</td>
<td>0.242</td>
<td>0.049–1.186</td>
</tr>
<tr>
<td>Fungal sepsis [n (%)]</td>
<td>0 (0)</td>
<td>1 (1.5)</td>
<td>0.330</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Secondary outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable sepsis [n (%)]</td>
<td>4 (6.3)</td>
<td>14 (20.9)</td>
<td>0.016</td>
<td>0.257</td>
<td>0.08–0.822</td>
</tr>
<tr>
<td>Any sepsis [n (%)]</td>
<td>6 (9.5)</td>
<td>23 (34.3)</td>
<td>0.001</td>
<td>0.201</td>
<td>0.076–0.537</td>
</tr>
<tr>
<td>Sepsis-attributable mortality [n (%)]</td>
<td>0 (0)</td>
<td>5 (7.5)</td>
<td>0.027</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

✓ Added value → they tested increasing dosing proportional to BW
✓ Weakness → Analysis ultimately conducted on neonates >1000 and <2000g
✓ NNT: 10
✓ Statistical power : 0.57
✓ Ideal sample size would have been 114 per arm in order to have 80% power

Barrington J Perinatol 2016
**OBJECTIVE** ➔ To determine tolerability of bovine lactoferrin (bLF) in very preterm infants, and whether the intervention can be adequately masked.

**STUDY DESIGN** ➔ a single-center, masked pilot trial of infants <31 weeks ga. The primary outcome was feeding tolerance, defined as time to achieve full feeds (140 ml kg(-1) per day). Parents answered a short questionnaire regarding acceptability of the intervention.

**RESULTS** ➔ 79 infants were enrolled and analyzed according to intention to treat. There was no effect of bLF on the primary outcome. In addition, mortality, late onset sepsis and other complications of prematurity were no different. Equal numbers of parents in both groups believed their infant received bLF.

**CONCLUSION** ➔ bLF is well tolerated, easy to administer and its presence in prepared milk is not evident.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sepsis in bLF–treated</th>
<th>Sepsis in Placebo</th>
<th>95% C.I.</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barrington K et al 2016</td>
<td>79 neonates &lt;31 wks g.a</td>
<td>17.5%</td>
<td>20.5%</td>
<td>No Report</td>
</tr>
</tbody>
</table>

**Sherman J Pediatr 2016**
Randomized Controlled Trial of Talactoferrin Oral Solution in Preterm Infants

Michael P Sherman, MD, PhD (hc)1,2, David H Adamkin, MD2, Victoria Niklas, MD3,†, Paula Radmacher, PhD2, Jan Sherman, PhD1,4, Fiona Wertheimer, DO5, and Karel Petrik, PhD6,†

Objective—To evaluate safety and explore efficacy of recombinant human lactoferrin (talactoferrin, TLf) to reduce infection.

Study design—Randomized, double blind, placebo-controlled trial in 60 infants with birth weights of 750 to 1500 grams. Each infant received enteral TLf or placebo on day 1 through 28 days of life; TLf dose was 150 mg/kg/12 hour. Primary outcomes were bacteremia, pneumonia, urinary tract infection, meningitis and necrotizing enterocolitis. Secondary outcomes were sepsis syndrome and suspected NEC. We recorded clinical, laboratory and radiologic findings, diseases, and adverse events in a database used for statistical analyses.

Results—Infants in the two groups had similar demographics. No enteral or organ-specific adverse events to TLf occurred. Hospital-acquired infections in the group with TLf were 50% of that observed in infants fed placebo (p<0.04), including fewer blood or line infections, urinary tract infections, and pneumonia. Non-infectious outcomes did not differ statistically in the two arms. No differences in growth or neurodevelopment occurred among infants treated with TLf and placebo during a one-year, post-hospitalization period.

Conclusion—We found no clinical or laboratory toxicity and a trend towards less infectious morbidity in infants treated with TLf.

Main results

<table>
<thead>
<tr>
<th>Patients</th>
<th>Sepsis in Talactoferrin group(s)</th>
<th>Sepsis in Placebo</th>
<th>95% C.I.</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 neonates&lt;1500g</td>
<td>17%</td>
<td>33%</td>
<td>0.26-0.99</td>
<td>0.04</td>
</tr>
</tbody>
</table>

However please note:

SECONDARY OUTCOMES IN BREAST-FED INFANTS

<table>
<thead>
<tr>
<th>Breast-fed</th>
<th>Talactoferrin (n = 45)</th>
<th>Placebo (n = 46)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary efficacy endpoints/outcomes</td>
<td>N</td>
<td>n (%)</td>
<td>N</td>
</tr>
<tr>
<td>Suspected NEC</td>
<td>45</td>
<td>6/46 (13)</td>
<td>46</td>
</tr>
<tr>
<td>Neonatal Sepsis Syndrome or Inflammatory Response Syndrome</td>
<td>45</td>
<td>3/46 (8)</td>
<td>46</td>
</tr>
</tbody>
</table>

10/08/18
Metanalysis of the existing RCTs: the COCHRANE Review

Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants (Review)

Pammi M, Suresh G. Cochrane Review of RCTs of lactoferrin in preterm infants.
Cochrane Database of Systematic Reviews: 2017

Six RCTs in 1071 preterm infants.
Three co-primary outcomes:

- Late Onset Sepsis (n=886)
- NEC ≥ stage II (n=750)
- Hospital mortality (n=1071)
### Effect of LF on Late Onset Sepsis.

**Cochrane Review of 6 RCTs in 886 infants**

**Risk Ratio 0.59 (95% CI 0.40 to 0.87; P=0.008)**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Lactoferrin Events Total</th>
<th>Control Events Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akim 2014</td>
<td>4</td>
<td>22</td>
<td>8</td>
<td>0.57 [0.20, 1.63]</td>
<td></td>
</tr>
<tr>
<td>Santhi 2016</td>
<td>7</td>
<td>40</td>
<td>10</td>
<td>0.90 [0.23, 1.61]</td>
<td></td>
</tr>
<tr>
<td>Kaur 2015</td>
<td>2</td>
<td>83</td>
<td>9</td>
<td>0.24 [0.05, 0.85]</td>
<td></td>
</tr>
<tr>
<td>Marzoni 2014</td>
<td>9</td>
<td>153</td>
<td>20</td>
<td>0.34 [0.17, 0.70]</td>
<td></td>
</tr>
<tr>
<td>Ochoa 2015</td>
<td>4</td>
<td>55</td>
<td>4</td>
<td>1.00 [0.28, 3.68]</td>
<td></td>
</tr>
<tr>
<td>Sherman 2016</td>
<td>10</td>
<td>59</td>
<td>4</td>
<td>2.54 [0.64, 1.25]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>432</td>
<td>454</td>
<td>100.0%</td>
<td>0.59 [0.40, 0.87]</td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>38</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 11.14$, $df = 5$ ($P = 0.60$); $I^2 = 65$

Test for overall effect: $Z = 2.96$ ($P = 0.003$)

**P = 0.05** - indicates significant heterogeneity between trials

**NNT 17**

Current available evidence graded as “low-moderate quality”

---

### Effect of LF on Necrotising Enterocolitis.

**Cochrane Review of 6 RCTs in 750 infants**

**Risk Ratio 0.40 (95% CI 0.18 to 0.86; P=0.02)**

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Lactoferrin Events Total</th>
<th>Control Events Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akim 2014</td>
<td>2</td>
<td>22</td>
<td>5</td>
<td>0.10 [0.01, 1.76]</td>
<td></td>
</tr>
<tr>
<td>Banington 2016</td>
<td>1</td>
<td>40</td>
<td>2</td>
<td>0.49 [0.05, 5.16]</td>
<td></td>
</tr>
<tr>
<td>Kaur 2015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Not estimable</td>
<td></td>
</tr>
<tr>
<td>Marzoni 2014</td>
<td>5</td>
<td>247</td>
<td>14</td>
<td>0.37 [0.14, 1.02]</td>
<td></td>
</tr>
<tr>
<td>Ochoa 2015</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Not estimable</td>
<td></td>
</tr>
<tr>
<td>Sherman 2016</td>
<td>2</td>
<td>59</td>
<td>1</td>
<td>2.03 [0.19, 21.83]</td>
<td></td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td>368</td>
<td>382</td>
<td>100.0%</td>
<td>0.40 [0.18, 0.86]</td>
<td></td>
</tr>
<tr>
<td><strong>Total events</strong></td>
<td>8</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: $I^2 = 27.4$, $df = 3$ ($P = 0.43$); $I^2 = 0$

Test for overall effect: $Z = 2.33$ ($P = 0.02$)

**NNT 25**

Current available evidence graded as “low quality”
**Effect of LF on Mortality.**  
Cochrane Review of 6 RCTs in 1071 infants  
**Risk Ratio 0.65 (95% CI 0.37 to 1.11; P=0.12)**

Conclusions of Cochrane Review

- Evidence of low quality suggests that lactoferrin supplementation to enteral feeds with or without probiotics decreases late-onset sepsis and NEC stage II or III in preterm infants without adverse effects.

- Completed ongoing trials will provide data from more than 6000 preterm neonates, which may enhance the quality of the evidence.

- Clarification regarding optimal dosing regimens, types of lactoferrin (human or bovine), and long-term outcomes is needed.
### Major ongoing LACTOFERRIN RCTs in infants

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Intervention Group</th>
<th>Control Group</th>
<th>Primary Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enteral Lactoferrin In Neonates (ELFIN);</td>
<td>UK Neonates &lt;32 wks g.a. first 72 h of age (n = 2,200)</td>
<td>Bovine LF 150mg/kg/day (max:300mg) until discharge</td>
<td>Milk with placebo</td>
<td>1. Culture-proven or clinically suspected LOS from trial entry until discharge.</td>
</tr>
<tr>
<td>ISRCTN88261002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactoferrin Infant Feeding Trial (LIFT) to</td>
<td>AUSTRALIA, INDIA, CANADA, ITALY Neonates with BW&lt;1,500 g and g.a. 22–28 wks first 7 days of age (n = 1,100)</td>
<td>Bovine LF 200 mg/kg/day until 34 weeks g.a. corrected or discharge</td>
<td>Breast milk or formula without BLF</td>
<td>1. Incidence of sepsis or brain injury or CLD or NEC or severe ROP</td>
</tr>
<tr>
<td>prevent sepsis and death in preterm infants; ACTRN12611000247976</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### NHMRC Lactoferrin Infant Feeding Trial

Presentation of Headline Results: Torino, Italy – ICCN - 24 and 25 May 2018

**NOT FOR CITATION, TRANSMISSION OR PUBLICATION**

William Tarnow-Mordi, on behalf of the LIFT Trial Management Group and the LIFT ANZ/ Canada Collaborative Study Group, with thanks to Andrew Martin, Rebecca Brown, John Simes, Adrienne Kirby for assistance with this analysis
BMJ Open
Protocol for the Lactoferrin Infant Feeding Trial (LIFT): a randomised trial of adding lactoferrin to the feeds of very low birth weight babies prior to hospital discharge

• LIFT is a blinded RCT to evaluate whether supplementing feeds in VLBW infants with once daily study supplement of lactoferrin vs no lactoferrin (control) reduces the

Primary composite outcome
— Death or Late onset sepsis or brain injury or NEC or ROP

and

Secondary outcomes including
- Death, late onset sepsis, brain injury, NEC, chronic lung disease, blood transfusions

Intervention:
Bovine lactoferrin in breastmilk or formula milk to a daily dose of 200 mg/kg (control group receive no bLF added to breast milk or formula milk), until 34 weeks corrected gestational age or for 2 weeks, whichever is longer, or until discharge home, if earlier.

Power and Sample Size:
A trial of 1,500 participants yields 85% power with 2-sided 5% significance to detect a difference in primary outcome from 26% in controls to 19.5% in the bLF group.

Pre-defined subgroups:
(i) birth-weight <1000 g and 1000-1499 g;
(ii) randomised ≤72 hr and >72 hr from birth;
(iii) those who received or did not receive probiotics
(iv) ≤ 28 weeks and >28 weeks gestation
Figure 1a
CONSORT Diagram – Participant Flow

Assessed for eligibility (n=3,409)

Excluded (n=1,857)
- Clinician refusal: 43
- Parent Refusal: 964
- Potential feeding intolerance: 9
- Congenital anomaly: 49
- Baby likely to transfer to another hospital <1 week: 186
- Long term follow up unlikely: 70
- Other*: 546

*mostly missed/not approached

Randomized (n=1,542)

Enrollment

Figure 1b
CONSORT Diagram – Participant Flow

Randomized (n=1,542)

Lactoferrin

Allocation

Allocated to BLF (n=771)
- Received allocated intervention (n=766)
- Did not receive any study intervention
  - 1 withdrew consent for treatment and follow-up
  - 2 withdrew consent for treatment
  - 1 death

Allocated to Control (n=771)
- Received allocated intervention (n=763)
- Did not receive any study intervention
  - 4 deaths
  - 2 withdrew consent (for treatment)
  - 2 Transfer to non-participating hospital

Control
So then….how Much Lactoferrin Do We Need? (1)

<table>
<thead>
<tr>
<th>Day of life</th>
<th>Human milk intakes (ml/feed) and no. of feeds</th>
<th>Concentration of LF in colostrums and early human milk (mg/ml)</th>
<th>Presumed weight in grams</th>
<th>Estimates of mean daily amounts of human lactoferrin ingested with feeds (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5 x 6</td>
<td>7</td>
<td>1,000</td>
<td>21</td>
</tr>
<tr>
<td>2</td>
<td>0.5 x 8</td>
<td>7</td>
<td>950</td>
<td>26.6</td>
</tr>
<tr>
<td>3</td>
<td>1 x 8</td>
<td>6.5</td>
<td>900</td>
<td>46.8</td>
</tr>
<tr>
<td>4</td>
<td>1 x 12</td>
<td>6.5</td>
<td>850</td>
<td>66.3</td>
</tr>
<tr>
<td>5</td>
<td>2 x 12</td>
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<td>267</td>
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<td>273</td>
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<td>4</td>
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<td>364.8</td>
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<tr>
<td>18</td>
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<td>4</td>
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<td>364.8</td>
</tr>
</tbody>
</table>

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


So then…..how Much Lactoferrin Do We Need? (2)

Table 2: Typical intake of hLF in a 1,000 g infant after starting trophic feeding with breastmilk

<table>
<thead>
<tr>
<th>Day</th>
<th>1-2</th>
<th>3-4</th>
<th>5-6</th>
<th>7-8</th>
<th>9-10</th>
<th>11-12</th>
<th>13-14</th>
<th>15-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>ml feed</td>
<td>0.5</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>no. of feeds</td>
<td>6-8</td>
<td>8-12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mean daily volume of feeds</td>
<td>3-4</td>
<td>8-12</td>
<td>24</td>
<td>36</td>
<td>48</td>
<td>60</td>
<td>72</td>
<td>96</td>
</tr>
<tr>
<td>hLF concentration [mg/ ml]</td>
<td>7</td>
<td>6.5</td>
<td>6</td>
<td>5.5</td>
<td>5</td>
<td>4.5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Presumed weight in grams*</td>
<td>1,000</td>
<td>900</td>
<td>850</td>
<td>870</td>
<td>870</td>
<td>890</td>
<td>920</td>
<td>950</td>
</tr>
<tr>
<td>Mean daily hLF (mg/ kg)</td>
<td>21-27</td>
<td>47-66</td>
<td>130</td>
<td>172</td>
<td>209</td>
<td>267</td>
<td>298</td>
<td>365</td>
</tr>
</tbody>
</table>

*assuming a typical weight loss of up to 15% in the first week

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

LIFT application, NHMHRC, 2014 - unpublished
In summary ......

These models suggest that an ideal newborn, with an ideal maternal fresh milk exposure since birth, would naturally ingest the following daily amounts of bioactive LF:

✓ At least 50 mg/kg at DOL 3
✓ At least 150 mg/kg at DOL 7
✓ Around 300 mg/kg at DOL 15 to 21

Please remember these figures !!!

A possible key to better understanding
⇒ see the following work and keep remembering the previous figures
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.

**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.
- 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.

Levels of LF in breast milk are higher at 7 days than at 21 days ($P<0.001$).
Sepsis in Infants and Levels/Intakes of LF

- 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.

- 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).

Antimicrobial Activity of Lactoferrin When Added to Infant Formula

- In a secondary experiment, it was determined if 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.
Wrapping-up and trying to overcome the existing inconsistencies-
Some speculation arising from the whole of the current data

- Lactoferrin (either human or bovine) supplementation looks providing clinically measurable benefits only when LF intakes from human milk are below a certain threshold.

- Possible protective threshold levels of LF intake (according to the experimental data, and to the natural breastfeeding trends) could be comprised between 50-150 mg/kg at 7 days of life, and between 300-400 mg/kg at 21 days of life.

- When breastfeeding is already providing these intakes, an external supplementation might be no more needed, nor effective, nor conferring additional advantages.

- However, in all situations where LF intake is not what theoretically needed, LF external supplementation could be considered.

Unanswered, pending questions on LF use

- **Dosages** → likely higher than 100 mg/kg, but how high? 150 or 200? 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** → which effects, if any, when added to HM? Which ones to Formula?

- **Interactions with probiotics** → which effects when added to probiotics? Which strains (BB or LB or what)?

BB = Bifidobacterium; LB = Lactobacillus.
Stability of lactoferrin in stored human milk

DE Rollens, PG Radmacher, RM Turcu, SR Myers and DH Adamkin

250mg/dL = 300-375 mg/kg/dia @120-150 mL/kg/dia

150mg/dL = 180-225 mg/kg/dia @120-150mL/kg/dia

Additional unmet needs / Gaps in the Current Knowledge

- Stability of HLF when stored
- Need for Drug-like preparations with fully reliable stability, purity and functional activity of BLF
- Effect on other outcomes of prematurity (e.g. ROP, BPD)
- Generalizability of the bovine LF findings also to Human Recombinant Lactoferrin (Talactoferrin), and also to toddler’s age
- Short-term and long-term safety

Thank you for your attention

Twenty-six children were enrolled in each group.
The overall incidence of diarrhea in the study was 1.3 episodes/child-year.
In the lactoferrin and placebo groups, the diarrhea incidence was 1.3 versus 1.2 episodes/child-year, and the mean prevalence of diarrhea was 0.7% versus 0.9% (p = NS).
The mean (SD) duration of diarrhea episodes was not significantly different in the lactoferrin and placebo groups (1.91 vs. 2.61 days).
Colonization rates with common pathogens were similar in both groups, with the exception of a lower frequency of Giardia-positive samples in the lactoferrin group (colonization rate for Giardia species: 9.6% in lactoferrin group vs. 17.2% in placebo group (p <0.05))

Results

Ochoa TJ et al. CID 2008
Impact of Lactoferrin Supplementation on Growth and Prevalence of Giardia Colonization in Children

Theresa J. Ochoa,1* Elia Cho Roces,2 Miguel Coppel,3 Iris Perzan,3 Ana Prada,4 Robert J. Mcmahon,5 and Thomas G. Cleary6
1Universidad Peruana Cayetano Heredia, Lima, Peru, 2Center for Infectious Diseases, University of Texas School of Public Health, Houston, and 3Mead Johnson Nutritionals, Evansville, Indiana

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Does daily intake of bovine lactoferrin-containing products ameliorate rotaviral gastroenteritis?

Egashira M, Takayanagi T, Moriuchi M, Moriuchi H.

The effects of the oral administration of lactoferrin against viral gastroenteritis, where rotavirus or norovirus was identified as a pathogen, have been reported. It had been speculated that a daily intake of bovine lactoferrin containing products may ameliorate the severity of the disease in children affected by rotaviral gastroenteritis.

This trial involved 238 children < 5 years of age. Children were randomized to LF (100 mg/day) daily for 12 weeks. Lactoferrin supplementation did not significantly reduce the incidence of rotavirus-induced gastroenteritis (5.1% in control vs 4.4% in treated group). However, lactoferrin did significantly reduce the frequency and duration of both vomiting and diarrhea (all P<0.05).

Conclusion: bovine lactoferrin may be beneficial in reducing the frequency and duration of vomiting and diarrhea from rotaviral gastroenteritis.