Published in final edited form as:
Biometals. 2014 October ; 27(5): 1007–1016. doi:10.1007/s10534-014-9754-3.

Lactoferrin for prevention of neonatal sepsis

Christie G. Turin[1], **Alonso Zea-Vera**[1], **Alonso Pezo**[1], **Karen Cruz**[1], **Jaime Zegarra**[1,2], **Sicilia Bellomo**[1,2], **Luis Cam**[3], **Raul Llanos**[4], **Anne Castañeda**[4], **Lourdes Tucto**[1], **Theresa J. Ochoa**[1,5], and **NEOLACTO Research Group**

[1]Universidad Peruana Cayetano Heredia, Lima, Peru

[2]Hospital Nacional Cayetano Heredia, Lima, Peru

[3]Hospital Nacional Alberto Sabogal, Lima, Peru

[4]Hospital Nacional Guillermo Almenara, Lima, Peru

[5]University of Texas Health Science Center at Houston, Houston, United States

Abstract

Preterm neonates are at risk to acquire infections. In addition to the high mortality associated with sepsis, these patients are at risk for long-term disabilities, particularly neurodevelopment impairment. Several interventions have been evaluated to reduce rates of infections in neonates but have not proven efficacy. Lactoferrin (LF), a milk glycoprotein with anti-inflammatory, immunomodulatory and anti-microbial properties, has the potential to prevent infections in young children. We performed a review of current and ongoing clinical trials of LF for prevention of neonatal sepsis, and found eleven registered clinical trials that include more than 6000 subjects. Few of these trials have finished; despite their small sample size, the preliminary results show a trend towards a positive protective effect of LF on neonatal infections. Larger trials are underway to confirm the findings of these initial studies. This information will help to define LF´s role in clinical settings and, if proven effective, would profoundly affect the treatment of low birth weight neonates as a cost-effective intervention worldwide.

Keywords

Lactoferrin; Bovine lactoferrin; Recombinant human lactoferrin; Neonatal sepsis; Prevention; Clinical trial

Introduction

Neonatal sepsis is a worldwide public health problem, with higher incidence in the developing world (Zaidi et al. 2005). Despite advances in diagnosis and treatment, infections in the neonatal period remain a major cause of death in newborns. Globally, 3.1 millions of neonates die per year, 12% of them due to sepsis or meningitis (Liu et al. 2012).

Corresponding author: Theresa J. Ochoa, MD, Department of Pediatrics, Instituto de Medicina Tropical “Alexander von Humboldt”, Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martin de Porras, Lima 31, Peru, Phone 51-1-319-0000 ext 2715; Fax 51-1-482-3404, Theresa.J.Ochoa@uth.tmc.edu.

Neonates are at risk to acquire infections, especially preterm and low-birth-weight newborns. In addition to the high morbidity and mortality associated with neonatal sepsis, these patients are at high risk for long-term disabilities, particularly neurodevelopment impairment (Shane and Stoll 2014). Therefore, several interventions, including intravenous immunoglobulin, glutamine, anti-staphylococcal monoclonal antibodies and granulocyte/granulocyte-macrophage colony-stimulating factors have been evaluated for reduction in rates of neonatal sepsis, but have not shown efficacy (Camacho-Gonzales et al. 2013). Given the failure of these approaches, lactoferrin (LF) prophylaxis, if effective, could be an important strategy to prevent infections in this period (Shane and Stoll 2013; Camacho-Gonzales et al. 2013).

The first trial testing LF for the prevention of late-onset sepsis was performed by Manzoni et al. in Italy (Manzoni et al. 2009). They found that the incidence of sepsis and death from sepsis were significantly lower in the LF-treated groups compared with the placebo. This study was met with great excitement; however, before LF becomes a standard of care, additional studies should confirm its potential to decrease infections and mortality in premature infants. This article provides an analysis of current and ongoing clinical trials of LF for prevention of neonatal sepsis and gives an update of experimental evidence that supports LF effect against neonatal infections.

Materials and Methods

A literature search using Medline, Embase and Lilacs was performed. The following MeSH search headings were used: “Lactoferrin”, “Sepsis”, “Infant, newborn”; these terms were also included as text words and “talactoferrin” was added. We also searched the abstract archives of the Pediatric Academic Societies (PAS) meeting, Infectious Diseases Society of America (IDSA) annual meeting, American Academy of Pediatrics (AAP) National Conference and Exhibition and the European Society for Pediatric Infectious Diseases meeting using the search term “lactoferrin”. In industry-funded trials we searched the sponsor web page for press releases and information about trials. Additionally, we searched for registered clinical trials (randomized and quasi-randomized studies) using the terms “lactoferrin” in the following electronic registries of published or unpublished clinical trials: Australian New Zealand Clinical Trial Registry, Brazilian Clinical Trials Registry, Chinese Clinical Trial Registry, Clinicaltrials.gov, Clinical Trial Registry-India, Cuban Public Registry of Clinical Trials, EU Clinical Trial Register, German Clinical Trials Register, Iranian Registry of Clinical Trials, International Standard Randomized Controlled Trial Number, Japan Pharmaceutical Information Center Clinical Trial Information, Japan Medical Association Center for Clinical Trials, Netherlands Trial Registry, Pan African Clinical Trials Registry, South African National Clinical Trials Register, Sri Lanka Clinical Trials Registry, University Hospital Medical Information Network Clinical Trial Registry (Japan) and WHO International Clinical Trial Registry Platform Search Portal. Finally, we contacted all main investigators by email and requested an update of the status of their respective trials. We selected all trial registries, abstracts and information provided by the sponsor or main investigator and discarded duplicated information. We collected data on the trial methodology using the “PICO” format (population, intervention, control and outcome), the current status and published results.

Results

In addition to the Manzoni study, we found 10 registered clinical trials of LF for prevention of neonatal sepsis worldwide (Table 1): three in Europe (one in Italy, one in the UK, one in The Netherlands), two in North America (one in US, one in Canada), two in South America (Peru), one in Asia (Turkey), one in Africa (Egypt) and one in Oceania (Australia). The trial from Turkey (Trial # 5 on Table 1) has been accepted for publication; four trials (# 1, 2, 4 and 8) have been completed but are not published yet; two studies (# 7 and # 10) are currently recruiting, and two (# 6 and 9) have not started recruitment yet. One trial registry (# 3) has not been updated in more than 2 years and their authors have not answered to our email; therefore, we have considered its status unknown.

Population

The sample size of each trial was between 50 to 2200 neonates, for a total of 5713 neonates randomized in eleven trials. The inclusion criteria were based on birth weight, gestational age and/or days of life. Three clinical trials (# 2, 4 and 7) based their eligibility criteria only on the newborn birth weight. Three studies (# 1, 8 and 10) included neonates only according to a maximum gestational age at birth and four (# 3, 5, 6, 9) used both parameters. The maximum birth weight accepted varied from 1500–2500g and the maximum birth gestational age varied from 28 to 36 weeks. The majority of studies (# 2, 4, 7, 8, 9, and 10) enrolled only neonates before 72 hours of birth, only 1 trial (# 6) included subjects who were within 7 days of life and 3 trials (# 1, 3, 5) did not specify a maximum age.

The most common exclusion criteria was the presence of a congenital underlying condition including chromosomal abnormalities in nine trials, gastrointestinal problems that prevent oral intake in seven trials and family history of milk allergy in five trials. Four trials included all of these exclusion criteria and three included only two.

Intervention

Bovine LF (BLF) was used in nine trials and recombinant human LF (talactoferrin) was used in one (# 2). Five trials (# 2, 4, 6, 7, and 10) gave the intervention according to the birth weight and the dose varied from 150 to 300 mg/kg/day. Three trials (#3, 5, and 8) used a fixed daily dose (from 100 to 200 mg/day), one used both fixed and weight based dosing (#9) and one (#1) used a dosing of milligram of LF per milliliter of milk (1mg/100ml). The duration of treatment was variable, based on weeks of live (4–8 weeks), gestational age (34–36 weeks) or patient discharge from hospital. Two trials did not specify the duration of the intervention.

Control

Four trials (# 1, 3, 6, and 8) did not administered a placebo; that is, the patient continued receiving the standard treatment and feeding (milk or formula without a supplement), without an additional supplementation. Clinical trials from Peru (#4 and 7) used maltodextrin as placebo. Other placebos used were oral saline (# 5) and distilled water (#9). Two trials (# 2 and 10) did not specify what was given as placebo.

Outcome

In all trials the main study outcome was the incidence of late-onset sepsis (LOS), sepsis after 72 hour of life, except for trial # 3 which evaluated neurodevelopment as the main outcome. Additional study outcomes were incidence of necrotizing enterocolitis (NEC) (# 2, 5, 6, 8 and 10) and neurodevelopment (# 1, 3 and 7). Sepsis was defined as culture-proven in five trials (# 2, 5, 6, 8 and 9) and clinically-suspected and/or culture-proven in three trials (# 4, 7 and 10). One trial (# 1) did not specify the sepsis definition. NEC was defined as Bell's stage II or greater in three trials (# 5, 8 and 10) and was not specified in two (# 2 and 6). One trial (# 7) evaluated neurodevelopmental outcome using the Mullen Scales; the other trials did not specify the method of neurodevelopmental evaluation.

Published Data

The first published trial included 472 very-low-birth-weight (VLBW) neonates, who were randomized to three groups: BLF, BLF + probiotic Lactobacillus rhamnosus (LGG) or placebo. They found a significant reduction of sepsis incidence in the BLF and BLF + LGG groups compared with the placebo group (5.9% and 4.6% vs. 17.3%) ($p=0.002$ for BLF vs control group and $p<0.001$ for BLF + LGG vs. control group). Death from sepsis also was reduced in both treatment groups compared with placebo (0% and 0.7% vs. 4.8%, $p=0.008$ and $p=0.04$, respectively). Extremely-low-birth-weight-neonates (<1000g) received the most benefit; neonates with a birth weight of 1000–1500g also had a protective effect, but did not reach statistical significance (Manzoni et al. 2009).

Later, Manzoni et al. performed a secondary analysis of data from the initial multicenter study to determine the effect BLF ± a probiotic on prevention of fungal infections (Manzoni et al. 2012). Invasive fungal infections were significantly decreased in LF groups (0.7% with BLF and 2.0% with BLF + LGG vs. 7.7% with placebo, $p<0.05$). The incidence of fungal colonization was the same in all three groups; however, the progression rate colonization-infection was significantly lower in the LF groups (3.7% with BLF and 12% with BLF + LGG vs. 41.9% with placebo, $p<0.05$).

The study performed in Turkey (# 5) has been accepted for publication (personal communication) in the American Journal of Perinatology. This trial included 50 VLBW infants born before 32 weeks gestational age who were assigned either to placebo (saline solution) or to LF (200 mg/day). The LF group developed fewer sepsis episodes than the placebo group (4.4 vs 17.3/1000 patient-days, $p=0.007$).

Preliminary Results

The LACUNA trial's (# 8) preliminary results were presented in the 2014 PAS/ASPR Joint Meeting. Seventy nine patients with less than 32 weeks of gestational age were included and randomized to LF (100mg/kg) or placebo. LF was well tolerated and the administration was successfully masked. Seven late-onset sepsis episodes were diagnosed in each arm; nevertheless the study was not powered to detect clinically relevant outcomes.

The talactoferrin clinical trial (# 2) preliminary results have been released in the 2013 PAS Annual Meeting. A total of 120 neonates were enrolled. Bacteremia was seen in 6.7% of

both groups. However, placebo-treated neonates were 2-times (OR: 1.6) more likely to develop an infection (bacteremia, meningitis, pneumonia, urinary tract infection and NEC), although the study was not powered to detect significant reductions in infection. No adverse events related to the intervention were reported.

Our team has completed a multicenter pilot study (# 4) using bovine LF; the preliminary results were presented at the 2012 PAS Annual Meeting and are currently under review for publication. We enrolled 190 neonates with a birth weight of less than 2500g (mean birth weight: 1591 ± 408g). BLF (200mg/kg/day) or placebo (maltodextrine) was administered for 4 weeks and infants were followed until 3 months of postnatal age. The cumulative incidence of late-onset sepsis was lower in the BLF group 12.6% (12/95) vs. 22.1% (21/95) in the placebo group, but did not reach statistical significance. In infants ≤1500g the sepsis rate was 20% (8/40) in the LF group vs. 37.5% (15/40) in the placebo. There were no adverse events related to the intervention.

We are currently conducting the NEOLACTO trial (# 7) in 3 neonatal units of Lima, Peru, on 414 infants with a birth weight <2000g to determine the effect of BLF on neonatal sepsis and neurodevelopment at 24 months of corrected age. We have recruited more than 350 patients currently, and more than 180 sepsis episodes have been evaluated. Since this is an ongoing and blinded trial, we do not have preliminary results yet; nevertheless, the study is being conducted as planned, and no adverse event has been reported related to the investigational product.

Discussion

After the publication of the first clinical trial by Manzoni et al., several ongoing studies are evaluating the same hypothesis in more than 5000 subjects. Few of these trials have finished and despite their small sample size the preliminary results show a trend towards a positive protective effect of LF on neonatal infections. Larger trials are on the way to confirm the findings of these initial studies.

Before LF could become a standard of care for premature infants, there are several important questions that should be answered. Is LF safe in preterm neonates? Most trials have focused on neonates with a birth weight of less than 1500g; therefore, it's likely that, if LF is proven effective, its use at least initially is going to be limited to VLBW neonates. An important concern regarding LF use in these small premature infants is the potential for adverse events, particularly allergic reactions, since the LF being used is purified from bovine milk. Some researchers have excluded neonates with a family history of cow´s milk allergy as a safety measure. However, allergy to cow´s milk protein is due mainly to caseine, alpha-lactalbumin and beta-lactalbumin, but not to LF (Sharma et al. 2001). Talactoferrin and other recombinant human LFs have the theoretical advantage of reducing this risk, but have the issue of a non-human potentially allergenic glycosylation pattern. So far, more than 400 neonates have received BLF as part of the sepsis trials with no adverse events reported. Additionally, many more infants have received BLF in the past on trials evaluating LF´s effect on anemia and fecal flora, with no adverse events (Ochoa et al. 2012). Nevertheless,

long term consequences remains to be assessed; studies performing long term evaluations for neurodevelopment should also look at this safety issue.

Which type of LF should be used in neonates? All, except one trial has used BLF. Bovine LF has >70% amino acid homology to human LF but has different glycans than human LF. Despite some structural differences, both LFs seem to preserve their functional properties (Baker and Baker 2012). On the other hand, recombinant human LF is produced in different systems (rice, cows, goats, and fungus - *Aspergillus niger var awamori*) (Sun et al. 1999) and therefore, the glycosylation pattern varies depending on each species source. Currently, there is not enough evidence to support that one type is better or safer than the others (Lingappan et al. 2013). Bovine LF has the benefit of being significantly less expensive than recombinant human LF, and if effective, is the form most likely to be used for pediatric care.

What is the right LF dose? Dosing regimens are highly heterogeneous between trials. This complicates the comparisons of results and dose selection if LF becomes a standard of care. Manzoni's trial used a fixed dose regimen (100 mg/day) for all infants; however the dosage may be insufficient in larger infants. The ongoing trials (LIFT, NEOLACTO and ELFIN) use a weight based dose ranging from 150 to 200 mg/kg/day. Although this seems to be more appropriate, for real life application (outside the context of a clinical trial) daily dose calculation would require frequent readjustments with growth. Therefore, since LF overdose seems unlikely, simplified dosing regimens should be established based on dosages used in clinical trials (for example: start at 200mg/day for neonates of less than 1000g and add 100mg/day for each 500g over 1000g of weight).

If effective, is LF effect on neonatal sepsis pathogen-specific? The main pathogens associated with late-onset sepsis in neonates in developed countries are Gram-positive organisms (coagulase negative *Staphylococci* [CoNS]*; Staphylococcus aureus, and Group B Streptococcus* [GBS]), which account for 70% of all episodes (Shane, 2013)(4). While in developing countries, the main pathogens are Gram-negative bacteria, mainly *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas*, which account for 60% of all episodes (Zaidi et al. 2005). *Candida* species are the third leading cause of late-onset sepsis in prematures and low-birth-weight infants (Camacho-Gonzales et al. 2013). The ongoing clinical trials are being performed in diverse locations providing the opportunity to test LF's effect on different microorganisms. In Manzoni's trial, LF was more effective in fungal and Gram-positive sepsis and was not significantly effective in Gram-negative sepsis, although the study was not powered for this comparison. Additionally, the incidence of fungal sepsis was high compared to other European countries. New trials will test LF effect in settings with higher incidences of Gram-negative sepsis, like developing countries, and with lower incidences of fungal sepsis, like the UK. Some clinical trials are evaluating LF effect on clinically-defined neonatal sepsis. Although, this is less reliable than culture-proven infections, and will not give information on pathogen-specific protection, these suspected infections accounts for an important percentage of sepsis-like episodes in developing countries (Thaver and Zaidi 2009).

What is the experimental evidence that supports LF effect against neonatal infections? The protective effect of LF on neonatal sepsis is attributed to its three main functional properties:

anti-microbial, anti-inflammatory and immunomodulatory. The role of LF in the immunomodulatory pathway and inflammatory response to infections have been extensively studied and reviewed (Vogel 2012; Legrand 2012; Embleton et al. 2013; Lingappan et al. 2013).

The potential for benefit against specific pathogens varies. Among Gram-positive bacteria, Leitch et al. demonstrated the anti-staphylococcal activity of LF plus lysozyme against *Staphylococcus epidermidis* (Leitch and Willcox 1999); in vitro studies showed the synergistic effect of recombinant human LF and antimicrobials against coagulase-negative *Staphylococci* and *Candida* (Venkatesh and Rong 2008); animal studies revealed the effectiveness of LF in reducing viable *Staphylococcus aureus* in mice (Nibbering et al. 2001; Artym et al. 2004). In general, LF binds to the lipoteichoic acid on the surface of Gram-positive organisms, disrupting the bacteria cell membrane and decreasing biofilm formation.

Among Gram-negative bacteria LF causes disruption of bacterial biofilms of specific microorganisms (Ammons and Copie 2013), such as *Pseudomonas* (Singh et al. 2002), *Escherichia coli* and *Klebsiella* (Sheffield et al. 2012); LF kills antibiotic-resistant *Klebsiella pneumoniae* in mice (Nibbering et al. 2001). BLF protects mice from a lethal dose of parenterally administered *E. coli* (Zagulski et al. 1989) and protects against endotoxin-induced lethal shock in piglets (Lee et al. 1998). Human LF neutralizes endotoxin (Zhang et al. 1999) and protects rats from gut-related *E. coli* systemic infections (Edde et al. 2001). LF has been thought to protect against Gram-negative bacteria in a variety of ways. It sequesters iron that is essential for bacterial growth and binds to the lipid A portion of LPS on the cell surface, disrupting the bacterial cell membrane (Ochoa and Cleary 2009). Additionally, exposure of BLF to pepsin (e.g., in gastric secretions) releases an N terminus peptide fragment (lactoferricin) that is bactericidal in vitro for Gram-positive and Gram-negative bacteria and yeast (Orsi 2004).

These laboratory observations suggest that LF is likely to be beneficial in humans for some infections depending on form of LF, timing, route and size of dose, agent causing infection, additional therapeutic measures, age of patients, and cofactors used in treatment. Additional variables may be important in the analysis, such as the use of carbohydrates (glucose or maltodextrin) as placebo, which may have a prebiotic effect on gut's microbiota and therefore, a possible a protective effect in neonatal sepsis (Watson 2013).

Conclusion

There are eleven trials evaluating LF effect on neonates. In future years we expect to see a significant amount of data published regarding LF protective effect in late-onset neonatal sepsis. This information will help to define its role in clinical settings and, if proven effective, may profoundly affect the treatment of low birth weight neonates by providing a low cost intervention potentially impacting neonatal morbidity and mortality worldwide.

Acknowledgments

Funding

This study was supported by the Public Health Service award (R01-HD067694) from the National Institute of Child Health and Human Development (NICHD), USA.

Bibliography

Ammons MC, Copie V. Mini-review: Lactoferrin: a bioinspired, anti-biofilm therapeutic. Biofouling. 2013; 29:443–455. [PubMed: 23574002]

Artym J, Zimecki M, Kruzel ML. Enhanced clearance of Escherichia coli and Staphylococcus aureus in mice treated with cyclophosphamide and lactoferrin. Int Immunopharmacol. 2004; 4:1149–1157. [PubMed: 15251111]

Baker HM, Baker EN. A structural perspective on lactoferrin function. Biobchem Cell Biol. 2012; 90:320–328.

Camacho-Gonzalez A, Spearman PW, Stoll BJ. Neonatal infectious diseases: evaluation of neonatal sepsis. Pediatr Clin North Am. 2013; 60:367–389. [PubMed: 23481106]

Edde L, Hipolito RB, Hwang FF, Headon DR, Shalwitz RA, Sherman MP. Lactoferrin protects neonatal rats from gut-related systemic infection. Am J Physiol Gastrointest Liver Physiol. 2001; 281:1140–1150.

Embleton ND, Berrington JE, McGuire W, Stewart CJ, Cummings SP. Lactoferrin: Antimicrobial activity and therapeutic potential. Semin Fetal Neonatal. 2013; 2013:143–149.

Lee WJ, Farmer JL, Hilty M, Kim YB. The protective effects of lactoferrin feeding against endotoxin lethal shock in germfree piglets. Infect Immun. 1998; 66:1421–1426. [PubMed: 9529062]

Legrand D. Lactoferrin, a key molecule in immune and inflammatory processes. Biochem Cell Biol. 2012; 90:252–268. [PubMed: 22136726]

Leitch EC, Willcox MD. Elucidation of the antistaphylococcal action of lactoferrin and lysozyme. J Med Microbiol. 1999; 48:867–871. [PubMed: 10482299]

Lingappan K, Arunachalam A, Pammi M. Lactoferrin and the newborn: current perspectives. Expert Rev Anti Infect Ther. 2013; 11:695–707. [PubMed: 23879609]

Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. The Lancet. 2012; 379:2151–2161.

Manzoni P, Rinaldi M, Cattani S, Pugni L, Romeo MG, Messner H, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birth-weight neonates: a randomized trial. JAMA J Am Med Assoc. 2009; 302:1421–1428.

Manzoni P, Stolfi I, Messner H, Cattani S, Laforgia N, Romeo MG, et al. Bovine lactoferrin prevents invasive fungal infections in very low birth weight infants: a randomized controlled trial. Pediatrics. 2012; 129:116–123. [PubMed: 22184648]

Nibbering PH, Ravensbergen E, Welling MM, van Berkel LA, van Berkel PH, Pauwels EK, et al. Human lactoferrin and peptides derived from its N terminus are highly effective against infections with antibiotic-resistant bacteria. Infect Immun. 2001; 69:1469–1476. [PubMed: 11179314]

Ochoa TJ, Cleary TG. Effect of lactoferrin on enteric pathogens. Biochimie. 2009; 91:30–34. [PubMed: 18472012]

Ochoa TJ, Pezo A, Cruz K, Chea-Woo E, Cleary TG. Clinical studies of lactoferrin in children. Biochem Cell Biol. 2012; 90:457–467. [PubMed: 22380791]

Orsi N. The antimicrobial activity of lactoferrin: current status and perspectives. Biometals. 2004; 17:189–196. [PubMed: 15222464]

Shane AL, Stoll BJ. Recent Developments and Current Issues in the Epidemiology, Diagnosis, and Management of Bacterial and Fungal Neonatal Sepsis. Am J Perinatol. 2013; 30:131–142. [PubMed: 23297182]

Shane AL, Stoll BJ. Neonatal sepsis: Progress towards improved outcomes. J Infect. 2014; 68:S24–S32. [PubMed: 24140138]

Sharma S, Kumar P, Betzel C, Singh TP. Structure and function of proteins involved in milk allergies. J Chromatogr B Biomed Sci App. 2001; 756:183–187.

Sheffield CL, Crippen TL, Poole TL, Beier RC. Destruction of single-species biofilms of Escherichia coli or Klebsiella pneumoniae subsp. pneumoniae by dextranase, lactoferrin, and lysozyme. Int Microbiol Off J Span Soc Microbiol. 2012; 15:185–189.

Singh PK, Parsek MR, Greenberg EP, Welsh MJ. A component of innate immunity prevents bacterial biofilm development. Nature. 2002; 417:552–555. [PubMed: 12037568]

Sun XL, Baker HM, Shewry SC, Jameson GB, Baker EN. Structure of recombinant human lactoferrin expressed in Aspergillus awamori. Acta Crystallogr D Biol Crystallogr. 1999; 55:403–407. [PubMed: 10089347]

Thaver D, Zaidi AK. Burden of neonatal infections in developing countries: a review of evidence from community-based studies. Pediatr Infect Dis J. 2009; 28:S3–S9. [PubMed: 19106760]

Venkatesh MP, Rong L. Human recombinant lactoferrin acts synergistically with antimicrobials commonly used in neonatal practice against coagulase-negative staphylococci and Candida albicans causing neonatal sepsis. J Med Microbiol. 2008; 57:1113–1121. [PubMed: 18719181]

Vogel HJ. Lactoferrin, a bird's eye view. Biochem Cell Biol. 2012; 90:233–244. [PubMed: 22540735]

Watson D, O'Connell Motherway M, Schoterman MH, van Neerven RJ, Nauta A, van Sinderen D. Selective carbohydrate utilization by lactobacilli and bifidobacteria. J Appl Microbiol. 2013; 114:1132–1146. [PubMed: 23240984]

Zagulski T, Lipinski P, Zagulska A, Broniek S, Jarzabek Z. Lactoferrin can protect mice against a lethal dose of Escherichia coli in experimental infection in vivo. Br J Exp Pathol. 1989; 70:697–704. [PubMed: 2690922]

Zaidi AK, Huskins WC, Thaver D, Bhutta ZA, Abbas Z, Goldmann DA. Hospital-acquired neonatal infections in developing countries. The Lancet. 2005; 365:1175–1188.

Zhang GH, Mann DM, Tsai CM. Neutralization of endotoxin in vitro and in vivo by a human lactoferrin-derived peptide. Infect Immun. 1999; 67:1353–1358. [PubMed: 10024582]

Table 1

Registered Clinical Trials on lactoferrina (LF) for prevention of neonatal sepsis[a]

N°	Study	Sponsor/ Country	Population	Intervention[b]	Control	Outcome
1	Effect of prebiotic or lactoferrin supplementation in formula on the gut flora of preterm infants ISRCTN71737811	Royal Friesland Foods B.V./ The Netherlands	Neonates with a gestational age between 26 to 35.6 weeks admitted to the NICU or High Care Unit of the hospital (n=80)	Standard preterm formula with addition of GOS OR Standard preterm formula with LF 1 mg/100 ml/day, for 6 weeks after start of ull enteral feeds.	Standard preterm formula without addition of prebiotics or LF for 6 weeks after start of full enteral feeds.	Primary outcome **1** Composition of gut flora **2** Incidence of infections **3** Oxidative stress and iron status Secondary outcome **1** Growth (head circumference, length, weight) at 6 months **2** Psychomotor development at 12 months
2	Study of talactoferrin oral Solution for nosocomial infection in preterm infants; NCT00854633	Agennix/ United States	Neonates with a birth weight between 750–1500 grams in the first 24 hours of age (n=120)	Talactoferrin alfa (recombinant human LF) (enteral) 300 mg/kg/day, twice per day, from birth to 29 days of life.	Placebo (not mentioned)	Primary outcome **1** Reduction in incidence of culture-proven and CRP elevated LOS Secondary outcome **1** NEC **2** Length of stay **3** Mortality during hospitalization
3	Supplementation with lactoferrin in preterm newborns (lactoprenew); NCT01172236	University of Siena/ Italy	Neonates with a birth weight ≤1500 grams or gestational age between 23–32 weeks (n=1300)	Bovine LF 100 mg/day + standard therapy. Unspecified duration.	Standard therapy	Primary outcome **1** Evaluate the antioxidant effect of LF and its ability to reduce free radicals related diseases in the newborn (neurodevelopment follow-up 12 months). Secondary outcome **1** Identify the panel of markers for assessing oxidative stress
4	Pilot study: lactoferrin for prevention of neonatal sepsis (NEOLACTO); NCT01264536	Universidad Peruana Cayetano Heredia - Bill and Melinda Gates Foundation/ Peru	Neonates with a birth weight between 500 and 2500 grams admitted to the NICU in the first 72 hours of age (n=190)	Bovine LF 200 mg/kg/day, three times per day for 4 weeks.	Maltodextrin 200 mg/kg/day, three times per day for 4 weeks.	Primary outcome **1** Number of confirmed episodes of LOS Secondary outcome **1** Incidence of Gram positive and Gram negative bacterial and fungal bouts of sepsis, pneumonia, diarrhea and mortality in the first month of life

N°	Study	Sponsor/ Country	Population	Intervention[b]	Control	Outcome
5	Lactoferrin prophylaxis in VLBW; NCT01287507	Ankarav University/ Turkey	Neonates with a birth weight <1500 grams and <32 weeks of gestational age (n=50)	Bovine LF 200 mg/day until discharge.	Oral saline daily until discharge	Primary outcome **1** Effect of oral LF in culture-proven sepsis **2** Effect of oral LF in NEC Secondary outcome **1** Safety of LF in VLBW infants: effect on feeding tolerance, abdominal distension, vomiting and gastric residuals **2** Duration of hospitalization
6	Lactoferrin Infant Feeding Trial (LIFT) to prevent sepsis and death in preterm infants; ACTRN12611000247976	University of Sydney/ Australia	Neonates with a birth weight ≤1500 grams and gestational age of 22–28 weeks in the first 7 days of age (n=1100)	Bovine LF 200 mg/kg/day until 34 weeks corrected age or discharge.	Breast milk or formula without BLF until 34 weeks corrected age or discharge	Primary outcome **1** Incidence of sepsis or brain injury or chronic lung disease or NEC or severe retinopathy Secondary outcome **1** Death related to culture-proven sepsis
7	Lactoferrin for prevention of sepsis in infants (NEOLACTO); NCT01525316	Universidad Peruana Cayetano Heredia - NICHD/ Peru	Neonates with a birth weight between 500 and 2000 grams admitted to the NICU in the first 72 hours of age (n=414)	Bovine LF 200 mg/kg/day, three times per day for 8 weeks.	Maltodextrin 200 mg/kg/day, three times per day for 8 weeks.	Primary outcome **1** First-episode of LOS or sepsis-associated death Secondary outcome **1** Neurodevelopment at 24 month of corrected age
8	Trial of lactoferrin for prevention of infections in very premature babies (LACUNA); ISRCTN66482337	Research Center of CHU Sainte-Justine/ Canada	Neonates with a gestational age of 23 to 30.6 weeks admitted to the NICU in the first 48 hours of age (n=79)	Bovine LF 100 mg/day, 2 doses per day until 36 weeks gestational age or dischage.	Milk without LF	Primary outcome **1** Death or at least one Health-care associated infections before discharge home. **2** Tolerance of LF Secondary outcome **1** Infections per 1000 patient day **2** NEC **3** Surgical intervention for NEC **4** Death ascribe to acute effects of sepsis
9	Oral lactoferrin supplementation for prevention of sepsis in preterm neonate; NCT01821989	Moosel Mokadem/ Egypt	Neonates with a birth weight between 500 and 2500 grams and ≤36 weeks of gestational	Two arms: LF 100 mg daily OR LF 150 mg/kg twice daily. Unspecified duration.	Placebo in form of distilled water	Primary outcome **1** Evaluate the effectiveness of oral LF in preventing culture-proven neonatal sepsis Secondary outcome

N°	Study	Sponsor/ Country	Population	Intervention[b]	Control	Outcome
			age admitted to the NICU in age admitted to the NICU in the first 48 hours of age (n=180)			**1** Complete blood count with differential leucocytic count. **2** Compare two dose regimen of LF supplementation **3** Study effect of LF supplementation on serum iron stones.
10	Enteral LactoFerrin In Neonates (ELFIN); ISRCTN88261002	University of Oxford - NIHR/ United Kingdom	Neonates with gestational age <32 weeks in the first 72 hours of age (n=2200)	Bovine LF 150 mg/kg/day (maximum: 300 mg) until discharge.	Milk with placebo	Primary outcome **1** Culture-proven or clinically suspected LOS from trial entry until discharge. Secondary outcome **1** All-cause mortality prior to hospital discharge **2** NEC: Bell's stage II or III **3** Severe retinopathy of prematurity treated medically or surgically **4** Bronchopulmonary dysplasia **5** A composite of invasive infection, major morbidity and mortality. **6** Total number of days of administration of antibiotics per infant from 72 hours until death or discharge from hospital **7** Total number of days of administration of antifungal agents per infant **8** Total length of stay until discharge home **9** Length of stay in (i) intensive care, (ii) high dependency care, (iii) special care.

[a] Trials are listed in the order of the date they were registered;

[b] All interventions are administered orally

CHU Centre Hospitalier Universitaire; *CRP* C-Reactive Protein; *GOS* galacto-oligosaccharides; *LGG Lactobacillus casei* subspecies *rhamnosus; LF:* Lactoferrin; *LOS:* late-onset-sepsis; *NEC* necrotizing enterocolitis*; NICHD* National Institute of Child Health and Human Development; *NICU* Neonatal Intensive Care Unit; *NIHR* National Institute for Health Research; *VLBW* Very Low Birth Weight.