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Perspective

Recombinant Human Lactoferrin: An Opportunity to Treat the Neonatal and Adult Severe Sepsis

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ABSTRACT

Lactoferrin, both human and bovine, acts against oxidative stress damage and has a range of beneficial activities such as anti-pathogenic, anti-cancer, anti-inflammatory, immunomodulatory, and deoxyribonucleic acid (DNA)-regulatory. In addition, clinical studies have shown human lactoferrin to attenuate inflammation and reduce mortality from neonatal and adult severe sepsis. However, the magnitude of the lactoferrin effect varies between studies. Given the demonstrated efficacy of recombinant human lactoferrin in sepsis, its lack of side effects, ease of administration, and relatively low cost, it is worth exploring whether the protein could be administered "preventively" as soon as the possibility of a patient's condition progressing to severe sepsis is likely.

Keywords
Severe sepsis; Preterm infants sepsis; Necrotizing enterocolitis; Sepsis prevention.

BACKGROUND

Human lactoferrin (hLF) has been described by Kowalczyk et al[1] as a "*miracle molecule*". Their justification for such an accolade is that lactoferrin (LF) acts against oxidative stress damage and has a range of beneficial activities such as anti-pathogenic, anti-cancer, anti-inflammatory, immunomodulatory, and deoxyribonucleic acid (DNA)-regulatory that play a role in health and pathology throughout life. hLF is an iron-binding glycoprotein and the second most abundant protein in human milk.[2,3] It is found on mucosal surfaces and in mature human milk, tears, saliva, seminal fluid, and secondary granules of neutrophils[4] and in many body fluids such as mucosal secretions, including tears, saliva, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, and urine, and bodily fluids such as blood plasma and amniotic fluid. In addition, the expression and secretion of hLF on mucosal surfaces and its release at inflammatory sites have established its role as an agent of innate immunity. hLF could be an attractive synergistic agent with antifungals[5] and probiotics.[6]

Bovine LF (bLF) has been investigated extensively.[6] It inhibits the growth of various bacteria, fungi, viruses, and parasites. A high homology between human and bovine LFs suggests that both forms may provide similar health benefits. hLF is glycosylated with highly branched complex/hybrid type N-glycans, mainly sialylated and fucosylated.[7] However, bLF contains predominantly high mannose.[8]

LFs are relatively resistant to proteolysis. They carry a net positive charge and perform physiological functions in various tissues, including regulating iron absorption in the bowel. The antimicrobial activity of LF is due to iron sequestration at sites of infection, depriving the microorganism of this nutrient. Another mechanism is the direct interaction of the LF molecule with the infectious agent. The positive amino acids in LFs can interact with anionic molecules on some bacterial, viral, fungal, and parasite surfaces, causing cell lysis.[9]

Recombinant human lactoferrin (rhLF) can be produced on a large scale by fermentation. Here we examine the current data supporting the possible clinical applications of human lactoferrin.

BIOLOGICAL FUNCTIONS OF LACTOFERRIN

Several functions have been attributed to LF. It is considered a key component in the host's first line of defense, as it can respond to various physiological and environmental changes.[10] In addition to the Fe^{3+} homeostasis, LF possesses functionalities common to all transferrins: intense antimicrobial activity against a broad spectrum of bacteria, fungi, yeasts, viruses[11] and parasites.[12]

Enzymatic treatment of bLF with pepsin produced a low-molecular-weight peptide with antibacterial properties against many Gram-positive and Gram-negative bacteria, including *Esch-*

erichia coli (*E. coli*), *Salmonella enteritidis*, *K. pneumoniae* and others.[13] Similar properties have been reported for human lactoferrin.[14]

Human recombinant lactoferrin (hrLF, Talactoferrin®) has been manufactured and tested for its efficacy in several indications.[15] Engelmayer et al[15] measured wound healing activity of topical talactoferrin in full-thickness wounds of normal mice and diabetic (db(-)/db(-)) mice, systemic bioavailability, and the potential to modulate inflammation through *in vitro* and *in vivo* binding assays and inflammatory mediator measurements. Talactoferrin® significantly increased the closure rate during 12 to 19 d (maximally on d 3 to 6), the 75% closure incidence, and the time to 50% closure *versus* vehicle or becaplermin (recombinant human platelet-derived growth factor). Talactoferrin bound local dermal cells *in vivo* and human dermal fibroblasts *in vitro* induced the migration of dermal fibroblasts, THP-1 macrophages, Jurkat T-cells, and mouse granulocytes *in vitro*. Competition binding assays suggested the involvement of interleukin-8RB (IL-8RB) and C-C chemokine receptor type 2 (CCR2) chemokine receptors in binding and/or cell migration. Consistently, the induction of migration was partially inhibited in IL-8RB deficient granulocytes. Talactoferrin also enhanced the production of crucial repair inflammatory mediators IL-8, IL-6, macrophage inflammatory protein-1 α and tumor necrosis factor-α in d 3 wounds and IL-8, IL-6 and monocyte chemotactic protein-1 in cultured dermal fibroblasts (Table 1).

CLINICAL APPLICATIONS OF LACTOFERRIN

Neonatal Sepsis

The availability of rhLF provided an opportunity to consider LF as a lead compound for drug development. Both bovine and human lactoferrins have been examined for their potential clinical utility. However, their safety and efficacy must be validated to receive market approval as a drug. Further, it needs to be demonstrated that the compounds would be manufactured consistently to meet the quality requirements set by the market approval. Since bovine lactoferrin is a natural product collected from cow milk produced under various conditions, its consistency may be difficult to maintain. Therefore, the following discussion focuses on the recombinant human lactoferrin.

LF's variety of functions attracted testing its utility for clinical use in disease prevention, treatment, and diagnosis. For example, it was observed in early studies that infants fed with infant formulas containing LF absorbed less iron from the intestine than breastfed infants.[16,17]

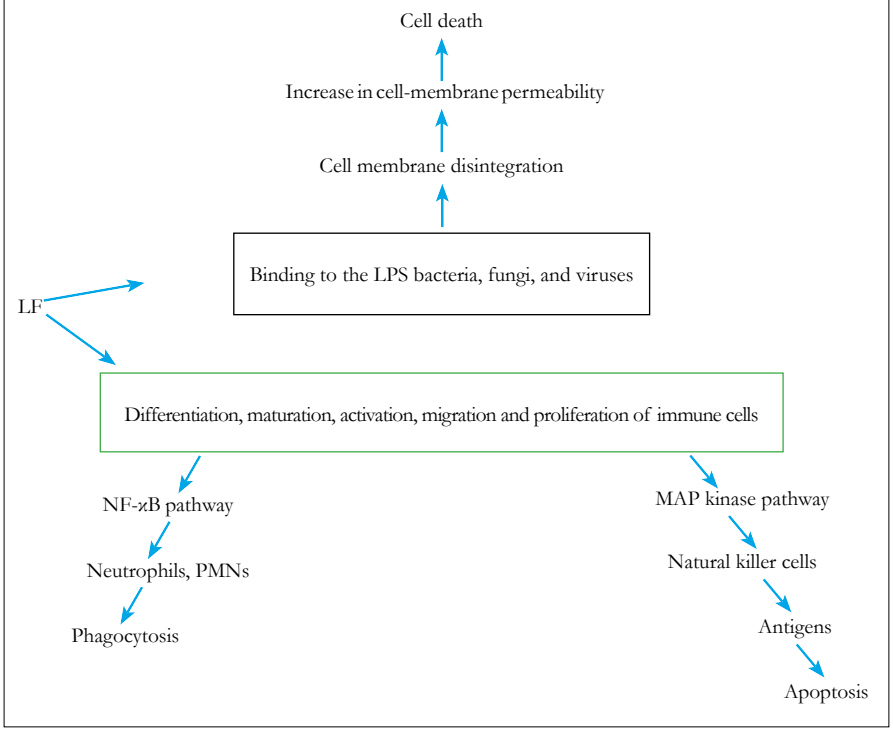
The Figure 1 shows lactoferrins' principal interactions and outcomes affecting infection, inflammation, and sepsis.

The immature infant's bowel allows intact LF to cross the gut wall and be absorbed and distributed by the bloodstream.[9] Caccavo et al[18] reported that lactoferrin binds with high affinity to lipid A, lipopolysaccharide's toxic moiety, and Gram-negative bacteria endotoxin. The binding modulates immune responses by decreasing the release of IL-1, IL-2, and tumor necrosis factor-alpha interferons-alpha (INF-α) and enhancing monocyte and natural killer cell cytotoxicity. These effects of LF may play an essential role in a protective role against lethal endotoxin shock, as demonstrated in animal models. Moreover, *in vitro* and *in vivo* neutralization of endotoxin by human lactoferrin-derived peptides may offer treatment of endotoxin-induced septic shock. Data indicate[19] that LF directly interacts with antigen-presenting cells (APCs), i.e., monocytes/macrophages and dendritic cells (DCs),

Table 1. *Reported Biological Activities of Lactoferrins*

Activity	Reference	Note
Attenuate oxidative stress damage. LFs act as anti-pathogenic, anti-cancer, anti-inflammatory, immunomodulatory, and DNA-regulatory agents.	1,4	
Found on mucosal surfaces and in mature human milk, tears, saliva, seminal fluid, and secondary granules of neutrophils.	4	Found in many body fluids, such as mucosal secretions, including tears, saliva, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, urine, blood plasma, and amniotic fluid.
A synergistic agent with antifungals and probiotics.	5,6	
Causes cell lysis on some bacterial, viral, fungal, and parasite surfaces.	9	
Respond to various physiological and environmental changes.	10	
Affect Fe3+ homeostasis and possess functionalities common to all transferrins: intense antimicrobial activity against a broad spectrum of bacteria, fungi, yeasts, viruses, and parasites.	11,12	
LFs low-molecular-weight peptides have antibacterial properties against many Gram-positive and Gram-negative bacteria, including *Escherichia coli*, *Salmonella enteritidis*, *K. pneumoniae* and others.	13,14,25	
Wound-healing activity.	15	
Bind with high affinity to lipid A and Gram-negative bacteria endotoxin. The binding modulates immune responses by decreasing the release of interleukin-l (IL-1), IL-2, and tumor necrosis factor-alpha INF-α and enhancing monocyte and natural killer cell cytotoxicity.	18	
Attenuate gut-related systemic infection by *E. coli* strain Ec5	21	Shown in orogastrically infected neonatal rats.
Alleviate or prevent life-threatening necrotizing enterocolitis.	24,27	In very-low-birth-weight preterm infants.
RhLF reduced mortality in severe sepsis patients by 46.5%.	31	

Figure 1. *Biological Interactions and Outcomes of Lactoferrins (Human, Recombinant and Bovine)*



modulates migration and cell activation, and affects the expression of soluble immune mediators, such as cytokines, chemokines, and other effector molecules. Drago-Serrano et al[20] review highlighted results from *in vitro* and *in vivo* models of the gut, lung, oral cavity, mammary gland, and liver infections supporting the therapeutic role of human and bovine LFs in modulating and protecting against the deleterious effects of bacterial, viral, fungal and protozoan-associated inflammation. It summarized the experimental evidence supporting LF's protective role against the deleterious effects of lipopolysaccharide (LPS)-induced pro-inflammatory cytokine response on gut-barrier function, diarrhea, bacterial translocation, and tissue damage. The authors concluded that the antimicrobial and LPS-binding protein activities of LFs may be a promising strategy for treating and preventing sepsis and endotoxic shock when administered alone or in combination with probiotics or antibiotics.

Subsequent *in vivo* and *in vitro* studies of Sherman et al[21] demonstrated the role of rhLF against gut-related systemic infection by *E. coli* strain Ec5 causing meningitis in orogastrically infected neonatal rats. This model mimics clinical observations in neonates that parenteral feeding is a risk factor for sepsis. Translocation of microbiota, e.g., Gram-negative enterobacteria, from the intestinal tract to systemic organs *via* the bloodstream may have fatal consequences. Treatment with rhLF decreased the clinical sepsis and bacterial loads in the kidney and blood. *In vitro* assays in macrophage cultures showed that levels of nitric oxide, tumor necrosis factor-alpha (TNF-α), and nuclear factor kappa B (NF-kB) expression elicited by LPS were even higher after adding rhLF. These findings suggest that the protective action of rhLF is due to an optimal activation of macrophages *via* pro-inflammatory cytokine elicitation to enhance their bacterial killing activity. The authors claimed that milk lactoferrin protects infants from gut-related, systemic infection. Neonatal rats pretreated orally with rhLF had less bacteremia and lowered disease severity scores ($p<0.001$) after intestinal infection with *E. coli*. An *in vitro* assay showed that rhLF did not kill *E. coli*, but a combination of rhLF+lysozyme was microbicidal. Also, rat macrophages *in vitro* released escalating amounts of nitric oxide and tumor necrosis factor-alpha when stimulated with increasing concentrations of rhLF, suggesting that rhLF may act with other "*natural peptide antibiotics*" or prime macrophages to kill *E. coli in vivo*. The seminal work of Michael Sherman and his colleagues paved the way for hLF and its peptides to be considered therapeutic agents.

Other reports also suggested that human milk protected newborn infants from infection by establishing a non-invasive bacterial flora in the intestines. Premature infants receiving cow milk-based formulas rather than human milk are exposed to the risk of gut-associated infections. Eddy et al[22] examined whether two doses of rhLF given 24-hours before *E. coli* infection by gastric gavage to 4-day-old newborn rats might decrease bacteremia and prevent illness or death after gut infection with *E. coli* (an infective dose of 1012 colony forming units (CFUs)/kg of body weight). Quantitative blood and liver cultures were obtained from surviving newborn rats 48-hours after infection. All blood cultures from control pups receiving oral NaCl (n=15) were positive (6.7×107±4.4×107 CFUs/mL). The rhLF group (n=22) showed decreased levels of positive blood cultures in 20 animals (7.0×104±3.3×104 colony-forming units per milliliter (CFUs/mL), mean standard error mean (SEM), $p<0.001$ *vs.* NaCl). The dead animals contained, on average, 4.5×108 CFU/mL in blood cultures. Aseptic liver touch cultures contained 241±31 CFU in the NaCl group compared to 114±33 CFU ($p<0.01$) in the rhLF

liver cultures. The two groups' differences in no illness and death or dying were highly significant (p<0.001). Co-administration of 5 mg/kg of $FeSO_4$ with NaCl or rhLF gavage showed that rhLF did not limit the growth of *E. coli* by iron restriction. The authors suggested that an alternative mechanism of rhLF action might involve an antibacterial effect of the lactoferricin domain in the rhLF N-lobe on *E. coli* or binding of endotoxin, limiting the pro-inflammatory effects of lipopolysaccharide. The results showed that rhLF reduced bacterial infection, illness, and death after massive gut infection with *E. coli*.

Similarly, Sherman et al[23] hypothesized that early colonization of the immature small intestine with lactobacillus rhamnosus GG (LGG), and the use of rhLF to promote the growth of LGG, would enhance gut defenses against enteroinvasive *E. coli*. Newborn rat pups were treated with nothing, intra-gastric LGG, or rhLF+LGG on days 3 and 4 of life. Gut colonization by LGG was quantified in lavaged jejunal and ileal fluids and gut wall homogenates on day 5 of life. Separate studies used similarly treated litters of newborn rats that were infected late on day 4 of life with *E. coli* (1012 CFU/kg). Sixteen hours later, the numbers of *E. coli* were measured in small bowel fluid and gut wall homogenates. Control pups initially had lactic acid bacteria colonize the bowel, but these bacteria were not LGG. Pups treated with LGG or rhLF+LGG had significantly higher numbers of LGG in the ileum *versus* jejunum. Contrary to our hypothesis, rhLF did not augment LGG colonization. After *E. coli*-related gut infection, planktonic [lavage fluid] and epithelia-adherent growth [gut wall homogenates] of *E. coli* in the small bowel were most effectively reduced by pre-treatment with rhLF and LGG (p<0.05). Prophylactic therapy with rhLF and Lactobacillus GG enhances defenses against invasive *E. coli* in the nascent small intestine. The data suggest that rhLF and LGG may reduce necrotizing enterocolitis and gut-related sepsis in preterm human infants.

Sherman et al[24] presented scientific and clinical evidence that lactoferrin alleviates or prevents life-threatening necrotizing enterocolitis. Preclinical studies in neonatal rats showed that oral lactoferrin given before enteral infection with pathogenic *E. coli* reduced bacteremia and mortality. A multicentered clinical trial found that very-low-birth-weight preterm infants given bovine lactoferrin had a significant reduction in late-onset sepsis; there was also a trend towards a diminished incidence of necrotizing enterocolitis. However, regulatory burdens required to bring bLF to the bedside may limit its availability.

The authors concluded that extremely preterm infants should receive colostrum, a natural lactoferrin concentrate, immediately after birth and, ideally, continue on breast milk throughout the hospital stay. This practice appears well-tolerated, but more data is needed to confirm that this practice reduces the prevalence of necrotizing enterocolitis.

This conclusion was further highlighted by Sherman,[25] adding that in the stomach, pepsin digests LF and releases a potent peptide antibiotic called lactoferricin, facilitating a healthy intestinal microbiome. Furthermore, the highest concentration of hLF is in colostrum. Therefore, feeding colostrum and also fresh mature milk offers a way to prevent necrotizing enterocolitis.

An intriguing observation was reported by Sherman et al[26] on lactoferrin acting as an adjuvant during influenza vaccination of neonatal mice. Since the health policy at the time precluded neonatal vaccination against influenza, morbidity and mortality rates were high for infants under 6-months of age. Aluminum (ALUM) hydroxide recruits neutrophils that secrete lactoferrin at deposition sites of antigen. Hence, LF may activate diminished numbers of dysfunctional dendritic cells. The authors theorized that lactoferrin+influenza might initiate an equivalent antibody response compared to ALUM. In their experiments, three-day-old mice received subcutaneously 30 µg of influenza A (H1N1) hemagglutinin+200 µg of bovine lactoferrin *versus* hemagglutinin+ALUM. Controls received hemagglutinin, lactoferrin, or ALUM. After 21-days, sera measured anti-H1N1 (enzyme-linked immunosorbent assay (ELISA)) and neutralizing antibodies (plaque assays). ELISA detected equal antibody production with lactoferrin+hemagglutinin compared to hemagglutinin+ALUM; both sera also neutralized H1N1 virus at a 1:20 dilution (p<0.01). Controls had no anti-H1N1 antibody. Neonates given lactoferrin had no anaphylaxis when challenged four weeks later. Therefore, lactoferrin appears to be a safe and effective adjuvant for inducing antibodies against influenza in neonates.

The extensive preclinical data generated by Sherman et al[27] strongly support the evaluation of the safety and efficacy of rhLF to reduce infection in preterm infants. The authors conducted a randomized, double-blinded, placebo-controlled trial in infants with a birth weight of 750-1500 g. Infants received enteral talactoferrin (TLF) (n=60) or placebo (n=60) on days 1 through 28 of life; the TLF dose was 150 mg/kg every 12-hours. Primary outcomes were bacteremia, pneumonia, urinary tract infection, meningitis, and necrotizing enterocolitis (NEC). Secondary outcomes were sepsis syndrome and suspected NEC. Recorded clinical, laboratory, and radiologic findings, along with diseases and adverse events, were submitted to statistical analyses. Demographic data were similar in the two groups of infants. The authors did not attribute any enteral or organ-specific adverse events to TLF. There were two deaths in the TLF group (1 each due to posterior fossa hemorrhage and post-discharge sudden infant death), and one death in the placebo group, due to NEC. The rate of hospital-acquired infections was 50% lower in the TLF group compared with the placebo group (p<0.04), including fewer blood or line infections, urinary tract infections, and pneumonia. Fourteen infants in the TLF group weighing <1 kg at birth had no Gram-negative infections, compared with only 3 of 14 such infants in the placebo group. Non-infectious outcomes were not statistically significantly different between the two groups, and there were no between-group differences in growth or neurodevelopment over a 1-year post-hospitalization period.

The authors identified no clinical or laboratory toxicity and observed a trend toward less infectious morbidity in the infants treated with TLF [Trial registration: ClinicalTrials.gov: NCT00854633].[28]

Clinical trials with lactoferrin in neonates were reviewed by Embleton et al.[29] The authors emphasized that especially preterm born infants are at risk of infections in early life. Necrotizing enterocolitis (NEC) and late-onset sepsis (LOS) are the most common reasons for death after the first week of life. Fresh breast milk from the infant's mother reduces the risks of these serious pathologies in a dose-dependent fashion. Studies show that lactoferrin impacts on immune function and, through a multitude of mechanisms, reduces the risk of viral, fungal, and bacterial infections. Enteral bovine lactoferrin has been tested in randomized clinical trials. Results suggested an important reduction in LOS in preterm or low-birth-weight infants. However, the largest trial, enteral lactoferrin in neonates (ELFIN), recruited 2,203 infants but did not show any significant reductions in LOS or NEC.

A comprehensive list of clinical studies involving lactoferrin can be found at https://www.ppt-health.com/clinical-studies-on-lactoferrin/.[30] Clinical studies[31-33] reported that human lactoferrin controlled inflammation in neonates and adults.

Adult Severe Sepsis

Guntupalli et al[33] reported a remarkable 46.5% relative reduction in mortality by rhLF in patients with severe sepsis. Talactoferrin alfa is a recombinant form of human lactoferrin. Its administration to experimental animal models reduced the translocation of bacteria from the gut into the systemic circulation and mortality from sepsis. This prospective, randomized, double-blinded, placebo-controlled, multicenter phase 2 trial clinical study aimed to determine if talactoferrin could reduce 28-day all-cause mortality in patients with severe sepsis and assess its safety.

The patients were one hundred ninety-four adults treated within 24-hours of the onset of severe sepsis. Talactoferrin 1.5 g or placebo was administered every 8-hours for up to 28-days or until discharge from the intensive care unit (ICU).

Modified intention-to-treat analysis was used to assess the primary (28-day all-cause mortality) and secondary endpoints. The all-cause mortality at 28-days was 26.9% in the placebo group and 14.4% in the talactoferrin group (two-sided p=0.052), representing a 12.5% absolute and a 46.5% relative reduction in mortality, meeting the protocol-specified primary endpoint. Reduction in all-cause mortality was sustained at 6-months (p=0.039). These reductions in mortality were observed across a wide spectrum of subgroups. The drug was well-tolerated, showing a safety profile similar to that of a placebo. Trial registration: ClinicalTrials.gov NCT00630656.[34]

McCulloh et al[35] asked whether the efficacy of rhLF to treat sepsis may be "*too good to be true*". The authors recognized that "*given these positive studies, it seems quite reasonable that lactoferrin would have significant potential in treating sepsis in older children and adults*". While the Guntupalli study[33] generated a highly promising outcome, a follow-up phase 2/3 trial with oral talactoferrin in adult sepsis was terminated since the placebo group showed better outcomes than the treated group.[36] McCulloh[35] and Opal stated: "*In all studies of sepsis treatment, the true devil in clinical trial design is in the details of diagnosis and assessment of illness severity*". Sepsis remains to be diagnosed by physical examination findings and general markers of organ function/dysfunction. Clinicians apply specific therapeutic protocols to patients with uncertain disease states and immunological backgrounds. Several studies highlighted the importance of early treatment to deliver better-than-expected mortality.[37-39] McCulloh et al[35] did not provide any data on this aspect of the above trials and the reasons for the two studies' results to differ.

Guntupalli et al[33] report the clinical trial results of the initial phase II trial with oral rhLF (talactoferrin) in adult patients with severe sepsis. Despite the complicating factor of mislabeled doses for a subset of patients, analysis of the data revealed a truly remarkable finding with a 12.5% absolute risk reduction in all-cause mortality in the talactoferrin group compared with the placebo group. This beneficial effect size in this study appeared to be more pronounced in the most severely ill patients and was effective in most subgroups, with the notable exceptions of patients septic from intra-abdominal infection and female patients. Data support the significance of lactoferrin in host immune and inflammatory control.

However, Vincent et al[36] reported that this study had unspecified "*drug allocation issues*" that may have affected the results. Therefore, a repeat Phase II trial followed by a phase III component was designed to confirm the effect of talactoferrin on sepsis mortality.[40] The oral talactoferrin in severe sepsis (OASIS) trial was a multicenter, randomized, placebo-controlled, double-blinded phase II/III trial to determine the effect of thrice-daily oral talactoferrin administration on 28-day all-cause mortality in patients admitted to the ICU with severe sepsis. Included patients had at least three of four systemic inflammatory response syndrome criteria and evidence of end-organ dysfunction. Patients were excluded if they met any of the following criteria: on immunosuppression medications, New York Heart Association (NYHA) class IV heart failure, severe liver disease, severe burns, poorly controlled human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS), or imminent death. Patients were randomized to limit the number required for enrollment and minimize confounders, using permuted block method stratification by the presence of septic shock, urinary source of infection, and geographic distribution. All-cause mortality endpoints were analyzed using a Cochran-Mantel-Haenszel test across the three stratifications. The trial was stopped early on recommendations of the drug monitoring safety board for futility and the potential hazard of the trial drug after 305 (153 talactoferrin, 152 placebo) patients were enrolled. The authors drew multiple parallels to a phenomenon found by the protein C worldwide evaluation in severe sepsis (PROWESS) group evaluating drotrecogin to explain the disparity of results between the two talactoferrin studies. The evaluation suggested that, in general, newer studies may show lower placebo mortality and concomitant negative study drug effect, driven by improving baseline sepsis care.[41]

However, although lower mortality rates could increase the requisite sample sizes to show a significant improvement, such an argument is substantially weakened in this case by the higher mortality in the talactoferrin group, which reached statistical significance for the in-hospital and 3-month values. Regardless, the

OASIS study adds another drug to the long list of ineffective pharmaceutical augmentation strategies for treating sepsis.

Based on the preclinical results,[15] talactoferrin was subsequently tested clinically in a Phase II trial in patients with diabetic ulcers and was found to be effective and safe. Talactoferrin should be further evaluated in patients with diabetic and other types of ulcers.

It is, however, essential that the past positive and negative results of clinical studies are evaluated by considering the trials' designs and implementation. Finding the effective manner in which rhLF needs to be administered is critical.

Rodriguez et al[42] aimed to determine if oropharyngeal therapy with a mother's own milk (OPT-MOM) reduces LOS; primary outcome), NEC, death, length of stay, time to full enteral nutrition (FEN) and full oral feeds in preterm infants (BW<1250 g). Infants (N=220) were treated with 0.2 mL of milk (Group A) or placebo (Group B) every 2 h for 48 h, then every 3 h until 32-weeks cervical gland area (CGA). OPT-MOM did not reduce LOS, NEC or death. Group A trended towards a reduced stay and better nutritional outcomes, but results were not statistically significant. Clinical trials: GOV: NCT02116699.[43]

Major efforts have been made to evaluate previous clinical studies and design better sepsis care. The Surviving Sepsis Campaign incorporated early goal directed therapy (EGDT) in their guidelines, based on a 2001 single-center, proof-of-concept study by Patel et al.[44] Their protocolized approach to sepsis management reduced hospital mortality. However, applying EGDT in general practice was difficult to generalize, costly, and complex.[45]

A similar conclusion was reached when comparing the following three studies: PROtocol-based Care for Early Septic Shock (ProCESS),[37] Australasian Resuscitation in Sepsis Evaluation (ARISE)[38] and Protocolized Management in Sepsis (ProMISe).[39] ProMISe) analyzed data on 1,243 patients with severe sepsis/septic shock from 56 emergency departments in the UK. This open, multicenter, parallel-group, randomized controlled trial of the clinical and cost effectiveness of protocolized approach included patients with at least two of four systemic inflammatory response syndrome criteria as well as either a lactate level greater than four mmol/L or systolic blood pressure less than 90 mm Hg after fluid challenge. Patients with pulmonary edema, stroke, major gastrointestinal bleeding, pregnancy, advanced HIV, or imminent death were excluded. Patients were randomized to the "usual" *vs.* algorithm-driven care similar to that used by Rivers and colleagues.[46] Patients received antibiotics before randomization.The study showed significantly higher use of central venouscatheters, arterial lines, transfusions, and dobutamine in the early goal-directed therapy (EGDT) arm. Patients in the EGDT arm had more ICU admissions, worse Sequential Organ Failure Assessments cores at 6 h, longer use of advanced cardiovascular support, and longer ICU length of stay. However, there was no significant difference in 90-day mortality between groups (29.5% EGDT *vs.* 29.2% in the usual care group (p=0.90). No meaningful difference in secondary outcomes was seen, including health-related quality of life or adverse events. There was a trend to higher 90-day hospital costs in the EGDT group. Together, the ProMISe, ProCESS and ARISE trials demonstrated that apart from early identification, fluid resuscitation, and antibiotics, a protocolized approach to sepsis using parameters such as central venous oxygen saturation did not improve outcomes. The ProMISe trial suggests that such criteria may overutilize resources. However, the three studies' results highlighted the importance of early administration of fluids and antibiotics, leading to better-than-expected mortality in the usual care groups. Further, inconsistencies in applying the protocols cause differences in mortality among the three studies.

The ProMISe trial, along with ProCESS and ARISE trials, demonstrated that beyond early identification, fluid resuscitation, and antibiotics, a protocolized approach to sepsis using parameters such as central venous oxygen saturation would not improve outcomes. In fact, the ProMISe trial implied that such criteria might even lead to the overutilization of resources. On the other hand, all three studies highlighted the importance of early administration of fluids and antibiotics, as noted by the better-than-expected mortality in the usual care groups. Inconsistencies in achieving these simple goals may be the reason for the differences in mortality among the three studies.[47]

Time and again, the only consistent management principle that improves outcomes in patients with sepsis is early identification and treatment with intravenous fluids and antibiotics. The sepsis alert system helps expedite this process, as shown in several studies. Although the decreased window to antibiotics time is an important finding, the lack of demonstrated mortality benefit could be due to the above-mentioned confounders, including the fact that more than half of the patients still did not get antibiotics promptly.

CONCLUSION

Lactoferrin is a multifunctional protein derived from milk; it has high affinity for iron ions. Iron is necessary for microorganisms to grow and reproduce, so the sequestration of iron significantly reduces their pathogenic potential. LF has numerous beneficial properties—antibacterial, antiviral, antifungal, and antiparasitic, as well as immunomodulatory, anti-inflammatory, and anti-cancer—that may play an important role in maintaining health from fetal life to old age. Currently, LF is an ingredient in many supplements and medicines, but a thorough understanding of the mechanisms of its beneficial effects requires further in-depth research.

Given the likelihood of rhLF efficacy in sepsis, its lack of side effects, ease of administration, and relatively low cost, it is worth exploring whether the protein could be administered "preventively" as soon as the possibility of a patient's condition progressing to severe sepsis is likely.

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