

Meta-Analysis of Enteral Lactoferrin Supplementation for Reducing the Risk of Preterm Infants Sepsis

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ABSTRACT

Introduction: Severe COVID-19 in pregnancy is strongly associated with preterm infant late-onset sepsis (LOS), which is a major cause of morbidity and mortality in infants. Recently, Lactoferrin, an iron-binding protein significantly discovered in human colostrum, shows potential effect on reducing the risk of preterm infant LOS and mortality by its immunomodulatory properties. Therefore, this study aimed to evaluate the effect of Lactoferrin on reducing the risk of preterm LOS, necrotizing enterocolitis (NEC), and mortality in preterm infants.

Methods: This study followed the guidelines provided by PRISMA. A literature search was conducted with PubMed, Cochrane Library, Google Scholar, and ScienceDirect. The combined effect of LOS, NEC and mortality incidence were presented as risk ratios (RR) with a 95% confidence interval (CI) using a random-effects model (REM) or fixed-effects model (FEM) forest plot. Furthermore, the heterogeneity level was checked by I^2 and the p-value of the chi² test.

Results: The incidence of LOS was significantly higher in the control than lactoferrin supplementation group [RR=0.65, 95% CI (0.56,0.77), p<0.00001, I^2 =56%]. However, there were no significant differences in NEC [RR=0.80, 95% CI (0.63,1.02), p=0.10, I^2 =39%] and mortality [RR=0.94, 95% CI (0.77,1.13), p=0.49, I^2 =39%], despite the trends are higher in the control group.

Conclusion: This meta-analysis showed that enteral lactoferrin supplementation in preterm infants was associated with a significant reduction in LOS, but not NEC stage II or III and all-cause mortality.

Registered Protocol: The protocol of this review was already registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number CRD42021279189.

Keywords: *lactoferrin; premature birth; neonatal sepsis; systematic review; meta-analysis*

INTRODUCTION

Preterm birth is a major cause of infant morbidity and mortality. It is responsible for 35% and 18% of all deaths in neonates and children under 5 years, respectively. Furthermore, it is defined by World Health Organization (WHO) as any live birth before 37 weeks of gestation. According to the gestation age, preterm birth can be further classified as extremely (<28 weeks), very (28

to <32 weeks), and moderate (32 to <34 weeks) to late preterm (34 to <37 weeks)¹. WHO reported that Indonesia ranked 9th for the highest number of preterm births globally. Meanwhile, some of its causes include repeated pregnancies, smoking, infections during pregnancy, and others².

Pregnant women are in a high-risk group of developing severe coronavirus (COVID-19) infection. The recent systematic

review and meta-analysis by Wei *et al.* showed that this virus in pregnancy is associated with preeclampsia, stillbirth, preterm birth, and low birth weight. Furthermore, its severe case increases the risk³. Villar *et al.* also stated that this infection increases preterm birth by 59%, primarily due to medical indications. This could result from an increased risk of severe pregnancy complications such as preeclampsia, eclampsia, and HELLP syndrome. Additionally, fever and shortness of breath increase the risk of preterm birth⁴.

The high risk of infection in neonates, especially premature infants, is caused by immune system immaturity. One of the mechanisms for the formation of the neonatal immune system is through the passive transfer of maternal antibodies through the placenta which occurs massively in the third trimester. Therefore, premature infants born earlier will lack protection from this mechanism⁵. Moreover in premature births, invasive procedures and the use of broad-spectrum antibiotics increase the risk of foreign bodies exposure and antibiotic resistance⁶.

The leading cause of preterm infant death is respiratory distress syndrome, necrotizing enterocolitis (NEC), sepsis which contributes to approximately 30% of the case, meningitis, and pneumonia. Neonatal sepsis can be further divided into early-onset (EOS) and late-onset sepsis (LOS)⁷. EOS occurs within the first 3 days of life and is usually caused by bacterial pathogens transmitted vertically from mother to infant before or during delivery. Meanwhile, LOS occurs after 72 hours of life and is associated with a postnatal nosocomial or community environment. It is a significant cause of mortality in the neonatal intensive care unit, specifically in preterm infants⁸.

The three types of pathogen that causes LOS are Gram-negative and -positive bacteria, as well as fungi. The distribution of these pathogens varies in different regions. In China, Gram-negative bacteria are responsible for

more than 50% of LOS, and fungi cause 17%. Meanwhile, its major cases in Italy are caused by Gram-positive bacteria. The predominant Gram-negative bacteria was *Klebsiella pneumoniae*, followed by *Escherichia coli*. Coagulase-negative staphylococci were the most frequent gram-positive bacteria, followed by *Staphylococcus aureus*, while the most common fungus was *Candida albicans*. Several pathogens associated with the highest fatality rates are *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter aerogenes*, *Escherichia coli*, and *Klebsiella pneumoniae*^{9,10}.

Prevention of LOS in preterm infants is essential due to its morbidity and mortality. Lactoferrin is an iron-binding protein significantly discovered in human colostrum (up to 9 mg/ml). Furthermore, it can also be observed in tears, saliva, and semen at lower concentrations¹¹. This protein has antimicrobial, antiviral, antifungal, and immunomodulatory properties. It works through several mechanisms, including iron sequestration, interfering with microbial adhesion to host cells, causing microbial cell membrane lysis, and preventing biofilm formation. It also promotes the growth of probiotic microorganisms, stimulates the differentiation and proliferation of intestinal cells, and increases the expression of intestinal digestive enzymes¹². Therefore, this systematic review and meta-analysis aimed to assess the effect of lactoferrin supplementation in reducing the risk of preterm infant sepsis and NEC, specifically in premature infants born to mothers with severe COVID-19.

MATERIALS AND METHODS

This review followed the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), and its protocol was already registered to PROSPERO (CRD42021279189). The following criteria were considered for studies'

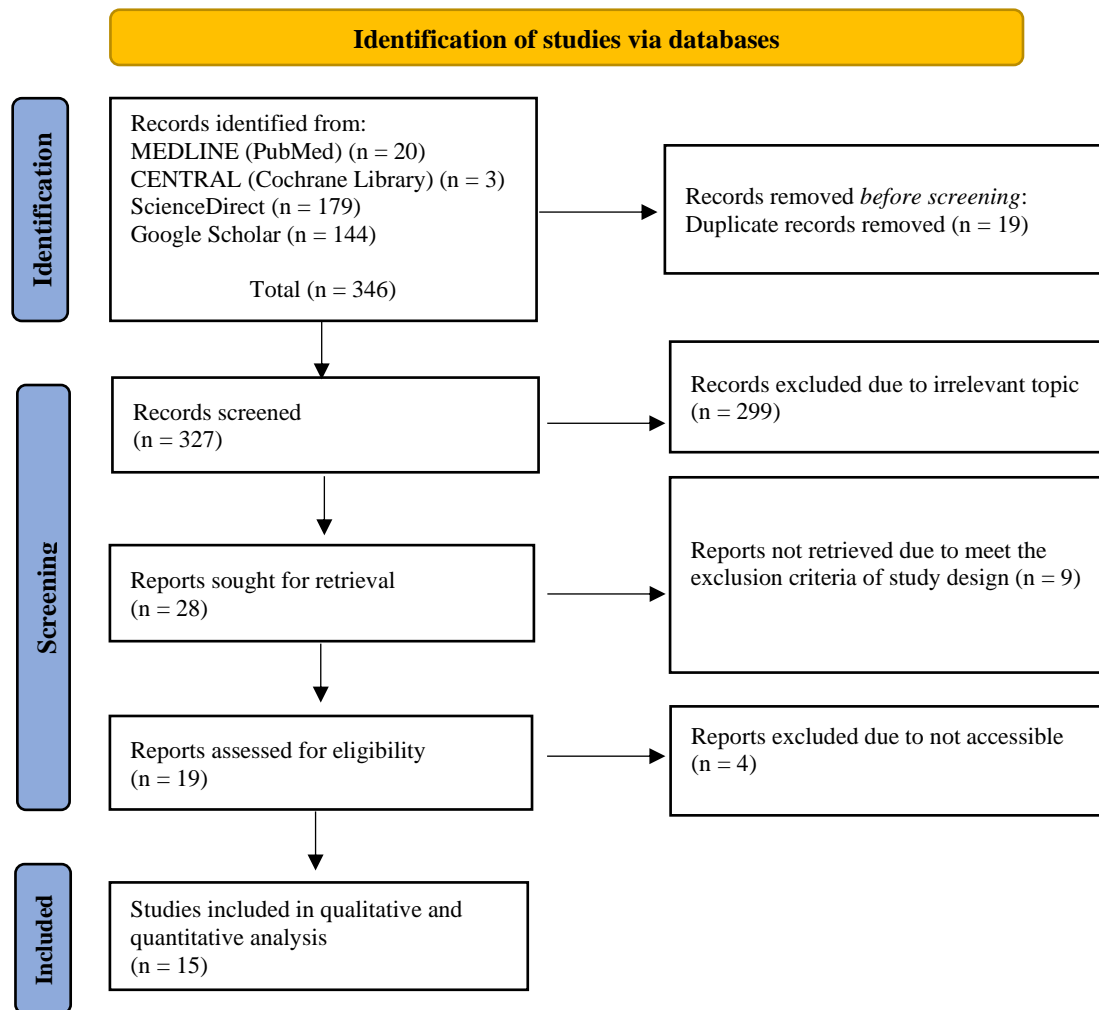


Figure 1. PRISMA Flow Diagram

eligibility: type of study, population samples, index test, and reference standards.

Type of Studies

Original studies or reports conducted with a population of extremely preterm infants administered enteral lactoferrin supplementation were included. In addition to articles without full-text access, irrelevant topics, non-English, narrative and systematic review with or without meta-analysis, non-comparative study, in vitro studies, technical reports, editor response, scientific posters, study protocols, and conference abstracts were excluded.

Samples

The population in this study is preterm infants and extremely infants admitted to the neonatal intensive unit (NICU) and perinatology unit. Those with severe congenital anomalies were excluded.

Outcomes

The results of this review were (i) confirmed sepsis cases in preterm or extremely preterm infants during the hospital stay period. This was determined by clinical signs and microbiologically proven by blood, cerebrospinal fluid, and urine cultures. (ii) Necrotizing enterocolitis (NEC) Bell's stage II or III during the hospital stay. (iii) All-cause and sepsis-attributable mortality.

Index Test

Studies evaluating the sepsis and NEC cases, as well as mortality rates in preterm infants post-enteral lactoferrin supplementation were included. Those without these outcomes were excluded.

Reference Standard

The reference standard was randomized controlled trial studies performed by qualified professionals evaluating the effect of enteral lactoferrin supplementation on preterm infants.

Data Sources and Search

The literature search was conducted using multiple electronic databases, including PubMed, ScienceDirect, Cochrane Library, and Google Scholar. The process was conducted from the inception of the database until August 2021. Furthermore, the keywords used were described using Boolean operators. The studies from these electronic databases were stored in the online software of Rayyan.ai.

Study Selection

After removing duplicates, four independent reviewers screened retrieved articles based on their topics, titles, and abstracts (YTNS, DCDT, AIT, and RNE). Potentially eligible full-text articles were assessed using the eligibility criteria described previously. Furthermore, emerging discrepancies were discussed by consensus among the review team. The study selection process was presented and recorded in the PRISMA flow diagram.

Data Extraction and Analysis

Data were extracted from the included studies and stored in Microsoft Excel 2016. The following were recorded: first author and year of publication, country, study design, sample size, sex, gestational age (weeks), birth weight, lactoferrin, dosage, start time, duration, administration route, and type of microorganism causing sepsis. The statistical

test for the primary outcomes was conducted using Review Manager (RevMan) v5.3.

Risk of Bias Assessment

Two reviewers (YTNS and AIT) independently assessed the risk of bias in individual studies based on the Cochrane Risk of Bias 2 tool (RoB 2 Tool), which included several domains such as randomization sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other sources. These studies were categorized as low, high, or unclear risk. Finally, conflicts were resolved through discussion with YTNS and AIT.

Quantitative Data Synthesis (Meta-Analysis)

This study calculated the risk ratio (RR) with a 95% confidence interval (CI). Random-effect model (REM) or fixed-effect model (FEM) was used to determine the forest plot pooled effect size based on the heterogeneity level. REM was used when the included studies were considered heterogeneous (high variability in studies' results), indicated by an I^2 value higher than 50%. Otherwise, a FEM forest plot was used.

Risk of Bias Across Studies (Publication Bias)

The publication bias was subjectively analyzed using a funnel plot generated by Review Manager (RevMan) v5.3. The asymmetrical shape of the funnel plot indicates the presence of publication bias. Meanwhile, its symmetrical shape shows the absence of publication bias.

Sensitivity Analysis

A sensitivity analysis was designed to determine which studies acted as confounding factors when the authors encountered the uncertain conclusion because of the

contradicting results from some included studies. This was performed by repeating the meta-analysis and trying to eliminate some studies.

RESULTS

Studies Selection

The search yielded 346 articles, after which nineteen duplicates were removed. The titles and abstracts of the remaining 327 were read for preliminary screening. Afterwards, those that did not meet the eligibility criteria were excluded. Full texts were retrieved for 28 articles, and 9 were excluded for reasons, but about 4 could not be accessed. Finally, 15 studies were included for qualitative and quantitative analysis. Figure 1 shows the study selection process presented in the PRISMA flow diagram.

Characteristics and Results of Individual Studies

Tables 1 and 2 show the details of extracted data from included studies. The subject of these studies was preterm infants receiving enteral lactoferrin supplementation to prevent sepsis, necrotizing enterocolitis, and mortality. The point of focus was the outcomes indicating the potential of lactoferrin as a new adjuvant supplement for preventing mortality in preterm infants. The studies included infants under 34 weeks of gestational age and 1609 grams of birth weight.

Out of the 15 studies in the qualitative synthesis, only one used talactoferrin (TLF), while the other used bovine lactoferrin (BLF). The initial dosage of lactoferrin varies from 100 to 300 mg/day starting from the first 12 or 72 hours of life until discharge. The administration route of lactoferrin supplementation is enteral and diluted using

breast milk formula. After those supplementations and standardized treatment, infants with clinical signs of sepsis were analyzed and treated with antibiotics or antifungal agents. The laboratory results showed that the majority of the etiologic agent causing sepsis were Gram-positive and -negative bacteria, as well as opportunistic fungi.

Risk of Bias in Individual Studies

Cochrane assessed the quality and risk of each study's bias using the Risk of Bias 2 Tool (RoB 2 Tool). Most of them had adequate information regarding the domains' judgement, leading to a low and moderate risk of bias. Also, there were 2 studies with high risk due to some unblinded outcome assessment. In the overall risk of bias, most included studies showed moderate risk accompanied by low risk.

Quantitative Data Synthesis (Meta-Analysis)

The pooled effect size of enteral lactoferrin supplementation on preterm infants or extremely preterm infants was compared to prevent sepsis, NEC, and mortality. The result event for each study was used to compare the intervention and control groups. Figure 3 shows a moderate pooled effect size of sepsis cases in preterm infants supplemented with enteral lactoferrin. The result indicated that the enteral lactoferrin supplementation for preterm infants and extremely preterm infants could reduce the risk of sepsis significantly [RR = 0.65, 95% CI (0.56, 0.77), $p < 0.00001$, $I^2 = 56\%$]. This showed a good effect of enteral lactoferrin supplementation in reducing the risk of preterm infants sepsis.

Table 1. Characteristics of Included Studies

Authors (Year)	Country	Study design	Sample size	Sex (M/F)	Gestational age (weeks)		Birth weight	
					Intervention	Control	Intervention	Control
Akin et al. (2014) ¹³	Turkey	RCT	50	23/27	29.5 ± 1.6	30.3 ± 2.5	1290 ± 346.7	1307 ± 262.1
Barrington et al. (2016) ¹⁴	Canada	RCT	79	46/33	28.0 ± 1.7	28.4 ± 2.1	1087 ± 315	1104 ± 320
Dai et al. (2015) ¹⁵	China	RCT	70	NR	30.03 ± 2.16	30.03 ± 2.16	<1500	<1500
ELFIN (2019) ¹⁶	United Kingdom	RCT	2199	1153/1046	29 (IQR 27- 30)	29 (IQR 27- 30)	1125.9 ± 356.2	11.43 ± 367.1
Farag et al. (2021) ¹⁷	Egypt	RCT	56	14/32	32.4 ± 1.9	32.7 ± 1.4	1300 ± 300	1300 ± 300
Kaur and Gathwala (2015) ¹⁸	India	RCT	130	73/57	34.4 ± 2.9	33.9 ± 2.5	1494.98 ± 240.87	1484.01 ± 224.86
Liu et al. (2016) ¹⁹	China	RCT	160	NR	26 - 33	26 - 33	NR	NR
Manzoni et al. (2009) ²⁰	Italy	RCT	505	264/241	29.7 ± 2.5	29.4 ± 3.1	1158 ± 422	1600 ± 395
Manzoni et al. (2012) ²¹	Italy	RCT	321	160/161	29.6 ± 2.5	29.5 ± 3.2	1142 ± 244	1109 ± 269
Manzoni et al. (2014) ²²	New Zealand	RCT	505	264/241	29.7 ± 2.5	29.4 ± 3.1	1158 ± 422	1600 ± 395
Ochoa et al. (2015) ²³	Peru	RCT	190	92/98	32.2 ± 2.6	32.0 ± 2.6	1582 ± 442	1600 ± 395
Ochoa et al. (2020) ²⁴	Peru	RCT	414	230/184	30.8 ± 2.8	30.8 ± 3.2	1382 ± 371	1378 ± 353
Sherman et al. (2016) ²⁵	United States	RCT	120	69/51	28.0 ± 6/7	28 ± 6/7	1152 ± 206	1143 ± 220
Tang et al. (2017) ²⁶	China	RCT	172	78/94	31.63 ± 2.50	31.55 ± 3.28	1542.34 ± 244.12	1509.17 ± 269.24
Tanrow-Mordi et al. (2020) ²⁷	Australia	RCT	1541	844/697	28.4 ± 2.4	28.4 ± 2.3	1068 ± 262	1063 ± 261

Table 2. Characteristics of Lactoferrin Administered and The Microbiology Outcomes

Authors (Year)	Type of Lactoferrin	Dosage	Start time, duration	Administration route	Type of microorganisms causing sepsis
Akin et al. (2014) ¹³	BLF	200 mg/day	Within the first 72 h of life; until death or discharge	Enteral, LF was diluted in milk or formula	MRCONS, <i>Enterococcus</i> , <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas</i> , <i>Candida</i>
Barrington et al. (2016) ¹⁴	BLF	100 mg/day	Within the first 48 h of life; until 36 weeks post-menstrual age or discharge	Enteral, LF was diluted in milk or formula	CONS, <i>Escherichia coli</i> , <i>Klebsiella</i> , <i>Candida</i>
Dai et al. (2015) ¹⁵	BLF	100 mg/day	Within the first 72 h of life; NR	NR	NR
ELFIN (2019) ¹⁶	BLF	150 mg/kg/day	Within the first 72 h of life; until death or discharge	Enteral, LF was diluted in milk or formula	NR
Farag et al. (2021) ¹⁷	BLF	100-200 mg/day	Within the first 24 h of life; until the 28 th day of life	Enteral, LF was diluted in milk or formula	<i>Klebsiella</i> and <i>Candida</i>
Kaur and Gathwala (2015) ¹⁸	BLF	100-250 mg/day	Within the first 12 h of life; until the 28 th day of life	Enteral, LF was diluted in milk or formula	<i>Pseudomonas aeruginosa</i> , <i>Acinetobacter baumannii</i> , <i>Klebsiella</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Candida</i>
Liu et al. (2016) ¹⁹	BLF	250 mg/day	Within the first 72 h of life; until death or discharge	Enteral, LF was diluted in milk or formula	NR
Manzoni et al. (2009) ²⁰	BLF	100 mg/day	Within the first 72 h of life; until day 30 (45 for neonates<1000 g at birth)	Enteral, LF was diluted in milk or formula	NR
Manzoni et al. (2012) ²¹	BLF	100 mg/day	Within the first 24 h of life; until death or discharge	Enteral, LF was diluted in milk or formula	<i>Candida albicans</i>
Manzoni et al. (2014) ²²	BLF	100 mg/day	Within the first 72 h of life; until day 30 (45 for neonates<1000 g at birth)	Enteral, LF was diluted in milk or formula	NR
Ochoa et al. (2015) ²³	BLF	200 mg/day	Within the first 72 h of life; until day 30	Enteral, LF was diluted in milk or formula	<i>Serratia</i> , <i>Enterobacter aerogenes</i> , <i>Klebsiella</i> , CONS, <i>Pseudomonas</i> , <i>Group B Streptococcus</i> , <i>Enterococcus faecalis</i>
Ochoa et al. (2020) ²⁴	BLF	100-200 mg/day	Within the first 24 h of life; until the 8 th week of life	Enteral, LF was diluted in milk or formula	Gram-negative bacteria, Gram-positive bacteria, CONS, <i>Candida</i>
Sherman et al. (2016) ²⁵	TLF	300 mg/day	Within the first 24 h of life; until the 28 th day of life or discharge	Enteral, TLF solution	NR
Tang et al. (2017) ²⁶	BLF	100 mg/day	Within the first 72 h of life; until death or discharge	Enteral, LF was diluted in milk or formula	NR
Tanrow-Mordi et al. (2020) ²⁷	BLF	200 mg/day	Within the first 24 h of life; until 34 weeks post-menstrual age or discharge	Enteral, LF was diluted in milk or formula	CONS and <i>Group B Streptococcal antigenuria</i>

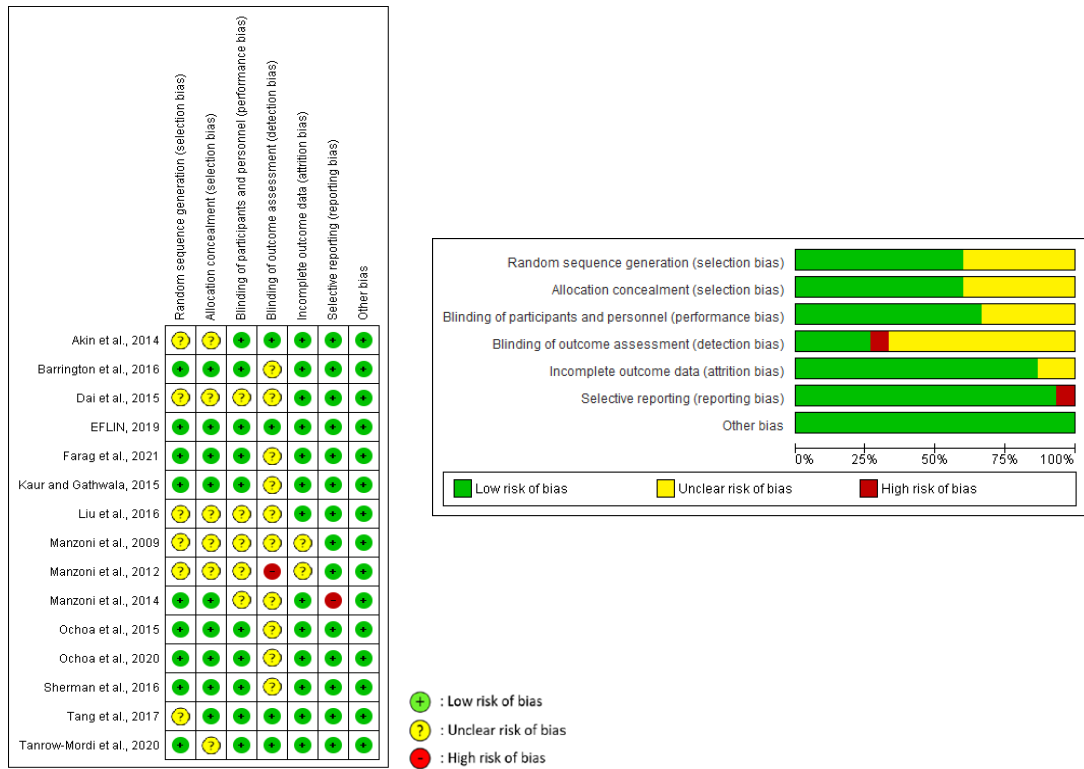


Figure 2. Risk of Bias Assessment In Individual Studies Using RoB 2

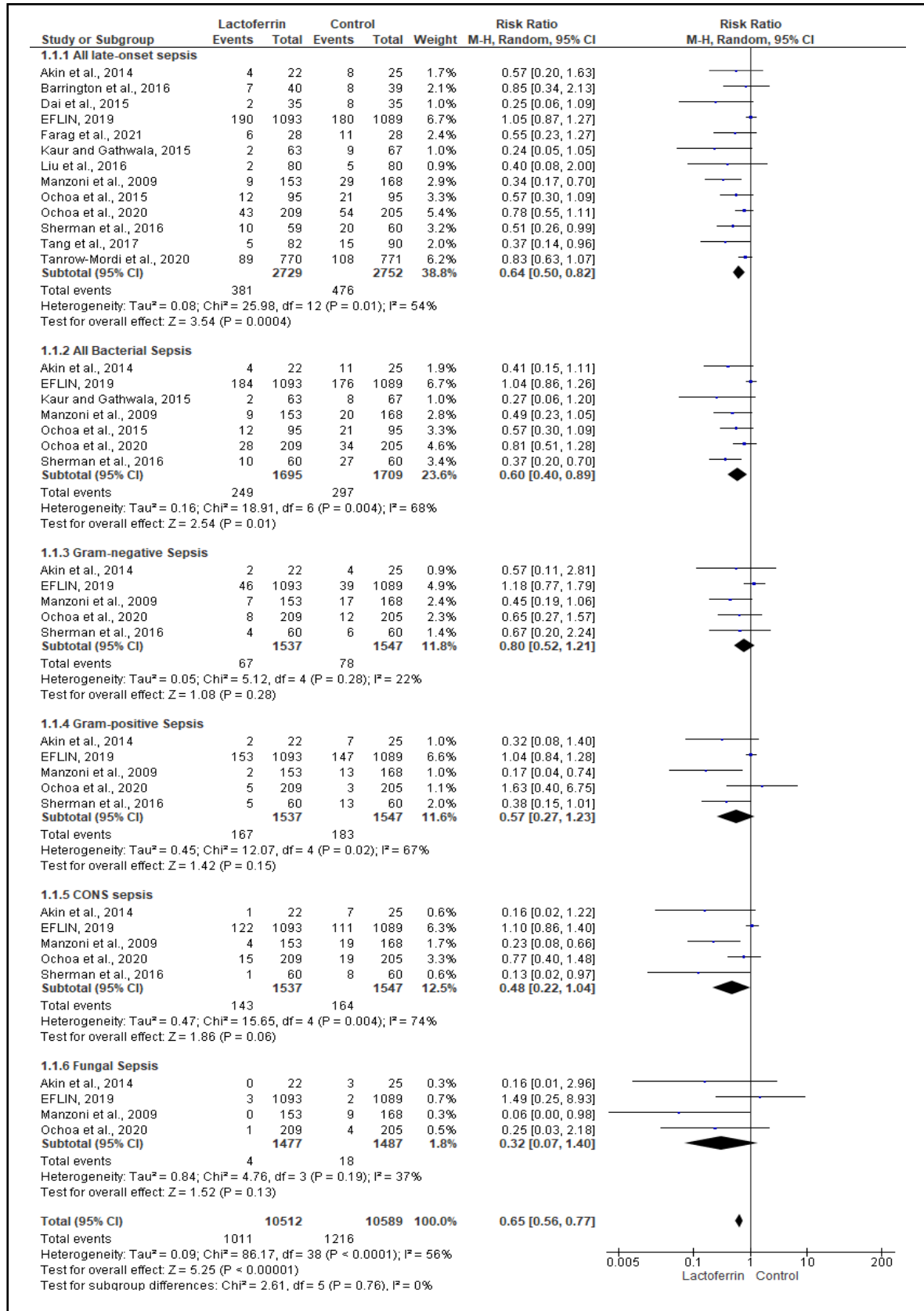


Figure 3. Forest Plot for Incidence of Late-Onset Sepsis (LOS) between Enteral Lactoferrin Supplementation and Control Groups

The risk of necrotizing enterocolitis in the preterm or extremely preterm infants was compared between the intervention and control groups. The results showed no significant difference in statistics, despite the existing trend that enteral lactoferrin supplementation

can reduce the risk of NEC in preterm infants or extremely preterm infants [RR = 0.80, 95% CI (0.63, 1.02), $p = 0.08$, $I^2 = 39\%$]. Figure 4 presents the result of a quantitative analysis of NEC risk in preterm infants between the intervention and control groups.

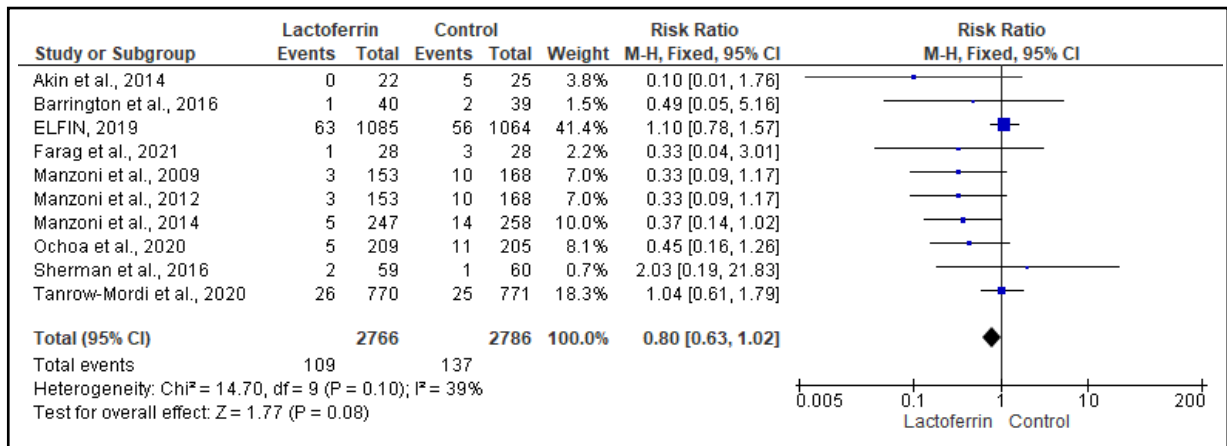


Figure 4. Forest Plot for Incidence Necrotizing Enterocolitis Stage II or III between Enteral Lactoferrin Supplementation and Control Groups

The mortality rate in the preterm or extremely preterm infants also compared between the intervention and control groups. All-cause and sepsis-attributable mortality was not statistically significant, despite the trend showing that mortality occur in control group

[RR = 0.94, 95% CI (0.77, 1.13), $p = 0.49$, $I^2 = 39\%$]. Figure 5 presents the result of a quantitative analysis of mortality in preterm infants between the intervention and control groups.

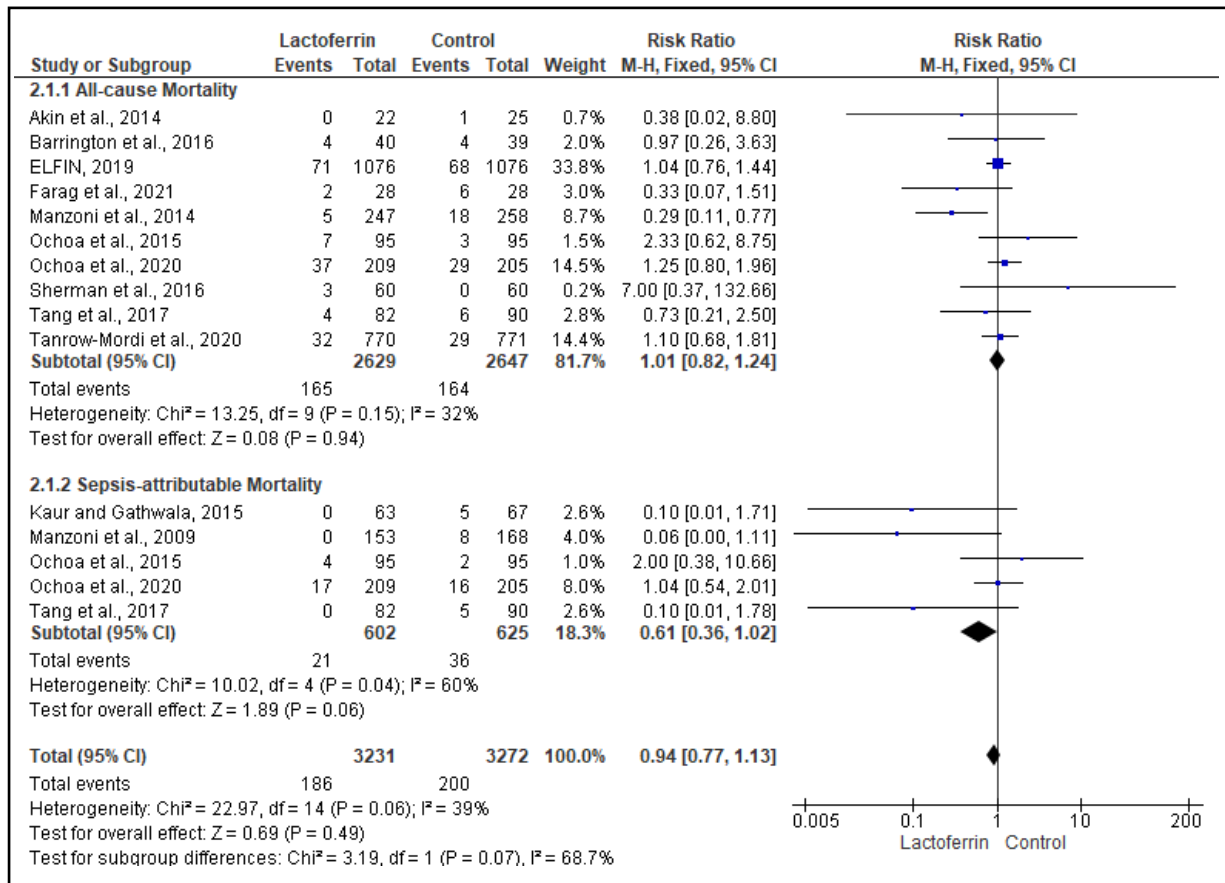


Figure 5. Forest Plot for Mortality between Enteral Lactoferrin Supplementation and Control Groups

Risk of Bias Across Studies (Publication Bias)

A publication bias was subjectively analyzed according to the symmetry of their shape using a funnel plot. This showed the absence of publication bias since the funnel

plot exhibit a symmetrical shape. Figure 6 indicates the funnel plot of the previous quantitative analysis.

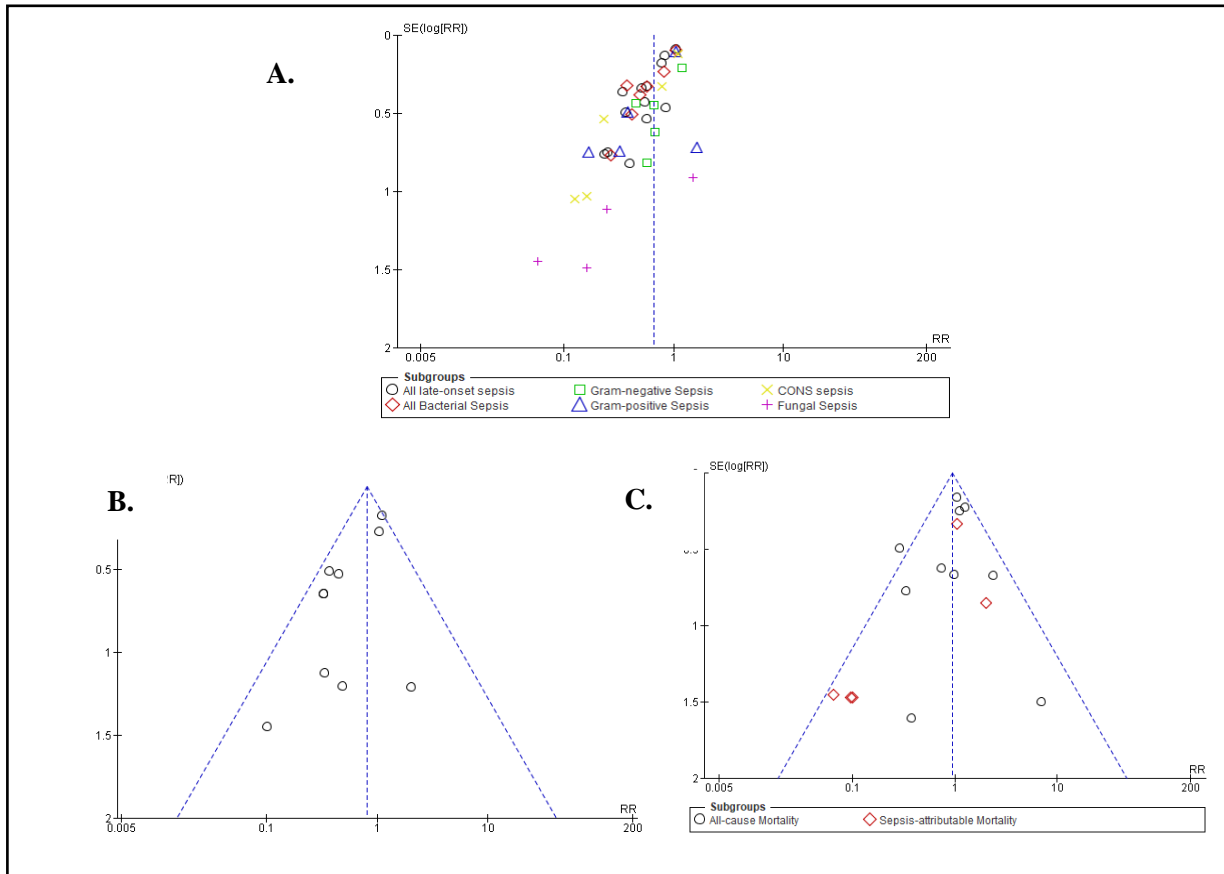


Figure 6. Funnel Plot for (A) Incidence of Late-Onset Sepsis (LOS), (B) Incidence Necrotizing Enterocolitis Stage II or III, (C) Mortality

DISCUSSION

The meta-analysis showed that enteral lactoferrin supplementation could reduce LOS incidence in preterm infants. The mechanism of action was associated with the protein's capability to bind to iron. Lactoferrin could act as antimicrobial, antiviral, antifungal, and immunomodulatory agents to prevent sepsis and mortality¹⁶. It directly binds with bacterial lipopolysaccharides (LPS) to inhibit the interaction between endotoxin-containing LPS binding protein (LBP) and CD14, which is essential to prevent the pathogenesis of sepsis²⁸. In addition, lactoferrin also has anti-inflammatory effects by increasing neutrophil degranulation activity and reducing the production of reactive oxygen species (ROS) and proinflammatory cytokines²⁹.

The other results showed that the enteral lactoferrin administration and control group showed no significant difference in preventing the incidence of NEC stage II or III. However, this could be due to the largest RCT study with contradictory results. ELFIN reported that NEC incidence was more significant in the enteral lactoferrin supplementation group than in the control group¹⁶. The sensitivity test was performed by excluding ELFIN 2019. Furthermore, it showed a significant difference that enteral lactoferrin supplementation could reduce the incidence of NEC stage II or III.

The incidence of total mortality increased in the control group. However, statistical analysis shows no significant difference between mortality in the enteral lactoferrin supplementation and control groups. Subgroup analysis was conducted to

determine which cause of death could prevent with enteral lactoferrin supplementation. Based on this analysis, the incidence of all-cause mortality is almost the same, despite the higher prevalence of sepsis. This result can occur because several diseases could not be prevented by lactoferrin supplementation, including congenital anomalies and other intrauterine infections.

The study by ELFIN stated that lactoferrin supplementation (150 mg/kg/day) for 34 weeks did not reduce the risk of LOS, NEC, and mortality in premature infants. It was also reported that the incidence of NEC and mortality was lower in control than in the enteral lactoferrin supplementation group¹⁶. Sherman *et al.* showed that the mortality rate was higher in the enteral lactoferrin supplementation group²⁵. This contradictory study is linear with Ochoa *et al.*, which reported a higher mortality rate in the group receiving enteral lactoferrin supplementation^{23,24}. Meanwhile, Tarnow - Mordi *et al.* showed the exact incidence of NEC and mortality between the two groups²⁷.

As a perspective review, lactoferrin may benefit preterm infants, specifically those born from mothers with severe COVID-19. It is already observed that these mothers have a high risk of preterm labour, sepsis, NEC, respiratory disorders, thrombocytopenia, and liver function abnormalities that can lead to the mortality of their newborns³⁰. Extremely premature birth (gestational age 25 weeks) is possible for mothers with severe COVID-19 in pregnancy, with a high risk of mortality³¹. Furthermore, lactoferrin might reduce the severity of this virus by down-regulating ACE2 and preventing viral adhesion to the host cell proteoglycans. Lactoferrin also plays an essential role as an IL-8 agonist in binding proteoglycans of immune cells to perform anti-inflammatory effects, thereby preventing the risk of morbidity and mortality in premature infants³².

This meta-analysis showed that enteral lactoferrin supplementation was associated with reduced late-onset sepsis in preterm infants. However, there were some limitations in this study. More RCT studies are required to suggest the advantage of this supplementation in preventing morbidity and mortality in preterm infants. Further studies with COVID-19-positive mothers are also required to prove the theory from this review.

CONCLUSION

This meta-analysis showed that enteral lactoferrin supplementation was associated with a significant reduction in LOS, but not NEC stage II or III and all-cause mortality, in preterm infants.

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