Systematic Review and Meta-Analysis

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# Prophylactic lactoferrin for preventing late-onset sepsis and necrotizing enterocolitis in preterm infants

## A PRISMA-compliant systematic review and meta-analysis

Yi He, MSc[a,b], Luying Cao, MSc[a,b], Jialin Yu, MD[a,b,c,*]

**Abstract**

**Background:** Currently, prophylactic use of drugs to promote a healthy gut microbiota and immune system in preterm infants is hot debated, among which lactoferrin is a promising supplementation. However, the effect and safety of lactoferrin to prevent late-onset sepsis (LOS) and necrotizing enterocolitis (NEC) in preterm infants remains controversial.[1]

**Methods:** Databases including Medline, Ovid-Embase, The Cochrane Library, CBM, CNKI, and VIP database of Chinese Journal were searched to collect randomized controlled trials (RCTs) about lactoferrin for preventing LOS and NEC in preterm infants. Languages of included RCTs were restricted to English and Chinese. Meta-analysis was conducted by Rev Man 5.3 software. The Mantel–Haenszel method with random-effects model was used to calculate pooled relative risks (RRs) and 95% confidence intervals (CIs).

**Results:** A total of 9 RCTs, involving 1834 patients, were included. Pooled analysis showed that prophylactic lactoferrin could significantly reduce the incidence all culture-proven LOS (41/629 [6.5%] vs 96/659 [15.3%]; RR 0.47; 95% CI 0.33–0.67; $P < .01$) and NEC (stage II or more) (9/448 [2.0%] vs 26/462 [5.6%]; RR 0.40; 95% CI 0.18–0.86; $P < .01$). Lactoferrin was also associated with a significantly decreased hospital-acquired infection (16/139 [11.5%] vs 35/140 [25%]; RR 0.47; 95% CI 0.27–0.80; $P < .01$); and infection-related mortality (4/474 [0.8%] vs 25/505 [4.9%]; RR 0.24; 95% CI 0.04–1.32; $P < .01$, $I = 53\%$). Lactoferrin could shorten time to reach full enteral feeding (weighted mean difference [WMD] = −2.11, 95% CI −3.12 to −1.10; $P < .01$) and showed a decreasing trend of duration of hospitalization (WMD = −1.69, 95% CI −6.87 to 3.50; $P < .01$; $I = 95\%$). Lactoferrin did not have a significant effect on all-cause mortality (22/625 [3.5%] vs 35/647 [5.4%]; RR 0.70; 95% CI 0.38–1.30; $P = .16$; $I = 13\%$). None of the included trials reported any confirmed adverse effects caused by the supplemented lactoferrin or probiotics.

**Conclusion:** Current evidence indicates that lactoferrin could significantly reduce the incidence of NEC and LOS, and decrease the risk of hospital-acquired infection and infection-related mortality in premature infants without obvious adverse effects.

**Abbreviations:** BW = birth weight, CI = confidence interval, ELBW = extremely low birth weight, FM = formula milk, GA = gestational age, HM = human milk, LBW = low birth weight, LGG = Lactobacillus rhamnosus GG, LOS = late-onset sepsis, NEC = necrotizing enterocolitis, NICU = neonatal intensive care unit, RCT = randomized controlled trial, RR = relative risk, TLF = talactoferrin, VLBW = very low birth weight, WMD = weighted mean difference.

**Keywords:** lactoferrin, meta-analysis, necrotizing enterocolitis, premature infant, randomized controlled trial, sepsis

## 1. Introduction

Infectious diseases attribute to most of deaths in neonate.[2] Although modest reductions have been seen for the last decade, late-onset sepsis (LOS) and necrotizing enterocolitis (NEC)—a serious inflammatory gut condition, are still among the leading cause of serious morbidity and mortality in preterm infants. Studies have shown that an increased rate of neonatal infection associated with lower gestational age (GA) and lower birth weight.[3,4] Premature infant are indeed highly prone to infection because of immature immune system, exposure to invasive procedures, and use of broad-spectrum antibiotics.

Studies have demonstrated that very low birth weight (VLBW) infants fed with human milk (HM) develop fewer sepsis, NEC, and cause less neonatal intensive care unit (NICU) costs.[5,6] However, maternal milk is limited for preterm infants, especially VLBW and extremely low birth weight (ELBW), because the production of maternal colostrum is limited after birth or intestinal immaturity hinders full enteral feedings. Various bioactive components in HM can promote the commensal

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*[a] Department of Neonatology, Children's Hospital of Chongqing Medical University, Ministry of Education Key Laboratory of Child Development and Disorders, Yuzhong, [b] China International Science and Technology Cooperation Base of Child Development and Critical Disorders, [c] Chongqing Key Laboratory of Pediatrics, Chongqing, China.*

*[*] Correspondence: Jialin Yu, Children's Hospital of Chongqing Medical University, No.136, Zhongshan 2nd Road,Yuzhong District, 400014 Chongqing, China (e-mail: yujialin486@126.com).*



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intestinal microbiome, nascent gut development, and host defenses establishment. Lactoferrin, one of the most important protein consumed by breast-fed infants immediately after birth, may be the major milk component responsible for decreasing infection, due to its antimicrobial, antioxidant, antifungal, anti-inflammatory, and immune-modulation properties.[7,8] Therefore, it is considered as a promising supplementation to promote the development of normal intestinal function and reduce the incidence of LOS and NEC in preterm infants.

Currently, the effect and safety of lactoferrin to prevent LOS and NEC in preterm neonates still remains controversial. Recently, many trials have been published investigating the protective effect of lactoferrin in preterm infants. The goal of our meta-analysis is to inform clinicians about the risks and benefits of lactoferrin.

## 2. Methods

This systematic review and meta-analysis was conducted and reported in adherence to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement[9] and the guidelines of the Cochrane Handbook for Systematic Reviews of Interventions.[10] Because our study was a review of previous published studies, ethical approval or patient consent was not required.

### 2.1. Search strategies and inclusion criteria

Medline, Ovid-Embase, The Cochrane Library, CBM, CNKI, and VIP were searched for records that compared enteral lactoferrin with or without probiotics to placebo or no intervention in preterm neonates in NICUs from May 1, 2017. The last search was conducted on February 1, 2018. The cited references of retrieved articles and previous reviews were also manually checked to identify any additional eligible trials. All citations were imported into a bibliographic database (End Note X7; Thomson Reuters), and 2 of the authors (YH and LC) independently screened the candidate articles to check their eligibility for inclusion. We searched for the following terms (Table 1).

We developed a Patient, Intervention, Comparators, Outcome, and Study design approach as the eligibility criteria—Population: preterm infants <37 weeks or birth weight (BW) <2500 g or both; infants with sever congenital anomalies (gastrointestinal problem, suspected congenital infections) were excluded. Intervention: lactoferrin with or without probiotics administered for >7 days; Comparators: placebo or no lactoferrin; Outcome: The primary outcome was NEC stage II or more (defined as clinical signs with the presence of pneumatosis intestinalis on abdominal X-ray, according to the modified Bell criteria[11]) and all culture-proven LOS (defined as a positive blood culture and/or cerebrospinal fluid culture obtained after 72 hours of life in the presence of clinical signs and symptoms of infection[12]). The secondary outcome was hospital-acquired infection (defined as hospital-acquired bacteremia, pneumonia, urinary tract infection, gastroenteritis and NEC[13]), infection-related mortality (defined as death within 5 days after the last positive culture result from any site without other causes), duration of hospitalization, days to achieve full enteral feeding, all-cause mortality, and adverse effects. Study design: only randomized controlled trials (RCTs) were eligible. Discrepancies regarding study inclusion between the 2 authors (YH and LC) were resolved through discussion with the correspondence author (JY), as required. Only published data were used for those studies. For duplicated publications of the same clinical trial, we choose the latest updated data.

**Table 1**

**Search strategy.**

| Search terms |
|---|
| Lactoferrin or talactoferrin or lactotransferrin or bovine lactoferrin or human lactoferrin or lactoferricin |
| Preterm infant or premature infant or neonate or newborn or very low birth weight infant or VLBW or low birth weight infant or extremely low birth weight infant or ELBW |
| Sepsis or late-onset sepsis |
| Necrotizing enterocolitis |
| Randomized clinical trials |

### 2.2. Date extraction and quality assessment

Two of the authors (YH and LC) independently extracted relevant data from each included trials by using a unified data form. Extracted data were entered into a standardized EXCEL file. The items included in the data form were as follows: source (first author), number of preterm infants enrolled, interventions, type of milk (HM or formula milk [FM]), and outcomes of interest. Discrepancies between authors were resolved by consensus. We used the Cochrane Risk-of-Bias Tool to assess the risk of bias for each RCT.[14]

### 2.3. Statistical analysis

Since the included RCTs were performed in different regions including developing countries and developed countries, we presume that there are variability in races, time of intervention, dosages, lab detection accuracy, and other unknown confounding factors. Owing to the assumption of within- and between-study heterogeneity, the Mantel–Haenszel method with random-effects model was used to calculate pooled relative risks (RRs) and 95% confidence intervals (CIs). Trials with uneven distribution of demographic characteristics and sepsis-related risk factors between study and control groups were not included in our meta-analysis, such as GA, BW, Apgar score, prenatal steroids, antimicrobial drugs, and use of invasive devices. Heterogeneity across studies was tested by using the $I^2$ statistic. Studies with an $I^2$ value of >50% were considered to have significant heterogeneity.[15] Subgroup analyses were conducted according to pathogen of sepsis and birth weight. Sensitivity analyses were conducted by exclusion of any single study to investigate the influence of a single study on the overall pooled RRs. However, we could not assess publication bias by visually inspecting funnel plot because of limited numbers of RCTs. A $P$ value $< .01$ was considered as statistically significant, except where otherwise specified. All the statistical analyses were performed using Rev Man 5.3 (The Nordic Cochrane Centre, Copenhagen, Denmark).

### 2.4. Search results

A total of 397 potentially relevant records were identified by our search strategy, of which 268 records remain after duplicates were removed; 247 records were excluded after the screening the titles and abstracts. The remaining 21 articles were assessed for eligibility and 12 articles[16–27] were considered eligible for

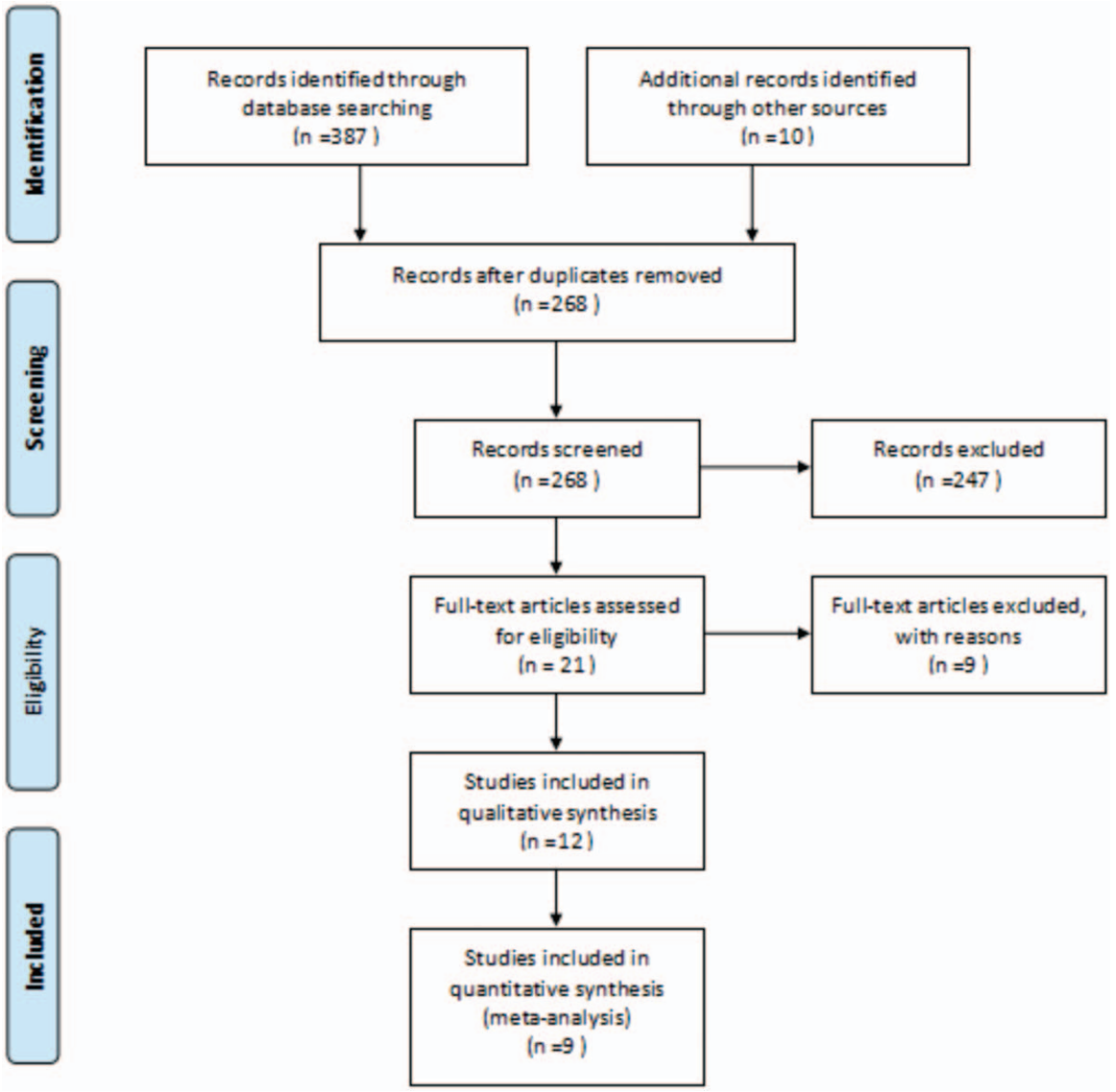


**Figure 1.** Selection process for the studies included in the meta-analysis.

inclusion after full-text reading. Three duplicate publications[19,20,24] were found. Finally, 9 RCTs were included in the systematic review. The flow diagram of the study selection process is given in Fig. 1. Hence, 9 trials were statistically analyzed. Characteristics of the 9 trials are summarized in Table 2. The quality of the trials assessed by the Cochrane Risk-of-Bias Tool is summarized in Figs. 2 and 3.

## 3. Outcomes

### *3.1. Comparison 1: Lactoferrin supplementation vs placebo*

***3.1.1. Outcome 1.1: NEC stage II or more.*** Out of the 9 included studies, NEC was the primary outcome of interest in 5 studies, whereas the remaining 4 did not mention the outcome. Figure 4 shows the efficacy of lactoferrin in the prevention of NEC in preterm neonates, using a random-effects model. Of the 5 estimates, the incidence of NEC was 9/448 (2.0%) versus 26/462 (5.6%); RR 0.40; 95% CI 0.18–0.86; $P < .01$; heterogeneity: $P = .57$, $I = 0\%$. Further exclusion of any single study did not materially alter the overall combined RR, with a range from 0.33 (95% CI 0.14–0.74) to 0.44 (95% CI 0.20–0.98).

***3.1.2. Outcome 1.2: LOS.*** Pooled results from 9 RCTs (N= 1834) using random-effects model meta-analysis showed that lactoferrin significantly decreased the risk of all culture-proven LOS (41/629 [6.5%] vs 96/659 [15.3%]; RR 0.47; 95% CI 0.33–0.67; $P < .01$; heterogeneity: $P = .66$, $I = 0\%$). Results of all the studies were homogeneous except Ochoa 2015. On sensitivity analysis, the beneficial effects continued to be observed after excluding studies with high risk of bias for random sequence generation and also for allocation concealment. Mazoni 2014 did not report on the result of sepsis, thus the study has high risk of reporting bias. We extracted data of sepsis from the previous study Mazoni 2009, which should minimize the effect of its reporting bias. Furthermore, after excluding the trial with high risk of reporting bias—Mazoni 2014, the RR of LOS was still

**Table 2**

**Characteristics of randomized controlled trials included in the meta-analysis.**

| Source | N | Participants | Type of milk | Interventions | Outcomes of interests* |
|---|---|---|---|---|---|
| Akin 2014 | 50 | BW < 2500 g or GA < 32 w | HM or FM | BLF (200 mg $d^{-1}$) | 1, 2, 4, 5, 6, 7 |
| Barrington 2016 | 79 | BW < 2500 g or GA < 31 w | HM or FM | BLF (100 mg $d^{-1}$) | 1, 2, 5, 7 |
| Dai 2015 | 105 | BW < 1500 g or GA 26–33 w | Not mentioned | BLF (100 mg $d^{-1}$) or BLF (100 mg $d^{-1}$) + LGG ($6 \times 10^9$ CFU $d^{-1}$) | 2 |
| Kaur 2015 | 130 | BW < 2000 g | HM or FM | BLF (80–142 mg $kg^{-1}$ $d^{-1}$) | 2, 4, 6, 7 |
| Liu 2016 | 160 | BW < 2500 g or GA 26–33 w | Not mentioned | BLF (250 mg $d^{-1}$) | 1, 2, 5, 6, 7 |
| Manzoni 2009/2012/2014 | 743 | BW < 2500 g | HM or FM | BLF (100 mg $d^{-1}$) or BLF (100 mg $d^{-1}$) + LGG ($6 \times 10^9$ CFU $d^{-1}$) | 1, 2, 4, 5, 6, 7 |
| Ochoa 2015 | 190 | BW < 2500 g | HM or FM | BLF (200 mg $kg^{-1}$ $d^{-1}$) | 2, 4, 7 |
| Sherman 2016 | 120 | BW 750–1500 g | HM or FM | TLF (300 mg $kg^{-1}$ $d^{-1}$) | 1, 2, 4, 5, 6, 7 |
| Tang 2017 | 257 | GA < 37 w | HM or FM | BLF (100 mg $d^{-1}$) or BLF (100 mg $d^{-1}$) + LGG ($6 \times 10^9$ CFU $d^{-1}$) | 2, 4, 7 |

BLF = bovine lactoferrin, BW = birth weight, FM = formula milk, GA = gestational age, HM = human milk, LGG = Lactobacillus rhamnosus GG, TLF = talactoferrin.
* 1: Necrotizing enterocolitis stage II or more; 2: all culture-proven late-onset sepsis; 3: hospital-acquired infection; 4: infection-related mortality; 5: days to achieve full enteral feeding; 6: duration of hospitalization; 7: all-cause mortality; 8: adverse effects relevant to LF.

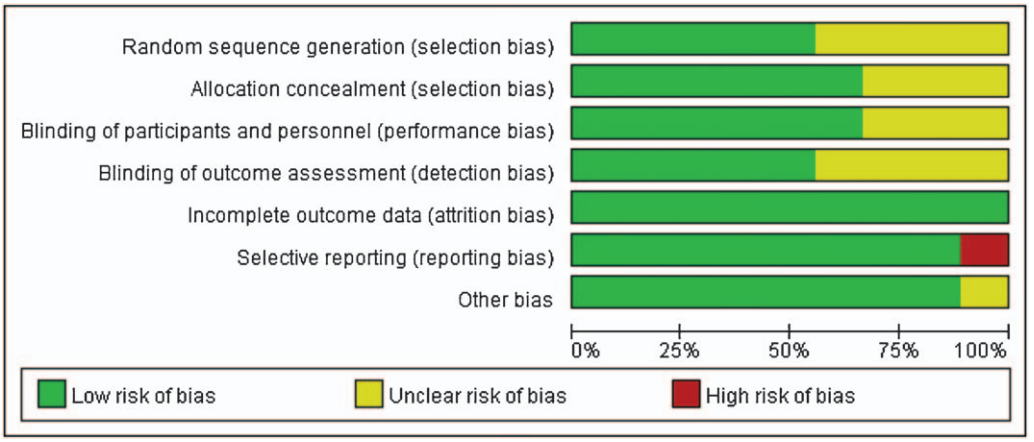


**Figure 2.** Risk of bias graph.

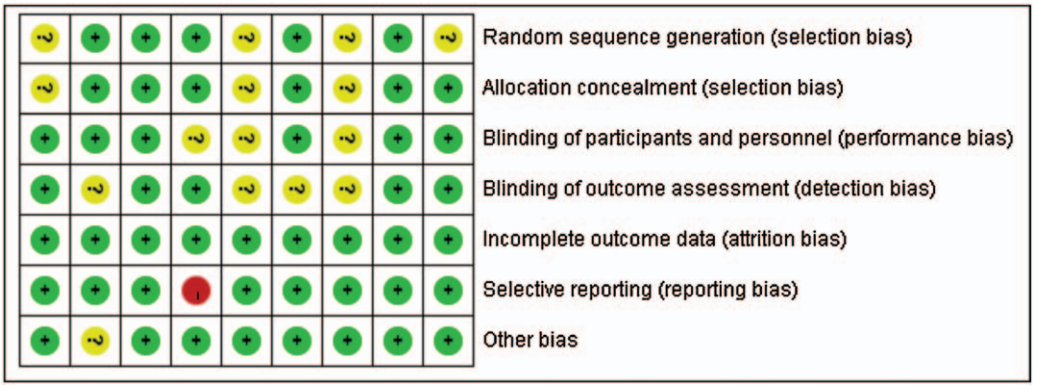


**Figure 3.** Risk of bias summary.

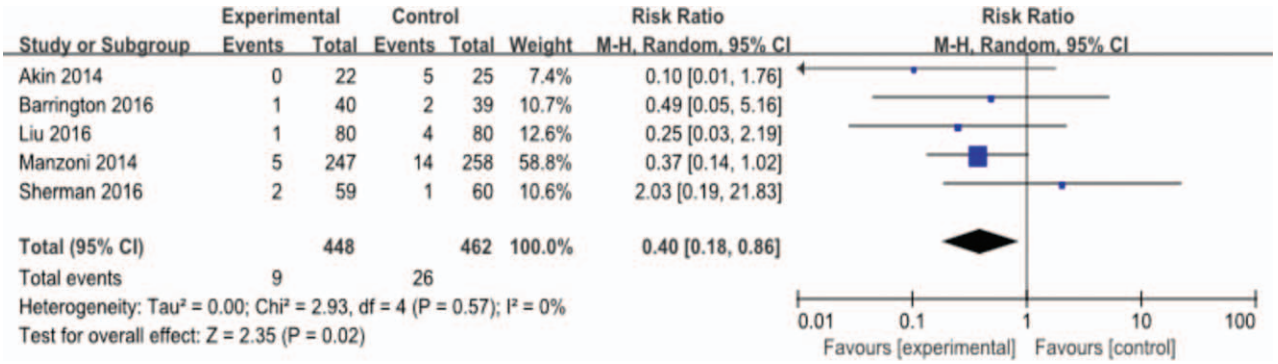


**Figure 4.** Outcome 1.1: Effect of lactoferrin on necrotizing enterocolitis in preterm neonates.

consistent with the main analysis (RR 0.52, 95% CI 0.35–0.78, $P<.01$, heterogeneity: $P=.68$, I=0%).

Subgroup analysis of the pathogen type of sepsis suggests that the beneficial effect of lactoferrin apply to all kinds of sepsis: Gram (−) sepsis (6 studies, 21/474[4.4%] vs 44/505 [8.7%]; RR 0.51; 95% CI 0.30–0.87; $P=.01$; heterogeneity: $P=.55$, I=0%), Gram (+) sepsis (6 studies, 13/474 [2.7%] vs 51/505 [10.1%]; RR 0.28; 95% CI 0.16–0.51; $P<.01$; heterogeneity: $P=1.0$, I=0%), bacterial sepsis (6 studies, 34/474 [7.2%] vs 95/505 [18.8%]; RR 0.39; 95% CI 0.27–0.56; $P<.01$; heterogeneity: $P=.77$, I=0%), and fugal sepsis (4 studies, 1/320 [0.3%] vs 22/350 [6.3%]; RR 0.13; 95% CI 0.03–0.47; $P<.01$; heterogeneity: $P=.89$, I=0%). Although the distribution of pathogens by type was not significantly different in the treated and control groups, lactoferrin tends to have better prevention efficacy in fungal sepsis compared to bacterial sepsis (RR 0.13 vs 0.39) and in Gram (+) sepsis compared to Gram (−) sepsis (RR 0.28 vs 0.51). It also detects a decrease in coagulase-negative staphylococci sepsis among preterm infants with the supplementation of lactoferrin, which is the most prevalent Gram (+) pathogen causing neonatal sepsis after GBS (2 studies, 7/99 [7.1%] vs 17/99 [17.2%]; RR 0.43; 95% CI 0.18–1.01; $P=.04$; heterogeneity: not applicable).

The benefit of lactoferrin is significant for VLBW (infants with BW < 1500 g) (4 studies, 22/297 [7.4%] vs 64/296 [21.6%]; RR 0.38; 95% CI 0.24–0.59; $P<.01$; heterogeneity: $P=.65$, I=0%). For low birth weight (LBW) (infants with BW 1500–2500 g), the efficacy of lactoferrin is not statistically significant (2 studies, 6/86 [7.0%] vs 9/92 [9.8%]; RR 0.71; 95% CI 0.26–1.90; $P=.49$; heterogeneity: not applicable).

The results of lactoferrin on LOS are shown in Fig. 5.

***3.1.3. Outcome 1.3: Hospital-acquired infection.*** The pooled meta-analysis showed that lactoferrin supplementation resulted in a statistically significant reduction in the incidence of hospital-acquired infection (2 studies, 16/139 [11.5%] vs 35/140[25%]; RR 0.47; 95% CI 0.27–0.80; $P<.01$; heterogeneity: not applicable). The results of lactoferrin are shown in Fig. 6.

***3.1.4. Outcome 1.4: Infection-related mortality.*** Figure 7 reveals that lactoferrin could reduce infection-related mortality, with statistical significance; however, the result is of high heterogeneity (6 studies, 4/474 [0.8%] vs 25/505 [4.9%]; RR 0.24; 95% CI 0.04–1.32; $P<.01$, heterogeneity: $P=.08$, I= 53%). After sensitivity analysis, we find that Ochoa 2015 led to the high heterogeneity. In this study, it should be noted that the treatment only began when oral or tube feeding started and 6 of 33 sepsis episodes occurred before starting the intervention. The author also reported that they have enrolled babies of high risk (500–1000 g). However, since they did not give explicit reasons of the death, we cannot find out if it is related to the timing of intervention and high risky basis of the babies. This may explain for the high heterogeneity of this study. The results were significant with low heterogeneity after excluding this study (5 studies, 0/379 [0%] vs 23/410 [5.6%]; RR 0.10; 95% CI 0.02–0.44; $P<.01$; heterogeneity: $P=.81$, I=0%).

***3.1.5. Outcome 1.5: Days to achieve full enteral feeding.*** The mean difference for days to achieve full enteral feeding in preterm infants was −2.19, with statistical significance (4 studies, weighted mean difference [WMD]=−2.19, 95% CI −2.97 to −1.42; $P<.01$; heterogeneity: $P=.37$, I=4%). Lactoferrin slightly shortened the time to achieve full enteral feeding as is shown in Fig. 8.

***3.1.6. Outcome 1.6: Duration of hospitalization.*** The mean difference for duration of hospitalization was −1.02 (5 studies, WMD=−2.11, 95% CI −3.12 to −1.10; $P<.01$; heterogeneity: $P<.1$, I=95%). Considering the different discharge requirement in China, which leads to longer hospitalization, the high heterogeneity of Liu 2016 with other studies is easy to explain. After exclusion the study Liu 2016 with high heterogeneity, the result is still significant (4 studies, WMD=−0.85, 95% CI −1.09 to −0.60; $P<.01$; heterogeneity: $P=.44$, I=0%).

***3.1.7. Outcome 1.7: All-cause mortality.*** We noted no statistically significant difference in all-cause mortality (7 studies, 22/625 [3.5%] vs 35/647 [5.4%]; RR 0.70; 95% CI 0.38–1.30; $P=.11$; heterogeneity: $P=.33$, I=13%). The result was not statistically significant.

***3.1.8. Outcome 1.8: Adverse effects related to lactoferrin.*** None of the included trials reported any confirmed adverse effects, treatment death, or feeding intolerance caused by the supplemented lactoferrin or probiotics. Only 1 study Sherman 2016 reported possibly related adverse effects of study drug (5/59 [8.4%] in talactoferrin [TLF] group and 7/60 [11.7%] in control group), which detects no statistically significant difference in 2 groups ($P=.57$). They reported that gastrointestinal (76%), blood and lymphatic (60%), nutrition and metabolism (72%), and respiratory disorders (72%) were the most common treatment emergent adverse effects.

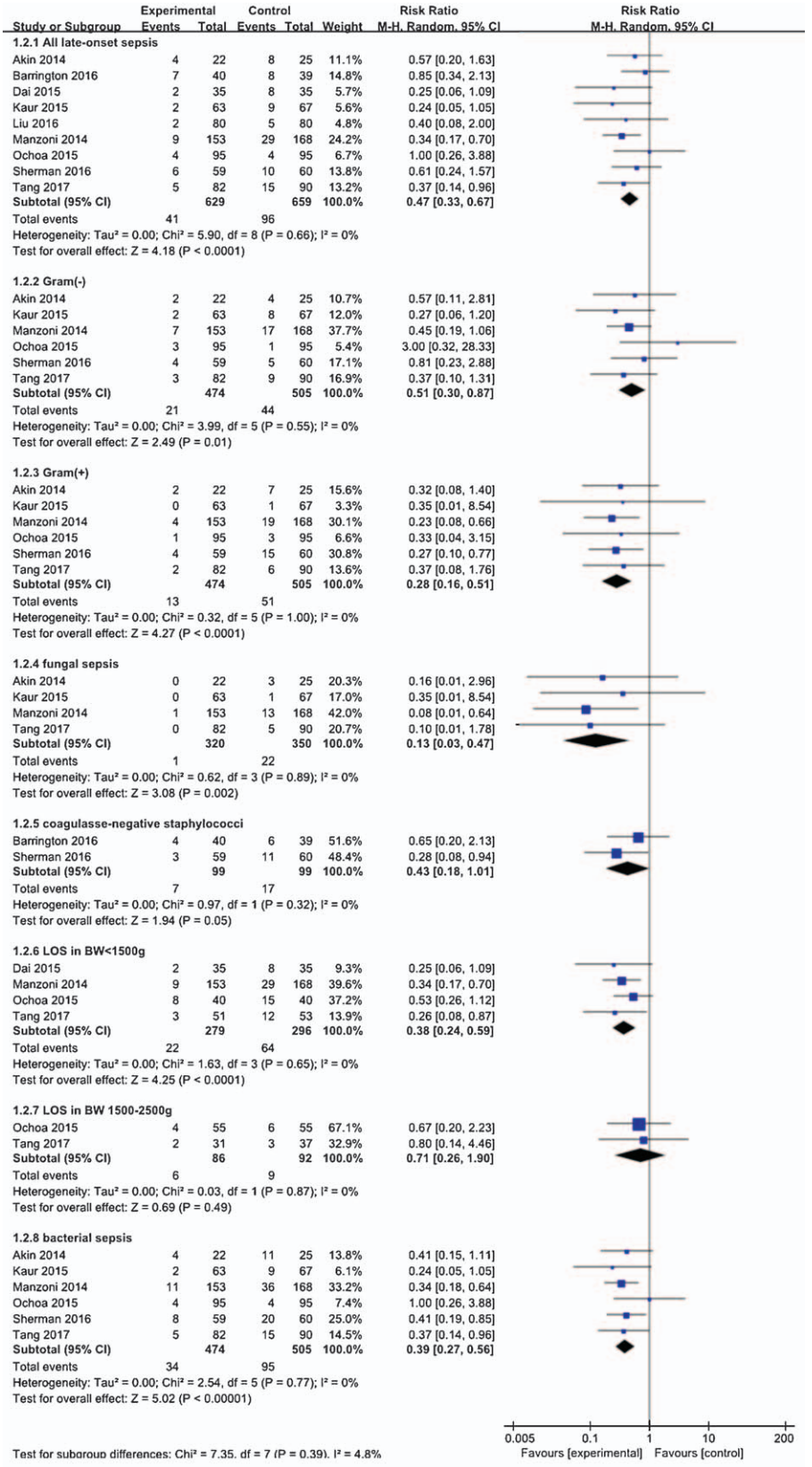


**Figure 5.** Outcome 1.2: Effect of lactoferrin on late-onset sepsis in preterm neonates.

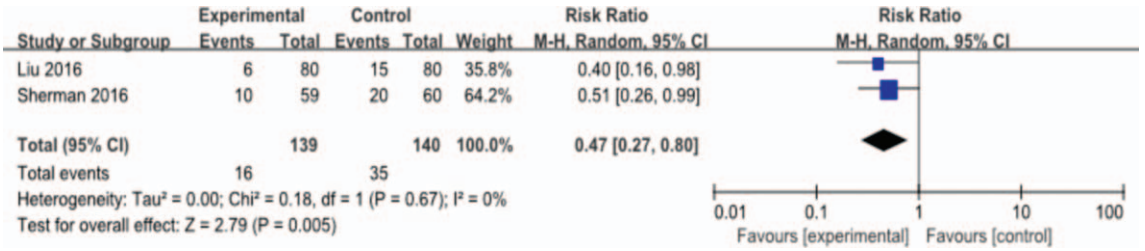


**Figure 6.** Outcome 1.3: Effect of lactoferrin on hospital-acquired infection in preterm neonates.

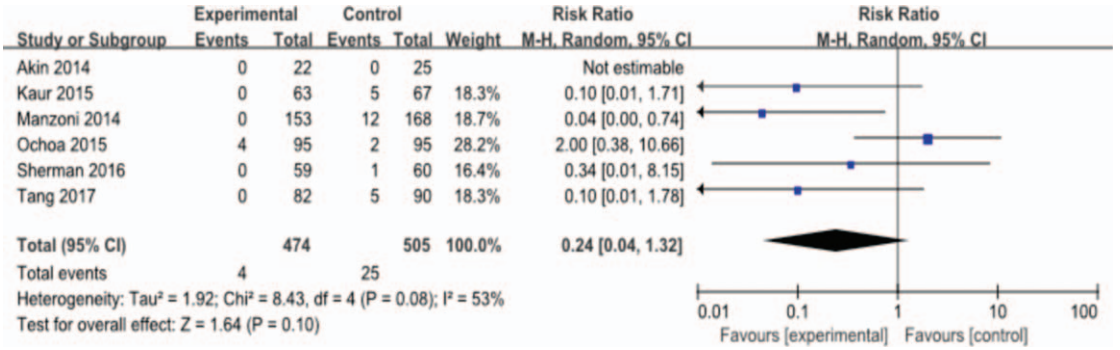


**Figure 7.** Outcome 1.4: Effect of lactoferrin on infection-related mortality in preterm neonates.

### *3.2. Comparison 2: Lactoferrin supplementation combining with LGG vs placebo*

***3.2.1. Outcome 2.1: NEC stage II or more.*** Lactoferrin supplementation with Lactobacillus rhamnosus GG (LGG) decreased the incidence of NEC stage II or III and the result is not statistically significant (1 study, 0/238 [0%] vs 14/258 [5.4%]; RR 0.04; 95% CI 0.00–0.62; $P=.02$; heterogeneity: not applicable).

***3.2.2. Outcome 2.2: LOS.*** Our results suggest a beneficial effect of lactoferrin with LGG in all kinds of sepsis: All LOS (3 studies, 12/271 [4.4%] vs 52/293 [17.7%]; RR 0.25; 95% CI 0.14–0.47; $P<.01$; heterogeneity: $P=.77$, $I=0\%$); bacterial sepsis (2 studies, 11/236 [4.7%] vs 51/258 [19.8%]; RR 0.24; 95% CI 0.13–0.45; $P<.01$; heterogeneity: not applicable); Gram (−) sepsis (2 studies, 8/236 [3.4%] vs 26/258 [10.1%]; RR 0.34; 95% CI 0.16–0.73; $P<.01$; heterogeneity: not applicable); Gram (+) (2 studies, 3/236 [1.3%] vs 25/258 [9.7%]; RR 0.13; 95% CI 0.04–0.44; $P<.01$; heterogeneity: not applicable); fugal (2 studies, 4/236 [1.7%] vs 18/258 [7.0%]; RR 0.24; 95% CI 0.08–0.71; $P=.01$; heterogeneity: not applicable).

***3.2.3. Outcome 2.3: Infection-related mortality.*** A reduction of infection-related mortality can be detected in infants supplemented with lactoferrin combined with LGG, with statistical significance (2 studies, 2/236 [0.8%] vs 13/258 [5.0%]; RR 0.17; 95% CI 0.04–0.75; $P=.02$; heterogeneity: not applicable).

***3.2.4. Outcome 2.4: Time to achieve full enteral feeding.*** Only 1 study assessed the outcome and lactoferrin supplementation with LGG shortened time to achieve full enteral feeding (1 study, WMD = −1.40, 95% CI −2.27 to −0.53; $P<.01$; heterogeneity: not applicable).

***3.2.5. Outcome 2.5: All-cause mortality.*** Our results detect no significant difference of all-cause mortality in 2 groups (2 studies, 13/323 [4.0%] vs 24/348 [6.9%]; RR 0.58; 95% CI 0.30–1.13; $P=.11$; heterogeneity: not applicable).

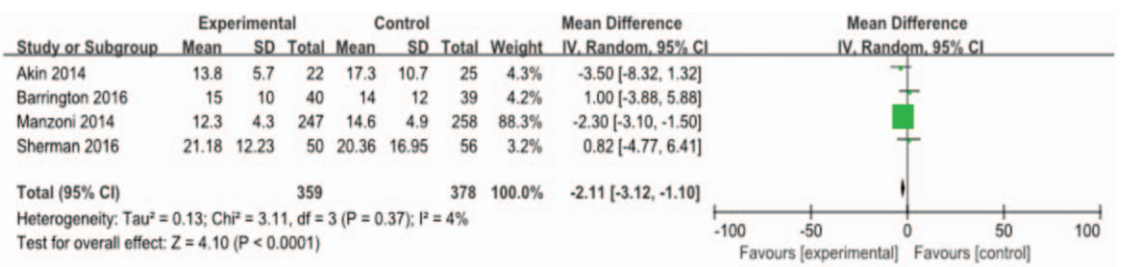


**Figure 8.** Outcome 1.5: Effect of lactoferrin on days to achieve full enteral feeding in preterm neonates.

**LF supplementation in premature neonates**

**Patient or population:** patients with LOS and NEC in premature infants
**Settings:**
**Intervention:** LF

| Outcomes | Illustrative comparative risks* (95% CI) Assumed risk Control | Corresponding risk LF | Relative effect (95% CI) | No of Participants (studies) | Quality of the evidence (GRADE) | Comments |
|---|---|---|---|---|---|---|
| NEC<br>Follow-up: 1 months | Study population | | RR 0.37<br>(0.18 to 0.78) | 910<br>(5 studies) | ⊕⊕⊖⊖<br>low[1,2] | |
| | 56 per 1000 | 21 per 1000<br>(10 to 44) | | | | |
| | Moderate | | | | | |
| | 51 per 1000 | 19 per 1000<br>(9 to 40) | | | | |
| LOS - All late-onset sepsis | Study population | | RR 0.46<br>(0.31 to 0.67) | 967<br>(9 studies) | ⊕⊕⊖⊖<br>low[1,3] | |
| | 157 per 1000 | 72 per 1000<br>(49 to 105) | | | | |
| | Moderate | | | | | |
| | 173 per 1000 | 80 per 1000<br>(54 to 116) | | | | |
| hospital acquired infection | Study population | | RR 0.46<br>(0.27 to 0.79) | 279<br>(2 studies) | ⊕⊖⊖⊖<br>very low[1,2] | |
| | 250 per 1000 | 115 per 1000<br>(68 to 198) | | | | |
| | Moderate | | | | | |
| | 260 per 1000 | 120 per 1000<br>(70 to 205) | | | | |
| infection related mortality | Study population | | RR 0.09<br>(0.02 to 0.36) | 789<br>(5 studies) | ⊕⊕⊖⊖<br>low[1,4] | |
| | 56 per 1000 | 5 per 1000<br>(1 to 20) | | | | |
| | Moderate | | | | | |
| | 38 per 1000 | 3 per 1000<br>(1 to 14) | | | | |
| days to achieve full enteral feeeding | | The mean days to achieve full enteral feeeding in the intervention groups was 2.19 lower<br>(2.96 to 1.41 lower) | | 737<br>(4 studies) | ⊕⊕⊖⊖<br>low[1,2] | |

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).

**CI:** Confidence interval; **RR:** Risk ratio;

GRADE Working Group grades of evidence
**High quality:** Further research is very unlikely to change our confidence in the estimate of effect.
**Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
**Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
**Very low quality:** We are very uncertain about the estimate.

[1] unclear risk of bias
[2] limited objectives and studies
[3] 1 study has high risk of publication bias
[4] Ocha 2015 has high heterogeneity of resultscompared with other studies probably due to different timing of intervention and high risky objectives

**Figure 9.** Quality of evidence using GRADE method.

## 4. Discussion

### 4.1. Comparison with previous study

In summary, our meta-analysis identified 9 trials that enrolled 1834 preterm infants to evaluate the safety and efficacy of lactoferrin to prevent LOS and NEC in preterm neonates. Current evidence revealed a significant reduction in the incidence of LOS and NEC without sever adverse effects, indicating a benefit of prophylactic lactoferrin supplementation in preterm infants.

Differences between our study and one latest Cochrane meta-analysis should be noted. The meta-analysis by Pammi and Suresh[28] included 6 trials involving 1041 subjects and concluded that lactoferrin decreased NEC stage II or III (RR 0.40, 95% CI 0.18–0.86) and LOS (RR 0.59, 95% CI 0.40–0.87). After the previous meta-analysis, several studies investigating the effects of lactoferrin in preterm infants were published. Our updated meta-analysis included maximum number of completed studies. Overall, our results are generally consistent with the previous study, with lower heterogeneity. In contrast with the previous study, the current one detects that lactoferrin supplementation not only decreased the incidence of LOS and NEC (low-quality evidence), but also significantly reduced the incidence of hospital-acquired infection and infection-related mortality (low- to very low-quality evidence). Additionally, lactoferrin also has benefit in the general condition of preterm infants by reducing the duration of hospitalization and the time to achieve full enteral feeding (low- to very low-quality evidence). The benefit for general conditions seems minor, probably because premature infants generally have feeding issues such as poor suck, gastroesophageal reflux, immature gut motility, and thus requires longer NICU care than term babies. We detect no significant difference in all-cause mortality between lactoferrin supplementation group and control group. Of interest, comparing to LBW, the protective effect of lactoferrin for LOS seems to be more distinct in ELBW and VLBW, with lower RR for LOS (RR 0.36 vs 0.71) and statistical significance. The possible reason for this difference is that ELBW and VLBW infants received higher per-kilogram lactoferrin doses. Because only 3 trials[16,21,22] administered lactoferrin based on weight, the pooled results of these 3 trials were under-powered to detect a greater benefit of weight-adjust dosage. Comparing neonates under the intervention of lactoferrin with and without LGG, the latter have lower RR for NEC, LOS, and infection-related mortality. This suggests a possible greater benefit of lactoferrin combined with probiotics LGG. However, whether probiotics could enhance the protective

effect of lactoferrin is hard to detect under current evidence. One reason was that current studies were not adequately powered to reveal its beneficial effect because of sample size.

### 4.2. Quality assessment

We assume the quality of evidence to be low using the GRADE method mainly because of unclear risk of bias and limited objectives (Fig. 9).

Current available studies had obvious risk of bias and incomplete data extraction that might have an influence on the final results and conclusion. Out of the 9 included trials, only 5 studies reported explicit randomization and 6 studies reported explicit allocation concealment and blinding. In Dai 2015 and Liu 2016, the investigator did not give explicit description of the selection and blinding process, which led to unclear risk of bias and downgraded the quality of evidence. In Manzoni 2014, the researchers only reported the incidence of NEC, causing high risk of selective reporting bias in LOS.

Besides, several potential limitations should also be taken into consideration when interpreting the results. First, the included RCTs were performed in different regions including developing countries and developed countries. Lower detection rates of culture proven sepsis and higher risk of sepsis attack in lower income countries can cause high heterogeneity of data. However, we detect low heterogeneity of the data when analyzing the benefit effect of lactoferrin in preventing LOS, NEC, and infection-related mortality. The heterogeneity of duration of hospitalization is high but it is most likely to be related to different discharge standards. Second, the initial time of intervention was different among studies. The concentration of lactoferrin is high in colostrum and relatively low in mature milk. It is proved that lactoferrin interacts with gut cells differently in a concentration-dependent manner in vitro studies, manifesting as lactoferrin-driven gut cell proliferation and maturation in high concentration and intestinal differentiation in lower concentration.[29] To mimic the property of lactoferrin in colostrum, the initial time of supplementing lactoferrin in preterm infants should be in the very first days of life.[30] Ochoa 2015 did not started the treatment until the start of oral or tube feeding on average at 4.0 $\pm$1.4 days of life. When analyzing the data, we do detect high heterogeneity of this study, which gives enhanced evidence about the timing of starting lactoferrin supplementation. Our further analysis of data indicates no significant different incidence of LOS when treatment initiated between the 1st and 3rd of life. Finally, HM lactoferrin may act as a confounding factor in our analysis, but since the distribution of feeding patterns were even in all the treated and untreated group, we assumed breastfeeding had little influence on the result. Hence, no subgroup analysis of HM and FM feeding was done. Besides, for infants fed with exclusive maternal milk the benefit of lactoferrin is still significant and the incidence of LOS in untreated infants was similar between HM and FM feeding.[13,18] This suggests that maternal milk alone does not confer the benefit of lactoferrin supplementation and premature infants need additional lactoferrin, specifically to prevent LOS in preterm infants. Although colostrum contains the highest lactoferrin content, the necessity for supplementing additional lactoferrin still matters considering that it can take 2 to 3 weeks until VLBW infants to receive full-volume enteral feedings and full amounts of protective components in HM.[31]

The efficacy of lactoferrin for preventing LOS and NEC still needs more high-quality RCTs to demonstrate. The long-term effects of lactoferrin also need to follow up. Current evidence is under-powered to detect. Future study should focus on the optimal duration, dosage, type (TLF or bovine lactoferrin), and long-term effects of lactoferrin.

## 5. Conclusion

In conclusion, the 9 included trials indicated that lactoferrin supplementation in preterm neonates is safe without obvious adverse effects. The results of our meta-analysis demonstrated that prophylactic supplementation of lactoferrin could significantly reduce the incidence of NEC, LOS, and hospital-acquired infection in preterm neonates. It also detects a trend of decreased infection-related mortality, with statistical significance. Our study also revealed that lactoferrin could slightly reduce the time to achieve full enteral feeding and duration of hospitalization of preterm infants, with statistical significance.

In addition, lactoferrin with LGG seems to have greater benefits in the prevention of NEC and LOS (very low evidence). However, there are not enough high-quality trials to determine whether the beneficial effects of lactoferrin could be enhanced by combining with probiotics.

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## Author contributions

**Formal analysis:** Yi He, Luying Cao.
**Software:** Yi He, Luying Cao.
**Supervision:** Jialin Yu.
**Writing – original draft:** Yi He.
**Writing – review and editing:** Yi He, Jialin Yu, Luying Cao.

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