The effect of lactoferrin supplementation on death or major morbidity in very low birthweight infants (LIFT): a multicentre, double-blind, randomised controlled trial

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Summary

Background Very low birthweight or preterm infants are at increased risk of adverse outcomes including sepsis, necrotising enterocolitis, and death. We assessed whether supplementing the enteral diet of very low-birthweight infants with lactoferrin, an antimicrobial protein, reduces all-cause mortality or major morbidity.

Methods We did a multicentre, double-blind, pragmatic, randomised superiority trial in 14 Australian and two New Zealand neonatal intensive care units. Infants born weighing less than 1500 g and aged less than 8 days, were eligible and randomly assigned (1:1) using minimising web-based randomisation to receive once daily 200 mg/kg pasteurised bovine lactoferrin supplements or no lactoferrin supplement added to breast or formula milk until 34 weeks' post-menstrual age (or for 2 weeks, if longer), or until discharge from the study hospital if that occurred first. Designated nurses preparing the daily feeds were not masked to group assignment, but other nurses, doctors, parents, caregivers, and investigators were unaware. The primary outcome was survival to hospital discharge or major morbidity (defined as brain injury, necrotising enterocolitis, late-onset sepsis at 36 weeks' post-menstrual age, or retinopathy treated before discharge) assessed in the intention-to-treat population. Safety analyses were by treatment received. We also did a prespecified, PRISMA-compliant meta-analysis, which included this study and other relevant randomised controlled trials, to estimate more precisely the effects of lactoferrin supplementation on late-onset sepsis, necrotising enterocolitis, and survival. This trial is registered with the Australian and New Zealand Clinical Trials Registry, ACTRN12611000247976.

Findings Between June 27, 2014, and Sept 1, 2017, we recruited 1542 infants; 771 were assigned to the intervention group and 771 to the control group. One infant who had consent withdrawn before beginning lactoferrin treatment was excluded from analysis. In-hospital death or major morbidity occurred in 162 (21%) of 770 infants in the intervention group and in 170 (22%) of 771 infants in the control group (relative risk [RR] 0·95, 95% CI 0·79–1·14; p=0·60). Three suspected unexpected serious adverse reactions occurred; two in the lactoferrin group, namely unexplained late jaundice and inspissated milk syndrome, but were not attributed to the intervention and one in the control group had fatal inspissated milk syndrome. Our meta-analysis identified 13 trials completed before Feb 18, 2020, including this Article, in 5609 preterm infants. Lactoferrin supplements significantly reduced late-onset sepsis (RR 0·79, 95% CI 0·71–0·88; p<0·0001; I^2=58%), but not necrotising enterocolitis or all-cause mortality.

Interpretation Lactoferrin supplementation did not improve death or major morbidity in this trial, but might reduce late-onset sepsis, as found in our meta-analysis of over 5000 infants. Future collaborative studies should use products with demonstrated biological activity, be large enough to detect moderate and clinically important effects reliably, and assess greater doses of lactoferrin in infants at increased risk, such as those not exclusively receiving breastmilk or infants of extremely low birthweight.

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Introduction

About a quarter of very low birthweight or preterm infants suffer major adverse outcomes such as late-onset sepsis, necrotising enterocolitis, brain injury, and retinopathy requiring treatment, or death.[1] Lactoferrin is an iron-binding, cationic glycoprotein found in the whey fraction of milk and in neutrophils, tears, saliva, sweat, cerebrospinal fluid, and mucosal secretions.[2] It contributes to innate immunity; has antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and anti-infective functions; and can promote iron absorption.[2–4] Bovine and human lactoferrin are highly homologous 80 kDa molecules.[2] Their shared N-terminal, 11-amino acid peptide has antibiotic properties.[5] This similarity and

Research in context

Evidence before this study

A Cochrane review identified trials published in MEDLINE and PreMEDLINE before Dec 31, 2016, on lactoferrin supplementation in infants born before 37 week's gestation, with no language restrictions, using the search terms "sepsis", "sepsis or septicaemia", "septic", "NEC", "necrotizing enterocolitis', "infant–newborn", "neonat*", "newborn*", "lactoferrin", and "talactoferrin". On meta-analysis, six trials suggested that lactoferrin supplementation reduced late-onset sepsis (relative risk [RR] 0·59, 95% CI 0·40–0·87; p=0·0079; 886 participants; low-quality evidence) and four suggested that lactoferrin supplementation reduced necrotising enterocolitis (RR 0·40, 0·18–0·86; p=0·02; 750 participants; low-quality evidence) but not all-cause mortality (low-quality evidence). However, ELFIN, a well done trial and the largest to date, in 2203 infants of less than 32 weeks' gestation, did not show significant benefit of supplementation. A meta-analysis of ten trials done in 3679 infants born at less than 37 weeks' gestation, including ELFIN, published before Feb 10, 2019, without language restriction, reported that lactoferrin supplementation without probiotics significantly reduced late-onset sepsis (moderate level of evidence), but not necrotising enterocolitis or mortality. However, most trials were small, and some contained other design and methodological weaknesses that might have introduced biases resulting in overestimation of the effect sizes.

Added value of this study

Our trial showed that lactoferrin supplementation had no significant effect on death or major morbidity. However, the 95% CI did not reliably exclude reductions of up to 21% or increases of up to 14% in the RR of this outcome. Before any data analysis, our statistical analysis plan had prespecified that the trial would be placed in context by including it in an updated meta-analysis of all available trials to assess the effects of lactoferrin supplementation on late-onset sepsis, necrotising enterocolitis, and all-cause mortality. Applying Cochrane Neonatal Review Group methods and PRISMA criteria, we identified 13 eligible trials, without language restriction, completed before Feb 18, 2020, in infants given lactoferrin up to 300 mg/kg per day. There were no safety concerns. Lactoferrin supplementation reduced the RR of late-onset sepsis by 21% with high precision (95% CI 12–29; p<0·0001) with no significant effect on necrotising enterocolitis or death. The highly precise reduction in late-onset sepsis (p<0·0001) remained after excluding three trials of lower quality. In all 13 trials, we observed funnel plot asymmetry and statistical heterogeneity between larger and smaller studies in the effect on late-onset sepsis which was unlikely to be due to the play of chance (p<0·0001). This heterogeneity could reflect one or more of the following reasons: reporting bias owing to missing or delayed publications, overestimation of treatment effects in smaller trials, true differences between trials in the effectiveness, dose, or duration of supplemental lactoferrin because the estimated benefit was greater assuming random versus fixed effects, or true differences in underlying population risk (eg, concomitant use of probiotic).

Implications of all the available evidence

Lactoferrin supplementation appears to reduce late-onset sepsis and to be safe. An individual participant meta-analysis of all trials is needed to explore whether in preterm infants at increased risk (eg, those not exclusively fed fresh mother's milk or infants of extremely low birthweight) supplementation using lactoferrin products with demonstrable biological activity reduces late-onset sepsis, anaemia, severe retinopathy, or other outcomes; and whether such effects are modified by differently manufactured lactoferrin products, the duration and dose of lactoferrin, or concurrent medications. If more infants and their mothers are to benefit (as patients in other specialties have done) from moderate, clinically relevant improvements (eg, reductions in the RR of key outcomes such as death, disability, or sepsis of ≤10%) it is important that the international community find ways to develop a new generation of simple and efficient perinatal trials, which can rapidly recruit tens of thousands of infants.

evidence showing that supplementation of enteral feeds with oral lactoferrin for 3 months reduces infections[3] and anaemia[4] in term infants provided a strong biological rationale to evaluate whether lactoferrin supplementation reduces late-onset sepsis, other morbidities, or mortality in preterm infants. In a Cochrane meta-analysis[6] of randomised controlled trials,[7–14] lactoferrin supplementation significantly reduced the relative risk (RR) of late-onset sepsis by 41% in 886 infants and of necrotising enterocolitis by 60% in 750 infants, but not of all-cause mortality.[6] In a systematic review of ten trials in 3679 preterm infants with or without probiotics, lactoferrin supplementation decreased the RR of late-onset sepsis by 44%.[15] However these data were of low to moderate quality[15] and included a randomised controlled trial of high quality, which reported no benefit in 2203 preterm infants.[16]

We did the lactoferrin infant feeding trial (LIFT) to evaluate whether enteral lactoferrin supplements improve morbidity-free survival in very low-birthweight infants. Trials or meta-analyses can require several thousand participants to detect moderate but important effects and avoid false negative results.[17–20] Accordingly, our peer-reviewed protocol included a statistical analysis plan,[20] which was finalised before any investigators or biostatisticians, except for an unblinded independent study statistician, were unmasked to the data. The statistical analysis plan[20] prespecified the synthesis of LIFT with all relevant randomised controlled trials in meta-analyses (appendix p 11) to estimate more precisely the effects of lactoferrin supplementation on late-onset sepsis, necrotising enterocolitis, and survival.

Methods

Study design and participants

We did a multicentre, double-blind, randomised controlled trial in 14 Australian and two New Zealand neonatal intensive care units. Infants born weighing less than 1500 g, aged less than 8 days, and without lethal anomalies were eligible. Infants who had late-onset sepsis before consent was obtained were ineligible. Families of eligible infants were approached for consent after birth. The protocol was approved by Northern Sydney Local Human Research Ethics Committee (version 2.0, reference 1003–118M), Women's and Children's Health Network Human Research Ethics Committee, Mercy Health Human Research Ethics Committee, and Southern Health and Disability Ethics Committeein in January, 2017.[20] Written informed consent was obtained from one or both parents by appropriately trained medical or nursing staff after parents had the opportunity to read the patient information and participants' consent form (appendix p 26).

Randomisation and masking

Infants were randomly assigned to daily feeds with or without pasteurised bovine lactoferrin (avoiding placebos, which might not be biologically inert in preterm infants). Central, web-based randomisation was employed in a 1:1 ratio using minimisation with a random component, stratifying by site, gender, birthweight (<1000 g *vs* ≥1000–1499 g), and multiple birth (yes or no). The central web based computer system at the NHMRC Clinical Trials Centre followed a randomisation algorithm, which included minimisation. Infants were randomly assigned to their study treatment in the first week after birth, aiming to start giving lactoferrin supplementation to the treatment group as soon as possible. Sometimes treatment was delayed due to feed intolerance. Infants from multiple births were randomly assigned individually. Selected night nurses, designated nurses, or members of the milk kitchen team who prepared the daily feeds were not masked (and were instructed not to disclose the allocated study treatment to parents or other nursing and clinical staff), but all other nurses and clinicians, as well as parents and caregivers of the infants and investigators were masked to study group assignment. Unmasking of the investigational product was allowed to manage serious adverse events. Coordinating centre staff were always available. We assessed adherence to protocol while remaining masked to treatment allocation.

Procedures

Lactoferrin was supplied by Australia's Own (The Entrance, NSW, Australia) (appendix p 36). Infants were given once daily study feeds of breast or formula milk supplemented by pasteurised bovine lactoferrin 200 mg/kg or once daily study feeds of breast or formula milk with no added lactoferrin. The dose of 200 mg/kg was calculated based on the infant's most recently recorded weight. Lactoferrin was provided as 200 mg capsules in bottles containing 60 capsules. For infants allocated to lactoferrin, capsules were opened, and their powder was dissolved in milk to a concentration of 100 mg/mL just before the feed.

Study treatment began immediately after randomisation and continued until 34 weeks' post-menstrual age or for 2 weeks, if that was longer, or until discharge from the study hospital if that occurred first. Assessment forms were completed at baseline, during treatment, at 36 weeks, and after discharge and follow-up assessments at 2 years are continuing.[20] Concomitant treatments were allowed, but only recorded if a suspected unexpected serious adverse reaction occurred.

Outcomes

The primary aim was to evaluate hospital survival of low-birthweight infants without morbidity by comparing rates of the primary composite outcome of death or major morbidity between study groups. Observing recommended approaches for composite outcomes,[21] components were prespecified, independently associated with later disability, and amenable to unbiased assessment. Major morbidities were defined according to the Australian and New Zealand Neonatal Network data dictionary (appendix pp 41–42) as brain injury on ultrasound, necrotising enterocolitis, late-onset sepsis at 36 weeks' post-menstrual age, or retinopathy treated before discharge. Brain injury required ventricular distension with blood, intra-parenchymal haemorrhage, parenchymal echodensity, periventricular leukomalacia, porencephalic cyst, or ventricular index 97th percentile or higher plus 4 mm.[34,35] Necrotising enterocolitis required three criteria: (1) either temperature instability, apnoea, bradycardia, or lethargy and either gastric residual volume containing 25% or more of each of two consecutive feeds, abdominal distension, vomiting, or faecal blood; (2) abdominal wall cellulitis and palpable mass, pneumatosis intestinalis, portal vein gas, dilated loop on serial radiographs, or surgical or post-mortem diagnosis; and (3) antibiotics while withholding enteral feeds. Clinically suspected sepsis alone was insufficient to diagnose late-onset sepsis, which required clinical signs after 48 h from birth and positive bacterial or fungal culture of blood, cerebrospinal fluid, or urine. Blood cultures were considered positive if organisms grew and antibiotics were given. Bloodstream cultures of coagulase negative staphylococci, potential contaminants, or group B *Streptococcal antigenuria* were considered positive with clinical sepsis and leucocytosis or thrombocytopenia. Viral infections required proven culture or suggestive haematological or serological results. Culturing the same organism within 14 days signified one episode of sepsis. We did not record the numbers of days infants received mother's milk, donor milk, or formula.

In pooled overviews of accumulating data, which we did while blinded to study group, substantially more infants

See Online for appendix

than expected met the criteria for chronic lung disease because of increasing use of nasal continuous positive airway pressure by prongs or high-flow cannula without supplemental oxygen,[22] potentially reducing the study's power. While remaining masked to study outcomes in each randomised group, the trial management committee therefore removed chronic lung disease from the primary composite outcome in July, 2016.[23]

The five pre-specified secondary outcomes, which were each components of the composite primary outcome, were survival to hospital discharge, brain injury, stage II or III necrotising enterocolitis, or late-onset sepsis by 36 completed weeks postmenstrual age, and retinopathy treated before hospital discharge according to local guidelines. As some components of the secondary outcomes, such as brain injury associated with periventricular leukomalacia, necrotising enterocolitis, sepsis, and retinopathy, are associated with free radical disease, inflammation, or infection, each might plausibly be improved by enteral supplementation with lactoferrin because of its antioxidant, anti-inflammatory, and antimicrobial properties. Other secondary outcomes, which were not part of the composite primary outcome, were chronic lung disease (having supplemental oxygen or nasal cannula at 36 weeks), days to full enteral feeds, days in hospital, number of red blood cell transfusions before 36 weeks, and, in Australia, costs. Survival to 24–36 months without disability was ascertained using Ages and Stages Questionnaires completed by parents, Short Health Status Questionnaires by medical practitioners, or Bayley-III Scales of Infant and Toddler Development by trained assessors.[30] These outcomes were not planned as part of the main study report. We assessed safety by suspected and unexpected serious adverse reactions. Prolonged hospitalisation, disability, incapacity, death, or risk of death were deemed serious.

Statistical analysis

We originally planned to enrol 1100 infants to provide more than 80% power to show increased survival without major morbidity from 68% in controls to 76% in the intervention group, with two-tailed α of 5% (two-sided p value 0·05), equivalent to a primary composite outcome rate of 32% in controls with a 25% relative risk (RR) reduction.[20] The control primary composite outcome rate was based on contemporaneous Australia and New Zealand data. Masked to study group, the Trial Management Committee reviewed pooled data in December, 2016, and concluded that it would be prudent to expect a primary outcome rate of 26% in controls. A target sample size of 1500 infants was re-estimated to provide 85% power to show a 25% RR reduction (from 26% to 19·5%) and generate a two-sided p value of 0·05. Initial and updated protocols are available in the published protocol[20] and in the appendix p 3.

An Independent Data and Safety Monitoring Committee did two prespecified interim analyses using Haybittle-Peto boundaries in an agreed charter (appendix p 30–34). The committee reviewed safety data twice (during interim analyses). The committee was unmasked to randomised outcomes; therefore, it was not consulted about updating the primary outcome or sample size.[23]

We employed the intention-to-treat principle unless comparing suspected unexpected serious adverse reactions, when infants were analysed by treatment received. Endpoints were analysed using linear models fitted using the generalised estimating equation approach to account for possible correlation of data between siblings from multiple births. A log-binomial regression model was applied to the primary endpoint and other binary secondary endpoints, a linear regression model was applied to continuous endpoints, and a negative binomial regression model was applied to count data. Prespecified analyses are presented in the statistical analysis plan (appendix p 3). Effects were summarised by relative risks and 95% CI. An economic evaluation was planned in Australia.

Consistency of the primary outcome across subgroups was tested using a treatment-by-subgroup interaction term with main effects as covariates. Prespecified subgroups were: birthweight less than 1000 g versus 1000–1499 g; randomised at 72 h or sooner versus at 72 h or later from birth; 28 weeks or less versus 28 weeks or more gestation at birth; receiving probiotics versus not by 36 weeks postmenstrual age. As probiotic use was a post-baseline covariate, we assessed its role as an effect modifier by evaluating randomised treatment effects across sites, categorised by tertile according to probiotic use. Sensitivity analyses of primary outcome rates adjusted by stratification factors and adherence to protocol were prespecified.[20]

As described in the Data and Safety Monitoring Committee charter (appendix 30–34), the Haybittle-Peto approach to early stopping required no final increase in p value to adjust for the two interim analyses done because it imposes such a stringent threshold for early termination of enrolment that a comparison between randomised groups in the primary endpoint, or death alone had to yield a two-sided p value of less than 0·0027 (equivalent to a deviation of more than three standard errors from the null, with a value of χ^2 test of 9·00 with 1 degree of freedom) to justify recommending that the Trial Management Committee consider stopping the study early. All p values were presented unadjusted for multiplicity. Only those p values from the analyses of the individual components of the primary composite endpoint were evaluated against critical values specified according to the Benjamini-Hochberg procedure to limit the false discovery rate to 5%. Other comparisons were interpreted with due consideration of type I error. Analyses were done using SAS (version 9.4)

Meta-analysis

We produced a meta-analysis, complying with PRISMA guidelines (appendix p 23),[24] of the effects of lactoferrin supplementation on late-onset sepsis, necrotising

enterocolitis and all-cause-mortality in LIFT and similar trials. We used the standard methods of the Cochrane Neonatal Review Group to search the Cochrane Central Register of Controlled Trials, MEDLINE via PubMed, PREMEDLINE, Embase, and CINAHL without language for relevant trials up to Feb 18, 2020. We also searched clinical trials databases, conference proceedings, and the reference lists of retrieved articles. Late onset-sepsis was defined as planned in each trial, including microbiologically proven and clinically suspected sepsis, if reported. We estimated levels of evidence for each outcome using the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) evaluation (appendix pp 11,13–15, 22), acknowledging its potential limitations.[25] We produced funnel plots of effects of trials of lactoferrin supplementation versus the reciprocal of its standard error and evaluated funnel plots for asymmetry using Egger's test.[26] As recommended by the Cochrane Collaboration, we did not use sequential methods to adjust meta-analyses for risk of false-positive or false-negative errors (appendix p 41). The trial is registered with the Australian Clinical Trials Registry, ACTRN12611000247976.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All co-authors vouch for the accuracy and completeness of data and analyses and the fidelity of reporting of the trial to its protocol. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 27, 2014, and Sept 1, 2017, we recruited 1542 infants and randomly assigned 771 to the lactoferrin supplementation group and 771 to the control group. One infant who had consent withdrawn before beginning lactoferrin treatment was excluded from analysis (figure 1), as prespecified in the statistical analysis plan.[20]

The treatment groups were similar and well balanced with respect to demographic and other baseline characteristics (table 1). Median treatment duration was 29 days (IQR 16–40) in the treatment group and 29 days (17–40) in the control group. 719 (93%) of 770 of infants in the treatment group had at least 7 days of treatment compared with 732 (95%) of 771 infants in the control group, 638 (83%) versus 665 (86%) had at least 2 weeks of treatment, and 603 (78%) versus 634 (82%) completed treatment (appendix p 5). Non-completion of treatment, which usually reflected transfer to non-participating sites, occurred in 152 (20%) infants in the treatment group versus 130 (17%) in the control group (appendix p 5). There were no crossovers from control to lactoferrin.

There was no significant difference between study groups in-hospital death or major morbidity (162 [21%] infants in the treatment group *vs* 170 [22%] in the control group; RR 0·95, 95% CI 0·79–1·14; p=0·60).

A sensitivity analysis using the original primary outcome, which included chronic lung disease, did not materially change this result (appendix p 6).

There were no significant differences in survival to hospital discharge, brain injury, stage II or III necrotising enterocolitis, late-onset sepsis, and retinopathy treated

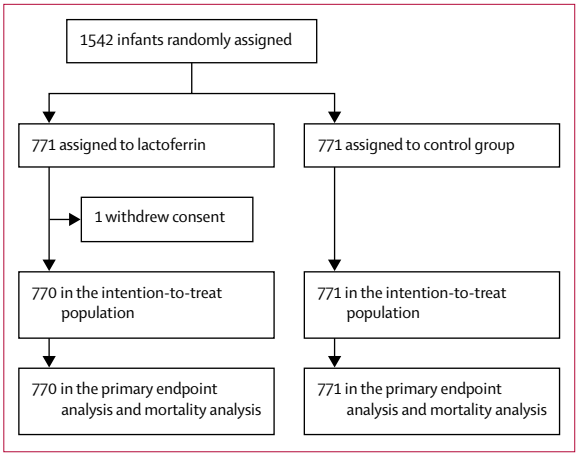

Figure 1: **Trial profile**

	Lactoferrin (n=770)	Control (n=771)
Sex		
Female	348 (45%)	349 (45%)
Male	422 (55%)	422 (55%)
Birthweight, g	1068 (262)	1063 (261)
Gestational age at birth, weeks	28·4 (2·4)	28·4 (2·3)
Birth <29 weeks	339 (44%)	324 (42%)
Multiple births	237 (31%)	237 (31%)
Inborn	700 (91%)	708 (92%)
Mode of delivery		
Cesarean section-in labour	156 (20%)	152 (20%)
Caesarean section-not in labour	378 (49%)	405 (53%)
Vaginal with instruments	32 (4%)	32 (4%)
Vaginal without instruments	202 (26%)	180 (23%)
Apgar score at 5 min		
Missing	6 (1%)	4 (1%)
<7	159 (21%)	138 (18%)
≥7	605 (79%)	629 (82%)
Initial feed type		
Mother's own breast milk*	733 (95%)	725 (94%)
Donor breast milk*	54 (7%)	53 (7%)
Formula milk*	64 (8%)	63 (8%)
Only donor or formula milk	36 (5%)	44 (6%)
>72 h interval from birth to randomisation	494 (64%)	490 (64%)

Data are mean (%) or mean (SD). *Not mutually exclusive.

Table 1: **Baseline characteristics**

	Lactoferrin (n=770)	Control (n=771)	Relative risk with Lactoferrin (95% CI)*	p value
In-hospital death or major morbidity*	162 (21%)	170 (22%)	0·95 (0·79–1·14)	0·60
Stage II or III necrotising enterocolitis*	26 (3%)	25 (3%)	1·09 (0·63–1·9)	0·75
Late-onset sepsis	89 (12%)	108 (14%)	0·83 (0·64–1·08)	0·16
Any brain injury*	50 (7%)	47 (6%)	1·06 (0·72–1·54)	0·78
Retinopathy treated before discharge*	29 (4%)	20 (3%)	1·43 (0·84–2·44)	0·19
In-hospital death*	32 (4%)	29 (4%)	1·12 (0·68–1·84)	0·66

Data are n/N (%). * Model fitted using generalised estimating equations to accommodate clustering of multiple births.

Table 2: **Outcome with respect to in-hospital death or major morbidity by 36 weeks' postmenstrual age**

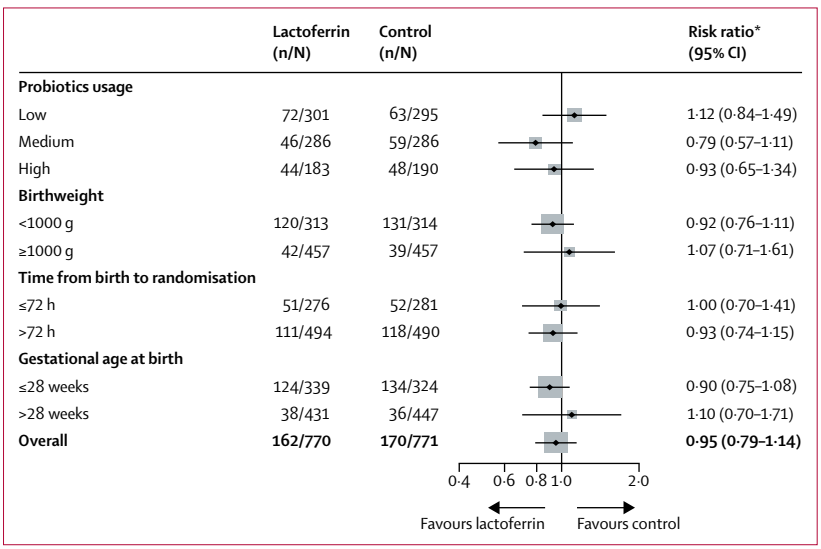

Figure 2: **Subgroup analyses of primary outcome**
n=number of events. N=group size. *Estimated from log-binomial model fitted using generalised estimating equations to accommodate clustering of multiple births.

before hospital discharge (table 2), or in provision of supplemental oxygen or blood transfusions by 36 weeks, days before full enteral feeds, or days in hospital (appendix p 6).

The effects of lactoferrin supplementation on the primary outcome did not differ by birthweight, age at randomisation, gestation, or probiotics use (figure 2). Two suspected unexpected serious adverse reaction occurred in the lactoferrin group, a case of late unconjugated jaundice and a case of inspissated milk syndrome, and one in controls, a fatal case of inspissated milk syndrome (appendix p 10). Conclusions were insensitive to adjustment for baseline covariates and treatment adherence, and other planned sensitivity analyses (appendix pp 7–8).

Our meta-analysis identified five new trials, including LIFT, completed before Feb 18, 2020.[27–30] Manzoni and colleagues have provided additional, previously unreported data, with rates of late-onset sepsis of 10 (11%) of 94 patients in the intervention group and 18 (20%) of 90 patients in the control group, in 184 infants (figure 3A, appendix p 14)[11,12] who were recruited after publication of the original trial.[10] We therefore evaluated a total of 5609 infants in 13 trials. The LIFT-Canada trial is continuing.[31] We assessed LIFT and nine other trials[7–14, 30] as being of low risk of bias and found no other completed, unpublished trials (appendix pp 11–17). Assuming fixed-effects in each trial, lactoferrin supplementation reduced late-onset sepsis (RR 0·79, 95% CI 0·71–0·88; p<0·0001; RR reduction 0·21, 95% CI 0·12–0·29) with moderate heterogeneity between trials (I^2=58%; figure 3A), but it did not significantly reduce necrotising enterocolitis (eight trials; RR 0·90, 95% CI 0·69–1·17; p=0·43; I^2=26%; figure 3B) or mortality (12 trials; RR 0·97, 95% CI 0·79–1·20; p=0·81; I^2=18%; figure 3C). The funnel plot was asymmetrical for late-onset sepsis (Egger's test Z=–4·18; p<0·0001) but not for necrotising enterocolitis or mortality (appendix p 19). Assuming random-effects in each trial, lactoferrin supplements reduced late-onset sepsis (RR 0·61, 95% CI 0·48–0·77; p<0·0001; RR reduction 0·39 (95% CI 0·23–0·52); I^2=58%), without significantly reducing necrotising enterocolitis or mortality (appendix p 20). These conclusions were unchanged after excluding three trials of lower quality because of increased risk of bias (appendix p 21).[27–29] The level of evidence for trials of the effect of lactoferrin supplementation on late-onset sepsis, necrotising enterocolitis, and mortality was rated as low (appendix p 22). Six trials proposing to enrol a total of 2520 infants remain unreported, of which at least two (representing 420 infants) are overdue (appendix p 17).

Discussion

In our trial, lactoferrin supplementation had no significant effect on the primary outcome of death or major morbidity (table 2). Lactoferrin did not appear to benefit any of the predefined subgroups (figure 2). There were no safety concerns (appendix p 10).

The antibiotic, antioxidant, and anti-inflammatory properties of lactoferrin[2–5] justified assessing its effects on late-onset sepsis, necrotising enterocolitis, and all-cause mortality, as prespecified[20] (appendix p 11). However, reliably detecting a 25% reduction in late-onset sepsis, for example, would require much greater numbers than we enrolled in LIFT, which included only 197 events with this outcome (table 2). The UK ELFIN Trial in 2203 infants reported a RR for late-onset sepsis of 0·95, a result associated with a 95% CI with a lower limit of 0·86 and an upper limit of 1·04, which does not conclusively rule out a risk reduction of up to 14% or an increase in RR of up to 4%.[16] By comparison, trials and meta-analyses of trials on safe and affordable treatments for trauma and cardiovascular disease in adults, which enrolled between 10 008 and 58 050 participants, have shown clinically important reductions in the RRs of their primary outcome of as little as 7%.[18,19]

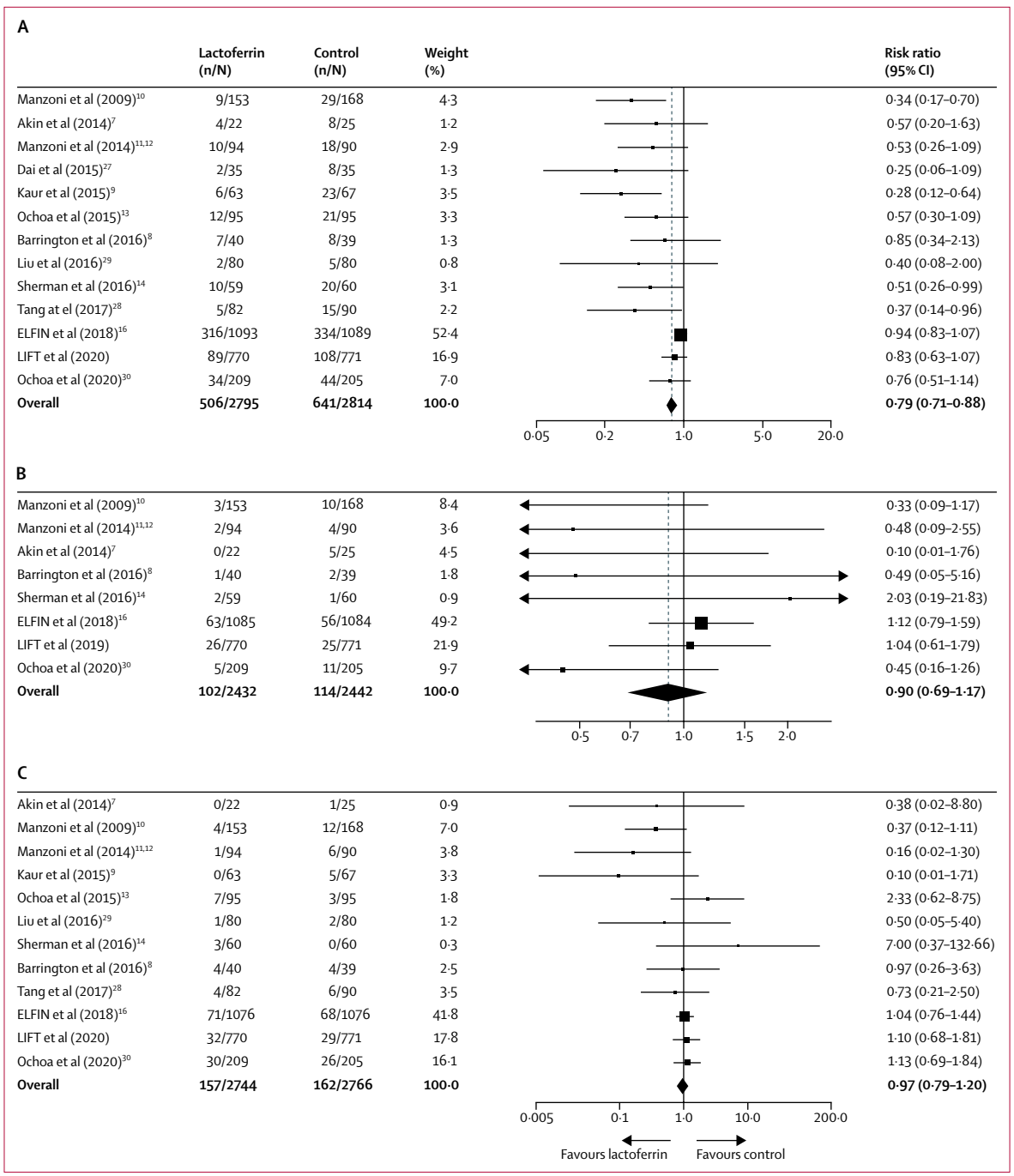

Figure 3: **Meta-analyses of the effects of lactoferrin supplementation**
(A) Forest plot of the effect of lactoferrin supplements on (A) late-onset sepsis, (B) necrotising enterocolitis, and (C) all-cause mortality. n=number of events. N=group size.

Our meta-analysis of trials of lactoferrin supplementation, which to our knowledge is the largest to date, included 5609 infants (figure 3, appendix 11) and complied with PRISMA reporting criteria.[24] Our meta-analysis findings confirm those of the most recent Cochrane systematic review,[33] which did not include previously unreported results for late-onset sepsis in 184 infants (appendix p 14).[11,12] Lactoferrin supplementation reduced the RR of late-onset sepsis by 21% (95% CI 12–29, p<0·0001), assuming fixed effects in each trial, but did not significantly reduce necrotising enterocolitis or all-cause mortality. In a sensitivity analysis, excluding three trials considered of lower quality because of risk of bias,[27–29] a highly precise overall effect of lactoferrin

supplementation on late-onset sepsis persisted (RR 0·81; 95%C 0·73–0·90; p<0·0001; appendix p 21).

How can the funnel plot asymmetry and statistical heterogeneity between larger and smaller trials for the effect of lactoferrin on late-onset sepsis (appendix p 19) be explained? Funnel plot asymmetry should not automatically be equated with small study bias, because it has other possible causes.[26,34] On the one hand, it can reflect publication bias from unreported so-called negative small trials, or bias from spuriously inflated effects in suboptimally done smaller trials. On the other hand, and this is less widely appreciated, statistical heterogeneity could reflect real differences in the effectiveness of lactoferrin products against sepsis, or real differences in underlying patient risk between trials.[26,34]

Real differences of both kinds seem plausible. First, variation in the effectiveness of lactoferrin products could reflect differences between bovine and human[14] lactoferrin, or between different processing and storage techniques, which can reduce the antimicrobial properties of bovine lactoferrin by 40–70%[35] or more.[36] Secondly pasteurised donor milk or formula, which contain little or no lactoferrin, might be less effective against late-onset sepsis than fresh mother's milk.[36–40] Hence, preterm infants who are not exclusively fed mother's own milk that is fresh could be at greater underlying risk. This hypothesis is supported by a cohort study[40] showing that the estimated intake of lactoferrin in mother's own milk was inversely associated with the composite outcome of late-onset sepsis, necrotising enterocolitis, and death; and by secondary analyses[41] of two trials in 1891 preterm infants[10,16] suggesting that lactoferrin supplementation reduced late-onset sepsis in those receiving mixed feeds, including formula or pasteurised donor milk. Also, in a case-control study preterm infants with late-onset sepsis had consumed less lactoferrin than those without late-onset sepsis, whose average lactoferrin intake was approximately 300 mg/kg per day, ranging up to approximately 800 mg/kg per day.[38] Hence, lactoferrin supplementation might provide greater protection for preterm infants who consume insufficient breast milk lactoferrin.

As recommended by Sterne and colleagues,[34] we further investigated the funnel plot asymmetry in the appendix pp 19–20 by comparing estimates of the effect of lactoferrin on late-onset sepsis assuming fixed versus random effects. The unexpectedly greater reduction in RR of late-onset sepsis of of 39% (95% CI 23% to 52%; p<0·0001), which was observed after assuming random effects in the trials (appendix p 20) compared with the reduction in RR of 21% (95% CI 12% to 29%; p<0·0001) assuming fixed effects, is unusual (figure 3A). It further supports the possibility that the lactoferrin products used in smaller studies, like the trial by Sherman and colleagues, which used human recombinant lactoferrin,[14] might have been truly more effective.[26]

How certain is the level of evidence that lactoferrin reduces late-onset sepsis when applying GRADE criteria? Because the asymmetrical funnel plot for late-onset sepsis (appendix p 19) showed statistical heterogeneity between larger and smaller trials that was unlikely to be coincidental (p<0·0001), we rated the certainty of the evidence of the effect on late-onset sepsis as low, even though a highly precise (p<0·0001) reduction in late-onset sepsis persisted in a sensitivity analysis restricted to ten trials of higher quality (appendix p 21). However, we note that GRADE does not allow upgrading for high precision, as seen in figure 3A and appendix pp 20–22, and that its practice of assigning equal weights to different types of methodological limitation is somewhat arbitrary.[25] Future evidence explaining this heterogeneity based on subgroup analyses by type or dose of lactoferrin product in individual participant meta-analyses could improve this grading.

Our study has several strengths. Central web-based randomisation ensured random sequence generation and concealment of allocation and preparation of study feeds only by designated staff resulted in a prospective, randomised, blinded design unlike many pragmatic trials in which staff were not blinded to study group or endpoints. Outcomes were reported in all infants whose parents gave consent and the trial is at low risk of bias (appendix pp 12,18). Our trial did not use a placebo because of practical and safety concerns, but the preparation of study feeds by only designated staff ensured masking of both treatment delivery and outcome assessment for all other staff, parents, and investigators. Any partial unblinding might have selectively increased the use of other interventions in the control group, potentially blunting a treatment benefit; however, there was no evidence of this. Because of a lower than anticipated incidence of the primary outcome and an unexpected increase in apparent chronic lung disease owing to prolonged use of nasal cannulae, the Trial Management Committee decided, appropriately, to increase the target sample size and remove chronic lung disease from the primary outcome, while remaining masked to randomised results.[23] Importantly, the committee did not seek advice on either decision from the Data and Safety Monitoring Committee, who were unmasked to randomised outcomes, and a sensitivity analysis of the primary outcome, including chronic lung disease, did not materially change our conclusions (appendix p 6).

Our study also has several limitations. Death or major morbidity occurred less frequently in the control group (22%) than expected (26%)[20] and the intervention was stopped after infants were transferred to non-participating sites, potentially reducing the power of the study. Although 85% of infants received probiotics (appendix p 5), LIFT was underpowered to assess the role of probiotics in modifying treatment effects. Our definition of brain injury included intraventricular haemorrhage,

which frequently preceded the onset of study treatment. As all comparisons were inconclusive, no cost-effectiveness analysis was done[37] (appendix p 35). We did not record the days on which infants received mother's milk, donor milk, or formula. Last, no trials have published evidence addressing whether concurrent medications, such as iron, interact with lactoferrin;[38] different manufacturing processes alter its effectiveness;[35–40] doses above 300 mg/kg per day are more effective;[38] or analysing recurrent sepsis is informative.

Available evidence cannot conclusively exclude clinical benefit from lactoferrin supplements in preterm infants. The ELFIN study[16] has not resolved this question because it did not place its results in the context of a PRISMA-compliant systematic review[16] of all available evidence; it did not conclusively rule out a 14% reduction in the RR of late-onset sepsis; and it only tested a single product, which might have been less effective than products used in smaller trials—perhaps explaining, in part, the funnel plot asymmetry and statistical heterogeneity shown in appendix p 19.

What further research is needed? A logical next step is a participant-level data meta-analysis of all known trials, as recommended by Doyle and Cheong in their commentary on the ELFIN trial.[16,42] This study could explore whether, in preterm infants not exclusively fed fresh mother's milk or in infants of extremely low birthweight, lactoferrin supplements reduce late-onset sepsis, anaemia, blood transfusions,[4] retinopathy[10] or other outcomes and whether such effects are modified by differently manufactured products;[2,35–41] duration and dose of lactoferrin;[35–41] concurrent medications;[38] or probiotic treatment.[36] If such hypotheses are corroborated, any new trials should be endorsed by stakeholders,[43] including parents, and evaluate different doses, perhaps up to 600 mg/kg per day,[38] using products of demonstrated biological activity with appropriate sample size and outcomes.

What is an appropriate sample size? Reliably detecting moderate reductions in key outcomes like mortality, disability, or sepsis, while minimising false-positive or false-negative results, requires a large sample size.[17–19] For example to show, with 90% power, a 10% reduction in the RR of mortality or major disability from 20% to 18% with a two-sided p value of 0·05 assuming a 10% drop-out rate requires over 17 000 (appendix p 25). Therefore, it seems premature to reject any possibility of benefit from a potentially safe, affordable intervention like lactoferrin based on data from fewer than 6000 participants.

How can we progress to trials with enough power to detect small or moderate, but clinically important, differences in key neonatal or perinatal outcomes reliably? Such trials might need very large numbers of individuals, which might be not be feasible in one country (appendix p 25). However, a very large study population could be achieved by prospective individual participant meta-analyses[44] of multiple concurrent, simple, and efficient[45–50] nationally funded studies using similar protocols, overseen by a single, international Data and Safety Monitoring Committee, who could advise when all studies can be stopped early if an answer emerges beyond reasonable doubt.[47]

Although this trial showed no significant effect of lactoferrin supplementation on death or major morbidity in very low birthweight infants, we cannot exclude the possibility that some lactoferrin products might be more effective than others because of the statistical heterogeneity observed between the trials in our meta-analysis.[34] An individual participant data meta-analysis may be warranted[42,49] to explore the potential effects of higher doses in lactoferrin in infants at increased underlying risk, using products with demonstrated biological activity.

Contributors

WOT-M was the chief investigator. MEA-L, AM, MPa, KR, PM, DO, KL, AK, WH, AG, JT, RB, BAD, HL, MPr , AK, DI, AG, LA, MC, TS, KD, GD, MT, DS, NA, JS, and RJS were co-investigators. AM and KR were trial statisticians. WOT-M, AM, PM, WH, AG, RB, BAD, MC, DS, and RJS designed the study. WOT-M, AM, WH, AG, RB, and DS collected and managed the data. WOT-M, AM, MEA-L, MP, KR, PM, DO, and RJS analysed the data. WOT-M, MEA-L, PM, AM, KR, DO, and RJS interpreted the data. WOT-M, MEA-L, AM, PM, KR, DO, JT, BAD, DS, and RJS wrote the article. All authors provided inputs for the study results and manuscript and approved the final version of the manuscript.

Declaration of interests

We declare no competing interests.

Data sharing

Individual de-identified participant data from the results reported in this Article will be available for 5 years after publication. Researchers will need to provide a methodologically sound proposal to lift@ctc.usyd.edu.au and this will be reviewed by the trial management committee. Researchers will need to sign a data access agreement.

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