Enteral lactoferrin to prevent infection for very preterm infants: the ELFIN RCT

James Griffiths,[1] Paula Jenkins,[1] Monika Vargova,[1] Ursula Bowler,[1] Edmund Juszczak,[1] Andrew King,[1] Louise Linsell,[1] David Murray,[1] Christopher Partlett,[1] Mehali Patel,[2] Janet Berrington,[3] Nicholas Embleton,[3] Jon Dorling,[4] Paul T Heath,[5] William McGuire[6]* and Sam Oddie[7] on behalf of the ELFIN Trial Investigators Group

[1]National Perinatal Epidemiology Unit, University of Oxford, Oxford, UK
[2]Bliss, London, UK
[3]Newcastle Neonatal Service, Royal Victoria Infirmary, Newcastle upon Tyne, UK
[4]Queen's Medical Centre, Nottingham, UK
[5]St George's, University of London and St George's University Hospitals NHS Trust, London, UK
[6]Centre for Reviews and Dissemination, University of York, York, UK
[7]Bradford Institute for Health Research, Bradford, UK

*Corresponding author william.mcguire@york.ac.uk

Declared competing interests of authors: Since November 2013, Edmund Juszczak has been a member of the National Institute for Health Research (NIHR) Health Technology Assessment (HTA) General Funding Committee and HTA Commissioning Funding Committee. William McGuire is a member of the NIHR HTA Commissioning Board and the NIHR HTA and Efficacy and Mechanism Evaluation Editorial Board. Jon Dorling is a member of the NIHR HTA General Board (from 2017) and the Maternal, Neonatal and Child Health Panel (from 2013). Nicholas Embleton reports grants from Prolacta Biosciences Inc. (Duarte, CA, USA), grants from Danone Nutricia Early Life Nutrition (Paris, France), personal fees from Nestlé Nutrition Institute (Vevey, Switzerland) and personal fees from Baxter Healthcare Ltd (Newbury, UK) outside the submitted work.

Published December 2018
DOI: 10.3310/hta22740

Scientific summary

Enteral lactoferrin to prevent infection for very preterm infants

Health Technology Assessment 2018; Vol. 22: No. 74
DOI: 10.3310/hta22740

NIHR Journals Library www.journalslibrary.nihr.ac.uk

Scientific summary

Background

Late-onset infection is the most common serious complication associated with hospital care for preterm infants. The reported incidence ranges from about 15% to 30% in very preterm infants, reflecting their high levels of exposure to invasive procedures and intensive care. Very preterm infants with late-onset infection have a higher rate of mortality and morbidities, such as bronchopulmonary dysplasia (BPD), necrotising enterocolitis (NEC) and retinopathy of prematurity (ROP), than infants without infections. Given this burden of mortality, morbidity and the associated costs for families and health services, the James Lind Alliance Preterm Birth Priority Setting Partnership (Southampton, UK) has identified the development and assessment of better methods to prevent infection in preterm infants as a research priority. One such promising intervention is enteral supplementation with the processed cow's milk protein, lactoferrin (The Tatua Cooperative Dairy Company Ltd, Morrinsville, New Zealand).

Lactoferrin is the major whey protein in breast milk and is a key component of the mammalian innate response to infection. It has broad microbiocidal activity via mechanisms, such as cell membrane disruption, iron sequestration, the inhibition of microbial adhesion to host cells and the prevention of biofilm formation. Lactoferrin has prebiotic properties, promoting the growth of beneficial bacteria and reducing colonisation by pathogenic species. It enhances intestinal mucosal integrity and immune function by modulating cytokine expression, suppressing free-radical activity and activating and mobilising leucocytes.

Bovine lactoferrin is 70% homologous with human lactoferrin, but has a higher antimicrobial activity. It has been a component of the human infant diet for thousands of years, is available as a food supplement in a powder form and is registered as 'Generally Recognised as Safe' by the US Federal Drug Administration.

The Cochrane review of lactoferrin supplementation for preterm infants includes six randomised controlled trials (RCTs) with 1071 participants in total (Pammi M, Suresh G. Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2017;**6**: CD007137). Meta-analyses suggest that lactoferrin reduces the incidence of late-onset invasive infection by about 40%. The effect size is similar whether infants are fed human milk or formula. The risk of NEC is decreased by about 60%. No evidence of adverse effects or intolerance exists. As the included trials were small and contained various methodological weaknesses, the evidence was considered to be of low quality and the review concluded that data from high-quality trials were needed to provide evidence of sufficient validity to inform policy and practice.

Objective

To assess the effect of enteral supplementation with bovine lactoferrin on the risk of late-onset infection and other morbidity and mortality in very preterm infants.

Methods

Study design

The Enteral Lactoferrin In Neonates (ELFIN) trial: a multicentre, randomised, placebo-controlled, parallel-group trial of prophylactic enteral lactoferrin supplementation to prevent late-onset invasive infection in very preterm infants (see www.npeu.ox.ac.uk/elfin).

Setting

Neonatal units in UK hospitals; participant recruitment and initial care in 37 units and continuing care during birth hospitalisation in a further 97 units.

Participants

Very preterm infants of < 72 hours old at randomisation. Infants with a severe congenital anomaly, without a realistic prospect of survival or who were likely to be fasted enterally for > 14 days were ineligible to participate. Written consent was sought from parents only after they had received a verbal and written explanation of the trial.

Interventions

Infants were allocated individually via a secure web-based randomisation portal in the ratio of 1 : 1, minimised for recruiting site, sex, gestational age at birth (completed weeks) and single versus multiple births, to receive either (1) bovine lactoferrin (150 mg/kg/day, up to a maximum of 300 mg/day) or (2) sucrose (British Sugar, Peterborough, UK) placebo (at the same dose). The lactoferrin powder or sucrose was mixed in sterile water plus either expressed breast milk or formula prior to administration via a nasogastric or orogastric tube or orally. The intervention was commenced when the infant's enteral feed volume reached 12 ml/kg/day and was continued once daily until the infant reached 34 weeks' postmenstrual age. Parents, clinicians, carers and those assessing the outcomes were unaware of group assignment.

Outcomes

Primary outcome

Microbiologically confirmed or clinically suspected late-onset infection (occurring > 72 hours after birth) from trial entry until hospital discharge.

Secondary outcomes

Microbiologically confirmed infection; all-cause mortality; severe NEC (Bell's stage II or III); ROP treated surgically or medically; BPD (receipt of supplemental oxygen or respiratory support at 36 weeks' postmenstrual age); a composite of infection, NEC, ROP, BPD and mortality; days of administration of antimicrobials until 34 weeks' postmenstrual age; duration of birth hospitalisation; and length of stay in intensive care, high-dependency care or special-care settings.

Statistics and analysis plan

Sample size

Calculations were based on a primary outcome event rate range of 18% to 24%. In summary, with 90% power and a two-sided 5% significance level, to detect an absolute risk reduction of 5–5.8% (relative risk reduction of 24–28%) required a trial with a total of up to 2200 participants (allowing for an anticipated loss to follow-up of up to 5%). This sample size was sufficient to exclude important effects on secondary outcomes with 90% power.

Statistical analyses

Demographic factors and clinical characteristics at randomisation were summarised with counts (%) for categorical variables, mean (standard deviation) for normally distributed continuous variables or median [interquartile range (IQR)] for other continuous variables.

Outcomes for participants were analysed in the groups to which they were assigned, regardless of deviation from the protocol or treatment received. Comparative analyses calculated the relative risk ratio (RR) with 95% confidence interval (CI) for the primary outcome (99% CIs for all other dichotomous outcomes), the mean difference (99% CI) for normally distributed continuous outcomes or the median difference (99% CI) for skewed continuous variables.

The groups were compared using regression analysis adjusting for the minimisation factors (recruiting hospital, sex, weeks' gestation at birth and single vs. multiple births) to account for the correlation between treatment groups introduced by balancing the randomisation. The crude unadjusted and adjusted estimates were calculated with the primary inference to be based on the adjusted analysis.

The consistency of the effect of lactoferrin supplementation on the primary outcome across specific subgroups of infants was assessed using the statistical test of interaction. Prespecified subgroups were (1) completed weeks' gestation at birth and (2) infants given human breast milk versus formula versus both human milk and formula during the trial period.

Results

The internal pilot was undertaken from May 2014 for 12 months in six neonatal units; 90 infants were recruited to participate. The main trial recruitment period ran from July 2015 to September 2017 in 37 neonatal units. In total, the trial recruited 2203 infants; 1099 infants were allocated to receive bovine lactoferrin and 1104 were allocated to receive sucrose placebo. Four infants had consent withdrawn or unconfirmed. In total, 1098 infants in the lactoferrin group and 1101 in the sucrose group were included in the intention-to-treat analyses.

Baseline characteristics were well balanced. The median gestation age at birth was 29 weeks in both groups (37% aged < 28 weeks). The median birthweight was 1126 g in the lactoferrin group and 1143 g in the placebo group. Overall, 91% of infants were exposed to antenatal corticosteroid, 57% were born via caesarean section, 25% were born following rupture of maternal amniotic membranes for > 24 hours and 12% had evidence of absent or reverse end diastolic flow in the fetal umbilical artery.

Primary outcome

Data were available for 2182 infants (99%). In the intervention group, 316 out of 1093 (28.9%) infants acquired a microbiologically confirmed or clinically suspected late-onset infection, compared with 334 out of 1089 (30.7) in the control group (adjusted RR 0.95, 95% CI 0.86 to 1.04).

Secondary outcomes

There were no significant differences in rates of:

- microbiologically confirmed infection: RR 1.05 (99% CI 0.87 to 1.26)
- all-cause mortality until hospital discharge: RR 1.05 (99% CI 0.66 to 1.68)
- NEC: RR 1.13 (99% CI 0.68 to 1.89)
- ROP: RR 0.89 (99% CI 0.62 to 1.28)
- BPD: RR 1.01 (99% CI 0.90 to 1.13)
- a composite of infection, NEC, ROP, BPD and mortality: RR 1.01 (99% CI 0.94 to 1.08).

There were no differences in the median number of days of receipt of:

- antimicrobials: median difference 0 (99% CI –1 to 1)
- hospital care: median difference 1 (99% CI –1 to 3)
- intensive care: median difference 0 (99% CI –1 to 1)
- high-dependency care: median difference 1 (99% CI –1 to 3)
- special care: median difference –1 (99% CI –3 to 1).

Subgroup analyses did not show any significant interactions for:

- completed weeks' gestation at birth: $p = 0.273$
- type of enteral milk received (human, formula, or both): $p = 0.400$.

Safety and adverse events

There were 16 serious adverse events (SAEs) reported for infants in the lactoferrin group (six severe) and 10 for infants in the sucrose group (three severe). Two SAEs, both in the lactoferrin group, were assessed as being 'possibly related' to the trial intervention. The remaining 24 SAEs were considered to be unrelated to the trial intervention.

Discussion

The ELFIN trial shows that enteral supplementation of bovine lactoferrin (150 mg/kg/day until 34 weeks' postmenstrual age) does not reduce the risk of late-onset infection, other morbidity or mortality in very preterm infants. This contradicts the existing trial evidence. The current Cochrane review includes six RCTs and meta-analyses that suggest substantial reductions in the risk of late-onset infection and NEC associated with lactoferrin supplementation in very preterm infants. The included trials were small and some contained other design and methodological weaknesses that may have introduced biases, thus resulting in overestimation of the effect sizes. The largest previous trial, in which 331 infants participated, showed a relative risk reduction of 66% for late-onset infection. This effect size estimate may have been inflated by performance and detection bias, and by methodological weaknesses, including the absence of predefined criteria for interim analyses. Given these concerns, the Cochrane review graded the quality of the existing evidence for effects on key outcomes as 'low' and concluded that data from methodologically rigorous RCTs were needed to generate evidence of sufficient validity to inform policy and practice.

The ELFIN trial provides these data. The validity of the findings is enhanced by the methodological quality and power of the trial. Best practices were used to limit bias, including central web-based randomisation for allocation concealment, blinding of parents, caregivers and investigators to the group allocation, and intention-to-treat analyses of outcomes based on a prespecified statistical analysis plan. The trial recruited > 2200 participants as per protocol and a priori sample size estimation. Demographic and prognostic characteristics were well-balanced between the two groups at randomisation with a minimisation algorithm, ensuring balance for known or putative prognostic indicators (completed weeks' gestation, sex, single vs. multiple births) or potential confounding influences (recruiting site). Interim analyses by the trial's independent Data Monitoring Committee (DMC) used criteria to minimise the chances of spurious findings caused by data fluctuations before a sufficient sample size was achieved. Adherence to the allocated interventions was high, the incidence of protocol violations was low and outcome data were available for > 99% of the trial cohort. Event rates for the primary and secondary outcomes were similar to those that were anticipated and as described in other cohort studies and RCTs involving very preterm infants.

The trial had sufficient power to detect important effects on the risk of late-onset infection and other morbidities. More precise estimates of effect were able to be generated than were available previously. The 95% CI for the relative risk estimate for the primary outcome excludes a > 14% risk reduction and a ≥ 4% increase in risk. These estimates were consistent across gestational ages at birth and were not affected by the type of enteral feeds that infants received during the trial period (human milk, formula or both). It is therefore unlikely that lactoferrin has any important benefits for subgroups of infants at higher risk of infection.

Estimates for the secondary outcomes indicated consistently that lactoferrin supplementation does not have important effects on the risk of major morbidities. An analysis was prespecified of the effect on a composite of infection, NEC, BPD, ROP and mortality. The adjusted RR point estimate for this secondary outcome was 1.01, with a 99% CI excluding a > 6% reduction and a ≥ 8% increase in risk. As these morbidities, particularly infection and NEC, are the major reasons for the receipt of invasive interventions and higher levels of care in very preterm infants, it is not surprising that there were no effects shown on the level of exposure to antimicrobial agents, or on the duration of hospitalisation or stay in intensive or high-dependency care settings.

Given that the ELFIN trial did not show any differences between groups in the risk of morbidity or on levels of care received, we do not plan to apply for permission and further funding to assess longer-term outcomes of trial participants. Any between-group differences in growth and neurodevelopmental outcomes are predicated largely on differences in the incidence of late-onset infections, NEC and associated morbidities. As these differences were not shown, there is no longer an impelling rationale for expecting lactoferrin supplementation to have an impact on long-term growth or development.

The ELFIN trial findings are applicable in the UK and internationally. Participants were enrolled in 37 neonatal units across the country, providing broad geographical, and social and ethnic representation. Many infants who were recruited were transferred subsequently to another neonatal unit, typically closer to the family home, for ongoing care. Trial participation continued in these additional 97 neonatal units and this practice mirrors managed clinical network care pathways for very preterm infants in the UK. The trial population was representative of very preterm infants cared for within health-care facilities in well-resourced health services and included a substantial proportion of extremely preterm infants (37%) and of infants with other putative risk factors for neonatal morbidity, such as prolonged rupture of maternal amniotic membranes (25%) and evidence of absent or reverse end diastolic flow in the fetal umbilical artery (12%). Overall, about 30% of participants acquired a microbiologically confirmed or clinically suspected late-onset infection, and about 17% in total had a microbiologically confirmed infection, consistent with rates reported from cohort studies and other RCTs. Similarly, the incidence of NEC (about 5%) was similar to rates reported in large, population-based surveillance studies and RCTs.

Conclusion

These findings do not support the use of enteral bovine lactoferrin supplementation to prevent late-onset infection or other morbidity in very preterm infants. Research efforts could continue to investigate the aetiology, epidemiology and pathogenesis of late-onset infection and related morbidities, and to develop, refine and assess other interventions that may prevent or reduce adverse acute and long-term consequences for very preterm infants and their families.

Trial registration

This trial is registered as ISRCTN88261002.

Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research. This trial was also sponsored by the University of Oxford, Oxford, UK. The funder provided advice and support and monitored study progress but did not have a role in study design or data collection, analysis and interpretation.

Health Technology Assessment

HTA/HTA TAR

ISSN 1366-5278 (Print)

ISSN 2046-4924 (Online)

Impact factor: 4.513

Health Technology Assessment is indexed in MEDLINE, CINAHL, EMBASE, The Cochrane Library and the Clarivate Analytics Science Citation Index.

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

Editorial contact: journals.library@nihr.ac.uk

The full HTA archive is freely available to view online at www.journalslibrary.nihr.ac.uk/hta. Print-on-demand copies can be purchased from the report pages of the NIHR Journals Library website: www.journalslibrary.nihr.ac.uk

Criteria for inclusion in the *Health Technology Assessment* journal

Reports are published in *Health Technology Assessment* (HTA) if (1) they have resulted from work for the HTA programme, and (2) they are of a sufficiently high scientific quality as assessed by the reviewers and editors.

Reviews in *Health Technology Assessment* are termed 'systematic' when the account of the search appraisal and synthesis methods (to minimise biases and random errors) would, in theory, permit the replication of the review by others.

HTA programme

The HTA programme, part of the National Institute for Health Research (NIHR), was set up in 1993. It produces high-quality research information on the effectiveness, costs and broader impact of health technologies for those who use, manage and provide care in the NHS. 'Health technologies' are broadly defined as all interventions used to promote health, prevent and treat disease, and improve rehabilitation and long-term care.

The journal is indexed in NHS Evidence via its abstracts included in MEDLINE and its Technology Assessment Reports inform National Institute for Health and Care Excellence (NICE) guidance. HTA research is also an important source of evidence for National Screening Committee (NSC) policy decisions.

For more information about the HTA programme please visit the website: http://www.nets.nihr.ac.uk/programmes/hta

This report

The research reported in this issue of the journal was funded by the HTA programme as project number 10/57/14. The contractual start date was in March 2013. The draft report began editorial review in July 2018 and was accepted for publication in August 2018. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The HTA editors and publisher have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this report.

Published by the NIHR Journals Library (www.journalslibrary.nihr.ac.uk), produced by Prepress Projects Ltd, Perth, Scotland (www.prepress-projects.co.uk).