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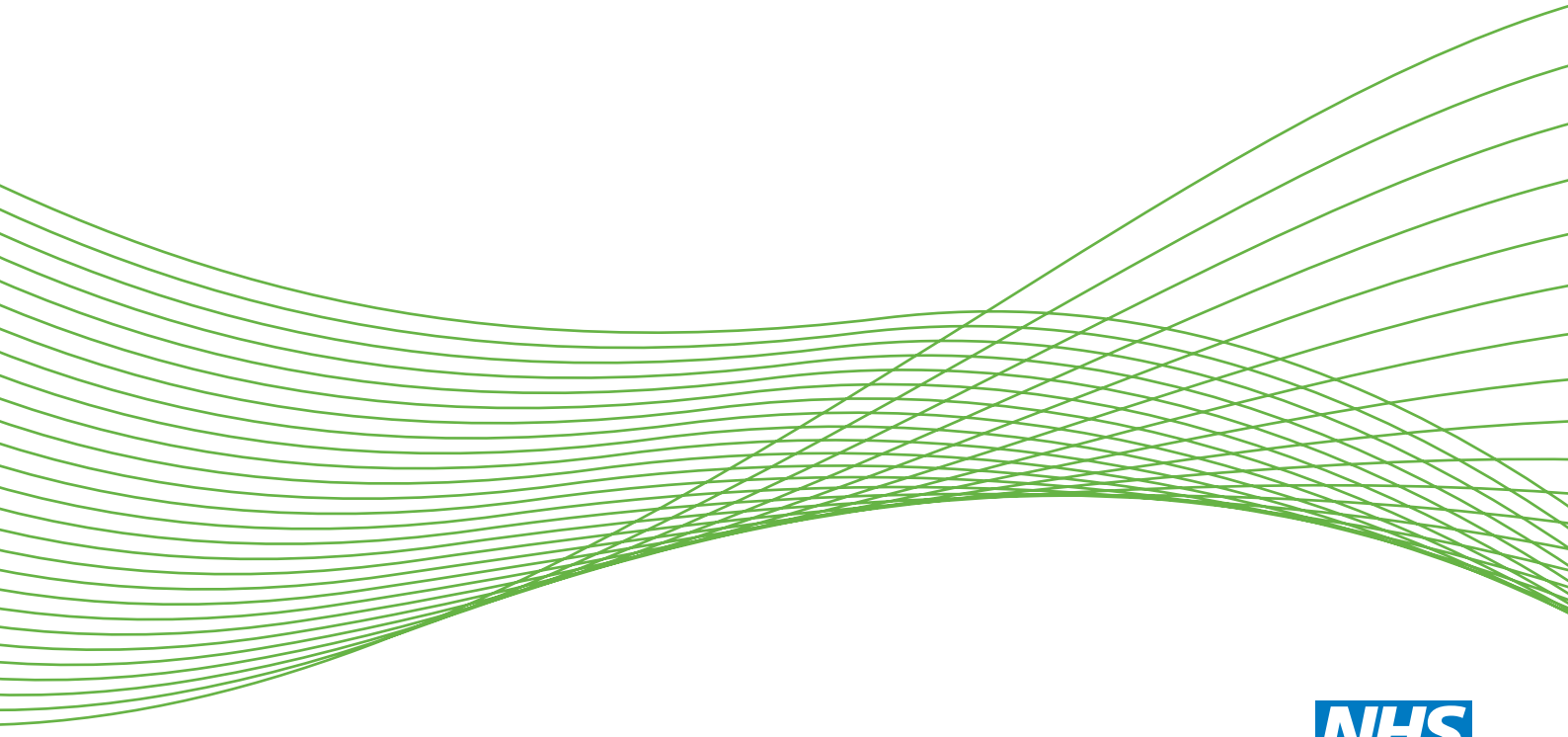

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Abstract

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

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Background: Infections acquired in hospital are an important cause of morbidity and mortality in very preterm infants. Several small trials have suggested that supplementing the enteral diet of very preterm infants with lactoferrin, an antimicrobial protein processed from cow's milk, prevents infections and associated complications.

Objective: To determine whether or not enteral supplementation with bovine lactoferrin (The Tatua Cooperative Dairy Company Ltd, Morrinsville, New Zealand) reduces the risk of late-onset infection (acquired > 72 hours after birth) and other morbidity and mortality in very preterm infants.

Design: Randomised, placebo-controlled, parallel-group trial. Randomisation was via a web-based portal and used an algorithm that minimised for recruitment site, weeks of gestation, sex and single versus multiple births.

Setting: UK neonatal units between May 2014 and September 2017.

Participants: Infants born at < 32 weeks' gestation and aged < 72 hours at trial enrolment.

Interventions: Eligible infants were allocated individually (1 : 1 ratio) to receive enteral bovine lactoferrin (150 mg/kg/day; maximum 300 mg/day) or sucrose (British Sugar, Peterborough, UK) placebo (same dose) once daily from trial entry until a postmenstrual age of 34 weeks. Parents, caregivers and outcome assessors were unaware of group assignment.

Outcomes: Primary outcome – microbiologically confirmed or clinically suspected late-onset infection. Secondary outcomes – microbiologically confirmed infection; all-cause mortality; severe necrotising enterocolitis (NEC); retinopathy of prematurity (ROP); bronchopulmonary dysplasia (BPD); a composite of infection, NEC, ROP, BPD and mortality; days of receipt of antimicrobials until 34 weeks' postmenstrual age; length of stay in hospital; and length of stay in intensive care, high-dependency and special-care settings.

Results: Of 2203 enrolled infants, primary outcome data were available for 2182 infants (99%). In the intervention group, 316 out of 1093 (28.9%) infants acquired a late-onset infection versus 334 out of 1089 (30.7%) infants in the control group [adjusted risk ratio (RR) 0.95, 95% confidence interval (CI) 0.86 to 1.04]. There were no significant differences in any secondary outcomes: microbiologically confirmed infection (RR 1.05, 99% CI 0.87 to 1.26), mortality (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), or 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 number of days of receipt of antimicrobials, length of stay in hospital, or length of stay in intensive care, high-dependency or special-care settings. There were 16 reports of serious adverse events for infants in the lactoferrin group and 10 for infants in the sucrose group.

Conclusions: Enteral supplementation with bovine lactoferrin does not reduce the incidence of infection, mortality or other morbidity in very preterm infants.

Future work: Increase the precision of the estimates of effect on rarer secondary outcomes by combining the data in a meta-analysis with data from other trials. A mechanistic study is being conducted in a subgroup of trial participants to explore whether or not lactoferrin supplementation affects the intestinal microbiome and metabolite profile of very preterm infants.

Trial registration: Current Controlled Trials ISRCTN88261002.

Funding: This project was funded by the National Institute for Health Research (NIHR) Health Technology Assessment programme and will be published in full in *Health Technology Assessment*; Vol. 22, No. 74. See the NIHR Journals Library website for further project information. 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.

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List of abbreviations

ARR	absolute risk reduction
BPD	bronchopulmonary dysplasia
CER	control event rate
CI	confidence interval
CPAP	continuous positive airways pressure
CRF	case report form
CSF	cerebrospinal fluid
DMC	Data Monitoring Committee
ELFIN	Enteral Lactoferrin In Neonates
GMP	good manufacturing practice
ID	identification
IMP	Investigational Medicinal Product
IQR	interquartile range
MHRA	Medicines and Healthcare products Regulatory Agency
NEC	necrotising enterocolitis
NIHR	National Institute for Health Research
NPEU CTU	National Perinatal Epidemiology Unit Clinical Trials Unit
PI	principal investigator
PIL	parent information leaflet
RCT	randomised controlled trial
REC	Research Ethics Committee
ROP	retinopathy of prematurity
RR	risk ratio
SAE	serious adverse event
SAR	serious adverse reaction
SD	standard deviation
SIFT	Speed of Increasing Milk Feeds Trial
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
TSC	Trial Steering Committee

Plain English summary

Babies who are born 'very prematurely' (i.e. > 8 weeks early) need specialist hospital care on a neonatal unit. These babies can develop serious infections and illnesses during their stay in hospital. The risk of developing such infections is highest in the most premature infants.

This study was designed to test whether or not giving lactoferrin (The Tatua Cooperative Dairy Company Ltd, Morrinsville, New Zealand), a naturally occurring milk protein (often used as a food supplement), to babies can help to protect them against infections. A small study was previously carried out in Italy and, although the results were promising, we needed to find out more. A large study was undertaken in neonatal units across the UK. More than 2200 very premature babies took part to find out whether or not lactoferrin is effective at preventing infections and other illnesses. With consent from babies' parents, clinicians randomly (by chance) allocated babies to receive either lactoferrin or sugar (sham treatment) mixed with milk once a day until they were no longer at a high risk of serious infections (the equivalent of 34 weeks' gestation). The babies' parents, nurses and doctors were not aware of whether each individual baby was receiving lactoferrin or sucrose (British Sugar, Peterborough, UK).

When all of the study data had been analysed, it was found that supplemental lactoferrin did not reduce the risk of infection or other serious illness or death, or affect the length of hospital stay, in very premature babies born > 8 weeks early. Because the study was large and used reliable methods, these results prove that lactoferrin supplementation does not have important benefits for very premature babies and that there is no need for any further research into the use of this treatment.

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.

Chapter 1 Introduction

Late-onset infection in very preterm infants

Late-onset invasive infection (occurring > 72 hours after birth) is the most common serious complication associated with hospital care for preterm infants. The UK James Lind Alliance Preterm Birth Priority Setting Partnership has identified the development and assessment of better methods to prevent infection in preterm infants as a research priority.[1]

The incidence of late-onset infection is typically estimated to be > 20% in very preterm infants, reflecting the level and duration of exposure to invasive procedures and intensive care.[2,3] Very preterm infants who acquire a late-onset bloodstream or deep-seated infection are at higher risk of mortality and a range of acute morbidities including necrotising enterocolitis (NEC), retinopathy of prematurity (ROP) and bronchopulmonary dysplasia (BPD) than comparable infants without infection.[4–6] Over the long term, late-onset infection is associated with higher rates of adverse neurodevelopmental outcomes, including visual, hearing and cognitive impairment, and cerebral palsy.[5]

Mortality and morbidity are usually associated with Gram-negative bacterial, *Staphylococcus aureus* or fungal bloodstream infections or meningitis.[7–9] Coagulase-negative staphylococcal infection, despite accounting for about half of all infections, is generally associated with a more benign clinical course.[10] Meningitis and other deep-seated infections are rare and the mortality rate is lower than that attributed to Gram-negative or other Gram-positive bacterial infections. However, even low-grade coagulase-negative staphylococcal bloodstream infections may generate inflammatory cascades that are associated with both acute morbidity (metabolic, respiratory or thermal instability, thrombocytopenia) and long-term white matter and other brain damage that may result in adverse neurodevelopmental outcomes.[11]

As a consequence of associated morbidities, very preterm infants with late-onset infection may spend about 20 more days in hospital than gestation-comparable infants without infection.[12] Late-onset infection and associated morbidities therefore have major consequences for perinatal health-care service management, delivery and costs.

Diagnosis, treatment and prevention of late-onset invasive infection

Clinical signs and laboratory markers may be unreliable predictors of true late-onset infection, especially in very preterm infants.[13,14] A policy of early empirical treatment of suspected infection is usually implemented. Most neonates who are treated as a result of 'sepsis evaluation', however, do not have infection confirmed subsequently.[15] This results in unnecessary exposure to antibiotics, which not only subjects very preterm infants to more interventions but may drive the emergence of antibiotic-resistant pathogens in the neonatal unit.[16,17] Although generic infection control measures, such as hand-washing and intravascular catheter 'care bundles', have helped to prevent some episodes of late-onset invasive infection in very preterm infants, benchmarking and quality improvement studies in neonatal networks have indicated that there is a need for measures to further reduce the incidence.[18]

Given this burden of mortality, acute and long-term morbidity, and costs to families and health services, there is a need to develop innovative strategies to prevent late-onset invasive infection in very preterm infants.[19]

Lactoferrin

Lactoferrin, a member of the transferrin family of iron-binding glycoproteins, is a key component of the mammalian innate response to infection.[20–22] It is the major whey protein in human colostrum, present at a concentration of about 6 mg/ml and is present in mature breast milk at a concentration of about 1 mg/ml.[23] Lactoferrin is also present in mammalian tears, saliva, cerebrospinal fluid (CSF) and other secretions.[22]

Lactoferrin has broad microbiocidal activity by mechanisms, such as cell membrane disruption, iron sequestration, the inhibition of microbial adhesion to host cells and the prevention of biofilm formation (*Box 1*).[20,21] Development of resistance to lactoferrin is improbable as it would require multiple simultaneous mutations. Lactoferrin remains a potent inhibitor of viruses, bacteria, fungi and protozoa after millions of years of mammalian evolution.[24,25]

Lactoferrin has prebiotic properties, creating an enteric environment that promotes the growth of beneficial bacteria and reducing colonisation with pathogenic species.[26,27] It has direct intestinal immunomodulatory and anti-inflammatory actions mediated by modulating cytokine expression, mobilising leucocytes into the circulation and activating T-lymphocytes.[28,29] At high concentrations, as in colostrum, lactoferrin enhances the proliferation of enterocytes and the closure of enteric gap junctions.[30] At lower concentrations, lactoferrin stimulates the differentiation of enterocytes and the expression of intestinal digestive enzymes.[31] Lactoferrin suppresses free-radical activity when iron is added to milk, suggesting that it may have further anti-inflammatory actions that could modulate the pathogenesis of diseases linked with free-radical generation, such as NEC, ROP and BPD.[32]

Bovine lactoferrin

Bovine lactoferrin (The Tatua Cooperative Dairy Company Ltd, Morrinsville, New Zealand) is > 70% homologous with human lactoferrin but has higher antimicrobial activity. It is inexpensive compared with

BOX 1 Lactoferrin: mechanisms of action

- Antimicrobial effects:
  - cell membrane disruption
  - iron sequestration
  - inhibition of microbial adhesion to host cells
  - prevention of biofilm formation.
- Prebiotic effects:
  - promote intestinal growth of beneficial bacteria (probiotics)
  - reduce colonisation with pathogenic species.
- Immune-modulatory and anti-inflammatory actions:
  - modulate cytokine expression
  - mobilise leucocytes into the circulation
  - activate T-lymphocytes
  - suppress free-radical activity.
- Intestinal integrity effects:
  - stimulate differentiation and proliferation of enterocytes
  - promote closure of enteric gap junctions
  - increase expression of intestinal digestive enzymes.

human or recombinant lactoferrin and is available commercially as a food supplement in a stable powder form.[33] The affinity of bovine lactoferrin for the human small intestine lactoferrin receptor is low and intact lactoferrin and digested fragments (lactoferricins), which also have high microbiocidal activity, are excreted enterally.[34,35] Bovine lactoferrin has been a component of the human infant diet for thousands of years and is registered as 'Generally Recognised As Safe' by the US Federal Drug Administration with no reports of human toxicity.[36] The 'no observed adverse effect level' is > 2 g/kg/day in rodents.[37] Given the absence of adverse effects, the European Food Safety Authority Panel concluded that bovine lactoferrin for infants is safe at the standard supplementation levels (up to about 210 mg/kg of body weight per day).[38]

Lactoferrin supplementation

Very preterm infants typically ingest little or no milk during the early neonatal period and thus have low lactoferrin intake. This deficiency may be further exacerbated by delays in establishing enteral feeding. Enteral lactoferrin supplementation has been proposed and assessed as a simple strategy to compensate for this gestational immunodeficiency.[39]

Existing evidence

The Cochrane review by Pammi and Suresh[40] identified six completed randomised controlled trials (RCTs), involving 1071 participants in total.[41–46] Meta-analyses suggest that enteral supplementation with lactoferrin reduces the incidence of late-onset invasive infection by about 40%; the effect size is similar whether infants are fed predominantly human milk or formula milk. The incidence of NEC is decreased by about 60%. No evidence of an effect on all-cause mortality was found and no adverse effects or intolerance were reported (*Box 2*). However, because the trials were generally small and contained various methodological weaknesses that increased the risk of selection and performance bias, and because meta-analyses were limited by data availability and heterogeneity, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group assessment of the quality of this evidence was 'low', meaning that further research was likely to have an important impact on the confidence in the estimates of effect and is likely to change these estimates. The Cochrane review concluded that additional data from large, good-quality RCTs of lactoferrin supplementation in very preterm infants were needed to enhance the validity and applicability of the evidence-base sufficiently to inform policy and practice.[40]

Objective

The study aimed to assess the effect of enteral administration of bovine lactoferrin on the incidence of late-onset infection, other morbidity and mortality in very preterm infants.

BOX 2 Summary findings of the Cochrane review meta-analyses[40]

- Late-onset infection: typical RR 0.59, 95% CI 0.40 to 0.87; typical RD –0.06, 95% CI –0.10 to –0.02; number needed to treat for an additional beneficial outcome was 17, 95% CI 10 to 50; six trials, 886 participants.
- Necrotising enterocolitis (Bell's stage II or III): typical RR 0.40, 95% CI 0.18 to 0.86; typical RD –0.04, 95% CI –0.06 to –0.01; number needed to treat for an additional beneficial outcome was 25, 95% CI 17 to 100; four trials, 750 participants.
- All-cause mortality: typical RR 0.65, 95% CI 0.37 to 1.11; typical RD –0.02, 95% CI –0.05 to 0; six trials, 1071 participants.

CI, confidence interval; RD, risk difference; RR, risk ratio.

Chapter 2 Methods

Design

The Enteral Lactoferrin In Neonates (ELFIN) trial was a UK, multicentre, parallel-group, placebo-controlled RCT (see www.npeu.ox.ac.uk/elfin).[47]

Ethics approval and research governance

The ELFIN trial protocol was approved by the National Research Ethics Service Committee East Midlands – Nottingham 2 on 2 April 2013 (reference number 13/EM/0118).

Local approval and site-specific assessments were obtained from the NHS trusts for trial sites.

The trial was registered with the International Standard Randomised Controlled Trial Register (https://doi.org/10.1186/ISRCTN88261002).

Patient and public involvement

During the development and delivery of the ELFIN trial, we engaged with infant and family representatives experienced in voicing service users' views (principally via Bliss, a UK national charity supporting preterm or sick newborn infants and their families: www.bliss.org.uk/). Parents with children who had received neonatal intensive care contributed via Bliss and directly to the development of trial materials (e.g. parent information and resources) and research staff training (e.g. in simulated sessions on 'seeking consent'). We adhered to INVOLVE good practice guidelines to ensure service-user leadership in the delivery of the trial and dissemination of the findings (www.invo.org.uk/).

Participants

Inclusion criteria

- Gestational age at birth of < 32 weeks.
- < 72 hours old at randomisation.
- Written informed parental consent.

Exclusion criteria

- Severe congenital anomaly.
- Anticipated enteral fasting for > 14 days.
- No realistic prospect of survival.

Infants receiving antibiotic treatment at randomisation were eligible to participate.

Setting

Neonatal units in the UK caring for very preterm infants:

- recruiting sites where parents' consent was obtained and infants could be recruited and randomised to commence participation in the trial ($n = 37$; see *Appendix 1*)
- continuing care sites where clinicians continued to administer the intervention and collect routine data if a participating infant of < 34 weeks' postmenstrual age was transferred from a recruiting site ($n = 97$; see *Appendix 2*).

Depending on the interventions being given, it was possible for an infant to participate in other clinical trials at the same time as participating in the ELFIN trial. The Speed of Increasing Milk Feeds Trial (SIFT) was designed to allow infants to be enrolled in both trials.[48] The ELFIN trial and SIFT shared procedures and, in some cases, joint data collection forms and other documentation. Other trials being run simultaneously in any units were discussed by the chief investigators or their delegated representative to agree whether or not joint recruitment was appropriate and likely to be acceptable.

Screening and eligibility assessment

Potential participants meeting the eligibility criteria were identified by the local health-care team. As the eligibility criteria did not require specific medical assessment, assessment of eligibility was accepted to be within the scope of competency of appropriately trained and experienced neonatal nurses, if so delegated by the principal investigator (PI).

Informed consent and recruitment

Consent was sought from parents of potential participants only after they had received a full verbal and written explanation of the trial [parent information leaflet (PIL); see www.npeu.ox.ac.uk/elfin/parent-resources (accessed 29 June 2018)]. Parents who did not speak English were approached only if an adult interpreter was available.

Informing potential participants' parents of possible benefits and risks occurred as a staged process.[49] If it was likely that the expected infant was eligible to participate in the trial, the PIL and preliminary verbal information was offered prior to birth. Further verbal information was provided after birth as it was to the parents of infants who were not identified antenatally.

Written informed parental consent was obtained by means of dated parental signature and the signature of the person who obtained informed consent: the PI or health-care professional with delegated authority (see www.npeu.ox.ac.uk/elfin/neonatal-staff). A copy of the signed informed consent form was given to the parents. A copy was retained in the infant's medical notes, a copy was retained by the PI and the original was sent to the Clinical Trials Unit.

Participants or parents were not given any financial or material incentive or compensation to take part. It was made clear that parents remained free to withdraw their infant from the trial at any time without the need to provide any reason or explanation. Parents were aware that this decision would have no impact on any aspects of their infant's continuing care.

The trial entry form was completed after informed consent had been given. The recorded information was entered on to the National Perinatal Epidemiology Unit Clinical Trials Unit (NPEU CTU) randomisation website [see https://rct.npeu.ox.ac.uk/ (accessed 29 June 2018)]. Infants were considered to have been enrolled once they have been given a study number and have been allocated a treatment pack number by the randomisation facility.

Intervention

Trial participants were allocated randomly to receive either:

- bovine lactoferrin or
- sucrose (British Sugar, Peterborough, UK).

The UK Medicines and Healthcare Regulatory Agency (MHRA) indicated that, for the purposes of the trial, the intervention and sucrose placebo were considered to be Investigational Medicinal Products (IMPs) and subject to good manufacturing practice (GMP) regulations. After discussion with MHRA, the IMP was considered to be 'category B' (risk slightly above routine practice because bovine lactoferrin is not a licensed product).

Bulk lactoferrin was imported from The Tatua Cooperative Dairy Company Ltd, a New Zealand-based company that manufactures highly purified powder (see www.tatua.com/specialty-nutritionals-ingredients/lactoferrin/).

Bulk sucrose was obtained in the UK from British Sugar (see www.britishsugar.co.uk/).

The IMP was packaged into individual doses in sealed opaque containers and assembled into participant packs to GMP in the MHRA-approved NHS clinical trials pharmacy unit at the Royal Victoria Hospital, Newcastle upon Tyne (www.newcastle-hospitals.org.uk/services/Pharmacy_services_newcastle-specials-pharmacy-production-unit.aspx).

Investigational Medicinal Product management

- Bovine lactoferrin was packaged into 25-ml opaque pharmacy pots (fill equivalent to 375 mg per pot) at the trials pharmacy in Newcastle Royal Infirmary, Newcastle upon Tyne, UK. Boxes containing 24 identically numbered pots were labelled with the same pack identification (ID) number to indicate that they all belonged to the same treatment course. At randomisation, infants were allocated a study number and a pack ID number; the study number was added to the label of the allocated pack with the infant's name and date of birth for checking before each administration of the IMP. Lactoferrin powder was stable within unopened pots and could be stored at room temperature. When the infant completed the course of treatment, any unused IMP pots were retained for accounting and destruction by the site pharmacist.
- Sucrose was processed, packaged and distributed as for bovine lactoferrin.

Investigational Medicinal Product prescription and preparation

Lactoferrin and sucrose were prescribed at a dose of 150 mg/kg body weight per day (up to a maximum of 300 mg/day). The IMP was prepared by neonatal nurses or clinicians on the neonatal unit in the unit milk kitchen or other appropriate area determined locally. The IMP powder was prepared for administration by mixing in sterile water plus expressed breast or formula (see *Appendix 3*).

The IMP was administered once daily by nasogastric or orogastric tube or orally once the enteral feed volume was > 12 ml/kg/day and continued until 34 weeks' postmenstrual age. Some small infants may have had the dose split at the discretion of the responsible clinical team. A maximum of 70 days of treatment was given.

All other aspects of care, including the timing of the commencement of enteral feeds and the type of milk feed used, were as per local policy, practice and discretion.

Randomisation

Randomisation of participants to receive either lactoferrin or sucrose was managed via a secure web-based randomisation facility hosted by the NPEU CTU, University of Oxford, Oxford, UK. Telephone assistance and randomisation back-up was available at all times.

To confirm eligibility, investigators needed to supply gestational age, sex and time of birth. To proceed to randomisation, investigators needed to confirm that signed informed consent was available. Infants were allocated to the lactoferrin versus sucrose groups in the ratio of 1 : 1 using a minimisation algorithm to ensure balance between the groups with respect to the recruiting site (neonatal unit), sex, single versus multiple births and gestational age in completed weeks. Twins or higher order multiple births were randomised individually.

Allocation concealment and blinding

Participating infants were randomly allocated a numbered pack containing either the lactoferrin or the placebo and allocated a unique study number. Parents, clinicians, investigators and outcomes assessors were unaware of the allocated treatment groups.

Stopping Investigational Medicinal Product

Administration of the trial IMP may have been stopped temporarily. Missed doses did not necessitate the removal of an infant from the trial. Data continued to be collected as per protocol if the trial medication was stopped temporarily or permanently in order to facilitate an unbiased treatment comparison via an intention-to-treat analysis.

Masking

Bovine lactoferrin has a pale pink/brown tinge, whereas sucrose was very light brown. The opaque containers did not allow the dry IMP to be seen unless the sealed stopper was removed intentionally. The lactoferrin powder had similar granularity to sucrose so that when the dry IMP was shaken within the opaque, sealed pots it was not possible to distinguish lactoferrin from sucrose by the sound generated. Mixing the IMP with sterile water plus breast milk or formula generated foam that settled within 30 minutes after shaking. When the mixed IMP was removed in a syringe with a purple plunger, the pink tinge to the lactoferrin was disguised by the colour of the breast milk or formula, which often resulted in a light brown colour (and this varied markedly between batches of milk). As lactoferrin was more likely than sucrose to retain a light pink tinge, all sites were supplied with a laminated picture of a range of possible colours for the IMP mixture in syringes, and it was stressed that this applied to both lactoferrin and sucrose.

Clinicians were able to request knowledge of the treatment allocation to guide the clinical management of the participant if it was deemed to be an emergency situation. In such instances, a single-use access code was provided in a sealed envelope and the participant's allocation was unmasked via the randomisation website.

Internal pilot

An internal pilot study was conducted in six neonatal centres within the Northern Region and Yorkshire Neonatal Networks ('operational delivery networks') to test whether or not the components and processes of the study worked together and ran smoothly. The main aims of the pilot were to:

1. confirm that regulatory processes were in order
2. ensure that the randomisation process was acceptable and effective
3. demonstrate efficient intervention and placebo preparation and distribution
4. determine that the anticipated acceptance rate (40% of eligible infants) was achievable

5. determine whether or not the projected recruitment rate was realistic – we set a target of a total of 4, 6 and 8 infants recruited in months 1, 2 and 3, respectively, expecting to reach a 'steady state' of a total of 10 infants (two per month per centre) by month 4 of the pilot phase
6. evaluate the delivery, management and acceptability (to families and staff) and ease of preparation and administration
7. assess the processes for collecting clinical outcomes and event rates and to determine that the predicted retention rate (> 95% of recruited infants) was attainable.

The decision to progress with the main trial was made in consultation with the Trial Steering Committee (TSC) and the funder.

The internal pilot phase was followed by a 3-year main recruitment phase in 37 recruiting centres (see *Appendix 1*).

Outcomes

Primary outcome

The number of infants who experience at least one episode of microbiologically confirmed (*Box 3*) or clinically suspected (*Box 4*) late-onset infection from trial entry until hospital discharge.

Secondary outcomes

- Microbiologically confirmed infection (see *Box 3*).
- All-cause mortality prior to hospital discharge.
- Necrotising enterocolitis: Bell's stage II or III (see *Appendix 4*).[52]
- Severe ROP treated medically or surgically.[53]
- Bronchopulmonary dysplasia: when the infant is still receiving mechanical ventilator support or supplemental oxygen at 36 weeks' postmenstrual age.[54]
- A composite of invasive infection, major morbidity (NEC, ROP or BPD) and mortality.
- Total number of days of administration of antimicrobials per infant from trial entry until 34 weeks' postmenstrual age.
- Total length of stay until discharge home.
- Length of stay in (1) intensive care, (2) high-dependency and (3) special-care settings (see *Appendix 5*).

BOX 3 Definition of microbiologically confirmed late-onset infection

Microbiological culture from blood or CSF sampled aseptically > 72 hours after birth of any of the following:

- potentially pathogenic bacteria (including coagulase-negative *Staphylococcus* species but excluding probable skin contaminants, such as diphtheroids, micrococci, propionibacteria or a mixed flora)
- fungi.

AND treatment, or clinician intention to treat, for ≥ 5 days with intravenous antibiotics (excluding antimicrobial prophylaxis) after investigation was undertaken. If the infant died or was discharged or transferred prior to the completion of 5 days of antibiotics this condition would still be met if the intention was to treat for ≥ 5 days.

Adapted from the UK Neonatal Infection Surveillance Network case definition.[2,3]

BOX 4 Definition of clinically suspected late-onset infection

Absence of positive microbiological culture, or culture of a mixed microbial flora or of likely skin contaminants (diphtheroids, micrococci, propionibacteria) only

AND treatment, or clinician intention to treat, for ≥ 5 days with intravenous antibiotics (excluding antimicrobial prophylaxis) after the above investigation was undertaken for an infant who demonstrates three or more of the following clinical or laboratory features of invasive infection:

- increase in oxygen requirement or ventilatory support
- increase in frequency of episodes of bradycardia or apnoea
- temperature instability
- ileus or enteral feeds intolerance or abdominal distension
- reduced urine output to < 1 ml/kg/hour
- impaired peripheral perfusion (capillary refill time of > 3 seconds, skin mottling or core–peripheral temperature gap > 2 °C)
- hypotension (clinician defined as needing volume or inotrope support)
- 'irritability or lethargy or hypotonia' (clinician defined)
- increase in serum C-reactive protein levels to > 15 mg/l or procalcitonin level of ≥ 2 ng/ml
- white blood cells count of < 4×10^9/l or > 20×10^9/l
- cells/l or platelet count of < 100×10^9/l
- glucose intolerance (blood glucose levels of < 40 mg/dl or > 180 mg/dl)
- metabolic acidosis (base excess of < –10 mmol/l or lactate level of > 2 mmol/l).

Adapted from the European Medicines Agency consensus criteria and predictive model.[50,51]

Sample size

The sample size estimate was informed by a range of plausible primary outcome control event rates (CERs) from 18% to 24%, based on surveillance reports from Europe, North America and Australasia (*Table 1*).[2,7,8,10,12] In summary, with 90% power and a two-sided 5% significance level, to detect an absolute risk reduction (ARR) of 5–5.8% (relative risk reduction of between 24% and 28%) would require a total of up to 2200 participants if the CER was 18%, 2070 if the CER was 21% and 2076 if the CER was 24%. This target sample size of 2200 allowed 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 [e.g. a 7% ARR in antibiotic exposure (from 45% to 38%)].

The participating recruiting neonatal units were estimated to admit 60 very preterm infants per annum on average. Based on 40% recruitment, 30 units were estimated to be able to recruit a total sample size of up to 2160 infants over 3 years (an average of two infants per unit per month).

TABLE 1 Participants required per arm by CER

Control event rate (%)	Treatment group event rate (%)	Absolute risk reduction (%)	Relative risk reduction (%)	Number required per arm	Total sample size required
24	18.2	5.8	24	1038	2076
21	15.5	5.5	26	1035	2070
18	13.0	5.0	28	1099	2200

Statistical analyses

Demographic factors and clinical characteristics at randomisation were summarised with counts (percentages) for categorical variables, mean [standard deviation (SD)] 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 centre, sex, weeks' gestation at birth and single vs. multiple births) to account for the correlation between treatment groups introduced by balancing the randomisation. We used random-effects models with centre fitted as a random effect and mother's ID number nested within this to take account of clustering within centre and within multiples. The other minimisation factors were fitted as fixed effects, with sex and multiplicity of birth included as binary variables and gestational age modelled as a continuous variable. 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 of gestation at birth and (2) infants given maternal or donated expressed breast milk versus formula versus both human milk and formula during the trial period (received on > 50% of days on which infant is fed enterally until developing late-onset infection or NEC, dying or reaching 34 weeks' postmenstrual age).

Data collection

All of the outcome data for this trial were routinely recorded clinical items that could be obtained from the clinical notes or local microbiology laboratory records. Information was collected using the data collection forms (see *Appendix 6*).

A 'blinded end-point review committee', masked to participant allocation, reviewed all case report forms (CRFs) reporting episodes of late-onset infection or necrotising enterocolitis or other gastrointestinal pathology. Two members independently assessed adherence to case definitions and resolved any disagreements or discrepancies by discussion or referral to a third committee member or both. Persisting uncertainties were discussed with the site PI or research nurse or both until resolved.

Adverse event reporting

Some adverse events were foreseeable (expected) because of the nature of the participant population, and their routine care and treatment. No adverse drug reactions were expected from bovine lactoferrin. Consequently, only those adverse events (or reactions) identified as serious were recorded for this trial (see *Appendix 7*).

Expected serious adverse events (SAEs) were recorded on the CRFs. All other SAEs were reported by trial sites to the NPEU CTU within 24 hours of the event being recognised. Information was recorded on a SAE reporting form and faxed to the NPEU CTU. Additional information (follow-up of or corrections to the original case) needed to be detailed on a new SAE form and faxed to the NPEU CTU. A standard operating procedure (SOP) outlining the reporting procedure for clinicians was provided with the SAE form and in

the trial handbook. The NPEU CTU processed and reported the event as specified in its own SOPs. All SAEs were reviewed by the Data Monitoring Committee (DMC) at regular intervals throughout the trial. The CI informed all investigators of information that could affect the safety of participants.

Suspected unexpected serious adverse reactions

Suspected unexpected serious adverse reactions (SUSARs) were reported to the MHRA and the approving Research Ethics Committee (REC) within 7 days, if life-threatening, and within 15 days for other SUSARs. In addition, a copy of the SUSAR form was forwarded to the chairperson of the DMC. The chairperson was provided with details of all previous SUSARs with their unmasked allocation. The chief investigator informed all investigators of any issues raised in a SUSAR that could affect the safety of participants.

Development safety update report

In addition to the expedited reporting above, the chief investigator submitted, once a year throughout the clinical trial, or on request, a development safety update report to the Competent Authority, the Ethics Committee and the sponsor.

Economic analysis (planned)

We planned to combine the health service resources used during an infant's hospital admission with clinical effectiveness data to conduct an economic evaluation to assess whether or not the intervention was likely to be cost-effective over the time horizon of the trial period. If appropriate, we intended to synthesise the costs and consequences of the intervention to generate an incremental cost-effectiveness ratio to inform any adoption decision. We planned to use regression models to allow for differences in prognostic variables, principally gestational age bands, and other sources of heterogeneity and to assess differences in the probable cost-effectiveness between the groups.

The primary outcome for an economic analysis was the incidence of late-onset invasive infection. As invasive infection is linked closely to morbidity and mortality, it is likely that the consequences will continue to appear over a longer time frame and may have an impact on both duration and quality of life. Therefore, as a second analysis, we intended to develop an economic model to account for projected longer-term costs and effects and to estimate the additional cost per quality-adjusted life-year gained of lactoferrin compared with placebo.

Governance and monitoring

At least one site initiation visit was conducted at all recruiting sites. The trial research nurse and the chief investigator or a delegated co-investigator provided structured training for site investigators, local research nurses and other clinical staff, including the site pharmacy team responsible for IMP management. Training focused on approaches to consent, protocol processes and governance requirements. These visits were supported with bespoke written and online training material available to all staff via the trial website (www.npeu.ox.ac.uk/elfin/training). Staff in continuing care sites did not have initiation visits unless requested but were directed to online training and access support from the chief investigator and trial research nurse as needed.

Ongoing monitoring included review of investigator site files, delegation logs, staff qualifications and training (good clinical practice certificates, curricula vitae) and pharmacy documentation. Quality assurance was achieved by following data management procedures at the study data centre and data monitoring at trial sites. Further site monitoring or audit was conducted if central monitoring exercises raised concern about patterns of recruitment or data reporting. This monitoring approach was justified by the level of risk associated with the trial and the intervention.

Data management was undertaken in accordance with NPEU CTU SOPs and a prespecified management plan. Data monitoring included the review of consent forms and participant eligibility. Additional data validation checks were carried out periodically, with data queries issued to study sites for resolution. Prior to database lock, final data validation checks were carried out and questions were resolved by discussion with the site PI or local research nurse, when possible.

During the trial, the study statisticians produced reports for the TSC and independent DMC. Issues of data quality identified by study statisticians were reported to study data management staff and queried when appropriate or included in future routing data validation checks, or both. TSC and DMC meetings provided opportunities for the external, independent review of summary data, with additional feedback on potential data quality issues being incorporated into ongoing data quality checks.

Summary of changes to the study protocol

A summary of the changes made to the original protocol is presented in *Appendix 8*.

Chapter 3 Results

Recruitment and retention

Recruitment and retention to the trial are summarised in the flow chart (*Figure 1*).

The internal pilot was undertaken in six neonatal units between May 2014 and April 2015. In total, 90 infants were recruited to participate. The main trial recruited infants from 37 neonatal units (including the six pilot sites) from July 2015 to September 2017 (when the recruitment target was reached). The trial randomised 2203 infants in total:

- 1099 infants were allocated to receive bovine lactoferrin
- 1104 infants were allocated to receive the sucrose placebo.

Four infants had consent withdrawn or unconfirmed. In total, 1098 infants in the lactoferrin group and 1101 in the placebo group were included in the intention-to-treat analyses (see *Appendix 9*).

Demographic and other baseline characteristics

The baseline characteristics and other demographic features of participating infants were well balanced between the two treatment allocation groups (*Table 2*). The median gestation age 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,

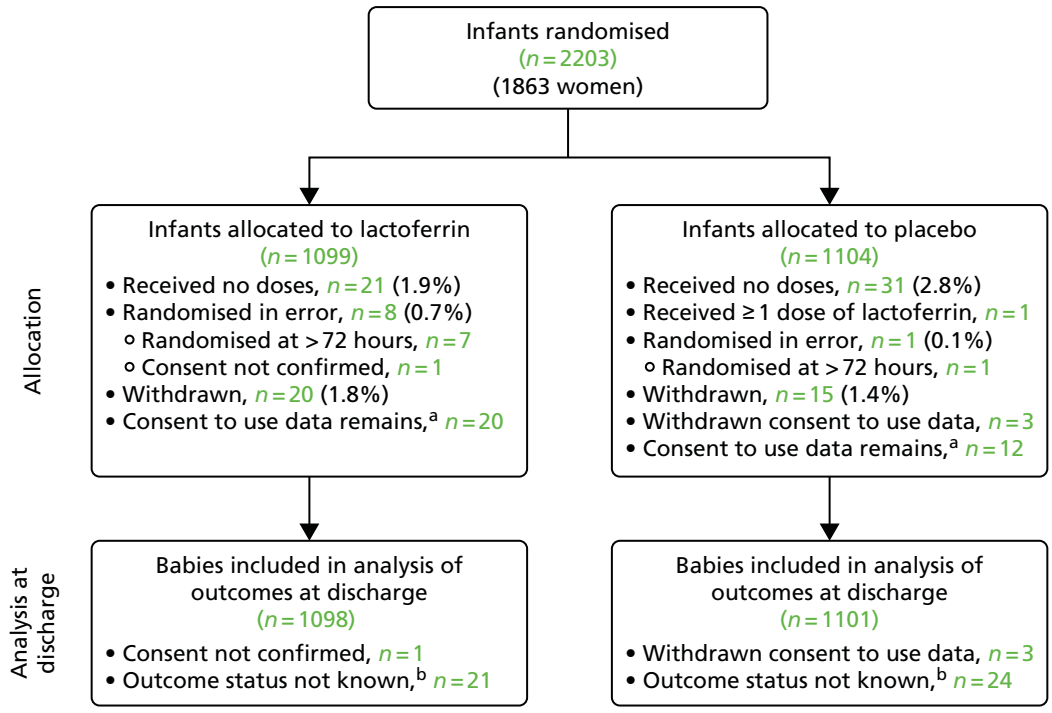

FIGURE 1 Flow of participants through the trial. a, Included in the analysis when data are available; b, included in analysis except when knowledge of discharge or discharge date is required.

TABLE 2 Infant and maternal characteristics at randomisation

Characteristic	Trial group	
	Lactoferrin ($n = 1098$)	Placebo ($n = 1101$)
Number of centres, *n*	37	37
Male sex, *n/N* (%)	575/1098 (52.4)	578/1099 (52.6)
Missing, *n*	0	2
Infant age at randomisation in days		
Median (IQR)	2 (2–3)	2 (2–3)
Birthweight (g)		
Mean (SD)	1125.9 (356.2)	1143.3 (367.1)
< 500, *n* (%)	8/1098 (0.7)	7/1101 (0.6)
500 to 749, *n* (%)	172/1098 (15.7)	172/1101 (15.6)
750 to 999, *n* (%)	254/1098 (23.1)	244/1101 (22.2)
1000 to 1249, *n* (%)	268/1098 (24.4)	255/1101 (23.2)
1250 to 1499, *n* (%)	199/1098 (18.1)	199/1101 (18.1)
≥ 1500, *n* (%)	197/1098 (17.9)	224/1101 (20.3)
Birthweight < 10th centile for gestational age, *n/N* (%)	175/1097 (16.0)	177/1098 (16.1)
Missing, *n*	1	3
Gestation at delivery (completed weeks)		
Median (IQR)	29 (27–30)	29 (27–30)
< 23, *n/N* (%)	1/1098 (0.1)	1/1101 (0.1)
23^{+0} to 23^{+6}, *n/N* (%)	33/1098 (3.0)	31/1101 (2.8)
24^{+0} to 24^{+6}, *n/N* (%)	73/1098 (6.6)	76/1101 (6.9)
25^{+0} to 25^{+6}, *n/N* (%)	73/1098 (6.6)	73/1101 (6.6)
26^{+0} to 27^{+6}, *n/N* (%)	227/1098 (20.7)	221/1101 (20.1)
28^{+0} to 29^{+6}, *n/N* (%)	315/1098 (28.7)	319/1101 (29.0)
30^{+0} to 31^{+6}, *n/N* (%)	376/1098 (34.2)	380/1101 (34.5)
Mother's age at randomisation (years)		
Mean (SD)	30.3 (6.1)	30.4 (6.0)
Multiple birth, *n/N* (%)	350/1098 (31.9)	346/1101 (31.4)
Caesarean section delivery, *n/N* (%)	635/1098 (57.8)	616/1101 (55.9)
Membranes ruptured before labour, *n/N* (%)	422/1093 (38.6)	428/1097 (39.0)
Missing, *n*	5	4
Membranes ruptured > 24 hours before delivery, *n/N* (%)	286/1092 (26.2)	264/1096 (24.1)
Missing, *n*	6	5
Mother received antenatal corticosteroids, *n/N* (%)	998/1093 (91.3)	997/1099 (90.7)
Missing, *n*	5	2
Infant heart rate of > 100 b.p.m. at 5 minutes, *n/N* (%)	995/1090 (91.3)	1010/1093 (92.4)
Missing, *n*	8	8

TABLE 2 Infant and maternal characteristics at randomisation (*continued*)

Characteristic	Trial group	
	Lactoferrin (*n* = 1098)	Placebo (*n* = 1101)
Infant temperature on admission (°C)		
Mean (SD)	36.9 (0.7)	37 (0.7)
Missing, *n*	4	10
Infant worst base excess within first 24 hours of birth		
Mean (SD)	–6.2 (3.9)	–6.3 (3.8)
Missing, *n*	9	12
Infant ventilated via endotracheal tube at randomisation, *n*/*N* (%)	338/1098 (30.8)	357/1101 (32.4)
Infant had absent or reverse end diastolic flow, *n*/*N* (%)	134/1079 (12.4)	130/1081 (12.0)
Missing, *n*	19	20

b.p.m., beats per minute.
Bold indicates minimisation factors.
Unless otherwise stated, the table gives the percentages of babies with data in that arm of the trial who had (or whose mother had) the stated characteristic.

and 12% had evidence of absent or reverse end diastolic flow in the fetal umbilical artery. The allocation arms were well-balanced in individual recruiting sites as per the minimisation algorithm (see *Appendix 10*).

Adherence

A total of 35 (1.6%) infants discontinued the intervention early: 20 in the lactoferrin group and 15 in the sucrose group. This includes a small number of infants who in the early stages of the trial discontinued the intervention because they were transferred to a hospital that did not have the regulatory approvals to administer the intervention. Parental consent remained for data collection for intention-to-treat analyses for 32 out of the 35 infants.

Adherence was high for infants who continued to receive the IMP (*Table 3*). The median percentage of days when an IMP dose was not given or not recorded was 4% in both treatment groups, and 0% of days in both groups for the dose not given or not recorded when those days where feeds were stopped or withheld for > 4 hours (for clinical reasons) were excluded. The median difference between expected dose and actual dose per day was 7 mg/kg/day lower in both groups and was 1 mg/kg/day (lactoferrin) or 2 mg/kg/day (sucrose) lower excluding those days where enteral feeds were stopped or withheld for > 4 hours.

Outcomes

The estimates of effect for the primary and secondary outcomes are presented in *Table 4*.

Primary outcome

Data were available for 2182 infants (99%). In the lactoferrin group, 316 out of 1093 (28.9%) infants acquired a late-onset infection versus 334 out of 1089 (30.7%) infants in the control (placebo) group (adjusted RR 0.95, 95% CI 0.81 to 1.10).

TABLE 3 Adherences measures

Measure	Trial group	
	Lactoferrin ($n = 1007$)[a]	Placebo ($n = 1011$)[a]
Percentage of days dose not given or not recorded[b]		
Median (IQR)	4 (0 to 18.18)	4 (0 to 16.22)
Range	0 to 100	0 to 100
Missing, *n*	10	13
Percentage of days dose not given or not recorded, excluding days where feeds were stopped or withheld for > 4 hours[b]		
Median (IQR)	0 (0 to 5.71)	0 (0 to 5.56)
Range	0 to 100	0 to 100
Missing, *n*	11	13
Difference between expected dose and actual dose per day (mg/kg/day)[c]		
Median (IQR)	–7 (–29 to 0)	–7 (–27 to 0)
Range	–150 to 253	–150 to 88
Missing, *n*	10	13
Difference between expected dose and actual dose per day, excluding days where feeds were stopped or withheld for > 4 hours (mg/kg/day)[c]		
Median (IQR)	–1 (–11 to 0)	–2 (–11 to 0)
Range	–150 to 271	–150 to 88
Missing, *n*	11	13

a Includes only infants who have completed at least one feed log, and doses are exclusively recorded on version 2 of the daily dosing log.
b The ratio of the number of days the dose was not given or recorded over the number of days in the expected treatment window.
c The average difference between the expected dose and actual dose over every day of treatment window. When necessary, the working weight is imputed using last observation carried forward.

TABLE 4 Primary and secondary outcomes

Outcome	Trial group		Unadjusted RR (CI)[a,b]	Adjusted RR (CI)[a,b,c]	*p*-value[d]
	Lactoferrin ($n = 1098$)	Placebo ($n = 1101$)			
Microbiologically confirmed or clinically suspected late-onset infection, *n/N* (%)	316/1093 (28.9)	334/1089 (30.7)	0.94 (0.83 to 1.07)	0.95 (0.86 to 1.04)	0.233
Missing, *n*	5	12			
Microbiologically confirmed late-onset infection, *n/N* (%)	190/1093 (17.4)	180/1089 (16.5)	1.05 (0.82 to 1.34)	1.05 (0.87 to 1.26)	0.490
Missing, *n*	5	12			
All-cause mortality, *n/N* (%)	71/1076 (6.6)	68/1076 (6.3)	1.04 (0.69 to 1.59)	1.05 (0.66 to 1.68)	0.782
Missing, *n*	22	25			
NEC (Bell's stage II or III), *n/N* (%)	63/1085 (5.8)	56/1084 (5.2)	1.12 (0.71 to 1.77)	1.13 (0.68 to 1.89)	0.538
Missing, *n*	13	17			

TABLE 4 Primary and secondary outcomes (*continued*)

Outcome	Trial group		Unadjusted RR (CI)[a,b]	Adjusted RR (CI)[a,b,c]	*p*-value[d]
	Lactoferrin (*n* = 1098)	Placebo (*n* = 1101)			
Severe ROP treated medically or surgically, *n/N* (%)	64/1080 (5.9)	72/1080 (6.7)	0.89 (0.58 to 1.35)	0.89 (0.62 to 1.28)	0.420
Missing, *n*	18	21			
BPD at 36 weeks' postmenstrual age, *n/N* (%)	358/1023 (35.0)	355/1027 (34.6)	1.01 (0.87 to 1.18)	1.01 (0.90 to 1.13)	0.867
Died before 36 weeks' postmenstrual age	64	60			
Missing, *n*	11	14			
Infection, NEC, ROP, BPD or mortality, *n/N* (%)	525/1092 (48.1)	521/1094 (47.6)	1.01 (0.90 to 1.13)	1.01 (0.94 to 1.08)	0.743
Missing, *n*	6	7			
Total number of days of administration of antimicrobials from commencement of IMP until 34 weeks' postmenstrual age					
Median (IQR)	2 (0–8)	3 (0–8)	0 (0 to 0)	0 (–1 to 1)	0.625
Missing, *n*	39	44			
Length of hospital stay (days) to discharge					
Median (IQR)	59 (40–85)	58 (40–84)	1 (–2 to 4)	1 (–1 to 3)	0.446
Missing, *n*	95	97			
Days in level 1 (intensive) care					
Median (IQR)	8 (4–16)	8 (4–16)	0 (–1 to 1)	0 (–1 to 1)	0.963
Missing, *n*	87	66			
Days in level 2 (high-dependency) care					
Median (IQR)	10 (3–30)	9 (3–29)	0 (–1 to 1)	1 (–1 to 3)	0.420
Missing, *n*	83	60			
Days in level 3 (special) care					
Median (IQR)	29 (21–39)	30 (22–39)	–1 (–2 to 1)	–1 (–3 to 1)	0.216
Missing, *n*	75	55			

a Risk ratios for binary outcomes and median differences for continuous outcomes.
b 95% CI for microbiologically confirmed or clinically suspected late-onset invasive infection, 99% CI for all other outcomes.
c Adjusted for minimisation factors: collaborating hospital, sex, gestational age at birth vs. single or multiple births, when technically possible.
d *p*-value for testing whether or not adjusted RR is equal to 1 or adjusted median difference is equal to 0.

Secondary outcomes

There were no significant differences in secondary outcomes: microbiologically confirmed infection (RR 1.05, 99% CI 0.80 to 1.37), mortality (RR 1.05, 99% CI 0.68 to 1.63), NEC (RR 1.13, 99% CI 0.70 to 1.82), ROP (RR 0.89, 99% CI 0.57 to 1.40), BPD (RR 1.01, 99% CI 0.83 to 1.22), or a composite of infection, major morbidity and mortality (RR 1.01, 99% CI 0.86 to 1.18). There were no differences in the number of days of administration of antimicrobials until 34 weeks' postmenstrual age, or in length of stay in hospital, or length of stay in intensive care, high-dependency care or special-care settings.

Subgroup analyses

Subgroup analyses did not show any significant interactions for completed weeks' gestation at birth or type of enteral milk received (*Table 5* and *Figure 2*).

Economic analysis

Given the absence of any effects on infant- or family-important outcomes (clinical effectiveness), and with the approval of the DMC and TSC, we did not undertake the proposed within-trial economic analyses or modelling (protocol amendment submitted).

Safety and adverse events

Table 6 summarises reported adverse events (definitions of adverse reactions and events are presented in *Appendix 7*).

There were 16 SAEs reported for infants in the lactoferrin group (six severe) and 10 for infants in the sucrose group (three severe). No infant had more than one reported event. Two SAEs, both in the lactoferrin group, were assessed as being 'possibly related' to the trial intervention: one case of blood in stool (expected) and one death following intestinal perforation likely associated with NEC (SUSAR). The remaining 24 SAEs were considered to be unrelated to the trial intervention.

TABLE 5 Subgroup analyses for confirmed or suspected late-onset infection

	Trial group				
Late-onset infection	Lactoferrin ($n = 1098$)	Placebo ($n = 1101$)	Unadjusted RR (95% CI)	Adjusted RR[a] (95% CI)	*p*-value[b]
Gestational age at delivery (completed weeks), *n/N* (%)					0.273
< 24	25/34 (73.5)	27/31 (87.1)	0.84 (0.66 to 1.07)	0.91 (0.69 to 1.20)	
24	46/73 (63.0)	56/75 (74.7)	0.84 (0.68 to 1.05)	0.84 (0.69 to 1.03)	
25	45/73 (61.6)	44/73 (60.3)	1.02 (0.78 to 1.34)	1.03 (0.73 to 1.45)	
26 to 27	107/227 (47.1)	99/220 (45.0)	1.05 (0.86 to 1.28)	1.04 (0.85 to 1.28)	
28 to 29	69/311 (22.2)	72/316 (22.8)	0.97 (0.73 to 1.30)	0.98 (0.74 to 1.29)	
≥ 30	24/375 (6.4)	36/374 (9.6)	0.66 (0.40 to 1.10)	0.66 (0.42 to 1.03)	
Type of milk, *n/N* (%)					0.400
Breast only	99/315 (31.4)	83/291 (28.5)	1.10 (0.87 to 1.40)	1.03 (0.88 to 1.21)	
Mixed	199/707 (28.1)	228/710 (32.1)	0.88 (0.75 to 1.03)	0.89 (0.79 to 1.01)	
Formula only	10/53 (18.9)	12/60 (20.0)	0.94 (0.45 to 2.00)	1.06 (0.58 to 1.91)	
Missing, *n*	18	29			

a Adjusted for minimisation factors: recruiting site, sex, gestational age at birth (completed weeks) and single vs. multiple birth.
b *p*-value for test for interaction from adjusted model.

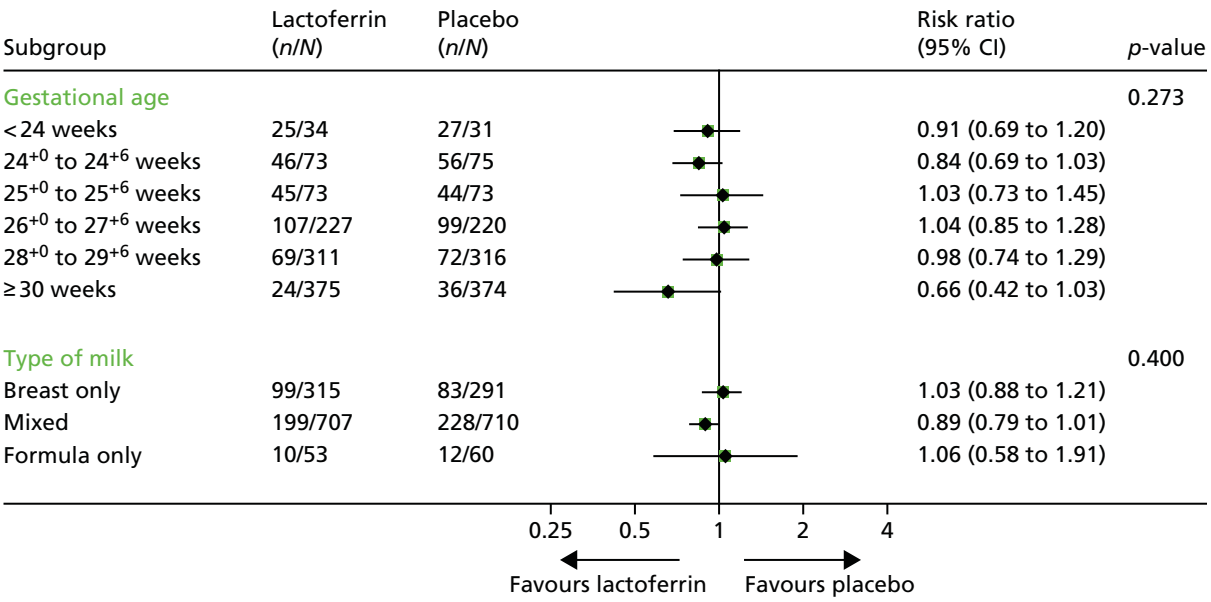

FIGURE 2 Subgroup analyses for confirmed or suspected late-onset invasive infection.

TABLE 6 List of SAEs reported by randomisation group

Trial group	SAE	Age (days)	Brief description of event	Severity	Related to trial
Lactoferrin ($n = 1098$)	1	12	Meconium ileus following one dose of IMP. Resolved with laparotomy, no bowel removed	Moderate	No
	2	30	Two episodes of clinical seizures, resolved with brief course of anticonvulsant	Moderate	No
	3	59	Cluster of seizures, probably related to severe Gram-negative bacteraemia and sepsis (ultimately fatal)	Severe	No
	4	12	Episode of supraventricular tachycardia, resolved with adenosine and propranolol	Mild	No
	5	49	Metabolic acidosis (likely renal tubular acidosis), resolved with sodium bicarbonate	Severe	No
	6	20	Episode of supraventricular tachycardia, resolved with face cooling	Mild	No
	7	19	Suspected NEC	Moderate	No
	8	18	Cluster of clinical seizures, resolved with magnesium sulphate and course of phenobarbital	Mild	No
	9	81	Infective exacerbation of chronic lung disease, resolved with antibiotics and corticosteroids	Severe	No
	10	17	Large inferior vena caval thrombus	Moderate	No
	11	68	Acute airway obstruction, resolved with respiratory support	Severe	No
	12	44	Aspiration pneumonitis resolved with respiratory support	Severe	No
	13	21	Blood in stool, unknown cause, resolved spontaneously	Moderate	Possibly (expected)
	14	19	Haemolytic anaemia, unknown cause, resolved spontaneously	Mild	No

continued

TABLE 6 List of SAEs reported by randomisation group (*continued*)

Trial group	SAE	Age (days)	Brief description of event	Severity	Related to trial
	15	10	Death following intestinal perforation secondary to NEC	Severe	Possibly (SUSAR)
	16	27	Death attributed to Gram-negative bacteraemia	Severe	No
Placebo ($n = 1101$)	1	61	Rib fracture secondary to osteopenia of prematurity, resolved with supportive care and nutrient supplementation	Moderate	No
	2	50	Superior sagittal sinus non-occlusive thrombus, resolved with heparin (6 weeks of treatment)	Moderate	No
	3	48	Hyperammonaemia, unknown cause, resolved with course of sodium benzoate	Moderate	No
	4	36	Death attributed to infection and sepsis	Severe	No
	5	24	Episode of tachycardia and ectopic beats, resolved with face cooling and reduction in caffeine dose	Mild	No
	6	37	Death secondary to exacerbation of chronic lung disease (severe BPD)	Severe	No
	7	26	Death attributed to severe BPD	Severe	No
	8	57	*S. aureus* bacteraemia and osteomyelitis, resolved with antibiotics	Moderate	No
	9	22	Episode of supraventricular tachycardia, resolved with adenosine	Moderate	No
	10	6	Episode of supraventricular tachycardia, resolved with carotid massage and adenosine	Mild	No

Post hoc analyses

1. Post hoc exploratory analyses did not show any differential effects depending on the infecting micro-organism identified for the outcome 'microbiologically confirmed late-onset infection' (*Table 7* and *Box 5*).
2. Post hoc exploratory analyses did not show any between-group differences in the risk of having more than one episodes of infection (*Table 8*).
3. Post hoc exploratory analyses did not show any differential effects for the primary outcome depending on whether infants had or had not received probiotics as part of their routine care (*Table 9*).

TABLE 7 Microbiologically confirmed late-onset infection by classification of micro-organism

Classification of micro-organism	Trial group	
	Lactoferrin ($n = 1098$)	Placebo ($n = 1101$)
Microbiologically confirmed late-onset invasive infection from trial entry until hospital discharge, *n/N* (%)	190/1093 (17.4)	180/1089 (16.5)
At least one Gram-positive organism confirmed, *n/N* (%)	153/1093 (14.0)	147/1089 (13.5)
At least one CoNS group organism, *n/N* (%)	122/1093 (11.2)	111/1089 (10.2)
At least one Gram-negative organism confirmed, *n/N* (%)	46/1093 (4.2)	39/1089 (3.6)
At least one fungal organism confirmed, *n/N* (%)	3/1093 (0.3)	2/1089 (0.2)
At least one other organism confirmed, *n/N* (%)	3/1093 (0.3)	2/1089 (0.2)
Missing, *n*	5	12

CoNS, coagulase-negative *Staphylococci*.

BOX 5 Classification of micro-organisms

1. *Staphylococcus epidermidis*.
2. *Staphylococcus capitis*.
3. Other coagulase-negative *Staphylococci*.
4. *S. aureus*.
5. *Enterococcus faecalis*.
6. Group B streptococci.
7. *Enterococcus* sp. (other).
8. *Streptococcus* sp. (other).
9. *Micrococcus* sp.
10. *Bacillus* sp.
11. Diphtheroids.
12. *Streptococcus pneumoniae*.
13. *Propionibacterium acnes*.
14. *Listeria monocytogenes*.
15. Other Gram-positive bacteria.
16. *Escherichia coli*.
17. *Klebsiella* sp.
18. *Enterobacter* sp.
19. *Pseudomonas* sp.
20. *Serratia* sp.
21. Coliforms (other).
22. *Acinetobacter* sp.
23. *Citrobacter* sp.
24. *Burkholderia* sp.
25. *Haemophilus* sp.
26. Other Gram-negative bacteria.
27. *Candida albicans*.
28. *Candida* sp. (other).
29. Other fungi.
30. Other organisms.

1–15, Gram positive; 1–3, coagulase-negative *Staphylococcus* group; 16–26, Gram negative; 27–29, fungi; 30, other.

TABLE 8 Number of episodes of confirmed or suspected sepsis

Number of episodes	Trial group	
	Lactoferrin ($n = 1098$)	Placebo ($n = 1101$)
Microbiologically confirmed or clinically suspected sepsis, *n*/*N* (%)		
None	777/1093 (71.1)	755/1089 (69.3)
1	258/1093 (23.6)	279/1089 (25.6)
2	46/1093 (4.2)	39/1089 (3.6)
3	9/1093 (0.8)	13/1089 (1.2)
4	3/1093 (0.3)	2/1089 (0.2)
5	0/1093 (0.0)	1/1089 (0.1)
Missing, *n*	5	12
Microbiologically confirmed infection, *n*/*N* (%)		
None	903/1093 (82.6)	909/1089 (83.5)
1	162/1093 (14.8)	155/1089 (14.2)
2	24/1093 (2.2)	23/1089 (2.1)
3	3/1093 (0.3)	2/1089 (0.2)
4	1/1093 (0.1)	0/1089 (0.0)

TABLE 9 Late-onset infection from trial entry until hospital discharge by exposure to probiotics

	Trial group	
	Lactoferrin ($n = 1098$)	Placebo ($n = 1101$)
Any record of probiotics being given, *n*/*N* (%)		
Yes	99/354 (28.0)	97/329 (29.5)
No	208/728 (28.6)	227/749 (30.3)
Missing,[a,b] *n*	16	23

a In the lactoferrin trial group, 15 babies had unknown probiotic use; nine had an episode of late-onset infection, two did not and four were unknown. One other baby with a record of probiotic use had unknown infection status.
b In the placebo trial group, 19 babies had unknown probiotic use; 10 had an episode of late-onset infection, one did not and eight were unknown. Four other babies with a record of probiotic use had unknown infection status.

Chapter 4 Discussion and conclusions

Summary of main findings

The ELFIN trial shows that enteral lactoferrin supplementation (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 finding contradicts the existing evidence base and illustrates why high-quality evidence from adequately powered RCTs is needed to inform policy and practice.[55] The current Cochrane review includes six RCTs, and meta-analyses of their data suggest substantial reductions in the risk of late-onset infection and NEC associated with lactoferrin supplementation in very preterm infants.[40] However, the trials included in the Cochrane review were small and some contained other design and methodological weaknesses that may have introduced biases resulting in overestimation of the effect sizes.[41–46] Given these concerns, the Cochrane review authors graded the evidence for key outcomes as being of 'low quality' and concluded that data from methodologically rigorous RCTs were needed to generate evidence of sufficient validity to inform policy and practice.[40]

The ELFIN trial provides these data. The validity of the findings is enhanced by the quality and power of the trial. We used best practices to limit bias, including central web-based randomisation for allocation concealment, blinding of parents, caregivers and investigators to the group allocation, and complete follow-up and assessment of the trial cohort with intention-to-treat analyses based on a prespecified statistical analysis plan. The trial achieved recruitment of 2203 participants as per protocol, based on the 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 major known or putative prognostic indicators (completed weeks of gestation, sex, single vs. multiple births) or potential confounding influences (recruiting site). Interim analyses by the trial's independent DMC used strict criteria to minimise the chances of spurious findings attributable to data fluctuations before a sufficient sample size was achieved.[56,57] 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 broadly similar to those that we anticipated and as have been described in other cohort studies and RCTs involving very preterm infants.[2,3] Consequently, the trial had sufficient power and internal validity to detect reliably modest yet important effects on the risk of late-onset infection and other morbidities.

Given the size of the ELFIN trial, with more than twice as many infants than had participated in all of the existing trials combined, we were able to generate more precise estimates of effect size 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 completed weeks of gestation at birth, making it unlikely that bovine lactoferrin has any important benefits for extremely preterm infants who have a higher risk of infection. Similarly, although it is plausible that lactoferrin may have had different effects in infants with lower levels of exposure to the immunoprotective factors present in human milk, we did not show any interaction with the type of enteral milk feeds received during the trial period (human milk, formula or both).[58]

The largest previous trial, in which 331 very low birthweight infants in neonatal units in Italy participated, showed a relative risk reduction of 66% for late-onset infection.[41] Although this estimate of effect may have been inflated by methodological weaknesses, such as the absence of predefined criteria for interim analyses, the Italian trial differed from the ELFIN trial in other ways that could have contributed to the divergence of findings. The participants and the intervention were broadly similar, as were enteral feeding practices, including receipt of human breast milk versus formula milk. However, key differences in the epidemiology of late-onset infection, as well as in infection-prevention practices and exposure to other interventions,

may have contributed to the difference in effects size estimates shown in the two trials. Notably, the incidence of invasive fungal infection was very high in the Italian trial (7.7% of the control group) and a substantial proportion of the overall effect on reducing late-onset invasive infection was due to the effect on preventing invasive fungal infection.[59] In contrast, the overall incidence of late-onset fungal infection was low in the ELFIN trial cohort (five episodes in total), consistent with that reported in UK surveillance studies.[9,60]

Given that a postulated mechanism of action of lactoferrin is to reduce bowel translocation of enteric pathogens, we assessed whether or not invasive infections with particular groups of enteric organisms were reduced.[58,61] In post hoc analyses, we did not show any evidence that lactoferrin supplementation affected the risk of late-onset infection with different groups of infecting micro-organism including Gram-negative bacteria (mainly *Escherichia coli* and other *Enterobacteriaceae*). This finding is consistent with the previously largest trial, which did not show an effect of lactoferrin supplementation on the incidence of infection with Gram-negative bacteria.[41]

The ELFIN trial did not show any difference in the effect of lactoferrin on the risk of late-onset infection in a post hoc subgroup analysis of infants who had or had not received probiotic supplementation during the trial period. A previous trial and the current Cochrane review had suggested that combining supplementation of lactoferrin with the probiotic micro-organism *Lactobacillus rhamnosus* GG was associated with a greater reduction in the risk of late-onset infection (> 70%) and NEC (> 90%) than lactoferrin alone.[40,41] This raised the possibility that the immunoprotective and prebiotic properties of lactoferrin might act synergistically with probiotic supplementation.[61] Although the ELFIN trial did not show any evidence of differential effects depending on whether or not infants had received probiotics during the trial period, the data are not sufficient to exclude the possibility that such prebiotic–probiotic synergism exists. A recent large cluster RCT in India has suggested that the prophylactic administration of an oral synbiotic (prebiotic fructo-oligosaccharide combined with probiotic *Lactobacillus plantarum*) reduces infection and mortality in late preterm or term newborn infants.[62] We are conducting a mechanistic study in a subgroup of ELFIN trial participants to analyse whether or not, and how, lactoferrin supplementation affects the intestinal microbiome and metabolite profile. The study will explore changes in microbiomic and metabolomic patterns preceding disease onset including NEC and late-onset infection.[61]

Limitations

The prespecified primary outcome included 'clinically suspected' and 'microbiologically confirmed' late-onset infection. We took this pragmatic approach because of concerns about the diagnostic accuracy of microbiological culture of blood in this population.[63] Standard microbiological culture may not detect cases of bacteraemia or fungaemia if an insufficient volume of the infant's blood is incubated ('false negative'). Conversely, microbiological cultures may also generate 'false-positive' results if blood sampling techniques allow entry of contaminating micro-organisms (typically from the infant's skin). To mitigate these potential sources of bias, we used an established consensus case definition that (1) required additional evidence of infection (clinical signs or biomarkers) and (2) mandated that clinicians indicate an intention to treat the infant with antibiotics or antifungals for at least 5 days.[2,3]

Typically, microbiological confirmation was obtained by culture of potentially pathogenic bacteria or fungi from an infant's blood or CSF sample, or from another normally sterile tissue space. The outcome definition included infection with coagulase-negative *Staphylococci*, provided that these were not a mixed flora but excluded micro-organisms that were likely to be skin contaminants (diphtheroids, micrococci or propionibacteria). This approach is consistent with standard clinical practice and surveillance protocols in the UK and elsewhere. The case definition of late-onset infection did not include urinary tract infection or radiologically confirmed pneumonia, as these are not accurate and reliable in very preterm infants in the absence of bacteraemia.[64]

Secondary outcomes

Estimates for the secondary outcomes indicated consistently that lactoferrin supplementation does not have important effects on the risk of major morbidities. We prespecified an analysis 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. We plan to increase the precision of these estimates of effect on rarer secondary outcomes by combining these data in a meta-analysis with other trials, including a recently completed Australasia RCT ($n = 1500$) of bovine lactoferrin supplementation for very low birthweight infants (Lactoferrin Infant Feeding Trial; see www.anzctr.org.au/ACTRN12611000247976.aspx).[65]

Cost analyses

As late-onset infection and NEC are the major reasons for receipt of invasive interventions and higher levels of 'categories of care' in very preterm infants, it is not surprising that we did not show any effects on the level of exposure to antimicrobial agents or on the duration of hospitalisation or stay in intensive or special 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 did not undertake detailed analyses of health-care costs as had been proposed in our approved funding application and trial protocol. We did not conduct a within-trial health economic analysis or use these data in a model to explore long-term family and health service costs, as these are driven mainly by the consequences of infection and other morbidity during the initial hospitalisation. Without evidence of clinical effectiveness on these infant-important outcomes, we considered a cost-effectiveness analysis of lactoferrin supplementation to be futile.[66]

Qualitative analysis and parent views

A qualitative analysis and exploration of participants' parents views and expectations has been undertaken in collaboration with the SIFT investigators.[48] Given that this study included SIFT participants predominantly (with few ELFIN trial participants), the findings will be reported within the SIFT report.

Long-term outcomes

We do not plan to apply for permission and additional funding to assess longer-term outcomes of trial participants. We specified in our funding application and protocol that if the trial did not detect statistically significant or clinically important differences in the in-hospital outcomes then follow-up will not be undertaken because 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.[5,6,11] As these were not shown, there is no longer an impelling rationale for expecting lactoferrin supplementation to have an impact on long-term growth or development.

Applicability

The ELFIN trial findings are likely to be applicable in the UK and internationally. Participants were enrolled in 37 neonatal units across the country, providing broad geographical, social and ethnic representation. Many infants who were enrolled in a recruiting site were transferred subsequently to another neonatal unit, which was typically closer to the family home, for ongoing care. Trial participation continued in another 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 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 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 and cohort studies and RCTs.[67]

Implications for practice

The ELFIN trial does not support the routine use of enteral bovine lactoferrin supplementation to prevent late-onset infection or other morbidity or mortality in very preterm infants.

Implications for research

Research efforts should 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.

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Richard Cooke (chairperson), Fan Hutchison (deputy chairperson), Andrew Ewer, Jennifer Hellier and Paul Mannix.

Independent Data Monitoring Committee

Henry Halliday (chairperson), Nim Subhedar, Michael Millar, Alison Baum and Mike Bradburn.

Ethics approval

National Research Ethics Service Committee East Midlands – Nottingham 2 (reference number 13/EM/0118, 02/04/2013).

Contributions of authors

James Griffiths (Trial Manager), **Paula Jenkins** (Trial Research Nurse), **Monika Vargova** (Administrator and Data Co-ordinator), **Ursula Bowler** (Senior Trials Manager), **Andrew King** (Head of Trials Programming), **David Murray** (Senior Trials Programmer), **Paul T Heath** (Co-investigator, chairperson of the blinded end-point review committee) and **William McGuire** (Chief Investigator) were responsible for the data collection and management.

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James Griffiths, **Edmund Juszczak**, **Louise Linsell**, **Christopher Partlett** and **William McGuire** were responsible for the report writing.

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Publications

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Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Please note that exclusive use will be retained until the publication of major outputs. Access to anonymised data may be granted following review.

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Appendix 1 Recruiting neonatal units

Recruiting sites	PI	Research nurse
Altnagelvin Area Hospital	Mary Ledwidge	Julie Brown
Birmingham Heartlands Hospital	Imogen Story	Natalie Albrighton
Birmingham Women's Hospital	Gemma Holder	Rachel Jackson/Elizabeth Simcox/ Heather Barrow
Bradford Royal Infirmary	Sam Oddie	Kelly Young/Trudy Booth
Calderdale Royal Hospital	Pamela Ohadike	Salamiah Burgess
University Hospital Coventry	Sarah Ellis	Kerri McGowan/Nicola Watts
Derriford Hospital, Plymouth	Rima Vaikute	Sarah-Jane Sharman
Great Western Hospital, Swindon	Girish Gowda	Rebecca Elliott-Jones
Hull Royal Infirmary	Helen Yates	Leanne Sherris
James Cook University Hospital, Middlesbrough	Shalabh Garg	Amanda Forster/Helena Smith
Jessop Wing – Sheffield Teaching Hospital	Liz Pilling	Pauline Bayliss
John Radcliffe Hospital, Oxford	Charles Roehr	Sheula Barlow/Sharon Baugh
Leeds General Infirmary	Kathryn Johnson	Suzanne Laing
Leicester Royal Infirmary	Elaine Boyle	Marie Hubbard/Rosalind Astles
Norfolk and Norwich University Hospital	Paul Clarke	Karen Few
Nottingham City Hospital	Dushyant Batra	Yvonne Hooton/Helen Navarra
Pinderfields Hospital, Wakefield	David Gibson	Gail Castle
Princess Anne Hospital, Southampton	Mark Johnson	Jenny Pond/Philippa Crowley/ Jane Rhodes-Kitson
Princess Royal Maternity Hospital, Glasgow	Helen Mactier	Isobel Crawford
Queen Alexandra Hospital, Portsmouth	Tim Scorrer	Michelle Pople/Michele Voysey
Nottingham University Hospital	Jon Dorling	Yvonne Hooton/Helen Navarra
Royal Cornwall Hospital, Truro	Yadlapalli Kumar	Barbara Bromage
Royal Devon and Exeter NHS Foundation Trust	David Bartle	Jacqui Tipper/Jenny Cunningham
Royal Hospital for Children, Glasgow	Colin Peters	Lorna McKay
Royal Infirmary of Edinburgh	David Quine	Lynn Clark
Royal Maternity Hospital, Belfast	Stanley Craig	Muriel Millar
Royal Preston Hospital	Richa Gupta	Claire Lodge
Royal Victoria Infirmary, Newcastle upon Tyne	Nick Embleton	Julie Groombridge
Singleton Hospital, Swansea	Jean Matthes	Amanda Cook
St George's Hospital, London	Nigel Kennea	Vana Wardley/Naomi Hayward
St Peter's Hospital, Chertsey	Peter Reynolds	Nicky Holland
Sunderland Royal Hospital	Ruppa Geethanath	Natalie Talbot
University Hospital of North Tees	Sundaram Janakiraman	Alex Ramshaw
Victoria Hospital, Kirkcaldy	Sean Ainsworth	Debbie Johnston
William Harvey Hospital, Ashford	Vimal Vasu	Shermi George

APPENDIX 1

Recruiting sites	PI	Research nurse
Wishaw General Hospital	CM Manjunatha	Denise Vigni
York District Hospital	William McGuire	Anna Clayton

Appendix 2 Continuing care sites

Accrington Victoria Hospital; Airedale General Hospital; Antrim Area Hospital; Barnsley Hospital; Basildon University Hospital; Basingstoke and North Hampshire Hospital; Bassetlaw Hospital; Birmingham Children's Hospital; Birmingham City Hospital; Borders General Hospital; Broomfield Hospital; Burnley General Hospital; Chesterfield Royal Hospital; Chorley and South Ribble Hospital; Colchester General Hospital; County Hospital, Stafford; Craigavon Area Hospital; Crosshouse University Hospital; Croydon University Hospital; Darlington Memorial Hospital; Diana Princess of Wales Hospital, Grimsby; Doncaster Royal Infirmary; Dorset County Hospital; Dumfries and Galloway Royal Infirmary; Forth Valley Royal Hospital; George Eliot Hospital, Nuneaton; Glangwili General Hospital; Gloucestershire Royal Hospital; Good Hope Hospital, Sutton Coldfield; Great Ormond Street Hospital for Children, London; Harrogate District Hospital; Horton General Hospital; Ipswich Hospital; James Paget University Hospital, Great Yarmouth; King's Mill Hospital, Sutton-in-Ashfield; Lincoln County Hospital; Liverpool Women's Hospital; Maidstone Hospital; Medway Maritime Hospital, Gillingham; Milton Keynes General Hospital; Musgrove Park Hospital, Taunton; Northampton General Hospital; North Manchester General Hospital; Northumbria Specialist Emergency Care Hospital, Cramlington; Pilgrim Hospital, Boston; Poole Hospital; Princess of Wales Hospital, Bridgend; Queen Elizabeth Hospital, Gateshead; Queen Elizabeth Hospital, King's Lynn; Queen Elizabeth The Queen Mother Hospital, Margate; Queen's Hospital, Burton on Trent; Queen's Hospital, Romford; Raigmore Hospital, Inverness; Rotherham General Hospital; Royal Berkshire Hospital, Reading; Royal Blackburn Hospital; Royal Bolton Hospital; Royal Derby Hospital; Royal Hampshire County Hospital, Winchester; Royal Oldham Hospital; Royal Shrewsbury Hospital; Royal Surrey County Hospital, Guildford; Russells Hall Hospital, Dudley; Salisbury District Hospital; Scarborough General Hospital; Scunthorpe General Hospital; Southend Hospital, Westcliff-on-Sea; Southport Hospital; South Tyneside District Hospital, South Shields; Stepping Hill Hospital, Stockport; St John's Hospital, Livingstone; St Mary's Hospital, Newport; Stoke Mandeville Hospital, Aylesbury; St Richard's Hospital, Chichester; The County Hospital, Hereford; The Cumberland Infirmary, Carlisle; Torbay Hospital, Torquay; Tunbridge Wells Hospital; Ulster Hospital, Dundonald; University Hospital Lewisham; University Hospital of North Durham, Durham; Warwick Hospital; Watford General Hospital; West Cumberland Hospital, Whitehaven; West Suffolk Hospital, Bury St Edmunds; Withybush General Hospital, Haverfordwest; Worcestershire Royal Hospital, Worcester; Worthing Hospital; and Wrightington Hospital, Wigan.

Appendix 3 Preparation of Investigational Medicinal Product for administration

1. Verify that the pack ID number on the pharmacy pot matches the pack ID allocated to the infant (stated on the randomisation confirmation e-mail and to be recorded on the daily dosing log).
2. Add 4 ml of sterile water (supplied in plastic vial) plus 1 ml of either expressed breast milk or formula (if expressed breast milk is not available) to the pharmacy pot, which contains 375 mg of either lactoferrin or sucrose placebo.
3. Seal the pot with the lid and shake vigorously by hand for 30 seconds.
4. Leave the pot at room temperature for 30 minutes.
5. Using a syringe, draw off suspension (2 ml/kg body weight up to a maximum of 4 ml) for nasogastric/orogastric or oral administration (via spoon/cup/syringe or bottle). [Participating centres were supplied with oral syringes if their standard oral syringe was not compatible with the lactoferrin/placebo pot insert].
6. Trial IMP normally to be given once daily. For very small infants, clinicians or caregivers may choose to administer the daily dose in two aliquots. If these are to be given > 30 minutes apart, then a fresh dose should be prepared as above for each.
7. If there was any concern about acute intestinal inflammation or perforation then the dose could be omitted. Whether or not doses were omitted at other times when the infant was unwell or demonstrated enteral feeds intolerance was at the discretion of the attending consultant paediatrician.

Appendix 4 Case definition of necrotising enterocolitis

Necrotising enterocolitis may be diagnosed at surgery, at post-mortem examination or clinically and radiologically using the following criteria.

At least one of the following clinical signs present:

- bilious gastric aspirate or emesis
- abdominal distension
- occult or gross blood in stool (no fissure).

In addition, at least one of the following radiological features present:

- pneumatosis intestinalis
- hepatobiliary gas
- pneumoperitoneum.

Infants who satisfy the definition of NEC above but are found at surgery or post-mortem examination for that episode to have a 'focal gastrointestinal perforation' should be coded as having 'focal gastrointestinal perforation', not as having NEC.

Appendix 5 British Association of Perinatal Medicine: 'categories of care'

URL: www.bapm.org/sites/default/files/files/CatsofcarereportAug11.pdf (accessed 29 June 2018).

Intensive care

General principle: this is care provided for infants who are the most unwell or unstable and have the greatest needs in relation to staff skills and staff-to-patient ratios.

Definition of intensive care day

Any day when an infant receives any form of mechanical respiratory support via a tracheal tube.

Both non-invasive ventilation [e.g. nasal continuous positive airways pressure (CPAP)] and parenteral nutrition.

- Day of surgery (including laser therapy for ROP).
- Day of death.
- Any day receiving any of the following:
  - presence of an umbilical arterial line
  - presence of an umbilical venous line
  - presence of a peripheral arterial line
  - insulin infusion
  - presence of a chest drain
  - exchange transfusion
  - therapeutic hypothermia
  - prostaglandin infusion
  - presence of replogle tube
  - presence of epidural catheter
  - presence of silo for gastroschisis
  - presence of external ventricular drain
  - dialysis (any type).

High-dependency care

General principle: this is care provided for infants who require highly skilled staff but where the ratio of nurses to patients is less than that in intensive care.

Definition of high-dependency care day

Any day when an infant does not fulfil the criteria for intensive care where any of the following apply:

Any day when an infant receives any form of non invasive respiratory support (e.g. nasal CPAP).

Any day receiving any of the following:

parenteral nutrition
continuous infusion of drugs (except prostaglandin and/or insulin)
presence of a central venous or long line (peripherally inserted central catheter)
presence of a tracheostomy
presence of a urethral or suprapubic catheter
presence of transanastomotic tube following oesophageal atresia repair
presence of nasopharyngeal airway/nasal stent
observation of seizures or cerebral function monitoring
barrier nursing
ventricular tap.

Special care

General principle: special care is provided for infants who require additional care delivered by the neonatal service but do not require either intensive or high-dependency care.

Definition of special care day

Any day where an infant does not fulfil the criteria for intensive or high-dependency care and requires any of the following:

oxygen by nasal cannula
feeding by nasogastric, jejunal tube or gastrostomy
continuous physiological monitoring (excluding apnoea monitors only)
care of a stoma
presence of intravenous cannula
phototherapy
special observation of physiological variables at least 4 hourly.

Appendix 6 Data collection forms

URL: www.npeu.ox.ac.uk/elfin/data-collection-forms (accessed 24 July 2018).

Form	Purpose
Trial entry form	The entry form contains sections to be completed before, during and after randomisation, and collects the infant's baseline characteristics
Daily dosing log	To be completed daily during the treatment period (once the infant receives milk feeds of 12 ml/kg/day until 34 weeks' postmenstrual age) to document the administration of lactoferrin or placebo, type of milk given and use of antibiotic and antifungal drugs
Late-onset infection form	To report each episode of microbiologically confirmed or clinically suspected late-onset invasive infection
Gut signs form	To report each episode whenever an infant has received $\geq$ 5 days of treatment for gut signs, if they are transferred with gut signs, or if they have died from gut signs
Hospital transfer and discharge form	To be completed by each recruiting, continuing care or data collection site whenever a participating infant is discharged home, is transferred to another unit, or has died
Discontinuation of intervention	To be completed if lactoferrin or placebo is permanently discontinued early (by clinician or parental decision) or where parents choose to withdraw their infant from the trial
SAE/SUSAR form	Should be completed for all SAEs that are 'unexpected' and sent to the NPEU CTU within 24 hours of becoming aware of the event
Incident form	To report any deviation from the protocol, trial-specific procedures or good clinical practice

Appendix 7 Safety reporting: definitions

Adverse event

Any untoward medical occurrence in a patient or clinical investigation participant who has been administered a medicinal product, which does not necessarily have to have a causal relationship with this treatment (the study medication).

An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of the study medication, whether or not it is considered to be related to the study medication.

Adverse reaction

All untoward and unintended responses to a medicinal product related to any dose.

The phrase 'responses to a medicinal product' means that a causal relationship between a study medication and an adverse event is at least a reasonable possibility (i.e. the relationship cannot be ruled out). All cases judged by either the reporting medically qualified professional or the sponsor as having a reasonable suspected causal relationship to the study medication qualify as adverse reactions.

Serious adverse event

Adverse events are defined as serious if they:

- result in death
- are life-threatening
- require inpatient hospitalisation or prolongation of existing hospitalisation
- result in persistent or significant disability/incapacity
- are a congenital anomaly/birth defect
- are other important medical events.

Note that other events that may not result in death, are not life-threatening or do not require hospitalisation may be considered SAEs when, based on medical judgement, the event may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above. The term 'life-threatening' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

Serious adverse reaction

A serious adverse reaction (SAR) is a SAE that is considered to have been caused by the administration of the trial medication. For a SAE to be considered a SAR, there must be a reasonable probability that it was related to the administration of the IMP.

Suspected unexpected serious adverse reaction

This is a SAR, the nature or severity of which is not consistent with the known safety profile of the trial medication (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics for an approved product).

Foreseeable ('expected') serious adverse events

The following are SAEs that could be reasonably expected to occur in this population of infants during the course of the trial or form part of the outcome data. They do not require reporting by the trial centres as SAEs:

- death (unless unexpected in this population)
- NEC or focal intestinal perforation
- BPD or chronic lung disease
- intracranial abnormality (haemorrhage or focal white matter damage) on cranial ultrasound scan or other imaging
- pulmonary haemorrhage
- patent ductus arteriosus
- ROP.

Appendix 8 Summary of changes to the study protocol

Protocol is available at www.npeu.ox.ac.uk/elfin/protocols (accessed 24 July 2018) and in the *Neonatology* article.[47]

Amendment	Date of REC favourable opinion	Date of MHRA approval	Document	Description
Amend 1	14 October 2013	– *(Prior to Clinical Trials Authorisation application)*	Protocol version 2	• The procedure for making up the intervention was changed to reduce the total fluid volume to 5 ml (1 ml milk + 4 ml water). The concentration of the solution to be administered (75 mg/ml) and dose (150 mg/kg/day) is unchanged from the original application • The exclusion criteria were clarified. One serious congenital anomaly is sufficient for exclusion (changed from *anomalies*) • Clarification was added that it is acceptable to recruit infants to both the ELFIN trial and SIFT • The definitions of microbiologically confirmed and clinically suspected late-onset infection were edited for clarity • 'Multiple births' was added as a minimisation factor at randomisation • Text was added to explain that participants will be 'flagged' by the Health and Social Care Information Centre • Changes to appendix 3: IMP management explaining the procedure for making up the intervention • The projected recruitment rate in appendix 4 was revised slightly upwards
			IMP dossier version 2	• Ampoules of sterile water will be sourced from clinical areas rather than being supplied with the IMP • Owing to total fluid volume per dose being reduced from 6 ml to 5 ml, the amount of lactoferrin in each individual dose was reduced to 375 mg so that the resulting solution remained 75 mg/ml as in the original application • Inclusion of a Press-In Bottle Adapter in each container of IMP, compatible with oral syringes • Updated the certificates and compliance statements included as appendices to the most recent available versions
			Consent form version 2	• Sections were added for the name of the participant from whom and hospital at which consent was taken
			PIL version 2	• Added the statement 'We will keep your name, address and other contact details. The Health and Social Care Information Centre and other central UK NHS bodies will be used to keep in touch with you and provide information about your baby's health status' • Added the statement 'Unidentifiable data from this study may be shared with other groups who are carrying out similar work' to cover anonymised data-sharing after the trial is over, in line with the requirements of the funder • Removed the word 'independent' in relation to the charity Bliss
			ELFIN statement of responsibilities version v1	• This document was introduced to describe the arrangements for recruiting, continuing care and data collection sites

Amendment	Date of REC favourable opinion	Date of MHRA approval	Document	Description
Amend 2	3 January 2014	17 January 2014	IMP dossier version 3	• A provision for the use of non-automated filling of doses for up to 82 subjects for the pilot phase of the trial. This is necessary because the auger filler to be used for automated filling will not be installed and commissioned in time for the proposed start of recruitment • A change to the HPLC assay for bovine lactoferrin
Amend 3	27 May 2014	–	ELFIN/SIFT summary leaflet version 1	• To add a joint summary leaflet to introduce both the ELFIN trial and SIFT to parents, where recruitment to both trials is being considered *(SIFT submitted an identical amendment to the ethics committee)*
Amend 4	11 July 2014	21 July 2014	–	• Notification of temporary halt to trial. The circumstances leading to the temporary halt are described fully in the amendment 4 covering letter
Amend 5	13 October 2014	3 November 2014	IMP dossier version 4	• Drug substance will be flushed with nitrogen when added to the hopper of the servo auger filler machine • Text for manual filling was amended to clarify that this process was used for batches IMPNS4B002 and IMPNS4C001. Further batches will be manufactured by automated process (using auger servo filler machine) • Six sealed containers will be packed in a nitrogen-flushed, labelled aluminium pouch, lined with polyethyltoluene and low-density polyethylene • Four pouches of six containers (24 containers total) will be packed into one labelled cardboard outer • Based on the data presented in *Table 2*, maximum expiry will be limited to 2 years from date of manufacture, assuming storage at ≤ 30 °C • The stability testing programme will be conducted at 30 °C and 40 °C, in line with the revised storage requirements of the product
			GMP label version 2	• Storage requirements have been amended to 'Store at or below 30 °C'. The original label specified to store at or below 25 °C
Amend 6	29 October 2014	–	NICU parent poster version 1, antenatal ward poster version 1	• Poster intended for viewing by pregnant women and/or their partners in antenatal areas • Poster intended for viewing by parents in the neonatal unit
Amend 7	23 April 2015	–	–	• Addition of further recruiting sites in England
Amend 8	31 July 2015	–	–	• Addition of recruiting sites in England, Northern Ireland and Scotland
			PIL version 3	• Added England/Northern Ireland/Scotland specific variants of version 3 • Updated contact details for the charity Bliss • *N.B. REC considered these changes non-substantial*
			Consent form version 3	• References PIL version 3 *(Considered by REC to be non-substantial)*

Amendment	Date of REC favourable opinion	Date of MHRA approval	Document	Description
Amend 9	10 September 2015	–	–	• Conversion of some continuing care sites in England to recruiting sites
Amend 10	–	16 December 2015	–	• Removed requirement for temperature monitoring of IMP at continuing care sites
Amend 11	18 December 2015	31 December 2015	Protocol version 2.1	• Single change to protocol to remove requirement for research nurse reports to project management group (not implemented as included in protocol version 3.0)
Amend 12	21 January 2016	–	–	• Conversion of additional continuing care sites in England to recruiting sites
Amend 13	8 March 2016	4 March 2016	Protocol version 3.0	• Section 4.7 describing existing RCTs of lactoferrin supplementation in preterm babies was updated to reflect the recent 2015 Cochrane review[68] • Section 7.2 was expanded to clarify that appropriately qualified and experienced neonatal nurses may be delegated by the PI to assess eligibility • Sections 7.8.1 and 7.8.2 were changed to clarify how doses of IMP should be calculated against an infant's current working weight and to better emphasise the pragmatic nature of this trial • Section 7.8.3 was added to clarify that independent nurse prescribers may be delegated by the PI to prescribe IMP, provided this is consistent with local trust policy • Section 7.10 was changed to reflect an updated procedure for unblinding a participant in the event of an emergency • Section 7.16 was changed so that the definition of end of trial is now the date at which the trial database is locked • Section 9.2 was added clarify the reference safety information to be used for the assessment of adverse drug reactions • Section 9.3.1 was changed to clarify that adverse events not meeting the criteria for seriousness will not be collected for this trial
Amend 14	9 August 2016	–	–	• Added additional recruiting sites • Change of some PIs at existing sites
Amend 15	22 November 2016	–	–	• Added a Wales-specific variant of ELFIN PIL version 3 • Added continuing care sites in Wales • Change of some PIs at existing sites
Amend 16	6 September 2017	–	–	• Change of a PI at an existing site

Amendment	Date of REC favourable opinion	Date of MHRA approval	Document	Description
Amend 17	15 March 2018	15 March 2018	Protocol version 4.0	• The addition of a secondary outcome: microbiologically confirmed late-onset invasive infection • Two secondary outcomes have been combined: total number of days of administration of antibiotics administered per infant from 72 hours until death or discharge from hospital, and total number of days of administration of antifungal agents per infant. The revised secondary outcome is as follows: total number of days of administration of antibiotics or antifungals (excluding prophylactic doses) from the commencement of dosing with the IMP until 34 weeks' postmenstrual age. The revised outcome more closely corresponds to the period when the IMP bovine lactoferrin is administered • An addition to the protocol to clarify that, if a participant is not discharged to home within 6 months (26 weeks) of the date of birth, data collection for that participant will be completed at 6 months (26 weeks) from the date of birth • Recruitment tables and graphs in appendix 4 were updated to reflect the actual recruitment and date that recruitment to the trial ended
Amend 18	Pending	Pending	See protocol version 5.0 for track changes	• Submitted 27 June 2018 • Declaration NOT to conduct previously specified health economics analysis

HPLC, high-performance liquid chromatography; NICU, neonatal intensive care unit.

Appendix 9 Withdrawals from intervention by randomisation group

Reason	Trial group, *n*	
	Lactoferrin (*n* = 1099)[a]	Placebo (*n* = 1104)[a]
Clinical decision	4	1
Consent remains	(4)	(1)
Consent completely withdrawn	(0)	(0)
Parental wish	15	14
Consent remains	(15)	(11)
Consent completely withdrawn	(0)	(3)
Other[b]	1	0
Total	20	15

a Includes all infants randomised.
b Baby transferred to a hospital that refused to accept or administer the study intervention.

Appendix 10 Group allocation per recruiting site

Centre	Trial group, *n* (%)	
	Lactoferrin (*n* = 1098)	Placebo (*n* = 1101)
Jessop Wing, Sheffield	24 (2.2)	24 (2.2)
Royal Infirmary of Edinburgh	25 (2.3)	26 (2.4)
Princess Royal Maternity Hospital, Glasgow	26 (2.4)	27 (2.5)
Wishaw General Hospital	20 (1.8)	23 (2.1)
Royal Maternity Hospital, Belfast	20 (1.8)	20 (1.8)
James Cook University Hospital	76 (6.9)	70 (6.4)
Nottingham City Hospital	21 (1.9)	19 (1.7)
Queen's Medical Centre, Nottingham	15 (1.4)	15 (1.4)
Birmingham Heartlands Hospital	16 (1.5)	14 (1.3)
Birmingham Women's Hospital	52 (4.7)	54 (4.9)
Sunderland Royal Hospital	21 (1.9)	25 (2.3)
Altnagelvin Area Hospital, Londonderry	5 (0.5)	5 (0.5)
University Hospital Coventry	33 (3.0)	35 (3.2)
Royal Victoria Infirmary, Newcastle	68 (6.2)	66 (6.0)
University Hospital of North Tees	32 (2.9)	32 (2.9)
John Radcliffe Hospital, Oxford	15 (1.4)	17 (1.5)
Hull Royal Infirmary	17 (1.5)	17 (1.5)
Bradford Royal Infirmary	109 (9.9)	109 (9.9)
Calderdale Royal Hospital	10 (0.9)	10 (0.9)
Derriford Hospital, Plymouth	4 (0.4)	4 (0.4)
Great Western Hospital, Swindon	3 (0.3)	3 (0.3)
Leeds General Infirmary	83 (7.6)	82 (7.4)
Leicester Royal Infirmary	52 (4.7)	53 (4.8)
Norfolk and Norwich University Hospital	33 (3.0)	30 (2.7)
Pinderfields General Hospital, Wakefield	6 (0.5)	4 (0.4)
Princess Anne Hospital, Southampton	28 (2.6)	32 (2.9)
Queen Alexandra Hospital, Portsmouth	83 (7.6)	86 (7.8)
Royal Cornwall Hospital, Truro	15 (1.4)	12 (1.1)
Royal Devon and Exeter Hospital	13 (1.2)	9 (0.8)
Royal Hospital for Children, Glasgow	18 (1.6)	20 (1.8)
Royal Preston Hospital	16 (1.5)	15 (1.4)
Singleton Hospital, Swansea	14 (1.3)	12 (1.1)
St George's Hospital, London	30 (2.7)	32 (2.9)
St Peter's Hospital, Chertsey	32 (2.9)	32 (2.9)
William Harvey Hospital, Ashford	26 (2.4)	29 (2.6)
York Hospital	15 (1.4)	15 (1.4)
Victoria Hospital, Kirkcaldy	22 (2.0)	23 (2.1)

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