TITLE PAGE

Incidence and enteral feed antecedents of severe neonatal necrotising enterocolitis in England 2012-13: a two-year, population surveillance study

Battersby C[1], BMedSci, Longford N[1], PhD, Mandalia S[1], PhD, Costeloe K[2], MD, Professor of Paediatrics, Modi N[1], MD, Professor of Neonatal Medicine, on behalf of the UK Neonatal Collaborative Necrotising (UKNC-NEC) Study Group*

[1]Neonatal Data Analysis Unit, Imperial College London, UK

[2]Barts and the London School of Medicine and Dentistry

Corresponding author

Professor Neena Modi, MD,

Professor of Neonatal Medicine, Room G.4.2 4th Floor, Lift Bank D, Department of Medicine, Section of Neonatal Medicine, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK

Email: n.modi@imperial.ac.uk

Tel: +44 (020) 3315 5101 Fax: +44 (020) 3315 8050

Co-authors

Cheryl Battersby, BMedSci, Neonatal Data Analysis Unit, Department of Medicine, Section of Neonatal Medicine, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK

Nick Longford, PhD, Neonatal Data Analysis Unit, Department of Medicine, Section of Neonatal Medicine, Imperial College London, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK

Sundhiya Mandalia, PhD, Neonatal Data Analysis Unit, Department of Medicine, Section of Neonatal Medicine, Chelsea and Westminster Hospital, 369 Fulham Road, London, SW10 9NH, UK

Kate Costeloe, MD, Professor of Paediatrics, Barts and the London School of Medicine and Dentistry, Neonatal Unit, Homerton Hospital, Homerton Row, London E9 6SRUK

Keywords: necrotising enterocolitis, incidence, population, preterm, United Kingdom, England, human milk, formula, bovine

Word count: Abstract (300 /300w)

Background: Necrotising enterocolitis (NEC), a feared neonatal gastrointestinal inflammatory disease with high mortality and morbidity, is growing in global relevance as birth rates and early survival of low gestational age (GA) infants increases. Population data are scant and pathogenesis is incompletely understood but enteral feed exposures are believed to influence risk.

Methods: We conducted a two-year national surveillance study to quantify the total population burden of severe NEC (confirmed at laparotomy and/or leading to death) in England, and a propensity score analysis of the impact of early feeds of maternal milk (MM), and avoidance of bovine-origin formula (BOF) and milk fortifier (BMF), on NEC risk in very preterm infants.

Findings: During the study period 118,073 infants (14,678 <32w GA) were admitted to 163 neonatal units across 23 networks; 531 (462 <32w GA) developed severe NEC; 247 died, 139 following laparotomy. Among infants <32w GA the adjusted network incidence ranged from 2·51% to 3·85% with no evidence of unusual variation in relation to the national incidence of 3·13% (95%CI 2·85, 3·42) despite variation in feeding practices. Also among infants <32w GA, commencing any MM within seven postnatal days resulted in an Absolute Risk Difference (ARD) of -0·88% (95% CI -1·15, -0·61), Relative Risk (RR) 0·69 (95% CI 0·60, 0·78), and Number Needed to Treat (NNT) 114 (95% CI 87, 136); equivalent figures for infants receiving no, compared to any bovine-origin products within 14 postnatal days were ARD -0·65% (95% CI -1·01, -0·29), RR 0·61 (95% CI 0·39, 0·83), NNT 154 (95% CI 94, 345).

Interpretation: Commencing MM early and avoiding bovine-origin products may reduce NEC but absolute risk reductions appear small. The rarity of severe NEC requires national and international collaboration for adequately powered preventive trials.

Funding: This study represents independent research funded by the National Institute for Health Research (NIHR).

Research in context

Evidence before this study

Necrotising enterocolitis (NEC) is a feared, acute neonatal gastrointestinal inflammatory disease with high mortality and morbidity. Reliable population incidence data are necessary for designing preventive and treatment studies. Enteral feeding stratagems are widely believed to influence NEC risk. When maternal milk (MM) is insufficient or unavailable some clinicians prefer pasteurised human donor milk (HDM) over bovine-origin formula (BOF) in the hope that avoidance of bovine-origin products will be protective and/or pasteurised HDM will retain some of the protective properties of MM. Others prefer BOF to HDM as the composition of the former is consistent, nutrient density is higher, and costs are lower. Other uncertainties relate to the timing of introduction of milk feeds in very preterm infants, and use of bovine-origin fortifier (BMF) (additional vitamins, minerals, and nutrients added to human milk).

We conducted a systematic search of studies from the 34 countries in the Organisation for Economic Co-operation and Development (OECD) (Medline: 1946 to 2015; Embase: 1974 to 2015; PubMed 1979 to 2016) using the terms “Necrotising Enterocolitis” (MESH) and “Country” (PROSPERO registration no CRD42015030046).[1] We identified 1554 records, reviewed 93 full text manuscripts and included 47 studies from 15 countries. We were unable to quantify burden of disease reliably because of lack of whole population data and use of varying case-definitions for NEC.

We also sought evidence of NEC risk in relation to enteral feeds. Quigley et al [2] identified two trials for inclusion in a Cochrane Library meta-analysis and showed no

strong evidence of benefit of HDM compared with BOF when used to supplement MM, on NEC risk (Risk Ratio (RR) 1·96 (95% CI 0·82, 4·67)), mortality and a range of morbidities. We identified two further randomised controlled trials [3, 4] and updated the meta-analysis, doubling the total sample size from 509 to 1089 infants; however conclusions remain unchanged (NEC RR: 1·62 (0·93, 2·82)).

Added value of this study

We provide reliable population data on the incidence of severe NEC in England. Despite differences in enteral feeding strategies across neonatal networks, we found no strong evidence of variation in disease burden. We employed a propensity score approach to observational data in which groups were matched on a large number of covariates to minimize confounding and mimic randomisation. We showed that in infants born <32 weeks GA, commencing any MM within 7 days of birth, and avoiding bovine-origin products for 14 days may reduce severe NEC, but absolute risk reductions are small. We were unable to separate the effects of HDM from MM because of low use of the former and high use of the latter in the study population.

Implications of all the available evidence

Severe NEC is a rare but devastating disease that is growing in importance as early preterm survival increases globally. Our large population study provides some evidence of a protective effect of early MM feeds and avoidance of bovine-origin products but the effect sizes appear small. Human milk banking has arisen largely as a result of voluntary, altruistic effort, but commercial HDM products are now becoming available. As the benefits of pasteurized HDM over BOF when MM is insufficient or unavailable remain unclear and as the products have different potential risk-benefit and cost profiles, adequately powered randomised controlled trials are

urgently required to reduce these pressing enteral feeding uncertainties that affect preterm infants around the world.

Introduction (3709/3000 words)

Globally each year, an estimated 15 million infants, representing around 10% of all births, are born preterm, and these numbers are rising.[5] As survival improves, the contribution of necrotising enterocolitis (NEC), an acute gastrointestinal inflammatory disease predominantly affecting preterm infants, has risen in importance as a major cause of mortality and morbidity.[4,5] Population incidence data on NEC are unreliable, largely because less severe disease has many non-specific features. Definitions vary between studies and include Bell's staging 1-3 (a combination of clinical and radiological features), and confirmation at laparotomy. Published rates, defined by Bell's staging ≥2, range from 1.7% in Japan to 5% in Canada for infants <32w gestational age (GA). [6-10] Among infants <32w GA or <1500g birth weight, around a third proceed to laparotomy (around 60% among the most immature). Overall mortality is around a third, rising to 50% following laparotomy.[6, 10] Variations in feeding practices are widely believed to influence the risk of NEC. Japan has the lowest reported rate, attributed to early and "aggressive" enteral feeding and high use of unpasteurized Human Donor Milk (HDM) though these practices have not been investigated with rigour.

The aetiology and pathophysiology of NEC are inadequately understood, preventive and treatment options are limited, and survivors have a three-fold increase in neurodisability. [11, 12] Surgical removal of affected bowel is a mainstay of treatment of

severe disease. Evidence to guide clinical practice is limited. Exposure to bovine-origin products (formula (BOF) or multicomponent fortifier (BMF) added to human milk to provide vitamins, minerals, and extra nutrients) are widely believed to increase risk, and human milk, whether maternal milk (MM) or HDM to decrease risk.[13, 14]

In England, all infants born <32 weeks GA are admitted to a National Health Service (NHS) neonatal unit, providing opportunity to conduct whole population surveillance. Neonatal services are organised into clinical networks, each comprising 6-8 neonatal units providing different levels of care. Infants are transferred between neonatal units in a network according to clinical need. Neonatal networks share management protocols and generally aim to provide a degree of consistency in clinical approach. We aimed to quantify the national burden of severe NEC, variation across neonatal networks, and identify enteral feed related antecedents.

Methods

Study design and participants

We conducted a prospective surveillance study over a two-year period. Daily clinical information on neonatal unit admissions is recorded in a point-of-care, clinician-entered Electronic Patient Record. A defined data extract, the Neonatal Dataset (NHS Information Standard SCCI595) is transmitted quarterly to the Neonatal Data Analysis Unit at Imperial College London and Chelsea and Westminster NHS Foundation Trust where patient episodes across different hospitals are linked, data are cleaned, and entered into the National Neonatal Research Database (NNRD).[15] Contributing neonatal units are known as the UK Neonatal Collaborative (UKNC).

The NNRD is approved by the National Research Ethics Service (10/H0803/151), Confidentiality Advisory Group of the Health Research Authority (8-05(f)/2010) and the Caldicott Guardians and Lead Clinicians of contributing hospitals. Neonatal units participating in this study (UKNC-NEC Study Group) committed prospectively to recording a complete set of pre-specified data in the neonatal EPR. This study is a component of the UKNC-NEC study (UK Clinical Research Network Portfolio ID 11853; National Research Ethics Service ref 11/LO/1430).

The study population comprised all live-born infants admitted to neonatal units in England between January 1st 2012 and December 31st 2013. We extracted the following variables from the NNRD: booking network, GA at birth, birth-weight, fetus number, sex, maternal age, race, antenatal corticosteroids, mode of delivery, outcome (discharge/died), cause of death, maternal pyrexia in labour, maternal antibiotics in labour, maternal infection, chorioamnionitis, NEC treatment (medical or surgical), diagnoses, procedures, abdominal X-ray (AXR) results, confirmation of NEC by visual inspection of bowel, histology and/or autopsy. We calculated birth-weight standard deviation Score (z score) [16] and for infants<32 weeks GA daily details of the milks and enteral products to which they were exposed (MM; HDM; BOF; BMF) together with total milk volumes.

Outcomes

We defined “severe NEC” as disease confirmed at laparotomy, histology or autopsy, or, if no tissue evidence was available, if reported on the death certificate as the primary cause of death. For cross validation, we used diagnostic codes, procedures, and AXR findings to identify infants that might have developed severe NEC, and verified these outcomes with study leads at each neonatal unit. We also obtained outcomes on infants transferred to any of four paediatric surgical centres in England

that do not use the neonatal EPR. Infants in whom a diagnosis of Spontaneous Intestinal Perforation was made at laparotomy were not included in the NEC group.

Statistical analyses

We identified infants with severe NEC across all gestations, their outcomes, postnatal and postmenstrual age at laparotomy, and determined the population incidence for infants born <32 weeks GA. We used the chi square test with Yates' correction to identify clinical characteristics associated with severe NEC. Candidate variables ($p<0.15$) were entered into the multivariate logistic regression model; the final model retained significant predictors of NEC and antenatal corticosteroids as this results in a significant reduction in neonatal death .[17]

We estimated the unadjusted and case-mix adjusted network incidence of severe NEC, assessing variation by comparing each network with the national rate. We calculated the standardised severe NEC ratio as the ratio of the observed and expected number of cases (predicted from the final multivariate model) with 95% confidence intervals estimated using Byar's approximation, [18] correction for multiple testing and controlling for the false discovery rate at 5%.[19]

We tested the null hypotheses "there is no variation in severe NEC at network-level" and "feeding exposures do not influence the risk of NEC". We identified the postnatal age at first enteral feed, and assessed variation between networks in type of feed (MM, HDM, BOF) and use of BMF, over the first two and 14 postnatal days by the chi-square test. We selected these periods a priori as early enteral feeding is considered standard of care in England. For infants with daily data for milk volumes available for at least 70% of days, we determined the postnatal age to reach an intake of 150ml/kg/day, and the volume of feed and postnatal age at which BMF was

introduced. For the propensity score analysis of the effect of early enteral feed exposures we excluded infants not admitted to neonatal care for the entire duration of the first 14 postnatal days, or in whom there had been clinical concern regarding NEC during this period GA

We imputed missing data using a pre-specified approach (see supplementary appendix for details). We created matched pairs using propensity scores estimated from a logistic regression model that included the covariates: maternal age, previous pregnancies, smoking, birth-weight, GA, birth-weight z score, mode of delivery, Apgar score at 1 minute, multiple birth and summary exposures to parenteral nutrition, MM, HDM, BOF and/or BMF. We split the propensity scores into deciles, performed one-to-one matching within deciles and within booking network and GA group (≤26, 27-29, 30-31 completed weeks). Within each analysis we replicated the matching procedure 20 times to estimate the standard errors of the parameters of interest. We incorporated interactions to obtain the best balance of each variable across the comparison groups. We compared the rate of severe NEC among i) infants who received early MM (commencement days 1-*7*) versus late or no MM (commencement day 8 or later, or never), regardless of whether feeds were supplemented by HDM or BOF/BMF); commencement was defined as the first day of a continual (uninterrupted until at least day 14) period of feeding MM; ii) those that received any bovine-origin (any exposure to BOF or BMF regardless of any other feeds received) versus no bovine-origin products (i.e. only human milk, whether MM or HDM) in the first 14 days; and iii) those that received HDM+/- BOF/BMF (no MM) versus BOF/BMF (no MM) in the first 14 days. The standard error of the estimate of the treatment effect was obtained by combining the within and between-replication standard errors [20]. All *p*-values reported are 2-sided. Analyses were performed using

SAS version 9.3 (SAS Institute Inc, Cary, North Carolina), except the propensity analysis which was performed in R.[21]

Role of the funding source

This work represents independent research funded by the National Institute for Health Research (NIHR) under its Programme Grants for Applied Research Programme (Reference RP-PG-0707-10010). The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR, or the Department of Health. The study was sponsored by Imperial College London. Neither funder nor sponsor had any role in study design, data collection, analysis, or interpretation, writing of the report, or the decision to submit for publication. All authors had access to the raw data. The corresponding author had full access to all of the data and the final responsibility to submit for publication"

Results

Study population

All 163 neonatal units in England participated in this prospective study (43 Special Care; 76 High Dependency; 44 Intensive Care) across 23 clinical networks. The NNRD contained details of 118,073 infants (14,678 < 32 weeks gestation) admitted to a neonatal unit in England over the two-year study period. We identified 531 (462 <32 weeks GA) infants with severe NEC; 423 underwent laparotomy and 108 died prior to laparotomy, of whom only 6·5% had a post-mortem examination. Of the infants that received a laparotomy, 139 (32·9%) died (Figure 1); NEC was the primary cause of death for over 80% who died following laparotomy. Details of survival of surgical NEC and severe NEC cases by gestational age are shown in Table 1. Earlier gestation was associated with later postnatal age at laparotomy (log

rank test <0·001). For infants <32 weeks GA, the median (IQR) postnatal and postmenstrual age at laparotomy was 22 (11, 37) days and 30.5 (27·9, 32·7) weeks, respectively. Supplementary Figure S1 shows transfer patterns of the 108 infants who died without laparotomy.

Population incidence and network-level comparison

The population incidence of severe NEC for infants <32 weeks GA was 3·15% (95% CI 2·87, 3·43); risk-adjusted incidence 3·13 (95% CI 2·85, 3·42). Incidence declined from 24 to 29 weeks GA, remaining largely unchanged thereafter (Figure 2). Infant characteristics associated with severe NEC in univariate analysis are shown in Supplemental Table 1. Following multivariate regression, only GA and growth restriction remained significant independent predictors. The odds of severe NEC were lower by around one-third for each additional gestational week and increased by around one-third for every unit reduction in birth-weight z score (Table 2). The unadjusted network incidence ranged from 2.0% (95% CI 0·5, 3·4) to 4·7% (95% CI 3·3, 6·2); adjusted 2.51 (1·13, 3·60) to 3·85 (2·37, 5·33). The funnel plot of standardised severe NEC ratio adjusted for GA, birth-weight z score and antenatal corticosteroids shows that all but two networks lie within the 95% control limits (Figure 3).

Description of enteral feeding patterns

From the total population of 14,678 infants <32 weeks GA, we excluded 12 transferred to non-English neonatal units and 441 with missing feeding data, resulting in 14,225 infants for the description of enteral feeding patterns. Of the 14·8% exclusively fed MM, 50% received BMF; 3·1% were exclusively fed formula. The median (IQR) time to first enteral feed was 3 (2, 4) days. During the first two and

first 14 postnatal days the proportion receiving MM, HDM and BOF were 66·5%, 14·5% and 28·9%; and 95·0%, 19·2%, and 41·4% respectively. Early feed exposures, whether assessed over the first two or first 14 postnatal days, were statistically significantly different at network-level (p<0·001). The median (IQR) time to reach an enteral intake of 150 ml/kg/day was 11 (8, 17) days, and to commence BMF, 20 (14, 32) days, at a median milk intake of 162 (149, 177) ml/kg/day. Feed exposures from birth to day of surgery or death were available for 441 infants with severe NEC. Of these 234 (53%) had received exclusive human milk feeds (176 exclusive MM; 57 MM and HDM; one exclusive HDM).

Propensity score analysis of enteral feeding antecedents of NEC

Of the 14,225 infants included in the description of feeding patterns, we excluded 1402 in whom length of stay was less than 14 days and 884 in whom there had been concern regarding possible NEC within the first 14 days. The final propensity score sample numbered 11,939 infants and 220 cases of severe NEC. Missing daily-records were few (1.6%) and of these we were able to impute 10,087 entries out of 28,379. Details of fitted propensities and balance plots are provided in Supplementary Figures S2a, b and S3a, b; baseline characteristics of the cohorts before and after propensity score matching in Supplementary Tables 2a, b. The absolute risk difference (ARD) for infants commencing MM ≤7 days compared to ≥8 days or never, regardless of other enteral feeds received, was -0·88% (95% CI -1·15, -0·61), RR 0·69 (95% CI 0·60, 0·78) and NNT 114 (95% CI 87, 136). The ARD for infants receiving no compared to any bovine products regardless of other enteral feeds received, was -0·65% (95% CI -1·01 to -0·29), RR 0·61 (95% CI 0·39 to 0·83), and NNT 154 (95% CI 99, 345). The sample size to conduct the third planned

analysis was insufficient as only 5·5% of infants received no MM during the first 14 postnatal days.

Discussion

In this complete population study we show that the severest form of NEC, requiring laparotomy and/or leading to death, affects 3·2% of infants below 32 weeks GA, with almost half of all deaths occurring before laparotomy. Commencing MM within 7 days of birth, regardless of other feeds received, and avoiding exposure to bovine-origin products within the first 14 days, reduced the relative risk of severe NEC by about a third, but the absolute risk reductions were small at less than 1%, and the NNT large. We were unable to assess the effect of pasteurised HDM, as use was low, involving less than a fifth of infants; in contrast, MM was received by 95% of infants in the first 14 days.

The strengths of our study are that it is based on a large population dataset with extensive patient-level and daily-level feeding information. Only a small fraction of data were missing, and of these we were able to impute over a third. We secured the participation of every neonatal unit in England and hence were able to ascertain the exact number of cases of severe NEC. Incomplete ascertainment is a common limitation of other studies [22, 23] compounded by selection bias when confined to tertiary referral centres. We applied a stringent definition and confirmed each case with clinical teams to minimise contamination from other diagnoses, such as spontaneous intestinal perforation.

Clinician choice of feeds is influenced by the perceived risk of NEC, hence the major limitation of observational studies is the non-random assignment of the intervention

which leads to confounding. Propensity analysis is used to mimic randomisation and minimise the risk of confounding by comparing groups of infants with similar propensity scores formed by matching on background characteristics that represent the probability of a particular exposure. We compared the rates of severe NEC between infants tightly matched on an extensive number of background variables, including booking network and GA as these strongly influence feeding practices. We did not include probiotics among the matching variables as use was not recorded on the NNRD during the study period. Probiotics are not considered standard of care in England, and were predominantly used only in the context of a double-blind placebo controlled randomised trial running concurrently during this study in 46 of the 163 neonatal units in England. [24] Although propensity matching discards a large fraction of data, our sample size of over 5000 neonates is much greater than in a typical clinical trial; for example the Cochrane Library meta-analysis comparing the risk of formula and human milk feeds on NEC involved only 869 neonates across six studies. [2]

Our study has limitations. Despite the extensive list of variables included in the propensity analysis there may be unmeasured confounders of relevance. The findings also relate only to infants that developed severe NEC and did not have any signs of the disease in the first 14 postnatal days which may have led to an under-estimate of the effect size. The selection of a cut-off of 14 days was a compromise; a larger number of days would address potential confounding more effectively but would increase the number of infants for whom NEC was a concern, and hence lead to an even greater number of exclusions. Limiting the analysis of feed exposures to 14 days also meant that we were unable to determine the specific effect of BMF on NEC as this was introduced relatively late, at a median age of 20 days. We

recognise that the burden of less severe disease is also important but given the subjective nature of the diagnosis of “medical-NEC” and the absence of a consistent case-definition, the interpretation of any analysis attempting to include these cases would be speculative. Our focus on *severe* NEC leading to death, and/or requiring surgery, aimed to provide greater objectivity though even surgical decision-making involves some subjectivity, and ~~that~~ so this approach will also underestimate the total burden of disease. We also acknowledge the difficulty of reliably separating spontaneous intestinal perforation from NEC. Quantification of total disease burden will require either a reliable clinical case-definition or a biomarker. Only a minority of infants that died without laparotomy had a post-mortem, therefore NEC as primary cause of death is based on clinical diagnosis and thus there is a small possibility of false attribution. Low post mortem rates are a recognised problem [25, 26]; our data reinforces the importance of current UK efforts to improve the situation, for example through less invasive examinations.[27]

Comparisons with other population based studies are hampered by the differences in case-definitions and inclusion criteria [6, 10, 28-31] In the United Kingdom there have been two studies in the last decade, EPICure 2, [30] restricted to neonates born up to 26 weeks GA, and a four month prevalence study with a low response rate.[23] Wide global variation is reported in the rate of NEC of all grades of severity, but our rates for laparotomy and mortality are similar to published data from Canada, [32, 33] Australia [6], and the United States. The finding that infants of lower GA develop NEC at a later postnatal age is also in accord with other studies [33-35] including the Probiotic in Preterms trial (PiPs) conducted in the south-east of England. [24]

Of note, over a third of the infants that died before surgery were transferred, the majority from a lower to a higher level neonatal unit. Almost a fifth died in non-tertiary

units. This highlights the potential for underestimation of NEC incidence in studies confined to referral centres. NEC can progress rapidly from non-specific signs to fulminant disease characterized by shock and multi-organ impairment within a matter of hours. Our data therefore also indicate that an important consideration for a networked model of care is whether back-transfer to a lower intensity neonatal unit, should be delayed, particularly for the most immature and growth restricted infants, until after the peak age of NEC onset, namely around 4 weeks postnatal or 33 weeks postmenstrual age.

Despite wide variation in feeding practices we did not identify strong variation in severe NEC incidence at network-level. Two networks fell outside the 95% prediction limits, though this is no more than might be expected by chance. Over half of all infants that developed severe NEC received only human milk prior to onset, confirming that this does not eliminate risk. This contrasts with widely held belief and findings from small, inadequately powered studies, [2] and justifies caution in the implementation of guidelines or quality-improvement approaches mandating specific enteral regimens without a firm evidence-base. [36] This notwithstanding, our finding of a small protective effect of MM adds to justification of this as a standard of care for NEC prevention. NEC requiring laparotomy is believed to account for 30-40% of all cases [6] hence the true impact of MM is likely to be greater. It is also possible that the small absolute risk reduction we identified in relation to avoidance of bovine-origin products was due to benefit from the human milk these infants would have received instead. Evidence from randomised controlled trials is limited [2] and suggests that BOF increases NEC risk in comparison to HDM only when used as sole diet but not when used as a supplement to MM, and does not affect mortality. Two recent additional studies, neither powered to detect a reduction in NEC, compared

supplementation of MM with pasteurised HDM or BOF; the DOMINO trial showed a reduction in NEC (Bell stage 2 or higher) but no benefit in the primary outcome, neurodevelopmental status at two-years. [37] The Early Nutrition Study showed no significant difference in a composite primary outcome of death, sepsis and NEC (Bell stage 2 or higher) but as MM comprised over four fifths of total milk intake during the intervention period in both groups, and power to detect a difference was limited. [3] As both studies involved the use of BMF, any positive effect of HDM may also have been negated. We identified low use of BMF in England, in contrast to practice in many parts of the world where this is considered a standard of care despite limited evidence of benefit to long term functional outcomes.[38]

NEC is a devastating disease that is set to grow in incidence as preterm survival increases around the world. Our study provides precise baseline data to inform the design of future clinical trials, and indicates that national and international collaboration will be necessary to achieve adequate power to test preventive stratagems. MM appears to confer some protection but a substantial proportion of mothers delivering preterm are unable to provide sufficient milk over the period of maximum NEC risk. For this reason whether HDM or BOF is the optimum product when MM is unavailable or insufficient remains an important ongoing uncertainty in global newborn care.

REFERENCES

1. PROSPERO. International prospective register of systematic reviews. Available from: http://www.crd.york.ac.uk/PROSPERO/.

2. Quigley M, McGuire W. Formula versus donor breast milk for feeding preterm or low birth weight infants. Cochrane Database Syst Rev. 2014;22(4).

3. Corpeleijn WE, de Waard M, Christmann V, et al. Effect of donor milk on severe infections and mortality in very low-birth-weight infants: The early nutrition study randomized clinical trial. Jama, Pediatr. 2016.

4. Sullivan S, Schanler RJ, Kim JH, Patel AL, Trawöger R, Kiechl-Kohlendorfer U, et al. An Exclusively Human Milk-Based Diet Is Associated with a Lower Rate of Necrotizing Enterocolitis than a Diet of Human Milk and Bovine Milk-Based Products. The journal of pediatrics. 2010;156(4):562-7.e1.

5. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012;379(9832):2162-72.

6. Luig M, Lui K. Epidemiology of necrotizing enterocolitis--Part II: Risks and susceptibility of premature infants during the surfactant era: a regional study. J Paediatr Child Health. 2005 Apr;41(4):174-9.

7. Guthrie SO, Gordon PV, Thomas V, Thorp JA, Peabody J, Clark RH. Necrotizing enterocolitis among neonates in the United States. J Perinatol. 2003 Jun;23(4):278-85.

8. Gagliardi L, Bellu R, Cardilli V, De Curtis M. Necrotising enterocolitis in very low birth weight infants in Italy: incidence and non-nutritional risk factors. J Pediatr Gastroenterol Nutr. 2008 Aug;47(2):206-10.

9. Moro M, Perez-Rodriguez J, Figueras-Aloy J, Fernandez C, Domenech E, Jimenez R, et al. Predischarge morbidities in extremely and very low-birth-weight infants in Spanish neonatal units. Am J Perinatol. 2009 May;26(5):335-43.

10. Shah TA, Meinzen-Derr J, Gratton T, Steichen J, Donovan EF, Yolton K, et al. Hospital and neurodevelopmental outcomes of extremely low-birth-weight infants with necrotizing enterocolitis and spontaneous intestinal perforation. J Perinatol. 2012;32(7):552-8.

11. Hintz SR, Kendrick DE, Stoll BJ, Vohr BR, Fanaroff AA, Donovan EF, et al. Neurodevelopmental and growth outcomes of extremely low birth weight infants after necrotizing enterocolitis. Pediatrics. 2005 Mar;115(3):696-703.

12. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed. 2007 May;92(3):F193-8.

13. Schanler RJ, Lau C, Hurst NM, Smith EOB. Randomized Trial of Donor Human Milk Versus Preterm Formula as Substitutes for Mothers' Own Milk in the Feeding of Extremely Premature Infants. Pediatrics. 2005 August 1, 2005;116(2):400-6.

14. Lucas A, Cole TJ. Breast milk and neonatal necrotising enterocolitis. Lancet. 1990 Dec 22-29;336(8730):1519-23.

15. Spencer A, Modi N. National neonatal data to support specialist care and improve infant outcomes. Archives of Disease in Childhood - Fetal and Neonatal Edition. 2012 January 3, 2012.

16. Pan H, Cole T. LMS Growth, a Microsoft Excel add-in to access growth references based on LMS method. Version 2.77 [Online]. 2014 [cited 2015 January 19]; Available from: http://www.healthforallchildren.com.

17. Roberts D, Dalziel S. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. Cochrane Database Syst Rev. 2006;19(3).

18. Breslow N, Day N. Statistical methods in cancer research International Agency for Research on Cancer 1994.

19. Benjamini Y, Hochberg Y. A Practical and Powerful Approach to Multiple Testing Journal of the Royal Statistical Society Series B(Methodological). 1995 1995;57(1):289-300.

20. Rubin DB. Multiple imputation for nonresponse in surveys. 2nd ed. New York: Wiley 2002.

21. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computering 2016; Available from: http://www.R-project.org.

22. Palmer SR, Biffin A, Gamsu HR. Outcome of neonatal necrotising enterocolitis: results of the BAPM/CDSC surveillance study, 1981-84. Arch Dis Child. 1989 Mar;64(3):388-94.

23. Rees CM, Eaton S, Pierro A. National prospective surveillance study of necrotizing enterocolitis in neonatal intensive care units. J Pediatr Surg. 2010 Jul;45(7):1391-7.

24. Costeloe K, Hardy P, Juszczak E, Wilks M, Millar MR. Bifidobacterium breve BBG-001 in very preterm infants: a randomised controlled phase 3 trial. The Lancet. 2015.

25. Berrington JE, Hearn RI, Bythell M, Wright C, Embleton ND. Deaths in Preterm Infants: Changing Pathology Over 2 Decades. The journal of pediatrics. 2012;160(1):49-53.e1.

26. McHaffie HE, Fowlie PW, Hume R, Laing IA, Lloyd DJ, Lyon AJ. Consent to autopsy for neonates. Arch Dis Child Fetal Neonatal Ed. 2001;85(1):F4-7.

27. Arthurs OJ, Thayyil S, Pauliah SS, Jacques TS, Chong WK, Gunny R, et al. Diagnostic accuracy and limitations of post-mortem MRI for neurological abnormalities in fetuses and children. Clin Radiol. 2015;70(8):872-80.

28. Ahle M, Drott P, Andersson RE. Epidemiology and Trends of Necrotizing Enterocolitis in Sweden: 1987–2009. Pediatrics. 2013 August 1, 2013;132(2):e443-e51.

29. Kastenberg ZJ, Lee HC, Profit J, Gould JB, Sylvester KG. Effect of deregionalized care on mortality in very low-birth-weight infants with necrotizing enterocolitis. Jama, Pediatr. 2015;169(1):26-32.

30. Costeloe KL, Hennessy EM, Haider S, Stacey F, Marlow N, Draper ES. Short term outcomes after extreme preterm birth in England: comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ. 2012;4(345).

31. Chen F, Bajwa NM, Rimensberger PC, Posfay-Barbe KM, Pfister RE. Thirteen-year mortality and morbidity in preterm infants in Switzerland. Archives of Disease in Childhood - Fetal and Neonatal Edition. 2016 April 8, 2016.

32. Sankaran K, Puckett B, Lee DS, Seshia M, Boulton J, Qiu Z, et al. Variations in incidence of necrotizing enterocolitis in Canadian neonatal intensive care units. J Pediatr Gastroenterol Nutr. 2004 Oct;39(4):366-72.

33. Yee WH, Soraisham AS, Shah VS, Aziz K, Yoon W, Lee SK, et al. Incidence and Timing of Presentation of Necrotizing Enterocolitis in Preterm Infants. Pediatrics. 2012 February 1, 2012;129(2):e298-e304.

34. Gordon PV, Clark R, Swanson JR, Spitzer A. Can a national dataset generate a nomogram for necrotizing enterocolitis onset? J Perinatol. 2014;34(10):732-5.

35. Sharma R, Hudak ML, Tepas JJ, III, Wludyka PS, Marvin WJ, Bradshaw JA, et al. Impact of gestational age on the clinical presentation and surgical outcome of necrotizing enterocolitis. J Perinatol. 2006;26(6):342-7.

36. Arslanoglu S, Corpeleijn W, Moro G, Braegger C, Campoy C, Colomb V, et al. Donor human milk for preterm infants: current evidence and research directions. J Pediatr Gastroenterol Nutr. 2013;57(4):535-42.

37. Unger S, O'Connor D, editors. DoMINO: Donor milk for improved neurodevelopmental outcomes Hot Topics 2015; Washington DC.

38. Kuschel CA, Harding JE. Multicomponent fortified human milk for promoting growth in preterm infants. Cochrane Database Syst Rev. 2004(1):CD000343.

Figures and tables

Figure 1 Study population by gestation (<32 and ≥32 completed weeks)

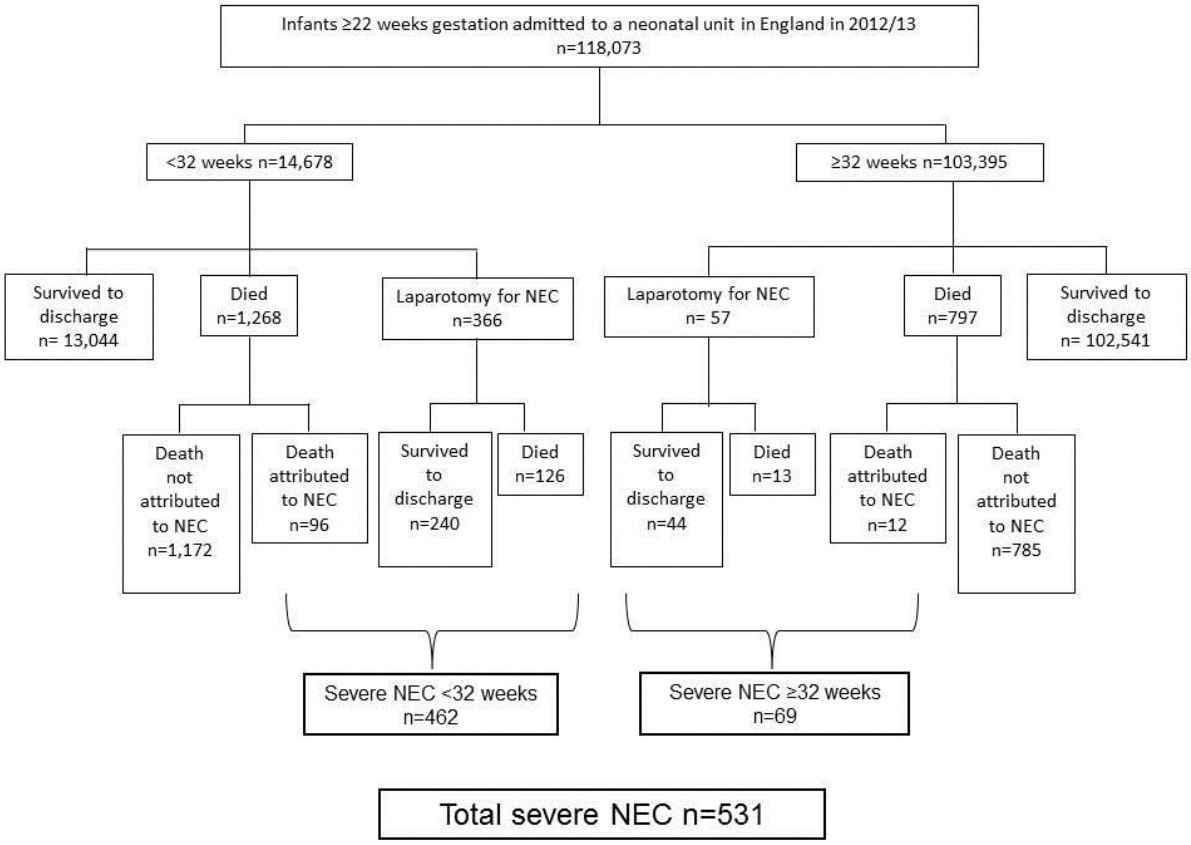


Table 1 Severe NEC: surgical NEC and deaths attributed to NEC by gestation (completed weeks)

	n	NEC confirmed at surgery n=423				NEC deaths no laparotomy (n=108)	Total severe NEC[§] (n=531)
Gestation (completed weeks)	Total n=118,073	Survived† (n=284)	Died[†] (n=139)	Postnatal age at laparotomy[‡]	Postmenstrual age at laparotomy[‡]		
22	14	0	0	—	—	0	0
23	370	13 (44·8%)	16 (55·2%)	25 (12-37)	27·3 (25·3-29·0)	8	37 (10·0%)
24	765	43 (65·2%)	23 (34·8%)	20 (12-38)	27·3 (26·0-30·0)	20	86 (11·2%)
25	886	45 (70·3%)	19 (29·7%)	31 (12-53)	29·6 (26·9-32·9)	13	77 (8·7%)
26	1121	37 (67·3%)	18 (32·7%)	29 (15-39)	30·6 (28·7-31·9)	11	66 (5·9%)
27	1417	25 (61·0%)	16 (39·0%)	13 (9-31)	29·6 (28·6-31·9)	18	59 (4·2%)
28	1793	35 (61·4%)	22 (38·6%)	24 (14-36)	31·9 (30·4-33·6)	13	70 (3·9%)

29	2108	16 (84·2%)	3 (15·8%)	18 (9-32)	32·1 (30·6-33·9)	4	23 (1·0%)
30	2623	17 (89·5%)	2 (10·5%)	11 (7-25)	32·1 (31·4-33·9)	6	25 (1·0%)
31	3581	9 (56·3%)	7(43·7%)	10 (8-17)	32·9 (32·4-33·8)	3	19 (0·5%)
<32	14,678	240 (65·6%)	126 (34·4%)	22 (11-37)	30·5 (27·9-32·7)	96	462 (3·15%)
32 to <37	42,169	29 (72·5%)	11 (27·5%)	9 (6-13)	35.3 (33·9-36·1)	*12	*52
≥37	61,226	15	2	6 (4-10)	40.3 (39·3-41·0)	0	*17

All values are n (%) unless otherwise indicated

† data are n (% of infants with NEC confirmed at surgery)

‡median (Interquartile range) for 422 infants whom for postnatal age (days) and postmenstrual age (weeks) at laparotomy were available. Postmenstrual age is the gestational age at birth plus the chronological age after birth.

§ n (% of total infants in gestational age group), severe NEC is defined as NEC confirmed at laparotomy, histology or autopsy, or resulting in death

*Raw numbers only

Figure 2 Incidence of severe NEC, infants <32 weeks gestation

Bars are 95% confidence interval; gestation category labelled 23 weeks represents 22 and 23 weeks (there were 14 infants at 22 weeks gestation with no cases of severe NEC)

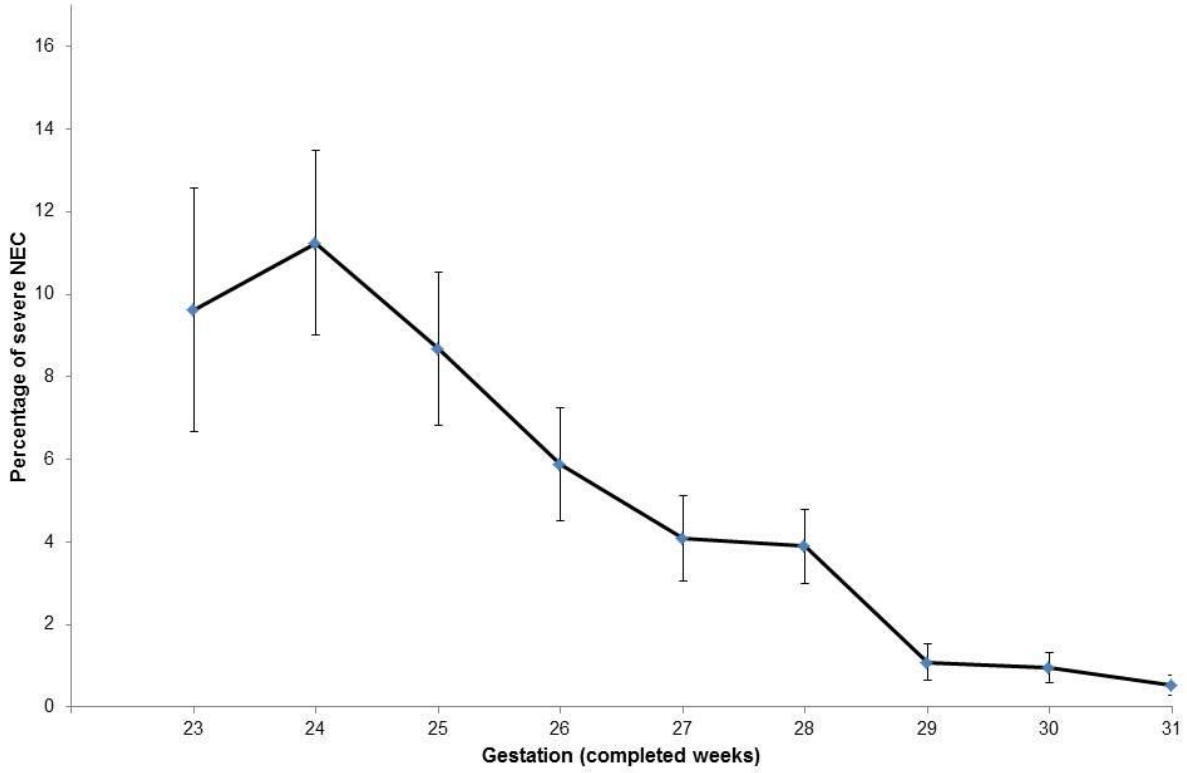


Table 2 Unadjusted and adjusted odds ratio (OR) for severe NEC for infants <32 weeks gestation

Variable		Unadjusted OR for Severe NEC	95% CI	Adjusted OR for Severe NEC	95% CI
Gestation (weeks)	Per additional week	0·68	0.65-0.70	0.67†	0·65-0·70 ***
Birth-weight z score	Per unit decrease in SDS	1·30	1·18-1·42	1·29‡	1·17-1·43***
Any antenatal corticosteroids	Yes	0·91	0·68 - 1·23	0.99§ *Reference*	0·75 to 1·34

†Adjusted for birth-weight z score (continuous) and antenatal corticosteroids

‡Adjusted for gestational age (completed weeks) and antenatal corticosteroids

§Adjusted for birth-weight z score (continuous) and gestation (weeks)

***p<0.05

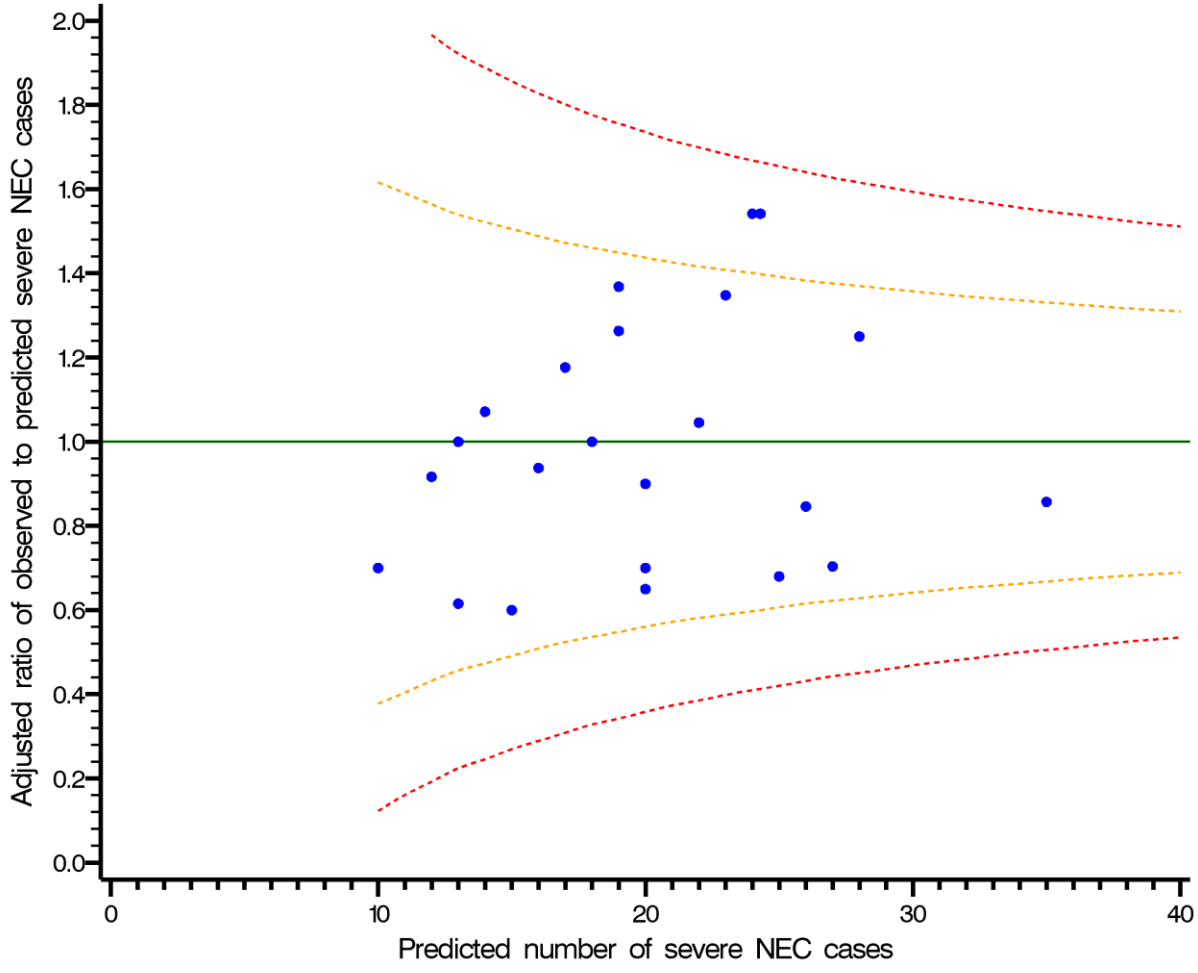
No significant interaction found between gestation and birth-weight z score

Infants with missing data for antenatal corticosteroids were assumed not to have received any antenatal corticosteroids

SDS standard deviation score

Figure 3

Standardised severe NEC ratio (ratio of observed to predicted rates of severe NEC) for infants <32 weeks gestation by neonatal network. Predicted rates are based on multivariate logistic regression (adjusted for gestation, birth-weight z score and antenatal corticosteroids)



Contributors

CB, KC and NM were involved in the study inception and protocol development; CB extracted and prepared the dataset; CB, SM and NL analysed the data; CB wrote the first draft of the paper. All authors reviewed the paper, suggested revisions, and approved the final version submitted; NM is the guarantor.

***Data from the following UK Neonatal Collaborative neonatal networks (neonatal units: UKNC-NEC Study leads) were included in this study:**

Cheshire & Merseyside (Arrowe Park Hospital: Dr O Rackham; Countess of Chester Hospital: Dr S Brearey; Leighton Hospital: Dr A Thirumurugan; Liverpool Women's: Dr N Subhedar; Macclesfield District General Hospital: Dr G Whitehead; Ormskirk District General Hospital: Dr T McBride; Warrington Hospital: Dr C Zipitis, Dr D Webb, Dr H Satish; Whiston Hospital: Dr L Chilukuri)

East of England Perinatal Network (Basildon Hospital: Dr N Sharief; Bedford Hospital: Dr R Kadalraja, Dr Mittal; Broomfield Hospital: Dr R N Mahesh Babu; Colchester General Hospital: Dr S Dalton; Hinchingbrooke Hospital: Dr H Dixon; Ipswich Hospital: Dr M James; James Paget Hospital: Dr V Jayalal; Lister Hospital: Dr J Kefas; Luton & Dunstable Hospital: Dr J Birch; Norfolk & Norwich University Hospital: Dr M Dyke; Peterborough City Hospital: Dr S Babiker; Princess Alexandra Hospital: Dr T Soe; Queen Elizabeth Hospital: Dr S Rubin; Rosie Maternity Hospital: Dr A Ogilvy-Stuart; Southend Hospital: Dr A Khan; Watford General Hospital: Dr S Narayanan; West Suffolk Hospital: Dr I Evans)

Greater Manchester (North Manchester General Hospital: Dr N Panasa; Royal Oldham Hospital: Dr J Moise, Dr N Maddock; Royal Albert Edward Infirmary: Dr C Zipitis; Royal Bolton Hospital: Dr C Turner; St Mary's Hospital: Dr N Edi-Osagie, Dr E

Gasiorowski; Stepping Hill Hospital: Dr C Heal; Tameside General Hospital: Dr J Birch, Dr A Date; University Hospital of South Manchester: Dr A Elazabi)

Kent & Medway (Darent Valley Hospital: Dr A Hasib; Maidstone, Tunbridge Wells Hospital: Dr H Kisat; Medway Maritime Hospital: Dr G Ramadan; Queen Elizabeth Margate, William Harvey Hospital: Dr V Vasu)

Lancashire & Cumbria (Furness General Hospital: Dr A Olabi; Royal Lancaster Infirmary: Dr J Fedee; Lancashire Women and Newborn Centre, Burnley: Dr S Sivashankar; Royal Preston Hospital: Dr R Gupta; Victoria Hospital: Dr Rawlingson)

Midlands Central (George Eliot Hospital, University Hospital Coventry: Dr P Satodia; Kettering General Hospital: Dr. P Rao; Northampton General Hospital, Warwick Hospital: Dr F Thompson, Dr S Gupta; Queen's Hospital Burton on Trent: Dr A Manzoor)

Midlands North Staffordshire, Shropshire and Black Country (Manor Hospital: Dr AK Bhaduri; New Cross Hospital: Dr A Skinner; Royal Shrewsbury Hospital: Dr S Deshpande; Russells Hall Hospital: Dr T Pillay; Staffordshire General Hospital: Dr KK Tewary; University Hospital of North Staffordshire: Dr K Palmer)

Midlands South West (Alexandra Hospital: Dr A Short; Worcestershire Royal Hospital: Dr A Gallagher; Birmingham City Hospital: Dr J Nycyk; Birmingham Heartlands Hospital: Dr R Mupanemunda; Good Hope Hospital: Dr J Meran; Birmingham Women's Hospital: Dr I Morgan, Dr A Bedford-Russell; Hereford County Hospital: Dr HC Underhill)

North Central London (Barnet Hospital, Chase Farm Hospital: Dr T Wickham; The Royal Free Hospital: Dr V van Someren; University College Hospital: Dr S Watkin; Whittington Hospital: Dr R Blumberg)

North East London (Homerton Hospital: Dr N Aladangady; King George Hospital, Queen's Hospital: Dr B Sharma; Newham General Hospital, North Middlesex University Hospital: Dr L Alsford; The Royal London Hospital, Whipps Cross University Hospital: Dr C Sullivan)

North Trent (Barnsley District General Hospital: Dr S Hamdan; Bassetlaw District General Hospital: Dr H Mulenga; Diana Princess of Wales Hospital: Dr P Adiotomre; Scunthorpe General Hospital: Miss A Jackson; Doncaster Royal Infirmary: Dr JS Ahmed; Chesterfield & North Derbyshire Royal Hospital: Dr A Foo; Rotherham District General Hospital: Dr C Harrison; The Jessop Wing, Sheffield: Dr E Pilling)

North West London (Chelsea & Westminster Hospital: Dr S Uthaya; Ealing Hospital: Dr R Mathur; Hillingdon Hospital: Dr M Cruwys; Northwick Park Hospital: Dr C Philipp, Dr R Nicholl; West Middlesex University Hospital: Dr E Eyre)

Northern (Cumberland Infirmary, West Cumberland Infirmary; Dr P Whitehead and M Ben-Hamida; Darlington Memorial Hospital, University Hospital of North Durham: D A Bowes; James Cook University Hospital, Friarage : Dr N Sabrine; Queen Elizabeth Hospital, Gateshead: Dr D Bosman; Royal Victoria Infirmary: Dr N Embleton; South Tyneside District Hospital: Dr R Bolton; Sunderland Royal Hospital: Dr M Abu-Harb; University Hospital of North Tees: Dr C Harikumar ; Wansbeck General Hospital: Dr J Olivier)

Peninsula (Derriford Hospital: Dr N Maxwell; North Devon District Hospital: Dr Y Cherinet; Royal Cornwall Hospital: Dr P Munyard; Royal Devon & Exeter Hospital: Dr N Osbourne; Torbay Hospital: Dr M Raman)

South East London (Guy's & St Thomas' Hospital: Dr K Turnock; King's College Hospital: Dr A Hickey; Princess Royal University Hospital, Queen Elizabeth Hospital: Dr O Banjoko; University Hospital Lewisham: Dr J Kuna)

South West London (Croydon University Hospital: Dr A Kumar; Epsom General Hospital: Dr K Watts; St Helier Hospital: Dr R Shephard; Kingston Hospital: Dr D Lindo; St George's Hospital: Dr L De Rooy)

South Central South Coast (North & South) (Basingstoke & North Hampshire Hospital: Dr R Wigfield; Dorset County Hospital: Dr P Wylie; Milton Keynes Foundation Trust Hospital: Dr I Misra; Oxford University Hospitals, Horton Hospital: Dr N Shettihalli; John Radcliffe Hospital: Dr E Adams; Poole Hospital NHS Foundation Trust: Dr M Khashu; Princess Anne Hospital: Dr F Pearson; Queen Alexandra Hospital: Dr C Groves; Royal Berkshire Hospital: Dr P de Halpert; Royal Hampshire County Hospital: Dr D Schapira; Salisbury District Hospital: Dr N Brown; St Mary's Hospital Isle of Wight: Dr C Burtwell; St Richard's Hospital: Dr N Brennan; Stoke Mandeville Hospital: Dr S Salgia; Wexham Park Hospital: Dr R Sanghavi)

Surrey and Sussex (Conquest Hospital: Dr G Whincup; East Surrey Hospital: Dr K Khader; Frimley Park Hospital: Dr A Mallik; Princess Royal Hospital, Royal Sussex County Hospital: Dr P Amess; Royal Surrey County Hospital: Dr M Hardo; St Peter's Hospital: Dr P Reynolds; Worthing Hospital: Dr E Vamvakiti)

Trent (King’s Mill Hospital: Dr V Noble; Lincoln County Hospital and Pilgrim Hospital: Dr AS Rao; Nottingham City Hospital, Nottingham University Hospital (QMC): Dr S Wardle, Dr J Dorling; Royal Derby Hospital: Dr M Ratnayaka)

Western (Gloucestershire Royal Hospital: Dr J Holman; Great Western Hospital: Dr S Zengeya; Royal United Hospital: Dr S Jones; Southmead Hospital: Dr P Mannix;

St Michael's Hospital: Dr P Cairns; Taunton & Somerset Hospital: Dr RJ Mann; Yeovil District Hospital: Dr M Eaton)

Yorkshire (Airedale General Hospital: Dr M Babirecki; Bradford Royal Infirmary: Dr S Oddie; Calderdale Royal Hospital: Dr K Schwarz; Dewsbury & District Hospital, Pontefract General Infirmary (Pinderfields): Dr D Gibson; Harrogate District Hospital: Dr C Jampala; Hull Royal Infirmary: Dr K Green, Dr J Preece; Leeds Neonatal Service: Dr K Johnson; Scarborough General Hospital: Dr A Hawkridge; York District Hospital: Dr G Millman)

Acknowledgements

We acknowledge assistance from Neonatal Data Analysis Unit analysts Eugene Statnikov and Daniel Gray, manager Richard Colquhoun and the support of members of the boards of the Neonatal Data Analysis Unit and Medicines for Neonates Programme (Jane Abbott, Deborah Ashby, Peter Brocklehurst, Kate Costeloe, Elizabeth Draper, Michael Goldacre, Jacquie Kemp, Azeem Majeed, Stavros Petrou, Andrew Wilkinson, Alys Young).

Declaration of interests: In the last 5 years NM has received grants from the NIHR, Medical Research Council, British Heart Foundation, Action Medical Research, HCA International, Westminster Medical School Research Trust, Bliss, Department of Health, NHS England, and NHS London, and consultancy fees from Ferring International. Other authors have no conflicts of interests to declare.