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Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: a protocol for a UK based observational study using routinely recorded data

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ABSTRACT

Introduction Necrotising enterocolitis (NEC) remains a major contributor to preterm mortality and morbidity. Prolonged duration of antibiotic therapy after delivery is associated with later NEC development but recent evidence suggests that absence of antibiotic treatment after delivery may also increase NEC risk. We will explore this controversy using a large pre-existing dataset of preterm infants in the UK.

Methods and analysis This is a retrospective cohort study using data from UK National Neonatal Research Database (NNRD) for infants born 1 January 2012 to 31 December 2020. Eligible infants will be <32 weeks gestation, alive on day 3. Primary outcome is development of severe NEC, compared in infants receiving early antibiotics (days 1–2 after birth) and those not. Subgroup analysis on duration of early antibiotic exposure will also occur. Secondary outcomes are: late onset sepsis, total antibiotic use, predischarge mortality, retinopathy of prematurity, intraventricular haemorrhage, bronchopulmonary dysplasia, focal intestinal perforation and any abdominal surgery. To address competing risks, incidence of death before day 7, 14 and 28 will be analysed. We will perform logistic regression and propensity score matched analyses. Statistical analyses will be guided by NEC risk factors, exposures and outcome presented in a causal diagram. These covariates include but are not limited to gestational age, birth weight, small for gestational age, sex, ethnicity, delivery mode, delivery without labour, Apgar score, early feeding and probiotic use. Sensitivity analyses of alternate NEC definitions, specific antibiotics and time of initiation will occur.

Ethics and dissemination We will use deidentified data from NNRD, which holds permissions for the original data, from which parents can opt out and seek study-specific research ethics approval. The results will help to determine optimal use of early antibiotics for very preterm infants.

Implications This data will help optimise early antibiotic use in preterm infants.

Trial registration number ISRCTN55101779.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Use of the National Neonatal Research Database gives access to a very large dataset of preterm infants.
⇒ The primary outcome (necrotising enterocolitis (NEC)) and the many contributory covariates are routinely recorded in this dataset.
⇒ Analysis by both regression and using propensity matching optimises learning from this large dataset.
⇒ Data entry may not always be as accurate as that collected specifically within a trial.
⇒ The diagnosis of NEC has no gold standard to allow standardisation across units.

INTRODUCTION

Around 3% of all babies are born very preterm (VPT, <32 weeks' gestation) and they require prolonged hospital stay, commonly including intensive care. Survival in these VPT infants (VPTI) has increased dramatically in recent years, but death is still common (~10% overall) as are life-long physical and cognitive impairment.[1] In the UK around 10 000 VPTI are born every year, representing an annual cost to the National Health Service (NHS) of ~£3 billion.[2] The most common cause of death or serious illness in preterm infants after the first few days are gut or infectious complications such as necrotising enterocolitis (NEC) or late onset sepsis.[3] Although knowledge around NEC, and preventive practices such as use of mothers own milk, donor human milk and probiotics are increasing, there has been little reduction in NEC incidence over recent years,[4 5] and mechanisms underlying the development of NEC are poorly understood. Antibiotic use as part of neonatal intensive care is common, particularly immediately after birth when infection

is implicated in preterm delivery—studies show more than half of infants weighing <1000 g routinely received more than 5 days antibiotics at birth.[6] Antibiotic use in VPTI has been implicated in NEC development in several ways. Studies show an increase in NEC incidence with increased duration of empirical early antibiotics[7 8] and alteration of the gut microbiotia (dysbiosis) has been mechanistically linked to NEC development.[9] However, recent observational data from 13 Neonatal Intensive Care Units (NICUs) from 5 continents (n=2831) identified that NEC incidence was higher in infants who did not receive empirical antibiotics early after birth, despite higher gestational age, compared with those receiving them (OR: 1.8 (95% CI 1.1 to 2.9)), with even higher OR when adjusted for relevant confounders (OR: 4.0 (95% CI 2.1 to 7.3)).[10] In contrast, results from a very recent study in preterm infants with low risk of infection shows opposite trends of lower odds in those not treated, but is underpowered for NEC as outcome (n=641, OR: 0.7 (95% CI 0.3 to 1.5)).[11] There is an increasing focus on antibiotic stewardship, and it can be expected that the proportion of infants that are not given antibiotics after preterm birth will increase in the coming years. Therefore, it is important to know if lower early usage of antibiotics will increase the incidence of NEC. While there have been calls for a trial of routine early antibiotic treatment[12] in VPT babies, and a single trial has so far attempted to do this, there are important logistical difficulties[13] with such an approach.

The National Neonatal Research Database (NNRD) provides a large, population level dataset that can be used to further test the hypothesis that early empiric antibiotic treatment reduces the incidence of NEC in preterm infants, and allows adjustment for confounding through the large number of patient level covariates recorded in the NNRD.

METHODS AND ANALYSIS

Design

Retrospective cohort study using routinely recorded clinical data held in the NNRD.

Data source

NNRD holds data from all infants admitted to NHS neonatal units in England and Wales around 90 000 infants annually. Neonatal units in England and Wales have contributed data since 2012. Data are entered by contributing units to a point-of-care electronic dataset and a defined dataset is extracted by NNRD. Data are extracted quarterly and sent to the Neonatal Data Analysis Unit, based at Imperial College, London.[14] The data include variables pertinent to the present analysis, including demographics, exposure and outcome variables.

Eligibility criteria

Eligible infants must have been born at <32 weeks gestation, be cared for in a unit contributing data to NNRD, and be alive at day 3. Infants will be excluded if they have a known severe congenital or gastrointestinal anomaly (excluding the presence of a patent ductus arteriosus, online supplemental etables 1 and 2) or have had abdominal surgery before day 3.

Time period

Infants born between 1 January 2012 and 31 December 2020 will be included.

Setting

UK neonatal units in England and Wales contributing to NNRD.

Definitions

Exposure (primary)

Receipt of any intravenous antibiotic drug (online supplemental appendix 1) for any of the first 2 days after birth.

Comparator: did not receive any antibiotics for any of the first 2 days after birth.

Primary outcomes

Severe NEC resulting in death or surgery as defined by Battersby *et al*.[4]

Secondary outcomes

Secondary outcomes for analysis are the effects of early antibiotic exposure on:

- Late onset sepsis (blood stream or cerebrospinal fluid (CSF) confirmed pure growth in culture (National Neonatal Audit Programme (NNAP) definition) after first 3 days and/or treatment with 5 days of antibiotics and a concurrent diagnosis of infection after the first 3 days).
- Total antibiotic use (number of days with any treatment of antibiotics during admission).
- Length of stay (postnatal age at discharge or death).
- Time to reach full feeding (first day of 3 consecutive days where parenteral nutrition or intravenous fluid are not recorded.
- Growth (change in SD score between birth and 36 weeks and discharge).

Further, we will analyse effects on some relevant adverse outcomes:

- Total predischarge mortality.
- Death prior to day 7, day 14, day 28.
- Bronchopulmonary dysplasia (respiratory support given at 36 weeks).
- Retinopathy of prematurity (ROP) (received treatment for ROP, according to NNRD definition).
- Brain injury (intraventricular haemorrhage grade 3 or above or cystic leukomalacia diagnoses recorded).
- Need for surgical procedures (online supplemental appendix 1).

Comparison of different durations of early antibiotic exposure will be performed based on the following categories:

- Antibiotic duration no longer than 3 days.
- Antibiotic duration 3–5 days.

- Antibiotic duration longer than 5 days without positive culture (blood stream or CSF confirmed pure growth in culture (NNAP definition) in the first 3 days.

For the above analyses, infants with a positive blood or CSF culture in the first 3 days will be excluded.

A specific subgroup of interest are the infants that are considered to have low risk of early onset sepsis (EOS), specified as fulfilling all of the following prenatal characteristics: no premature rupture of membranes, no labour and no (suspected) chorioamnionitis. Additional subgroup analyses will be performed for infants with gestation age <28 weeks and birth weight <1000 g.

Sample size

Observed NEC incidence noted in a previous study on a total 2831 infants from five different continents, using criteria for NEC diagnosis in keeping with pragmatically defined NEC, was 9% when early antibiotic treatment was absent and 4% when antibiotic was provided in the first 3 days.[10] We hypothesise to find a similar antibiotic related proportional reduction in incidence of severe NEC in this study, based on data collected over 9 years (2012–2020) from around 45 000 infants. In an earlier report based on an NNRD subgroup, the incidence of severe NEC was 3.2% for infants born <32 weeks.[4] The cohort event estimate is 1440 cases.

Data required

Online supplemental appendix 1 carries the full list of variables considered relevant for extraction from NNRD including definitions of constructed items/variables.

Potential confounders

Several covariates are relevant to include in the analysis as potential confounders. We will take a hypothesis driven approach to the selection of covariates. A causal diagram (directed acyclic graphs, DAG, figure 1) is drawn and analysed with relevant variables and potential confounders related to antibiotic exposure and NEC outcome. Nodes and edges are determined based on literature and subject matter knowledge. The selected covariates are considered to reflect conditions prior to the defined exposure (ie, within day 1–2 after birth). For several variables, only proxies will be available (table 1).

STATISTICAL ANALYSES

Primary analyses

Previous work using logistic regression included the following covariates in the model for the hypothesis: NICU (random effect)+gestational age+birthweight+-sex+delivery mode+APGAR scores+antenatal steroids+-feeding type. We aim to test the hypothesis with data from NNRD using the same regression model as used in the previous work (variables 1–8 in table 2) and also an expanded regression model with inclusion of all potentially relevant variables (table 2). Results will be presented as adjusted ORs with 97.5% CIs and Bonferroni-adjusted p values (unadjusted p values multiplied by 2). To better quantify the causal effect of antibiotics, standardised risk differences with 97.5% bootstrap CIs will also be presented.

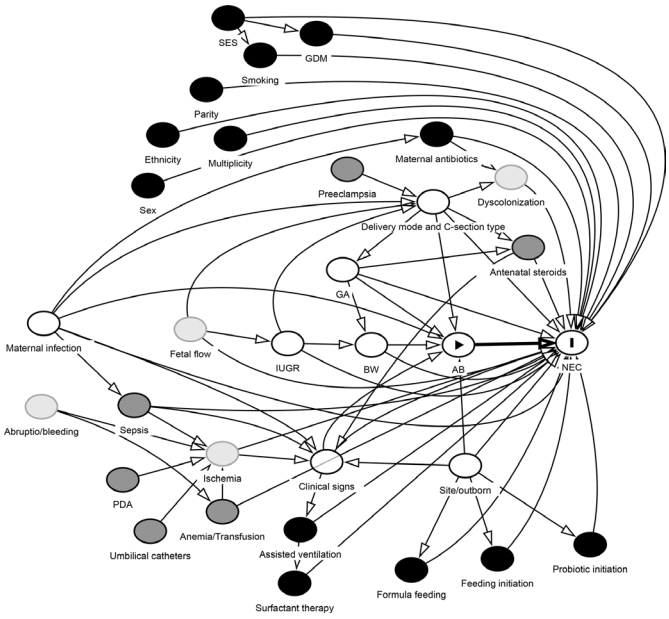

Figure 1 Directed acyclic graphs (DAG) diagram of causal assumptions related to the hypothesis based on subject-matter knowledge, used for confounder selection. Model code text for figure and interactive diagram analysis on dagitty.net is available in online supplemental appendix 2. Node with arrowhead: exposure; node with I: outcome; black nodes: ancestor of outcome; dark grey nodes: ancestor of exposure and outcome; white nodes: adjusted variables (primary analysis); thick arrow: causal path; thin arrows: non-biasing paths. AB, early antibiotics; BW, birth weight; GA, gestational age; GDM, gestational diabetes mellitus; IUGR, intrauterine growth restriction; NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; SES, socioeconomic status.

Priority of covariates

Covariates to include in the model are listed and prioritised in table 2. Confounders are ranked higher based on importance, that is, variables which arguably have effect on both outcome (NEC) as well as exposure (decision to start antibiotic treatment, which relates to infection risk/concern). Assessment of covariate importance is based on subject matter knowledge and scientific literature (references in table 2). For several variables, it is unclear whether there is a relevant effect on NEC and a conservative approach is employed to include potential confounders in the model.[15] Similar considerations apply for assessment of variables with relevant effect on decision to treat with early antibiotics. These variables will be included in the regression for propensity score calculation and subsequent matching. For highly similar variables, the lower priority or quality variables may be omitted if necessary (eg, multi-collinearity issues). Variables with very low quality (eg, too many missing values) will be omitted. For categorical variables, groups with very few observations will be removed (eg, separation issues).

Table 1 Overview of variables

Variable	Class/type	Expected availability in NNRD	Importance for effect estimation
AB	Exposure	Available (definable)	
NEC	Outcome	Available (definable)	
Site	Confounder	Available	Minimal sufficient adjustment set to model the direct and total effect of AB on NEC according to proposed DAG
BW	Confounder	Available	
Delivery mode*	Confounder	Available (categories)	
Clinical signs†	Confounder	Available (proxies)	
Maternal infection	Confounder	Available (clinical)	
GA	Confounder	Available	
IUGR	Confounder	Available (definable)	
Fetal flow	Ancestor of exposure and outcome (indirect)	Unobserved	Blocked by IUGR and delivery mode
Ischaemia	Ancestor of exposure and outcome (indirect)	Unobserved	Blocked by clinical signs
Pre-eclampsia	Ancestor of exposure and outcome (indirect)	Available	Blocked by delivery mode
Antenatal steroids	Ancestor of outcome	Available	Blocked by clinical signs
Sepsis	Ancestor of exposure and outcome (indirect)	Available (definable/proxy)	Blocked by clinical signs
PDA	Ancestor of exposure and outcome (indirect)	Available (definable)	Blocked by clinical signs
Umbilical catheters	Ancestor of exposure and outcome (indirect)	Available	Blocked by clinical signs
Anaemia/transfusion	Ancestor of exposure and outcome (indirect)	Available (proxy, that is, transfusions)	Blocked by clinical signs
Sex	Ancestor of outcome	Available	Precision variable
Ethnicity	Ancestor of outcome	Available	Precision variable
Multiparity	Ancestor of outcome	Available	Precision variable
Smoking	Ancestor of outcome	Available	Precision variable
GDM	Ancestor of outcome	Available	Precision variable
Socioeconomic status	Ancestor of outcome	Available (proxy that is, deprivation score)	Precision variable
Maternal antibiotics	Ancestor of outcome	Available (intra partum)	Precision variable
Dyscolonisation	Ancestor of outcome	Unobserved	Precision variable
Assisted ventilation	Ancestor of outcome	Available	Precision variable
Surfactant therapy	Ancestor of outcome	Available	Precision variable
Formula feeding	Ancestor of outcome	Available	Precision variable
Feeding initiation	Ancestor of outcome	Available	Precision variable
Probiotic initiation	Ancestor of outcome	Available	Precision variable

*Specification of different clinical conditions with important impact on decision to treat with AB, categorised as: vaginal AND spontaneous, vaginal AND induced, emergency caesarean AND labour, emergency caesarean AND no labour, elective caesarean AND labour, elective caesarean AND no labour.
†Respiratory/circulatory/unspecific signs/symptoms/parameters used clinical assessment and decision making related to decision to treat with antibiotics.
AB, early antibiotics; BW, birth weight; DAG, directed acyclic graphs; GA, gestational age; GDM, gestational diabetes mellitus; IUGR, intrauterine growth restriction; NEC, necrotising enterocolitis; NNRD, National Neonatal Research Database; PDA, patent ductus arteriosus.

Estimated effects of each variable included in the model included will be reported. Based on the recommendation to have at least 10 events per variable,[16] with the event estimate approximately 1500 cases, this will provide 150 df in the model. Based on the proposed covariates listed in table 2, the required df for analysis is 108. If the actual

Table 2 Priority of covariates to include in model based on DAG and availability from NNRD

		Influence on NEC[27–29]	Influence on AB-start (decision to treat based on sepsis risk)[30 31]	**Potential repetition/ redundancy**	**Relation to node in DAG**	**Structure (continuous or number of categories)**
1	Neonatal Intenisve Care Unit/site	Yes	Yes		Site	Random
2	GA	Yes	Yes		GA	Continuous
3	BW	Yes	Yes		BW	Continuous
4	Sex	Yes	No?		Sex	Dichotomous
5	APGAR5	Yes	Yes		Clinical signs	11 categories (0–10)
6	Delivery mode+expanded (6 categories)	Yes	Yes		Delivery mode and type	6 (see table 1)
7	Maternal antenatal steroids	Yes	Yes? (indicator of fetal status/delivery conditions)		Antenatal steroids	None/incomplete/ complete
8	Feeding first day	Yes	No		Feeding	1: Enteral feeding on day 1–2, human milk only 2: Enteral feeding on day 1–2, formula only 3: Enteral feeding on day 1–2, mix 3: No enteral feeding on day 1–2
9	IUGR	Yes	Yes		IUGR	Dichotomous (less than −2SDS)
10	APGAR1	Yes	Yes?			11 categories (0–10)
11	APGAR10	Yes	Yes		Clinical signs	11 categories (0–10)
12	EOS	Yes?	No		Sepsis	Dichotomous
13	Birth year (epoch)	Yes	Yes		(Similar to site/ standards)	4–5
14	Transfer on first day	Yes	Yes		Site/outborn	Dichotomous
15	Level of initial unit	Yes	Yes		Site	Dichotomous
16	Maternal pre-eclampsia requiring preterm birth	Yes?	Yes		Pre-eclampsia	Dichotomous
17	Prolonged ROM	Yes?	Yes		Maternal infection	Dichotomous
18	Maternal suspected chorioamnionitis	Yes?	Yes	Defined by antibiotics and fever	Maternal infection	Dichotomous
19	Intrapartum antibiotics	Yes?	Yes (in relation to chorioamnionitis)		Maternal antibiotics	Dichotomous
20	Maternal fever	Yes?	Yes (untreated chorioamnionitis)		Maternal infection	Dichotomous
21	Maternal GBS	Yes?	Yes		Maternal infection	Dichotomous
22	Umbilical cord pH	Yes	Yes		Clinical signs	Dichotomous: <7.00 yes or no
23	Umbilical cord lactate	Yes	Yes	Resembles pH	Clinical signs	Cont/Di/tri?
24	Base excess 12 hours worst	Yes	Yes		Clinical signs	Dichotomous: <−5 yes/no

Continued

Table 2 Continued

		Influence on NEC[27–29]	Influence on AB-start (decision to treat based on sepsis risk)[30 31]	Potential repetition/ redundancy	Relation to node in DAG	Structure (continuous or number of categories)
25	Umbilical cord base excess	Yes	Yes	Resembles BE 12 hours worst	Clinical signs	Dichotomous: <–5 yes/no
26	Blood transfusion day 1–2	Yes	Yes?		Anaemia	Dichotomous
27	Chest compressions	Yes	Yes?		Clinical signs	Dichotomous
28	Resuscitation drugs at delivery	Yes	Yes?		Clinical signs	Dichotomous
29	Ventilation at delivery	Yes?	Yes? (clinical status at birth)		Assisted ventilation	Dichotomous
30	Spontaneous respiration time	Yes?	Yes?		Clinical signs	3 categories: <1 min, 1–5 min, >5 min
31	Admission temp	Yes	Yes?		Clinical signs	3 categories: <36.5, 36.5–37.5, >37.5
32	Admission oxygen SAT	Yes	Yes		Clinical signs	3 categories: >94, 90–94, <90
33	Inotropes on first day	Yes	Yes?		Clinical signs	Dichotomous
34	Admission mean BP	Yes?	Yes/no?	Resembles inotropes	Clinical signs	Dichotomous: below GA yes/no
35	Ethnicity	Yes	Yes? (risk of inf)		Ethnicity	4 categories as suggested in appendix
36	Maternal deprivation score	Yes?	Yes? (risk of inf)		SES	Deprivation centiles?
37	Intubation first day	?	Yes?		Assisted ventilation	Dichotomous
38	Intubation at delivery	?	Yes?	Resembles intubation d1	Assisted ventilation	Dichotomous
39	Surfactant first day	Yes?	Yes?		Surfactant therapy	Dichotomous
40	Surfactant at delivery	?	Yes?	Resembles intubation d1	Surfactant therapy	Dichotomous
41	Time of cord clamp	Yes/No??	Yes? (clinical status at birth)		Clinical signs	Dichotomous: >60 s yes/no
42	Probiotics	Yes	No		Probiotic initiation	Dichotomous
43	PDA identified day 1–2	Yes	No		PDA	Dichotomous
44	PDA treatment day 1–2	Yes	No		PDA	Dichotomous
45	Multiplicity	?	No?		Multiplicity	Dichotomous
46	Smoking	Yes?	No?		Smoking	Dichotomous
47	Parity	?	No?		Parity	Dichotomous
48	Umbilical catheters	Yes?	No		Umbilical catheters	Dichotomous
49	Parenteral nutrition d1-2	?	?			Dichotomous

Continued

Table 2 Continued

		Influence on NEC[27–29]	Influence on AB-start (decision to treat based on sepsis risk)[30 31]	Potential repetition/ redundancy	Relation to node in DAG	Structure (continuous or number of categories)
50	Admission heart rate	?	?		Clinical signs	3 categories: >200, 100–200, <100
51	Maternal antenatal magnesium sulphate	No?	Yes/no ?	Resembles pre-eclampsia	Pre-eclampsia	Dichotomous
52	Maternal gestational hypertension	No?	No			Dichotomous
53	Maternal diabetes	No?	No		GDM	Dichotomous

BW, birth weight; DAG, directed acyclic graphs; EOS, early onset sepsis; GA, gestational age; GDM, gestational diabetes mellitus; IUGR, intrauterine growth restriction; NEC, necrotising enterocolitis; PDA, patent ductus arteriosus; ROM, rupture of membranes; SES, socioeconomic status.

number of cases in the obtained dataset is much lower than expected, thus providing insufficient df, covariates may be excluded in reverse order of priority. See detailed specifications of listed covariates/items in online supplemental appendix 1.

Sensitivity analyses

The following sensitivity analyses will be performed:

Early antibiotic exposure only with ampicillin or penicillin plus gentamicin, early antibiotic exposure defined by other timings after birth (later initiation and lasting until 4–6 days after birth) and alternative methods for diagnosing NEC (as standards for NEC diagnosis are unclear). For the latter analyses, we will define and reanalyse NEC diagnosis as 'pragmatic NEC' (5 days of nil by mouth and antibiotics and a diagnostic code of NEC) and NEC including focal intestinal perforation diagnosis (FIP). This condition is sometimes difficult to separate from NEC. We will also record infants with laparotomy-confirmed FIP (intestinal perforation, classified as non-NEC) in addition to the primary NEC (Battersby *et al*). definition. The statistical analyses will be repeated using propensity score matching (with propensity scores based on exposure regression), as an alternative approach to logistic regression.

Secondary analyses

We intend to use the same logistic regression models for secondary outcomes, as those specified for the primary outcome. The most important confounders (or proxies) for the secondary outcomes are included in this model. Detailed model specification for each specific secondary outcome as done for the primary outcome is beyond the aim and scope of this study (focusing on NEC). With propensity score matching, direct comparison between antibiotic exposure vs controls can in principle be performed for any outcome, assuming correct model specification for the propensity score.

Exploratory analyses

Additional non-defined exploratory analyses based on findings from the dataset may be performed.

Missing data

We assume that missing data occur randomly between groups and will be imputed 10-fold using multiple imputation by chained equations. Results will be pooled according to Rubin's rule.

Multiple testing

Adjusted p values will be reported with Bonferroni correction of the two primary analyses (along with corresponding 97.5% CIs) and Benjamini-Hochberg adjusted p values from the secondary analyses. Post hoc exploratory analyses will be reported without adjustment of p values and should be interpreted with corresponding caution.

ETHICS AND DISSEMINATION

The study will be registered with International Standard Randomised Controlled Trials Number before opening and is sponsored by Newcastle Hospitals NHS Foundation Trust and the protocol with statistical analysis plan will be uploaded to the Open Science Framework website osf.io prior to data analysis initiation. We will apply for HRA/REC approvals. The study is observational and uses deidentified data that is already collected. Dissemination will be by presentation and publication in peer-reviewed journals.

PATIENT PUBLIC INVOLVEMENT AND IMPORTANCE TO THE NHS

We have worked closely with parents on all our studies. The NEC UK parent group and other parent groups and representatives continue to assert that better understanding of NEC is a key priority. The NHS, parents and babies experience significant burden from NEC in terms of adverse outcome, prolonged hospitalisation,

developmental impact and NHS costs. There is a significant concern related to use of antibiotics in the neonatal population and it is important that studies help optimal use of early antibiotics.

DISCUSSION

This study aims to add relevant scientific information to an important clinical decision made for every preterm infant admitted to a neonatal unit: the use and duration of antibiotics in the absence of clear signs of bacteraemia or EOS. Cases of culture-proven EOS are relatively few, with rates being one to seven per 1000 live births in high-income countries.[17] There are potentially large numbers of infants where a clinical choice is available to withhold early antibiotic treatment. Data are currently conflicting as to the overall impact on NEC of receiving (or withholding) antibiotics in the first days of life. Early bacterial nature and load in the preterm gut have been linked to NEC development.[18 19] Use of intravenous antibiotics shortly after birth may slow colonisation, allowing the gut immune system a short period of adaption that reduces the risk of TLR4 mediated NEC.[20] The integrity of the mucosal barrier has been shown to improve significantly in the first days after preterm birth in humans.[21] Thus, potentially only short duration of very early antibiotic treatment may be relevant for such effect, in contrast to prolonged treatment which have been shown to cause persistent gut dysbiosis[22] that may instead increase NEC risk.[7 8] Data from a piglet model of NEC suggests that antibiotic use is mechanistically linked to preterm NEC development[23] and preterm immune development.[24] However, no difference was seen in total bacterial load of stool in preterm infants who did and did not go on to develop NEC.[25] Given the conflicting data Clinicians need better information to help guide early antibiotic treatment in relation to NEC, especially important as NEC rates in premature infants may actually be increasing.[26] The proposed study using NNRD benefits from access to large numbers of infants with recorded relevant risk factors and outcomes. Large datasets offer the advantage of including many NEC cases, and we anticipate around 1500 informative cases of NEC. These data are increasingly well-validated by individual units at the point of data entry, but are potentially less well-validated than infants with trial data collected within specific trials.

We have in this study given careful thought to handling confounding factors. Analysis of the current understanding of NEC and the use of directed acyclic graph to guide analysis have been undertaken to attempt to control for what are highly complex clinical factors.[27–29] As demonstrated in the DAG many factors, including those on a causal pathway to NEC, impact the decision to administer early antibiotics.[30 31] The aim to analyse this data using both propensity scoring and logistic regression is a major strength for this study and for future analyses using large databases to address complex questions. Propensity scoring has recently been used to address feeding during hypothermia[32] and the impact of early parenteral nutrition on preterm outcomes[33] using the NNRD, but without alternate statistical approach. While both propensity scoring and regression analysis have strengths and weaknesses to the best of our knowledge direct comparison of these methodologies has not been undertaken within large neonatal datasets, and is important methodologically for future neonatal studies. The data generated by this study will thus inform important aspects of wider neonatal care and in relation to early neonatal use of antibiotics and later occurrence of NEC.

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Correction notice This article has been corrected since it published online to reflect the correct author name and affiliations for author Gorm Greisen.

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Contributors NE had the original idea for the study. RS undertook the DAG. RS, JLF, JB, PTS planned statistical analysis. RS, NE, JLF, JB, PTS, CG, GG and SU all contributed to overall study design, protocol development and the writing and review of this paper. JB submitted for registration and approvals.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

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