PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Early antibiotic use and incidence of necrotising enterocolitis in very preterm infants: a protocol for a UK based observational study using routinely recorded data
AUTHORS	Shen, Rene; Embleton, Nicholas; Lyng Forman, Julie; Gale, Chris; Griesen, Gorm; Sangild, Per; Uthaya, Sabita; Berrington, Janet

VERSION 1 – REVIEW

REVIEWER	Seliga-Siwecka, Joanna Warszawski Uniwersytet Medyczny, Neonatology and Neonatal Intensive Care
REVIEW RETURNED	10-Aug-2022

GENERAL COMMENTS	Dear Authors, Congratulations on submitting a very interesting protocol for a study on early use and incidence of necrotizing enterocolitis in very preterm infants. Title The title of the manuscript draws attention of the reader, defines the study population, and outcomes but is not clear on the type of manuscript. Please consider the following title: “Early antibiotic use and incidence of necrotizing enterocolitis in very preterm infants: A protocol for an UK based observational study using routinely recorded data.” Abstract The authors provide a concise overview of the study. It is clearly stated why the study will be undertaken, and how the data will be collected. However, the researchers may consider adding a short summary of possible implications for the future at the end if the abstract. Introduction The authors provide enough contextual information for the journal’s neonatal-paediatric readership to understand the context. They accurately describe up to date research, with is properly referenced. Additionally, limitations and controversies are identified. As stated by the study group, this is a necessary study, and the results will influence antibiotic stewardship of extremely preterm infants within the first 24 hours. Methods Methods and statistical analysis are adequately planned. Nevertheless, given the antibiotic intervals in extremely preterm infants I would suggest changing 1-2 days as indicated by the authors to 36-48 hours. A 12-hour difference in stopping antibiotics will increase the number of antibiotic doses, which might influence NEC incidence? I would also consider excluding infants with “blood negative sepsis”. These patients will most probably represent infants with

	“sepsis-like syndrome”, unnecessarily treated for bacterial infection. Hence, including these infants may skew the results[1; 2; 3; 4]. Please provide the definition of chorioamniotis, as there is a high chance that different definitions will be used between the participating units. The controls are proper, and the outcomes are clearly defined. The statistics are sound, and the study will be conducted under ethical guidance. Discussion The discussion addresses the research problem. Other relevant studies are discussed. I look forward to reading the final manuscript with the study results! [1] J.B. Cantey, and P. Prusakov, A Proposed Framework for the Clinical Management of Neonatal "Culture-Negative" Sepsis. J Pediatr 244 (2022) 203-211. [2] B.R. Hadfield, and J.B. Cantey, Neonatal bloodstream infections. Curr Opin Infect Dis 34 (2021) 533-537. [3] J.B. Cantey, and A.L. Shane, Neonatal-Perinatal Infections: An Update. Clin Perinatol 48 (2021) xix-xx. [4] J.B. Cantey, and J.H. Lee, Biomarkers for the Diagnosis of Neonatal Sepsis. Clin Perinatol 48 (2021) 215-227.

REVIEWER	Lin, Jing Mount Sinai Medical Center, Department of Pediatirics
REVIEW RETURNED	10-Aug-2022

GENERAL COMMENTS	NEC remains a significant cause for mortality and morbidity of very premature infants. Several observational studies have demonstrated that prolonged duration of initial antibiotic therapy after birth is a risk factor associated with later NEC development in premature infants. However, a recent study suggests that absence of antibiotic treatment after delivery may be associated with increased risk for NEC. In the current manuscript, authors present a research proposal aim to explore this controversy by using a large pre-existing dataset of preterm infants in the UK. They propose to perform a retrospective cohort study using routine data from the UK National Neonatal Research Database (NNRD). From the database, they will select premature infants with gestational age <32 weeks, alive on day three, without major anomaly, and then determine and compare the difference of primary outcome (development of severe NEC) in infants receiving early antibiotics (days 1-2 after birth) and those not. Subgroup analysis on duration of early antibiotic exposure will also occur. They described in detail the sophisticated statistical methods for analysis. Both propensity scoring and logistic regression analysis will be used for identifying possible confounding factors. Since this is a retrospective cohort study using routinely recorded clinical data held in the NNRD. There is no need to design a study protocol to collect data prospectively, rather they just need to have a method for correct data retrieval and analysis. Therefore, the key for success of this research proposal is the quality of their database. In theory all the data are available from the medical

	records and therefore they should have no problem completing the study. Assuming the database they maintain has all the data needed, they can start and complete the study in a short period of time. However, if they need to collect data from medical records, it will take much longer time to complete the data collection. Another question I have is that authors appear to emphasize the first 2 days' antibiotic exposure. From medical point of view, the history of exposure to antibiotics in the first week of life may be more important since very few premature infant with gestation age< 32 weeks develop NEC in the first week of life. As authors described in the manuscript, antibiotic use as part of neonatal intensive care is common, particularly immediately after birth when infection is implicated in preterm delivery, more than half of infants weighing <1000g routinely received more than 5 days antibiotics at birth. Therefore, most premature infants with gestational age < 32 weeks will have some exposure to antibiotics in the first week of life. Those have no exposure to antibiotics in the first week of life usually are those with lower risk for early onset sepsis. They may represent a unique group of premature infants as author described in the manuscript. It will make more sense that based on their history of antibiotics exposure in the first week of life, premature infants be divided into 3 groups, i.e. no antibiotics exposure, antibiotics < 72 hours, antibiotics >72 hours. Analysis therefore should be performed as such to compare the length of exposure and their associated risk with NEC.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Joanna Seliga-Siwecka, Warszawski Uniwersytet Medyczny Comments to the Author:

Dear Authors,
Congratulations on submitting a very interesting protocol for a study on early use and incidence of necrotizing enterocolitis in very preterm infants.
Title
The title of the manuscript draws attention of the reader, defines the study population, and outcomes but is not clear on the type of manuscript. Please consider the following title: "Early antibiotic use and incidence of necrotizing enterocolitis in very preterm infants: A protocol for an UK based observational study using routinely recorded data." – **amended thank you**
Abstract
The authors provide a concise overview of the study. It is clearly stated why the study will be undertaken, and how the data will be collected. However, the researchers may consider adding a short summary of possible implications for the future at the end if the abstract. – **added thank you**
Introduction
The authors provide enough contextual information for the journal's neonatal-paediatric readership to understand the context. They accurately describe up to date research, with is properly referenced. Additionally, limitations and controversies are identified. As stated by the study group, this is a necessary study, and the results will influence antibiotic stewardship of extremely preterm infants within the first 24 hours.
Methods
Methods and statistical analysis are adequately planned. Nevertheless, given the antibiotic intervals in extremely preterm infants I would suggest changing 1-2 days as indicated by the authors to 36-48 hours. A 12-hour difference in stopping antibiotics will increase the number of antibiotic doses, which might influence NEC incidence? **Thank you for this observation. Whilst we do not disagree the**

database holds data by 24 hour period only, and so we can only do as we have indicated with whole days, and not by 12 hour periods.
I would also consider excluding infants with “blood negative sepsis”. These patients will most probably represent infants with “sepsis-like syndrome”, unnecessarily treated for bacterial infection. Hence, including these infants may skew the results[1; 2; 3; 4]. **We include analysis of the secondary outcomes LOS with positive cultures and treatment for 5 days as the most inclusive way to analyse the overall impact of early antibiotic exposure (but we will also have culture +ve separately).**
Please provide the definition of chorioamniotis, as there is a high chance that different definitions will be used between the participating units. **(as per Table 2 – fever and antibiotics)**
The controls are proper, and the outcomes are clearly defined. The statistics are sound, and the study will be conducted under ethical guidance.

Discussion
The discussion addresses the research problem. Other relevant studies are discussed.
I look forward to reading the final manuscript with the study results! **Thank you**

[1] J.B. Cantey, and P. Prusakov, A Proposed Framework for the Clinical Management of Neonatal "Culture-Negative" Sepsis. J Pediatr 244 (2022) 203-211.
[2] B.R. Hadfield, and J.B. Cantey, Neonatal bloodstream infections. Curr Opin Infect Dis 34 (2021) 533-537.
[3] J.B. Cantey, and A.L. Shane, Neonatal-Perinatal Infections: An Update. Clin Perinatol 48 (2021) xix-xx.
[4] J.B. Cantey, and J.H. Lee, Biomarkers for the Diagnosis of Neonatal Sepsis. Clin Perinatol 48 (2021) 215-227.

Reviewer: 2
Dr. Jing Lin, Mount Sinai Medical Center Comments to the Author:
NEC remains a significant cause for mortality and morbidity of very premature infants. Several observational studies have demonstrated that prolonged duration of initial antibiotic therapy after birth is a risk factor associated with later NEC development in premature infants. However, a recent study suggests that absence of antibiotic treatment after delivery may be associated with increased risk for NEC. In the current manuscript, authors present a research proposal aim to explore this controversy by using a large pre-existing dataset of preterm infants in the UK. They propose to perform a retrospective cohort study using routine data from the UK National Neonatal Research Database (NNRD). From the database, they will select premature infants with gestational age <32 weeks, alive on day three, without major anomaly, and then determine and compare the difference of primary outcome (development of severe NEC) in infants receiving early antibiotics (days 1-2 after birth) and those not. Subgroup analysis on duration of early antibiotic exposure will also occur. They described in detail the sophisticated statistical methods for analysis. Both propensity scoring and logistic regression analysis will be used for identifying possible confounding factors.
Since this is a retrospective cohort study using routinely recorded clinical data held in the NNRD. There is no need to design a study protocol to collect data prospectively, rather they just need to have a method for correct data retrieval and analysis. Therefore, the key for success of this research proposal is the quality of their database. In theory all the data are available from the medical records and therefore they should have no problem completing the study. Assuming the database they maintain has all the data needed, they can start and complete the study in a short period of time. However, if they need to collect data from medical records, it will take much longer time to complete the data collection. **Thank you – the data is a National resource to which ‘all’ neonatal units in**

the UK contribute standardised data with standardised definitions, and quality assurance steps.

Another question I have is that authors appear to emphasize the first 2 days' antibiotic exposure. From medical point of view, the history of exposure to antibiotics in the first week of life may be more important since very few premature infant with gestation age< 32 weeks develop NEC in the first week of life. As authors described in the manuscript, antibiotic use as part of neonatal intensive care is common, particularly immediately after birth when infection is implicated in preterm delivery, more than half of infants weighing <1000g routinely received more than 5 days antibiotics at birth. Therefore, most premature infants with gestational age < 32 weeks will have some exposure to antibiotics in the first week of life. Those have no exposure to antibiotics in the first week of life usually are those with lower risk for early onset sepsis. They may represent a unique group of premature infants as author described in the manuscript. It will make more sense that based on their history of antibiotics exposure in the first week of life, premature infants be divided into 3 groups, i.e. no antibiotics exposure, antibiotics < 72 hours, antibiotics >72 hours. Analysis therefore should be performed as such to compare the length of exposure and their associated risk with NEC. **Thank you this is included in our analysis plans, as indicated.**

Reviewer: 1
Competing interests of Reviewer: none to declare

Reviewer: 2
Competing interests of Reviewer: None

VERSION 2 – REVIEW

REVIEWER	Seliga-Siwecka, Joanna Warszawski Uniwersytet Medyczny, Neonatology and Neonatal Intensive Care
REVIEW RETURNED	17-Sep-2022

GENERAL COMMENTS	I am happy to recommend this manuscript for publication.