PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Comparison of diagnoses of early onset sepsis associated with use of Sepsis Risk Calculator versus NICE CG149: a prospective, population-wide cohort study in London, UK, 2020-21
AUTHORS	Piyasena, Chinthika; Galu, Sorana; Yoshida, Rie; Thakkar, Devangi; O'Sullivan, Joanna; Longley, Catherine; Evans, Katie; Sweeney, Suzanne; Kendall, Giles; Ben-sasi, Khadija; Richards, Justin; Harris, Chris; Jagodzinski, Jenni; Dermirjian, Alicia; Lamagni, Theresa; Le Doare, Kirsty; Heath, Paul; Battersby, Cheryl; Group, NeoTRIPs

VERSION 1 – REVIEW

REVIEWER	Kuzniewicz, Michael Kaiser Permanente
REVIEW RETURNED	26-Apr-2023

GENERAL COMMENTS	The study by Piyasena et al conducted a prospective cohort study comparing the incidence of missed culture-positive and culture-negative EOS between hospitals in the UK managing newborns with the SRC compared to NICE guidelines. They report lower antibiotic use in the SRC hospitals and no statistically significant differences in missed culture-positive or culture-negative EOS, although there was a lower incidence of culture-positive EOS in the SRC hospitals. Overall, missed cases were rare using both management strategies. The study has value in providing additional safety data of these two EOS management approaches and the largest study in the UK with nearly 100,000 infants included. Larger studies like this are useful in providing this safety data which has often not been included in smaller studies because of the impracticality since the incidence is rare. While the study does provide some important data, there are some issues with the comparisons being made as well as the value of examining an outcome of “culture negative sepsis”. Specific comments/recommendations below: Background: • Informative, no specific recommendations Methods: • The study did not make exclusions for significant congenital anomalies that may have predisposed infants to postnatally acquired infection. Two infants in the missed culture-positive EOS cases had impaired skin integrity (Harlequin ichthyosis and Collodion baby). Including these infants perhaps gives an artificially elevated risk of missed EOS cases. One might consider not including these cases as EOS since these infections were

	more likely obtained postnatally. This is important as they represent 2/7 (29%) of all their missed cases. • An outcome of missed “culture-negative” EOS is problematic. While it is true others have defined this similarly using duration of empiric antibiotics, it is has been increasingly acknowledged that this is a misnomer (Ending the Culture of Culture-Negative Sepsis in the Neonatal ICU – Cantey et al Pediatrics 2017). With modern blood culture techniques and an adequate volume of culture, blood cultures are highly unlikely to miss even very small bacterial loads. The prolonged treatment of infants with blood cultures showing no growth more likely reflects physician practices/opinions rather than a true outcome of an EOS management strategy. The authors do urge caution in interpreting these results in their discussion and postulate that findings could have been caused by physicians being more cautious after instating the SRC. Furthermore, this is also likely influences by the percentage of infants already receiving antibiotics in the first 24 hours after birth; thus, with nearly double the rate of empiric antibiotics in the first 24 hours in the NICU hospitals (15 vs 7%). Give the problematic nature of this outcome, the authors may consider dropping this outcome completely or at least deemphasizing it or making it a secondary outcome. Results • The comparison of the SRC and NICE hospitals is perhaps problematic since the incidence of culture-proven EOS is different between the two cohorts (0.49/1000 vs 0.81/1000). It seems like it may be hard to interpret missed rates directly if the overall incidence differs between these two cohorts. Could the authors account for this difference in groups, perhaps maybe by the percentage of EOS cases missed rather than an absolute incidence. The results will be similar but may be a better comparison. • In Table 2, the number of infants with clinical signs at birth, signs developed before discharge, and never had clinical signs (10+7+3=20 and 11+14+18=430 don’t add up t0 the totals of 21 and 46. Is there another category that I am missing? It wasn’t clear from the table. Discussion: • Nicely written and informative. Supplemental material: • sTable 5: for case #7 Moraxella and Corynebacterium seem like odd EOS organisms and leads to the question of how the investigators differentiated true pathogens from contaminants. Could the authors provide further detail on this topic and perhaps particularly in this case • sFigure 1: Using a figure here adds no more information and is harder to read than a simple table or text. Consider removing this pie figure

REVIEWER	Puopolo, Karen M University of Pennsylvania
REVIEW RETURNED	04-May-2023

GENERAL COMMENTS	In this manuscript, the authors compare the observed outcomes of newborns managed with NICE guidelines vs. the Kaiser Sepsis Risk Calculator (SRC) for risk of early-onset sepsis (EOS). This was an observational study of practice at birth hospitals in Greater London. The study was a pragmatic one, but carefully designed,

	and the authors acknowledge the limitations of the design. The authors conclude that use of the SRC, compared to use of NICE guidelines, resulted in lower use of empiric antibiotic therapies without increases in “missed” cases of culture-confirmed or “culture-negative” EOS. Overall this manuscript is acceptable for publication. There are items to be addressed to improve the manuscript. (1) In using the term “missed cases” the authors perpetuate the misconception that all infants with EOS can be identified at the moment of birth. Risk stratification tools are as their name implies: the tool provides an estimate of risk of the outcome, either in a categorical or continuous manner. Such tools do not diagnose disease, and generally do not identify zero risk with statistical certainty. With EOS, there is the additional biologic issue that the pathogenesis of infection (generally thought of as colonization of the fetus/newborn with maternally-derived flora, and transition from mucosal colonization to invasive infection) may not be “complete” at the moment of birth. Particularly with GBS (the most common pathogen identified), the newborn may be born colonized with the pathogen, and not infected. The identification of an ill-appearing infant identified as infected at 36 hours of age may not be a case present at birth that was “missed,” but in fact a case that only occurred after birth. I would suggest that the authors strongly consider rephrasing this throughout the manuscript. From the Abstract: “We sought to compare the incidence of missed early-onset sepsis (EOS) in infants ≥34 weeks’ gestation in hospitals using the Kaiser Permanente sepsis risk calculator (SRC) with hospitals using the NICE guidance.” This could be rephrased, “We sought to compare the incidence of early-onset sepsis (EOS) in infants ≥34 weeks’ gestation identified at >24 hours after birth, in hospitals using the Kaiser Permanente sepsis risk calculator (SRC) with hospitals using the NICE guidance.” (2) Culture-negative sepsis lies in the eyes of the beholder. I noted with interest that the authors defined this entity in terms of process: a blood culture was obtained, and antibiotics were administered for at least 5 days in the absence of an identified pathogen in blood or CSF culture. They carefully avoided the controversies around the use of CRP and other inflammatory markers; I think this was a good decision. But the authors should change their language here, too. They present no evidence that “sepsis” was present in these antibiotic-treated infants; they are only measuring clinical-decision making. That is actually quite appropriate in this study. In measuring decision-making, they capture not only the proportion of infants that are perceived as unwell, but the level of uncertainty held by the treating clinician, as well as the personal, internalized risk-acceptance of the clinician. A core aspect of the clinical use of the SRC is that a proportion of infants evaluated are placed in a management category of close clinical observation. It is anticipated that some of those infants will later present as unwell and require evaluation. It also could be anticipated that the threshold for intervention will be lower (and therefore, the rate at which such infants are administered antibiotics later in the birth hospitalization will be higher) than using the NICE guidance because of what I would term “change bias.” A bias toward evaluating newborns later after birth may be present in the months/years after changing from NICE to SRC, as some clinicians will view a potentially unwell infant as one who would have received empiric antibiotics after birth under NICE – and therefore may have a lower threshold to intervene if there is

	any clinical concern. Be this all as it may: I would recommend rephrasing this objective as well. From the Abstract: “Culture-proven missed EOS was defined as isolation of a bacterial pathogen in the blood or CSF culture of an infant from 24 hours of age up to 7 days of age. Culture-negative missed EOS was defined as an infant commencing intravenous antibiotics from 24 hours of age up to 7 days of age, for at least 5 days, but with negative blood or CSF cultures.” This could be rephrased as: “EOS was defined as isolation of bacterial pathogen in blood or CSF culture from birth to 7 days of age. We evaluated the incidence of EOS identified by culture obtained from 24 hours – 7 days after birth. We also evaluated the rate at which empiric antibiotics were commenced from 24 hours - 7 days after birth, for a duration of ≥5 days, with sterile blood or CSF cultures.” (3) The Supplemental Data describes the 7 “missed cases.” The studies that developed the SRC specifically excluded infants with congenital anomalies (Puopolo, et al Pediatrics 2011). I would add that exclusion to this study. The cases of infection in the 2 infants with congenital skin conditions are particularly problematic, as the defect in such newborns presents an important risk for invasive infection – meaning the pathogenesis of infection may not be that of EOS. This would eliminate 1 case each in SRC and NICE centers (Case 1 and Case 4). Case 5 is a terrible tragedy, but the absence of a culture-confirmed infection in the infant (or even current pregnancy colonizing data for the mother) means it must be eliminated from consideration. Sudden unexpected infant death has many etiologies; unless the coroner based the diagnosis on postmortem culture or histopathology showing GBS in blood or lungs, I would eliminate this from consideration. Case 7 is curious: the Moraxella isolate is a slug parasite-associated species and a very rare cause of human infection. In any case, I would suggest that the authors report 1 case of EOS diagnosed after 24 hours of age in SRC centers (Case 2) and 3 in NICE centers (Cases 3, 6 and 7) – and I am sure that is not a statistically different incidence. (4) Supplementary Table 1 shows an interesting variation in implementation. The reader should be directed to this Table and the variation should be emphasized in the 3rd paragraph of the Discussion as one reason why the SRC centers had a 7% rate of empiric antibiotic use. Another issue may be that American reports (Kuzniewicz, et al JAMA Pediatr 2017 and Dhudasia, et al. Hosp Pediatr 2018 are examples) often report a cohort of infants born ≥35 or ≥36 weeks’ gestation) and the current report includes infants to 34 weeks at birth, the latter being at somewhat higher overall estimated risk of infection.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Michael Kuzniewicz, Kaiser Permanente
Comments to the Author:
The study by Piyasena et al conducted a prospective cohort study comparing the incidence of missed culture-positive and culture-negative EOS between hospitals in the UK managing newborns with the SRC compared to NICE guidelines. They report lower antibiotic use in the SRC hospitals and no statistically significant differences in missed culture-positive or culture-negative EOS, although there

was a lower incidence of culture-positive EOS in the SRC hospitals. Overall, missed cases were rare using both management strategies. The study has value in providing additional safety data of these two EOS management approaches and the largest study in the UK with nearly 100,000 infants included. Larger studies like this are useful in providing this safety data which has often not been included in smaller studies because of the impracticality since the incidence is rare. While the study does provide some important data, there are some issues with the comparisons being made as well as the value of examining an outcome of "culture negative sepsis". Specific comments/recommendations below:

Background:
• Informative, no specific recommendations

Methods:
• The study did not make exclusions for significant congenital anomalies that may have predisposed infants to postnatally acquired infection. Two infants in the missed culture-positive EOS cases had impaired skin integrity (Harlequin ichthyosis and Collodion baby). Including these infants perhaps gives an artificially elevated risk of missed EOS cases. One might consider not including these cases as EOS since these infections were more likely obtained postnatally. This is important as they represent 2/7 (29%) of all their missed cases.
- Thank you for this suggestion. We agree that there is rationale to exclude these two cases as it is likely these infections were obtained postnatally. We have removed the 2 cases with congenital skin anomalies as suggested. We have included an explanation in the results "Two infants were excluded because of congenital anomalies predisposing to reduced skin integrity and the pathogenesis of invasive infection probably postnatal rather than that of EOS. These were Bacillus cereus and Acinetobacter baumanii isolated at 28 hours in an infant with harlequin ichythosis (SRC), and Staphylococcus aureus isolated at 91 hours in a collodion infant (NICE).

This reduces the cases of EOS ≤7 days to 65 (20 in SRC hospitals and 45 in NICE hospitals). We have recalculated the numbers in Table 1 and Table 2 accordingly and changed the corresponding numbers in the manuscript. Under the section "Incidence of EOS identified >24 hours of age, we have changed this from 7 to 5 cases.

• An outcome of missed "culture-negative" EOS is problematic. While it is true others have defined this similarly using duration of empiric antibiotics, it is has been increasingly acknowledged that this is a misnomer (Ending the Culture of Culture-Negative Sepsis in the Neonatal ICU – Cantey et al Pediatrics 2017). With modern blood culture techniques and an adequate volume of culture, blood cultures are highly unlikely to miss even very small bacterial loads. The prolonged treatment of infants with blood cultures showing no growth more likely reflects physician practices/opinions rather than a true outcome of an EOS management strategy. The authors do urge caution in interpreting these results in their discussion and postulate that findings could have been caused by physicians being more cautious after instating the SRC. Furthermore, this is also likely influences by the percentage of infants already receiving antibiotics in the first 24 hours after birth; thus, with nearly double the rate of empiric antibiotics in the first 24 hours in the NICU hospitals (15 vs 7%). Give the problematic nature of this outcome, the authors may consider dropping this outcome completely or at least deemphasizing it or making it a secondary outcome.
- We have described this outcome as recommended by Dr Puopolo (Second reviewer), removed mention of this outcome in several places throughout the text to de-emphasise. We have also removed "missed" from the title of the manuscript. Table 3 describing this outcome has been moved to the supplementary material to de-emphasise.

Results
• The comparison of the SRC and NICE hospitals is perhaps problematic since the incidence of culture-proven EOS is different between the two cohorts (0.49/1000 vs 0.81/1000). It seems like it may be hard to interpret missed rates directly if the overall incidence differs between these two cohorts. Could the authors account for this difference in groups, perhaps maybe by the percentage of EOS cases missed rather than an absolute incidence. The results will be similar but may be a better comparison.
-Thank you. We have compared the percentage of cases missed rather than the absolute incidence and reported this statistic. The result is similar.

• In Table 2, the number of infants with clinical signs at birth, signs developed before discharge, and never had clinical signs (10+7+3=20 and 11+14+18=430 don't add up t0 the totals of 21 and 46. Is there another category that I am missing? It wasn't clear from the table.
-This discrepancy is due to missing data, which has been declared immediately below the table. The numbers have been checked again.

Discussion:
• Nicely written and informative.

Supplemental material:
• sTable 5: for case #7 Moraxella and Corynebacterium seem like odd EOS organisms and leads to the question of how the investigators differentiated true pathogens from contaminants. Could the authors provide further detail on this topic and perhaps particularly in this case
- We agree that Corynebacterium can be considered a contaminant. However, the Vermont Oxford Network's Manual of Operation lists Moraxella species under Bacterial Pathogens, hence the reason for including this case. The infant received 5 days of antibiotics. We included this case for the purpose of reporting comprehensively. Text to explain this has been added to Supplementary File 1.

• sFigure 1: Using a figure here adds no more information and is harder to read than a simple table or text. Consider removing this pie figure
- The pie figure has been removed.

Reviewer: 2
Dr. Karen M Puopolo, University of Pennsylvania
Comments to the Author:
In this manuscript, the authors compare the observed outcomes of newborns managed with NICE guidelines vs. the Kaiser Sepsis Risk Calculator (SRC) for risk of early-onset sepsis (EOS). This was an observational study of practice at birth hospitals in Greater London. The study was a pragmatic one, but carefully designed, and the authors acknowledge the limitations of the design. The authors conclude that use of the SRC, compared to use of NICE guidelines, resulted in lower use of empiric antibiotic therapies without increases in "missed" cases of culture-confirmed or "culture-negative" EOS. Overall this manuscript is acceptable for publication. There are items to be addressed to improve the manuscript.

(1) In using the term "missed cases" the authors perpetuate the misconception that all infants with EOS can be identified at the moment of birth. Risk stratification tools are as their name implies: the tool provides an estimate of risk of the outcome, either in a categorical or continuous manner. Such tools do not diagnose disease, and generally do not identify zero risk with statistical certainty. With EOS, there is the additional biologic issue that the pathogenesis of infection (generally thought of as colonization of the fetus/newborn with maternally-derived flora, and transition from mucosal colonization to invasive infection) may not be "complete" at the moment of birth. Particularly with GBS

(the most common pathogen identified), the newborn may be born colonized with the pathogen, and not infected. The identification of an ill-appearing infant identified as infected at 36 hours of age may not be a case present at birth that was "missed," but in fact a case that only occurred after birth. I would suggest that the authors strongly consider rephrasing this throughout the manuscript. From the Abstract: "We sought to compare the incidence of missed early-onset sepsis (EOS) in infants ≥34 weeks' gestation in hospitals using the Kaiser Permanente sepsis risk calculator (SRC) with hospitals using the NICE guidance." This could be rephrased, "We sought to compare the incidence of early-onset sepsis (EOS) in infants ≥34 weeks' gestation identified at >24 hours after birth, in hospitals using the Kaiser Permanente sepsis risk calculator (SRC) with hospitals using the NICE guidance."
-We have made this change.

(2) Culture-negative sepsis lies in the eyes of the beholder. I noted with interest that the authors defined this entity in terms of process: a blood culture was obtained, and antibiotics were administered for at least 5 days in the absence of an identified pathogen in blood or CSF culture. They carefully avoided the controversies around the use of CRP and other inflammatory markers; I think this was a good decision. But the authors should change their language here, too. They present no evidence that "sepsis" was present in these antibiotic-treated infants; they are only measuring clinical-decision making. That is actually quite appropriate in this study. In measuring decision-making, they capture not only the proportion of infants that are perceived as unwell, but the level of uncertainty held by the treating clinician, as well as the personal, internalized risk-acceptance of the clinician. A core aspect of the clinical use of the SRC is that a proportion of infants evaluated are placed in a management category of close clinical observation. It is anticipated that some of those infants will later present as unwell and require evaluation. It also could be anticipated that the threshold for intervention will be lower (and therefore, the rate at which such infants are administered antibiotics later in the birth hospitalization will be higher) than using the NICE guidance because of what I would term "change bias." A bias toward evaluating newborns later after birth may be present in the months/years after changing from NICE to SRC, as some clinicians will view a potentially unwell infant as one who would have received empiric antibiotics after birth under NICE – and therefore may have a lower threshold to intervene if there is any clinical concern. Be this all as it may: I would recommend rephrasing this objective as well. From the Abstract: "Culture-proven missed EOS was defined as isolation of a bacterial pathogen in the blood or CSF culture of an infant from 24 hours of age up to 7 days of age. Culture-negative missed EOS was defined as an infant commencing intravenous antibiotics from 24 hours of age up to 7 days of age, for at least 5 days, but with negative blood or CSF cultures." This could be rephrased as: "EOS was defined as isolation of bacterial pathogen in blood or CSF culture from birth to 7 days of age. We evaluated the incidence of EOS identified by culture obtained from 24 hours – 7 days after birth. We also evaluated the rate at which empiric antibiotics were commenced from 24 hours - 7 days after birth, for a duration of ≥5 days, with sterile blood or CSF cultures."
- We have made this change and changed the language as advised consistently throughout the text. The word "negative" rather than "sterile" was used to include blood cultures isolating organisms considered as contaminants. The term "culture-proven" has been removed throughout the text as we now we state up front with the EOS definition as advised above i.e. with bacterial pathogen isolated.

(3) The Supplemental Data describes the 7 "missed cases." The studies that developed the SRC specifically excluded infants with congenital anomalies (Puopolo, et al Pediatrics 2011). I would add that exclusion to this study. The cases of infection in the 2 infants with congenital skin conditions are particularly problematic, as the defect in such newborns presents an important risk for invasive infection – meaning the pathogenesis of infection may not be that of EOS. This would eliminate 1 case each in SRC and NICE centers (Case 1 and Case 4). Case 5 is a terrible tragedy, but the absence of a culture-confirmed infection in the infant (or even current pregnancy colonizing data for the mother) means it must be eliminated from consideration. Sudden unexpected infant death has many etiologies; unless the coroner based the diagnosis on postmortem culture or histopathology

showing GBS in blood or lungs, I would eliminate this from consideration. Case 7 is curious: the Moraxella isolate is a slug parasite-associated species and a very rare cause of human infection. In any case, I would suggest that the authors report 1 case of EOS diagnosed after 24 hours of age in SRC centers (Case 2) and 3 in NICE centers (Cases 3, 6 and 7) – and I am sure that is not a statistically different incidence.
- We have chosen to include the case 5. The diagnosis of GBS was based on the postmortem, was stated as the diagnosis on the Medical Cause of Certified Death by the Coroner, and this was the diagnosis given to the parents. This further text has been added to the manuscript. We have also retained cases 2, 3, 6 and 7 as advised.
The list of "Bacterial Pathogens" in the Vermont Oxford Network Manual was used to determine whether an organism was considered a pathogen. This list includes "Moraxella species [M.catarrhalis and others]". We wanted to be as comprehensive as possible in our reporting.
As advised, the two cases of infection in babies with reduced skin integrity (cases 1 and 4) have been removed from the table and also removed from the total EOS number. Thus the incidence of EOS is revised and the results in the text and in the Tables 1, 2, supplementary table 4. The need to exclude babies with congenital anomalies is now mentioned in the text.
The remaining cases in Supplementary table 5 have been re-numbered, and the text altered in Supplementary file 1.

(4) Supplementary Table 1 shows an interesting variation in implementation. The reader should be directed to this Table and the variation should be emphasized in the 3rd paragraph of the Discussion as one reason why the SRC centers had a 7% rate of empiric antibiotic use. Another issue may be that American reports (Kuzniewicz, et al JAMA Pediatr 2017 and Dhudasia, et al. Hosp Pediatr 2018 are examples) often report a cohort of infants born ≥35 or ≥36 weeks' gestation) and the current report includes infants to 34 weeks at birth, the latter being at somewhat higher overall estimated risk of infection.
-We have emphasised the variation in implementation and the supplementary table more in the discussion. We have added "Almost all hospitals implemented a variation of the SRC with differences across hospitals (Supplementary table 1)." This is also included as a limitation and in the previous version of the manuscript. We have also noted the inclusion of infants born at 34 weeks' gestation as one reason for higher antibiotic usage overall.

VERSION 2 – REVIEW

REVIEWER	Kuzniewicz, Michael Kaiser Permanente
REVIEW RETURNED	10-Jul-2023

GENERAL COMMENTS	In review, the study by Piyasena et al conducted a prospective cohort study comparing the incidence of EOS diagnosed after 24 hours and the rate of treatment 24hrs -7 days with antibiotics >5 days in the absence of a positive culture between hospitals in the UK managing newborns with the SRC compared to NICE guidelines. They report lower antibiotic use in the SRC hospitals in the first 24 hours and no statistically significant differences in EOS cases diagnoses after 24 hours. Overall, cases diagnosed after 24 hours were rare using both management strategies. The study has value in providing additional safety data of these two EOS management approaches and the largest study in the UK with nearly 100,000 infants included. Larger studies like this are useful in providing this safety data which has often not been included in

	smaller studies because of the impracticality since the incidence is rare. The authors have responded to all the points raised in the initial review. They removed some problematic language around “culture-negative sepsis” and “missed cases”. In addition, they corrected some minor methodological issues. I thank the authors for their responsiveness.

REVIEWER	Puopolo, Karen M University of Pennsylvania
REVIEW RETURNED	09-Jul-2023

GENERAL COMMENTS	The authors have been appropriately responsive to the prior detailed reviews and this manuscript if now acceptable for publication.