Predicting neonatal early onset sepsis a 14-year cohort study

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Predicting Neonatal Early Onset Sepsis

A 14-Year Cohort Study

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Background: In many infants, treatment is started for suspicion of early onset sepsis (EOS), of whom the majority do not have an infection. Early prediction of the absence of a culture-proven sepsis (CPS) would significantly reduce the time of antibiotic treatment and hospitalization. Our objective was to analyze 3 criteria in infants with CPS: positive blood culture (BC) at 24 hours after the onset of suspicion of EOS (OSEOS), C-reactive protein (CRP) ≥10 mg/L and clinical signs of infection, so we can consequently consider to stop antibiotic treatment in infants without these criteria.

Methods: We included all infants with suspicion of EOS from 2007 until 2020. The proportion was calculated of (1) infants with CPS with, at 24 hours, a positive BC and/or CRP ≥10 mg/L and/or clinical signs of infection and (2) infants without CPS with CRP <10 mg/L between 12 and 24 hours after OSEOS.

Results: The BC showed growth of a pathogenic microorganism in 50 of 4120 included infants (1.2%). Time to positivity was ≥24 hours in 8 (16%) infants, of whom 7 infants had a raised CRP and/or clinical symptoms of infection within 24 hours. In 1095 (74%) of infants without CPS in whom CRP was measured between 12 and 24 hours after OSEOS, CRP was <10 mg/L.

Conclusion: A combination of BC, CRP, and clinical signs of infection can diagnose 98% (49/50) of infants with CPS 24 hours after OSEOS. Based on normal CRP and the absence of a positive BC, the decision to stop antibiotics could have been brought forward to 24 hours in 74% of infants.

Key Words: neonatal, early onset sepsis, time to positivity, blood culture, C-reactive protein

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Early onset sepsis (EOS), an invasive bacterial infection in infants in the first days after birth, is a rare but life-threatening condition with high mortality if treatment is not initiated very early after the start of infection. However, initial signs and symptoms in infants can be very subtle and nonspecific for infection. Therefore, current guidelines recommend to start antibiotics in the presence of risk factors for EOS without clinical signs of infection, resulting in a high number needed to treat.[1–5] The continuation of antibiotics is usually evaluated 36–72 hours after starting antibiotics. In the vast majority of infants, it is decided upon a combination of clinical signs, C-reactive protein (CRP) and blood culture (BC) results, to stop antibiotic treatment. Since there is a high number needed to treat, a reduction in antibiotic treatment duration would significantly reduce the time infants are unnecessarily exposed to antibiotics.[6] In addition, the time of hospital admission of most term infants treated for a possible infection would be reduced as well.

The Dutch guideline "Prevention and treatment of early onset sepsis" published in 2017 advises, in concordance with the NICE guideline published in 2012, to evaluate the need for antibiotics after 36–48 hours of antibiotic treatment.[4,5] However, the evidence for this time period of 36–48 hours is rather weak and might be too long.[7–9] Studies show that the vast majority of BCs become positive within 24 hours of incubation.[7–13] It is, however, unknown if the infants with BCs that became positive after 24 hours could have been diagnosed as EOS based on evaluation of clinical signs and inflammatory parameters at 24 hours after taking the BC and starting antibiotics.

Most studies thus far only take either CRP or another inflammatory marker (procalcitonin, interleukin-6 and white blood cells) and/or the BC into consideration. Good cutoff values of CRP in infants are not known, a substantial proportion of healthy infants has a CRP ≥10 mg/L at 24 and 48 hours of age.[14–16] CRP has a low sensitivity at the start of infection, making it unreliable for early diagnosis of neonatal sepsis; however, the sensitivity increases over time.[17–19]

Our objective was to analyze what proportion of infants with culture-proven EOS, at 24 hours after taking the BC because of suspicion of infection, have an elevated CRP, clinical signs of infection and/or a positive BC. Also, the proportion of infants without culture-proven sepsis (CPS) with normal CRP was calculated. The aim of our study was to analyze if the decision to stop antibiotic treatment in infants with a favorable clinical course, low CRP and negative BC could be brought forward to 24 hours after onset of suspicion of sepsis, instead of after 36–48 hours, resulting in a reduction of antibiotic exposure and duration of hospital stay in term infants.

MATERIALS AND METHODS

Setting and Study Design

This retrospective study was conducted at the Leiden University Medical Center, an academic tertiary care center. Infants born at a gestational age <32 weeks and/or severely ill were admitted to the neonatal intensive care unit (NICU), other infants with suspicion of EOS were admitted to the medium care unit (NMCU). The Institutional Ethics Review Board of the Leiden University Medical Center waived the need for formal approval because of the retrospective nature of the study. Infants whose parents chose to opt-out of evaluation studies were excluded.

Subjects and Data Collection

Infants admitted to the NICU or the NMCU with a BC taken as a sign of suspicion of EOS between January 1, 2007, and November 15, 2020, were identified. Only BCs collected on the first and/

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or second day of life were included, to exclude hospital acquired sepsis. If more than one BC was collected from an infant, only the first BC was analyzed. All relevant patient data were retrieved from the electronic patient data management system. Microbiologic data were retrieved from the laboratory information system. Clinical parameters were only retrieved from infants with a BC with a time to positivity (TTP) ≥24 hours. The primary objective was to analyze what proportion of infants with culture proven EOS, at 24 hours after the onset of suspicion of EOS, had CRP ≥10 mg/L, clinical signs of infection and/or a positive BC. The secondary objective was to calculate the proportion of infants with suspicion of EOS but without CPS that had CRP <10 mg/L and a negative BC results at 24 hours after culture taking and would have been a candidate for cessation of antibiotic therapy at 24 hours after the onset of sepsis suspicion.

Sepsis Work-up

Evaluation of sepsis, based on clinical signs of infection or the presence of risk factors, was performed at the discretion of the treating clinician, and included starting antibiotics (broad-spectrum penicillin with an aminoglycoside) after taking a BC. CRP values were obtained at the onset of suspicion of EOS and during treatment, but not at fixed time points.

BC Handling Procedure and Laboratory Techniques

The institutional protocol was to collect 0.5–2 mL of blood in a Peds Plus/F vial (Becton Dickinson B.V., Breda, The Netherlands), which can be used for culturing aerobic as well as anaerobic bacteria. The time of placing the order for BC collection was recorded in the laboratory information system as part of the ordering procedure. Cultures were transported to the in-hospital medical microbiology department day and night, by dedicated hospital transportation employees. Upon arrival at the department of Medical Microbiology, the BCs were directly placed in the BACTEC FX continuous monitoring system (Becton Dickinson B.V.). The time of the positive signal was automatically recorded and sent to the laboratory information system with a delay of a few minutes. During evening and night hours and in the weekends after 10:00 AM, BCs were directly placed in the BACTEC, but registration in the laboratory information system was performed the next morning around 8:30 AM. If the threshold for positivity was reached between placement and this registration, the culture was recorded positive at the time of registration, instead of upon positive signaling. This procedural limitation led to an overestimation of the TTP in bottles that started as "anonymous," with a hypothetically maximum of 22.5 hours (10:00–8:30). Therefore, BCs with TTP ≥24 hours did not have this overestimation.

Definitions

Pathogenic microorganisms: *Listeria monocytogenes, Streptococcus pyogenes, Streptococcus agalactiae, Enterobacterales, Pseudomonas aeruginosa, Haemophilus (para)influenzae, Streptococcus pneumoniae, Neisseria meningitidis* and *Staphylococcus aureus*.

Nonpathogenic microorganisms: all other microorganisms, including coagulase-negative staphylococci because line-associated infections are very uncommon in the first 2 days of life and these were not in the scope of our study.

Polymicrobial positive BCs were considered positive if at least one of the cultured microorganisms was pathogenic.

Standard incubation time of BCs was 7 days.

Onset of suspicion of EOS (OSEOS): the recorded timepoint of ordering the BC, when the clinician decided upon clinical signs or risk factors that there was a suspicion of EOS, collected a BC and started antibiotic treatment.

TTP: the time between OSEOS and the positive signal in the laboratory information system, including transportation time to the laboratory.

Data Analysis

Predictors studied were TTP, CRP obtained between 12 and 24 hours after OSEOS and clinical signs of infection. Data were presented as median with interquartile range (IQR) where appropriate. Nonnormally distributed numerical data were compared with the Mann–Whitney *U* test, categorical outcome data were compared using the Fisher exact test. Data were analyzed using R, version 3.6.3 (Vienna, Austria).[20]

RESULTS

Patient Demographics

From January 1, 2007, to November 15, 2020, BCs were collected from 4120 infants, of whom 3260 infants (79%) were admitted to the NICU. The BC remained negative in 97% (n = 3982) and became positive with a nonpathogenic microorganism, suggestive of contamination, in 2.1% (n = 88) (Fig. 1). In 50 BCs (1.2%), there was growth of pathogenic microorganisms. The microorganisms found were *S. agalactiae* (n = 28), *Escherichia coli* (n = 10), *H. influenzae* (n = 2), *Klebsiella pneumoniae* (n = 2), *S. aureus* (n = 2), *Klebsiella oxytoca* (n = 1), *L. monocytogenes* (n = 1), *S. pneumoniae* (n = 1), *H. parainfluenzae* (n = 1), *S. pyogenes* (n = 1) and *N. meningitidis* (n = 1). All cultures were monomicrobial, except 2 cultures, 1 with *N. meningitidis* and *S. anginosus* and 1 with *E. coli* and viridans group streptococci. Of the infants with a positive BC, 39 infants (78%) were admitted to the NICU and 11 infants (22%) to the NMCU.

C-Reactive Protein

In 1513 infants (52% of infants with CPS, 37% of all infants), a CRP value was available between 12 and 24 hours after OSEOS. This time frame was chosen because the sensitivity for an increased CRP in children with CPS found in this period was higher than <12 hours after OSEOS (Figs. 2 and 3). Within this timeframe, in 22/26 (85%) infants with CPS, CRP was raised ≥10 mg/L compared with 392/1487 (26%) of infants without positive BC ($P < 0.001$) (Fig. 2). In 1095 (74%) of infants without positive BC of whom CRP was measured between 12 and 24 hours after suspicion of EOS, CRP was <10mg/L (74% of NICU infants and 73% of NMCU infants) (Fig. 2).

TTP and Clinical Evaluation at 24 Hours

The median TTP for all BCs with a pathogenic microorganism was 14.4 hours (IQR 8.7, range 8.4–44.0). Excluding the 5 BCs with a procedural overestimation of TTP, the median TTP for BCs with a pathogenic microorganism, was 14.3 hours (IQR 8.8, range 8.4–44.0). Median TTP in NICU infants was 13.2 hours (IQR 9.2) and in NMCU infants 17.2 hours (IQR 7.2) (n.s.).

Eight of the 50 infants (16%) with culture-proven EOS had a TTP ≥24 hours. Table 1 shows the infants with TTP ≥24 hours with cultured microorganisms, TTP, CRP, ward of admittance and clinical signs of infection (if present). The microorganisms found in this group were *S. agalactiae* (n = 3), *H. influenzae* (n = 2), *K. oxytoca* (n = 1), *L. monocytogenes* (n = 1) and *N. meningitidis* (n = 1). In 5 of these 8 infants CRP was raised ≥10 mg/L within 24 hours after OSEOS. In the remaining 3 infants with normal CRP, 2 had clinical signs of infection present and treating physicians decided before the BC became positive, to continue the antibiotic treatment for a total of 7 days for suspected infection. In 1 infant TTP was ≥24 hours, no clinical signs of infection were present nor the CRP was elevated. Empiric antibiotics were still being administered atw

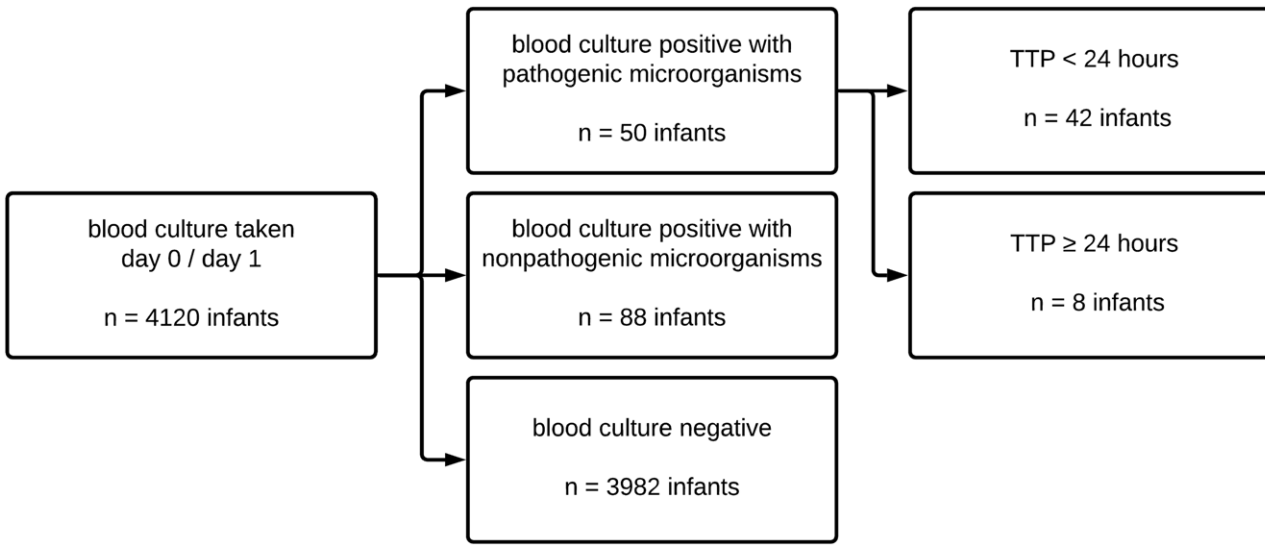

FIGURE 1. Flowchart.

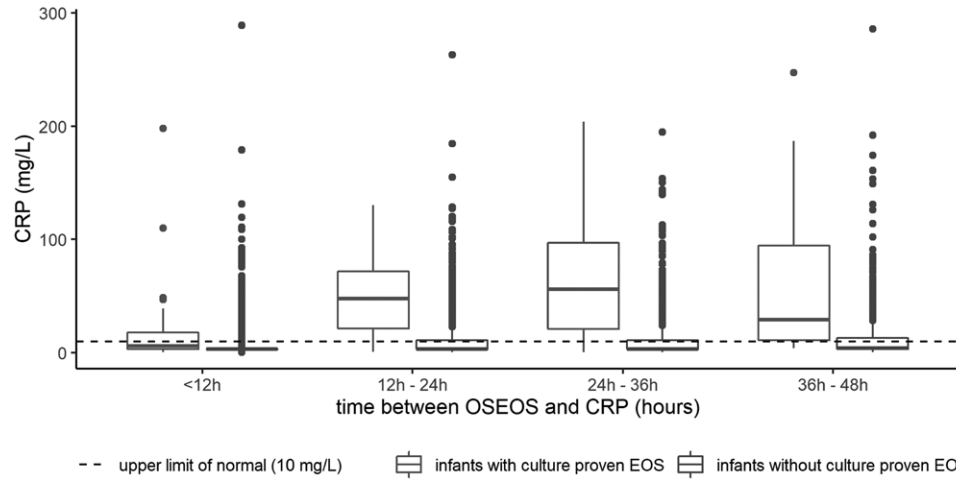

FIGURE 2. CRP in infants with suspicion of EOS: boxplot of highest CRP value per infant per time period. full color online

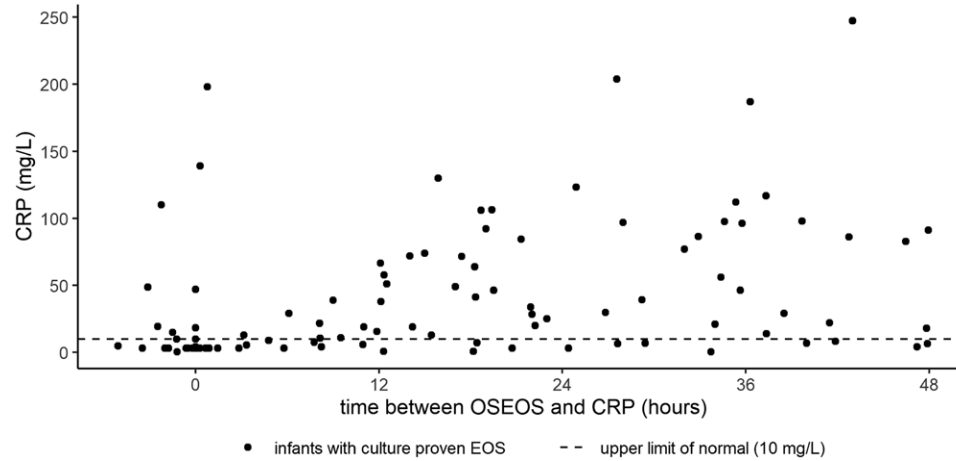

FIGURE 3. CRP in infants with culture proven EOS.

TABLE 1. Overview of Infants With Culture Proven Sepsis With TTP ≥ 24 Hours

	Gestational Age at Birth (wks)	Microorganism	TTP (h)	Maximum CRP (mg/L) <24 h After OSEOS	Clinical Signs of Infection*	Ward of Admittance
1	Term	*Streptococcus agalactiae*	24.03	<3 (t = 0 h)	Yes	NMCU
2	26	*Klebsiella oxytoca*	24.6	67 (t = 12 h)	Yes	NICU
3	28	*Haemophilus influenzae*	25.0	19 (t = 14 h)	Yes	NICU
4	Term	*S. agalactiae*	25.4	<3 (t = 0 h)	Yes	NICU
5	31	*Listeria monocytogenes*	26.3	198 (t = 1 h)	Yes	NICU
6	30	*S. agalactiae*	29.7	<3 (t = 0 h)	No	NICU
7	24	*H. influenzae*	34.3	11 (t = 10 h)	Yes	NICU
8	Term	*Neisseria meningitidis*	44.0	34 (t = 22 h)	Yes	NICU

*Clinical signs of infection and follow-up:

1. Prolonged rupture of membranes, maternal fever, tachycardia. In the first 24 hours development of fever, no other signs of infection. Initial CRP was low, follow-up CRP 39 hours after birth was 29 mg/L.

2. Maternal fever, prematurity and respiratory insufficiency, for which the infant needed continuous positive airway pressure. Initial CRP was raised.

3. Prematurity and respiratory insufficiency with hypercapnia, for which the infant was intubated, and surfactant was administered. Feeding retentions were present and initial CRP was raised.

4. Respiratory distress, low saturations, meconium-stained amniotic fluid, distended abdomen and low Apgar scores.

5. Reduced fetal movements, tachycardic and decelerative cardiotocography, after birth respiratory insufficiency for which the infant was intubated. The infant was in shock, and there were petechiae and thrombocytopenia. Initial CRP was raised.

6. Prematurity, prolonged rupture of membranes (4 days). Maternal urinary tract infection with *S. agalactiae*, treatment with clindamycin. There were no signs of infection after birth, initial CRP was low and the maximum CRP was 7 mg/L 29 hours after birth.

7. Prematurity, prolonged rupture of membranes and respiratory insufficiency, for which the infant was intubated, and surfactant was given, with increasing oxygen needs, high-frequency oscillatory ventilation was started. Initial CRP at birth was 10 mg/L, follow-up CRP 9 hours after birth was 11 mg/L. The infant died within 24 hours after birth because of respiratory failure.

8. Circulatory failure and abdominal problems (feeding retentions, vomiting and distended abdomen), suspicion of bowel obstruction/ischemic bowel. Initial CRP was low, and follow-up CRP after 22 hours was raised (34 mg/L).

30 hours when the positive BC results became available, the infant was then treated with a 2-week course of penicillin for *S. agalactiae* bacteremia.

TTP included transportation time to the in-hospital laboratory. The time between OSEOS and incubation was analyzed in a subgroup of 652 BCs (all BCs taken from June 1, 2017, to July 31, 2019), showing a median time of 1.5 hours (IQR 2.5).

Ruling Out Sepsis

Overall, 49 of 50 infants (98%) with CPS had one or more criteria suggestive of sepsis; 42 had positive BCs at 24 hours, 5 had negative BCs at 24 hours, but CRP ≥10 mg/L and clinical signs of infection and 2 had negative BCs and no CRP taken at 12–24 hours after OSEOS but clinical signs of infection.

DISCUSSION

In this 14-year cohort study, we identified 3 criteria of which at least one was present in 49 of 50 infants (98%) with a culture-proven EOS; a positive BC at 24 hours after OSEOS, CRP ≥10 mg/L or clinical signs of infection. If any of these criteria is present in an infant, 24 hours after starting antibiotics, a possible infection cannot not be ruled out and antibiotics would have to be continued. In 74% of the infants without a culture-proven EOS and an available CRP, the decision to stop antibiotics could have been brought forward to 24 hours instead of 48 hours as is currently advised in the guidelines, based on normal CRP levels and a negative BC result at 24 hours if there are no clinical signs of sepsis. The BC positivity rate in our cohort is low, with 1.2% of all BCs growing pathogenic microorganisms, consistent with other publications confirming the difficulty to diagnose EOS and a high number needed to start treatment for a possible EOS.[1–3] Seventy-three percent of NMCU infants with an available CRP between 12 and 24 hours after OSEOS had a CRP <10 mg/L and a negative BC at 24 hours and thus stopping antibiotics 24 hours after OSEOS would have resulted in less exposure to antibiotics and shorter duration of hospital stay in the vast majority of these infants. For the remainder of infants, more research is needed to guide timely and optimal decision-making regarding continuing or stopping treatment for possible sepsis.

Most other studies report that >95% of BCs with pathogens of infants with suspicion of EOS have a TTP <24 hours.[7,11,12,21] Adapting and extending the literature review of Marks et al,[12] selecting papers which presented data on the proportion of BCs of infants with suspicion of EOS positive at 24 hours, is shown in Table 2. Three of these studies define the start of TTP with incubation instead of inoculation or ordering the BC, and as such the reported TTP will be shorter.[7,12,21] For clinical decision-making, relevant timing is the time between blood draw and the moment the BC can be evaluated to stop antibiotics. Clinicians should be aware of the time of transportation to the laboratory when interpreting such studies. Laboratory and transportation logistics probably play a relevant role in determining TTP.[24]

In the largest study, including 594 infants with culture-proven EOS, TTP was analyzed including transportation and preanalytic time, and 68% of the BCs became positive within 24 hours.[24] In our population, including preterm infants, 84% of infants with culture proven EOS had a TTP <24 hours. We expected a longer TTP in prematurely born infants due to lower volumes of inoculated blood and more frequent need for maternal peripartum antibiotics. However, our results and the study of Kuzniewicz et al[24] suggested that these factors do not prolong TTP.

In our population, in 85% of infants with CPS and an available CRP between 12 and 24 hours after OSEOS, CRP was raised ≥10 mg/L. However, CRP was not determined in all children in this time period and selection bias may have occurred towards sicker infants. An increased CRP value might be an indicator of sepsis, but clinicians should be aware it can be low in infants with CPS.[18] In our study, 26% of infants without CPS had a raised CRP 12–24 hours after OSEOS. CRP is not only a laboratory sign of infection, it can be raised by different factors, such as low gestational age,

TABLE 2. Literature Review of Studies With Reported TTP <24 Hours in Infants With EOS

Study	Start of TTP	Number of Infants With BC-proven EOS, Excluding Contaminants	Percentage TTP <24 h	Study Population	CRP Taken into Account	Clinical Signs of Infection Taken into Account
Devlin and Malley[22] *	Unknown	6	100	Low-risk infants, gestational age not mentioned	All infants had a raised CRP	Yes, but not mentioned for the 6 children with culture-proven EOS
Al-Fifi et al[21]	Incubation	50	98	NICU and non-NICU	No	No
Khan et al[23] *	Inoculation	3	100	Term and preterm (<37 wks)	No	Yes
Ur Rehman Durrani et al[11]	Inoculation	44	100	NICU	No	No
Marks et al[12]	Incubation	40	97.5	Infants born at a gestational age of > 34 wks	No	Yes, of the infant with TTP ≥ 24 h
Kuzniewicz et al[24]	93% inoculation, 7% BC order time	594	68	71% ≥ 35 wks	No	No
Jardine et al[7]	Incubation	21	95.2	NICU	No	Yes, of the infant with TTP ≥ 24 h

*Conference abstract only, no full-text article available.

prolonged rupture of membranes or maternal pregnancy-induced hypertension.[14,15] In contrast, as shown by Stocker et al,[25] a normal CRP within 36 hours after start of antibiotic therapy can exclude the presence of EOS in infants born after 34 weeks' gestation with a high probability.

By combining CRP with BC results and clinical evaluation 98%, but not all infants with culture-proven EOS were correctly recognized at 24 hours, illustrating the common dilemma in the neonatology ward, especially if there is no other reason for hospital admission than suspected EOS. Missing sepsis is the widely feared scenario. Using BCs and CRP only, sepsis would have been missed in 3 infants at 24 hours, illustrating the necessity to take into account the clinical evaluation as well. Nevertheless, all common pathogenic microorganisms of EOS in the infant are acquired during birth and represent maternal colonization. It could be possible, by analogy with contamination of BCs with skin flora, that sporadically pathogenic bacteria are also contaminants. Especially when an infant is not clinically ill, nor has an elevated CRP, a low load of bacteria due of skin/BC contamination could lead to a prolonged TTP, which could have happened in the infant with TTP of 29.7 hours, low CRP, no clinical signs of infection, and proven maternal colonization with *S. agalactiae.*

Our proposed approach of evaluating to stop antibiotics 24 hours after OSEOS based on a negative BC, low CRP and favorable clinic would have resulted in one NICU infant (with a TTP of 30 hours) in whom the antibiotics would have been stopped prematurely. However, antibiotic treatment can be restarted in infants in whom the BC became positive after 24 hours, and antibiotics given at 24 hours will be effective for another 6 hours limiting the consequences of a prolonged TTP.

The strength of our study is combining TTP with CRP and clinical signs of infection. It shows that other inflammatory markers, such as procalcitonin and interleukin-6, are not needed to decide to stop antibiotic treatment 24 hours after OSEOS. Our study has several shortcomings, mainly due to its retrospective nature and because it was performed at a single center, including infants enrolled over a large period of time. Therefore, results can only be extrapolated with caution to other centers, because patient population, antibiotic protocols and laboratory and transportation logistics may vary. Second, the number of infants with a positive BC was low despite our range of 14 years. Hence, our data were only used to rule out sepsis at 24 hours of OSEOS and cannot be used to predict or rule out sepsis at any other timepoint. Also, CRP measurements were not performed at fixed time points, limiting our analysis of its predictive value.

We conclude that a combination of BC results, CRP and clinical signs of infection at 24 hours after OSEOS can rule out culture-proven early onset neonatal sepsis. This should reduce the exposure to antibiotics in all new born children significantly and reduce the time of hospital stay in the majority of term infants.

REFERENCES

1. Escobar GJ, Puopolo KM, Wi S, et al. Stratification of risk of early-onset sepsis in newborns ≥ 34 weeks' gestation. *Pediatrics.* 2014;133:30–36.
2. Fjalstad JW, Stensvold HJ, Bergseng H, et al. Early-onset sepsis and antibiotic exposure in term infants: a nationwide population-based study in Norway. *Pediatr Infect Dis J.* 2016;35:1–6.
3. Stocker M, van Herk W, El Helou S, et al; NeoPInS Study Group. Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). *Lancet.* 2017;390:871–881.
4. Kornelisse RF, Tuut MK, Venmans LMAJ, et al. Prevention and treatment of early onset sepsis. 2017. Available from: https://www.nvog.nl/wp-content/uploads/2018/02/Preventie-en-behandeling-van-early-onset-neonatale-infecties-1.0-07-06-2017.pdf. Accessed October 1, 2019.

5. National Institute for Health and Clinical Excellence. Antibiotics for early-onset neonatal infection. CG149. National Institute for Health and Clinical Excellence; 2012.
6. Malley M, Bruce M, Devlin H, et al. It is time to standardise the length of postnatal antibiotic administration nationally. *Arch Dis Child Fetal Neonatal Ed.* 2019;104:F225–F226.
7. Jardine L, Davies MW, Faoagali J. Incubation time required for neonatal blood cultures to become positive. *J Paediatr Child Health.* 2006;42:797–802.
8. Guerti K, Devos H, Ieven MM, et al. Time to positivity of neonatal blood cultures: fast and furious? *J Med Microbiol.* 2011;60(Pt 4):446–453.
9. Biondi EA, Mischler M, Jerardi KE, et al; Pediatric Research in Inpatient Settings (PRIS) Network. Blood culture time to positivity in febrile infants with bacteremia. *JAMA Pediatr.* 2014;168:844–849.
10. Garcia-Prats JA, Cooper TR, Schneider VF, et al. Rapid detection of microorganisms in blood cultures of newborn infants utilizing an automated blood culture system. *Pediatrics.* 2000;105(3 Pt 1):523–527.
11. Ur Rehman Durrani N, Rochow N, Alghamdi J, et al. Minimum duration of antibiotic treatment based on blood culture in rule out neonatal sepsis. *Pediatr Infect Dis J.* 2019;38:528–532.
12. Marks L, de Waal K, Ferguson JK. Time to positive blood culture in early onset neonatal sepsis: a retrospective clinical study and review of the literature. *J Paediatr Child Health.* 2020;56:1371–1375.
13. Huggard D, Powell J, Kirkham C, et al. Time to positivity (TTP) of neonatal blood cultures: a trend analysis over a decade from Ireland. *J Matern Fetal Neonatal Med.* 2021;34:780–786.
14. Chiesa C, Signore F, Assumma M, et al. Serial measurements of C-reactive protein and interleukin-6 in the immediate postnatal period: reference intervals and analysis of maternal and perinatal confounders. *Clin Chem.* 2001;47:1016–1022.
15. Chiesa C, Natale F, Pascone R, et al. C reactive protein and procalcitonin: reference intervals for preterm and term newborns during the early neonatal period. *Clin Chim Acta.* 2011;412:1053–1059.
16. Perrone S, Lotti F, Longini M, et al. C reactive protein in healthy term newborns during the first 48 hours of life. *Arch Dis Child Fetal Neonatal Ed.* 2018;103:F163–F166.
17. Hofer N, Zacharias E, Müller W, et al. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. *Neonatology.* 2012;102:25–36.
18. Lai MY, Tsai MH, Lee CW, et al. Characteristics of neonates with culture-proven bloodstream infection who have low levels of C-reactive protein (≦10 mg/L). *BMC Infect Dis.* 2015;15:320.
19. Tessema B, Lippmann N, Willenberg A, et al. The diagnostic performance of interleukin-6 and C-reactive protein for early identification of neonatal sepsis. *Diagnostics (Basel).* 2020;10:E978.
20. R Core Team. *R: A Language and Environment for Statistical Computing.* R Foundation for Statistical Computing. Available from: https://www.R-project.org/ [program], 2019.
21. Al-Fifi SH AWW, Asiri MH, Otaif MY, et al. When to stop antibiotics in clinically suspected neonatal sepsis with a negative blood culture? *Med J Cairo Univ*. 2010;78:601–605.
22. Devlin H ND, Malley M. Should we consider switching to a 24-hour course of antibiotics for asymptomatic, low risk babies with suspected early onset neonatal sepsis. *Arch Dis Child* 2019;104(Suppl 2):A1–A279.
23. Khan A TJ, Oo SY, O'Kane G. Timing of blood culture positivity in early neonatal sepsis. *J Paediatr Child Health* 2016:95.
24. Kuzniewicz MW, Mukhopadhyay S, Li S, et al. Time to positivity of neonatal blood cultures for early-onset sepsis. *Pediatr Infect Dis J.* 2020;39:634–640.
25. Stocker M, van Herk W, El Helou S, et al. C-reactive protein, procalcitonin, and white blood count to rule out neonatal early-onset sepsis within 36 hours: a secondary analysis of the neonatal procalcitonin intervention study. [published online ahead of print September 3, 2020] *Clin Infect Dis*. doi: 10.1093/cid/ciaa876.