**C-reactive Protein, Procalcitonin and White Blood Count to rule out neonatal early-onset sepsis within 36 hours: A secondary analysis of the NeoPInS study**Martin Stocker, Wendy van Herk\*, Salhab el Helou, Sourabh Dutta, Frank ABA Schuerman, Rita K van den Tooren-de Groot, Jantien W Wieringa, Jan Janota, Laura H van der Meer-Kappelle, Rob Moonen, Sintha D Sie, Esther de Vries, Albertine E Donker, Urs Zimmerman, Luregn J Schlapbach, Amerik C de Mol, Angelique Hoffman-Haringsma, Madan Roy, Maren Tomaske, René F Kornelisse, Juliette van Gijsel, Eline G Visser, Frans B Plötz, Paul Heath, Niek B Achten, Dirk Lehnick, Annemarie MC van Rossum

\* Martin Stocker and Wendy van Herk contributed equally to this manuscript

**Affiliations**

Martin Stocker, Department of Paediatrics, Neonatal and Paediatric Intensive Care Unit, Children’s Hospital Lucerne, Lucerne, Switzerland

Wendy van Herk, Department of Paediatrics, Division of Paediatric Infectious Diseases & Immunology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, the Netherlands

 Salhab el Helou, Division of Neonatology, McMaster University Children’s Hospital, Hamilton Health Sciences, Hamilton, ON, Canada

Frank ABA Schuerman, Department of Neonatal Intensive Care Unit, Isala Women and Children’s Hospital, Zwolle, the Netherlands

Rita K van den Tooren-de Groot , Department of Paediatrics, Haaglanden Medical Centre, ‘s Gravenhage, the Netherlands

Jantien W Wieringa, Department of Paediatrics, Haaglanden Medical Centre, ‘s Gravenhage, the Netherlands

Jan Janota, Department of Obstetrics and Gynocology, Motol University Hospital, Second Medical Faculty, Prague, Czech Republic and Institute of pathological Physiology, First Medical Faculty, Prague, Czech Republic

Laura H van der Meer-Kappelle, Department of Neonatology, Reinier de Graaf Gasthuis, Delft, the Netherlands

Rob M Moonen, Department of Neonatology, Zuyderland Medical Centre, Heerlen, the Netherlands

Sintha D Sie, Department of Neonatology, Amsterdam UMC, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands

Esther de Vries, Department of Paediatrics, Jeroen Bosch Hospital, ‘s-Hertogenbosch, the Netherlands Albertine E Donker, Department of Paediatrics, Maxima Medical Centre, Veldhoven, the Netherlands

Urs Zimmermann, Department of Paediatrics, Kantonsspital Winterthur, Winterthur, Switzerland

Luregn J Schlapbach, Paediatric Critical Care Research Group, Child Health Research Centre, University of Queensland, and Padiaitric Intensive Care Unit, Queensland Children's Hospital, Brisbane, Australia; and University Children's Hospital Zurich and University of Zurich, Zurich, Switzerland

Amerik C de Mol, Department of Neonatology, Albert Schweitzer Hospital, Dordrecht, the Netherlands Angelique Hoffman-Haringsma, Department of Neonatology, Sint Franciscus Gasthuis, Rotterdam, the Netherlands

Madan Roy, Department of Neonatology, St. Josephs Healthcare, Hamilton Health Sciences, Hamilton, ON, Canada

Maren Tomaske, Department of Paediatrics, Stadtspital Triemli, Zürich, Switzerland

René F Kornelisse, Department of Paediatrics, Division of Neonatology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, the Netherlands

Juliette van Gijsel, Therapeuticum Utrecht, Utrecht, the Netherlands

Eline G Visser, Department of Paediatrics, Division of Paediatric Infectious Diseases & Immunology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, the Netherlands

Frans B Plötz, Department of Pediatrics, Tergooi Hospital, Blaricum, the Netherlands

Paul Heath, Department of Paediatric Infectious Disease, St George’s University Hospital, London, UK

Niek B Achten, Department of Pediatrics, Tergooi Hospital, Blaricum, the Netherlands

Dirk Lehnick, Department of Health Sciences and Medicine, Head Biostatistics and Methodology, University of Lucerne, Lucerne, Switzerland

Annemarie MC van Rossum, Department of Paediatrics, Division of Paediatric Infectious Diseases & Immunology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, the Netherlands

**Key words:** neonatal early-onset sepsis, C-reactive protein, procalcitonin, white blood count, negative predictive value

**Running title:** Biomarker-guidance to rule out EOS

**Corresponding author:** Martin Stocker MD, Department of Paediatrics, Division of Neonatal and Paediatric Intensive Care, Children's Hospital, Lucerne, Switzerland. Mail: martin.stocker@luks.ch

**Alternate corresponding author:** Annemarie MC van Rossum, Department of Paediatrics, Division of Paediatric Infectious Diseases & Immunology, Erasmus MC University Medical Centre-Sophia Children's Hospital, Rotterdam, the Netherlands. Mail: a.vanrossum@erasmusmc.nl

**Summary:** Normal serial CRP and PCT measurements within 36h after start of empiric antibiotic therapy allow neonatal EOS to be ruled out with a high probability. The negative predictive values of CRP and PCT do not increase after 36 hours.

**ABSTRACT
Background**
Neonatal early-onset sepsis (EOS) is one of the main causes of global neonatal mortality and morbidity and initiation of early antibiotic treatment is key. But antibiotics may harm: Increasing resistance due to overuse of antibiotics and alteration of the individual microbiome are the potential downside of unnecessary antibiotic use.  **Methods**
Secondary analysis of NeoPInS, a prospective, multi-centre, randomised controlled intervention study: Primary outcome was the diagnostic accuracy of serial measurements of C-reactive protein (CRP), procalcitonin (PCT), and white blood count (WBC) within different time windows to rule out culture-positive EOS (proven sepsis).  **Results**
We analysed 1678 neonates with 10,899 biomarker measurements (4654 CRP, 2047 PCT and 4198 WBC measurements) obtained within the first 48 hours after start of antibiotic therapy due to suspected EOS. The area under the curve (AUC) comparing no sepsis versus proven sepsis for maximum values of CRP, PCT and WBC within 36 hours were 0.986, 0.921, and 0.360, respectively. The AUC for CRP and PCT increased with extended timeframes up to 36 hours but there was no further difference between start to 36 versus start to 48 hours. Cut-off values at 16mg/l for CRP and 2.8ng/l for PCT provided a sensitivity of 100% for discriminating no sepsis versus proven sepsis.  **Conclusions**
Normal serial CRP and PCT measurements within 36h after the start of empiric antibiotic therapy can exclude the presence of neonatal EOS with a high probability. The negative predictive values of CRP and PCT do not increase after 36 hours.

**INTRODUCTION**

Sepsis may kill: neonatal sepsis is one of the main reasons for global neonatal mortality, in low-income as well as high-income countries.1 Early diagnosis and prompt antibiotic therapy are key to prevent morbidity and mortality.2 Initial clinical signs and biomarkers are non-specific,3,4 which drives the massive use of antibiotics for suspected early-onset sepsis (EOS).5 The number needed to treat for one proven EOS in term and late-preterm infants varies in the literature between 40 and over 100.6 The use of antibiotics in neonates with sepsis is essential, but may also harm. The problem of increasing resistance due to overuse of antibiotics is well known and the World Health Organization has declared antibiotic resistance as one of the main problems to be focused on within the next decade.7 In addition, evidence is growing that antibiotic therapy early in life may change the individual microbiome with possible consequences for individual developmental origins of future health and disease.8

There is a broad agreement that biomarkers are not helpful regarding the decision to start antibiotic therapy because of a poor positive predictive value.4 In contrast, biomarkers may provide guidance on the duration of antibiotic therapy. The call to end the acceptance in treating culture-negative sepsis is distinct and reasonable.9 Although a positive blood culture is still the gold standard for diagnosis of sepsis, many clinicians are hesitant to stop antibiotic treatment early on the basis of negative cultures alone, due to concerns around culture-negative sepsis.10 Recently, we published the Neonatal Procalcitonin Intervention Study (NeoPInS) showing that PCT-guided decision-making reduces duration of antibiotic treatment significantly, with a low rate of re-infections and no study-related mortality.11 Nevertheless, overtreatment still remains and controversy around the most suitable marker persists.

Therefore, we conducted secondary analyses of the NeoPInS-cohort, consisting of 1678 neonates with 10'899 biomarker measurements within the first 48 hours after start of antibiotic therapy because of suspected EOS. We have analysed the diagnostic accuracy of serial measurements of C-reactive protein (CRP), procalcitonin (PCT), and white blood count (WBC) to rule out EOS. We aimed to focus on the negative predictive value within different timeframes to answer the question when it is safe to stop antibiotic therapy started due to suspected EOS. Results of this analysis may guide the design of new prospective studies aiming to further reduce exposure to antibiotics within the first week of life.

**METHODS**

This is a secondary analysis of biomarkers in the population of NeoPInS.11 The local institutional review boards and the national ethical committees of each site approved the study. Written informed consent was obtained from all parents or caregivers.

**Summary of the study design of NeoPInS**

NeoPInS was an investigator-initiated, multicentre, randomised controlled intervention study aiming to reduce duration of antibiotic therapy.11 The trial was registered at Clinicaltrials.gov (NCT00854932). The detailed methods of the study were reported in the published protocol and the study publication.11,12 Participants were neonates born after completion of 34 weeks of gestational age, who were suspected of EOS within the first 72 hours of life and were started on antibiotic therapy. The probability of infection was assessed within 12 hours after initiation of antibiotic therapy with a scoring system using risk factors, clinical signs, and the blood markers CRP and WBC, but was independent of PCT (first measurement of PCT 12 hours after start of antibiotic therapy). Risk factors were defined as maternal group B streptococci carriage, clinical signs of chorioamnionitis, premature rupture of membranes longer than 18 hours, and gestational age less than 37 0/7 weeks. Clinical signs possibly related to infection included respiratory signs, heart rate abnormalities, perfusion problems, temperature deviations, neurological signs, and abdominal signs. Abnormal biomarkers at start of suspected EOS were defined as CRP >10 mg/l and leucocytopenia < 5 G/l (< 5000 cells/mm3).

Neonates were randomised to PCT-guided therapy or standard care. Neonates with proven or probable infection were treated with antibiotics for at least seven days according to local policy in each participating centre, independent of randomisation. For neonates with a low or medium risk for infection in the PCT-group, duration of antibiotic treatment was PCT-guided, with a minimum of 24 hours. Neonates with a low or medium risk of infection in the standard group were treated for 36 to 72 hours and five to seven days, respectively. As in current daily practice, the decision to discontinue antibiotic therapy in the standard group was made by the treating physician based on blood culture results, clinical signs, and conventional laboratory test results (WBC, CRP). Physicians were at all times allowed to overrule the recommendation and continue antibiotic therapy based on other reasons, such as clinical symptoms or other laboratory investigations.

Within the first 48 hours of suspected EOS, biomarkers were analysed at start (WBC, CRP), 12 hours (PCT in the PCT-group), 24 hours (WBC, CRP, PCT) and 48 hours (WBC, CRP, PCT) after start of antibiotic therapy. Blood cultures were drawn before starting antibiotic therapies, and other cultures (e.g., cerebrospinal fluid cultures) and/or other additional diagnostic tests (e.g., radiography) were performed on indication. Follow-up information regarding recurrence of infection, re-hospitalisation, additional courses of antibiotics, and death was obtained by interviewing the parents during their follow-up visits, or by telephone interview at least one month after discharge.

**Participants and data acquisition**

The NeoPInS-cohort included neonates after completion of 34 weeks of gestational age receiving antibiotic therapy due to suspected EOS. All data for this secondary analysis were derived from the original NeoPInS-database. Availability of blood culture results, exact duration of antibiotic treatment and results of biomarker measurements (CRP, PCT, WBC) within the first 48 hours after start of antibiotic treatment were defined as key variables.

**Definitions of infection groups**

For this secondary analysis, we stratified infants into four groups based on risk of sepsis: sepsis proven, sepsis probable, sepsis uncertain, and no sepsis (table 1). Proven sepsis was defined as positive blood culture and at least one abnormal finding out of the three areas of risk factors, clinical signs, and biomarkers (CRP > 10mg/l or leucocytopenia < 5 G/l at start of suspected sepsis). We excluded infants from the group of proven infection if positive blood cultures were considered to be contaminated (growth of normal skin flora and antibiotic therapy of less than 48 hours). Sepsis probable was defined as neonates with high risk of sepsis due to risk factors, clinical signs and abnormal biomarkers at start of suspected sepsis, and with treatment duration of more than 5 days, but negative blood cultures. In literature, for infants with a sepsis probable the term culture-negative sepsis has been used.10 Whereas the term "culture-negative sepsis" implies that an infection is really present, we emphasise that this is unknown. Sepsis uncertain was defined as neonates with low to moderate risk of sepsis (none to two abnormal findings out of the three areas of risk factors, clinical signs, and biomarkers) and antibiotic therapy for more than 48 hours, and a negative blood culture. No sepsis was defined as neonates with low to moderate risk of sepsis (none to two abnormal findings out of the three areas of risk factors, clinical signs, and biomarkers), and antibiotic therapy of less than 48 hours without recurrence of infection, and a negative blood culture. Whereas the term "no infection" is not completely correct for this group, due to the fact that an infection is never completely excluded even after a short course of antibiotics, we used this term in order to make a clear distinction from the “infection unlikely” infants in the original NeoPInS study.

**Outcomes**

All outcomes were predefined before starting the secondary analyses. The primary outcome is the analysis of the area under the curve (AUC) of CRP, PCT, and WBC for the discrimination of no sepsis versus proven sepsis. The analyses were done for the maximal values within the first 48 hours after start of antibiotics for suspected EOS. The National Institute for Health and Care Excellence (NICE)-guidelines recommend re-evaluation of EOS management using CRP measurements repeated 18 and 36 hours after the start of antibiotics, thus we have analysed the biomarkers with increasing timeframes from start of antibiotics to 18 hours, 36 hours, and 48 hours, respectively.3 Optimal cut-off values were determined with an aimed sensitivity at 100% discriminating no sepsis versus proven sepsis. Secondary outcomes included the discrimination of no sepsis versus proven and probable sepsis, and no sepsis versus proven, probable and uncertain sepsis. This was done to support a pragmatic aspect to the analyses; in reality, we do not know if neonates with probable or uncertain categories of sepsis really have an infection or not. Optimal cut-off values were determined with an aimed sensitivity at 95% discriminating no sepsis versus proven or probable sepsis. Finally, to analyse the potential impact of additional biomarkers to the published PCT-guided algorithm of NeoPInS, the group of patients with antibiotic treatments below 48 hours in the PCT group in the original study were compared with the group of patients with CRP and/or WBC below the optimal cut-off values in the population without proven or probable sepsis.

**Statistical methods**

Data are presented as median (interquartile range, IQR) for continuous and as numbers and frequencies for categorical variables. Primary and secondary outcomes, i.e. discrimination of no sepsis versus proven sepsis (or further categories including probable and ambiguous sepsis) by means of WBC, CRP or PCT criteria, have been analysed utilizing ROC (receiver operating characteristics) curves and corresponding AUCs (areas under the curve) were evaluated. WBC, CRP or PCT criteria selected for the determination and comparison of ROC curves were maximum values of the respective biomarker between start of antibiotic therapy and 18, 36 or 48 hours after start. The ROC curves were compared using graphical displays and the determination of ROC AUCs incl. 95% confidence intervals. Potential differences between AUCs were analysed utilizing the test procedure according to DeLong.13 Statistical analyses were conducted with STATA (Version 15.2 or later, StataCorp, College Station, Texas, USA).

**RESULTS**

We analysed 1678 neonates with 10,899 biomarker measurements obtained within the first 48 hours after start of antibiotic therapy because of suspected EOS. The population is described in table 2. The chronological distribution of biomarker measurements after start of antibiotic therapy is shown in figure 1.

The ROC AUCs comparing no sepsis versus proven sepsis, no sepsis versus probable/proven sepsis, and no sepsis versus uncertain/probable/proven sepsis for maximum values of CRP, PCT, and WBC within 36 hours are shown in table 3. For all comparisons, the AUC for CRP and PCT increased with extended timeframes up to 36 hours, whereas there was no difference between start to 36 versus start to 48 hours (figure 2). The AUC for WBC was significantly lower (p<0.001) than for CRP and PCT for all comparisons and timeframes. Determination of cut-off values with a sensitivity of 100% discriminating no sepsis versus proven sepsis within 36 hours was at 16mg/l for CRP and 2.8ng/l for PCT (table 3).

The combination of CRP and PCT did not significantly increase the ROC AUC for any comparison. In the original NeoPInS study, 49.7% of infants in the PCT group with low or moderate risk of infection were treated for less than 48 hours with PCT-guidance. 65.1% of participants with a low or moderate risk of infection had a maximum CRP below the cut-off of 13mg/l discriminating with a sensitivity of >95% no sepsis versus probable/proven sepsis within 36 hours. Among the infants in the PCT group with low or moderate risk of infection who were treated for less than 48 hours, 84.7% had a maximum CRP <13mg/l.

**DISCUSSION**

The results of our analysis of CRP, PCT, and WBC within the first 48 hours after start of antibiotics for suspected EOS showed good accuracy to exclude culture-positive sepsis for CRP and PCT, but not for WBC. The high negative predictive value for CRP and PCT is in line with the current literature.14,15 The low predictive value of WBC for EOS early after delivery is in line with the literature as well. Only a leukocytopenia < 5 G/L at start of antibiotic therapy has a reasonable positive predictive value.16–18

The question of when is it safe to stop antibiotic treatment started for suspected EOS is an important one. Splitting the time period of 48 hours after start of antibiotic therapy into periods of 18, 36, and 48 hours, we were able to show that the performance of the negative predictive values of CRP and PCT didn’t increase from 36 to 48 hours. This is an important hint that we do not have to wait 48 hours to use these biomarkers to support the decision to stop antibiotic therapy. If CRP or PCT remain below the thresholds of 16mg/l and 2.8ng/l, respectively within 36 hours after start of antibiotics, antibiotic treatment can be stopped safely. This timeframe is as well supported by the overall reported time-to-positivity of blood cultures in neonates with EOS: In a recently published study, the median time to positivity in EOS was 12 hours and all cultures were positive within 24 hours.19 The NICE-guideline recommends to consider stopping antibiotic treatment after 36 hours if CRP is reassuring.3 The NICE-guideline has been criticized due to a higher rate of investigations and prolonged duration of antibiotic therapy after implementation.20 A possible reason for this may be found in the heuristic way that clinicians make a decision in such a situation. The cut-off value for CRP of 16mg/L has a high negative predictive value for culture-proven sepsis and helps regarding decisions to stop antibiotic treatment. On the other hand, it is not a useful cut-off value regarding the positive predictive value as up to 20% of newborns have a physiological rise of CRP above 10mg/l after birth and the positive predictive value was reported to be only 14%.21–23

Interestingly, the negative predictive values of CRP and PCT remain reasonable in the comparison of no sepsis versus proven/probable sepsis and no sepsis versus proven/probable/uncertain sepsis. This is important because we really do not know the true infection status of infants in the two groups of probable and uncertain sepsis. This is a common, daily dilemma for clinicians. Whereas it is relatively straightforward regarding antibiotic management of infants who clearly have no sepsis or have culture-proven sepsis, there remains a clinical challenge in how long to treat infants without a positive culture but with clinical signs possibly related to sepsis, and/or risk factors and/or elevated biomarkers. The call to end the era of acceptance to treat culture-negative sepsis and to justify antibiotic treatment solely on blood culture results sounds consequential, but past experiences of clinicians of apparent truly culture-negative sepsis cases may hinder the implementation of this strict approach.9,10 Nevertheless, the relation of culture-proven to culture-negative sepsis reported in the literature with ratios of 1:6 to 1:16 remains highly questionable.10

There remains the question, which biomarker is best to rule out EOS within the first 48 hours. At the first sight, PCT has the disadvantage to be more expensive and to have the need to use a nomogram due to PCT kinetics. On the other hand, CRP as well has a physiological rise within the first few days of life.21,24,25 A recently published meta-analysis regarding CRP-guided duration of antibiotic therapy showed a decreased duration of therapy for the neonatal population.26 However, no single study included in this meta-analysis was powered to prove safety. In our study, CRP performed slightly better than PCT, whereas PCT performed slightly better than CRP in recently published meta-analyses.14,15 As a limitation of our study, CRP was part of the definition of uncertain and probable sepsis cases, which may have apparently increased the diagnostic performance of CRP in comparison to PCT. Based upon our data and sampling scheme, a combination of CRP and PCT could not yet prove to increase the performance in this analysis. Nevertheless, to estimate the benefit of adding CRP to the PCT-guided NeoPInS algorithm with time-dependent cut-off values (nomogram), we compared the percentage of patients in the PCT-group treated less than 48 hours in the original study with the percentage of patients with a maximum CRP below 13mg/l without probable or proven sepsis. Close to 50% of PCT-guided neonates were treated less than 48 hours, and 85% of this group had a CRP below 13mg/l. 65% of the group with low or moderate risk of infection had a CRP below 13mg/l. Therefore, there is a potential benefit to increase the number of newborns treated for less than 48 hours from 50% to around 70% with implementation of CRP into the NeoPInS-Algorithm.

Beside the question of when is it safe to stop, we have to ask when do we have to start antibiotic therapy for suspected EOS. The use of the sepsis calculator may reduce the use of unnecessary antibiotics significantly.27,28 One of the largest published study using the sepsis calculator reported that 2.6% of all term and late-preterm neonates received antibiotics due to suspected EOS.29 Therefore, the discrepancy between the rate of neonates started on antibiotic therapy and the reported rate of proven EOS of 0.1 to 0.8 out of 1000 live births remains highly significant.6

The main limitation of this study is that it is a secondary analysis of the NeoPInS-cohort and the study was not designed for these analyses with a potential bias due to unblinded CRP, PCT and WBC measurements. In addition, there was only a low number of proven sepsis cases and we were not able to reasonably analyse positive predictive values. Nevertheless, with over 10'000 biomarkers our study is one of the largest biomarker analyses for suspected EOS.

Conclusions: Normal serial CRP and PCT measurements within 36h after start of empiric antibiotic therapy allow neonatal EOS to be ruled out with a high probability. The negative predictive values of CRP and PCT do not increase from 36 to 48 hours. One decade ago, there was the call to reduce duration of empiric treatment for suspected EOS from 72 to 48h. Now it's time for the next step: to stop unnecessary antibiotic therapies within 36h.

**Contributors:**

*Principal investigators*: AvR, MS. *Study concept and design:* AvR, MS, WvH, EV, JvG which was approved by all authors. *Enrolment of patients and data collection:* SeH, SD, MF, FS, RvdT, JW, JJ, LvdM, RM, SS, EdV, AD, UZ, LS, AdM, AH, MR, MT, RK (all local investigators). *Study supervision:* WvH, MS, and AvR. *Supervision & monitoring data entry and checking database for accuracy*: WvH, MS. *Statistical analysis:* MS, DL. *Analysis and interpretation of data:* MS, WvH, AvR, DL, FP, NA, PH. *Obtained funding:* AvR, MS, WvH. All authors read/ critically revised/ approved the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

**FUNDING**

This Work was supported byThe Thrasher Foundation [9143]; The NutsOhra Foundation [1101-059]; and The Sophia Foundation for Scientific research [681]

**ACKNOWLEDGEMENTS**

We thank the patients and their families for participating in this trial. We thank Jurgen Reimer, all physicians, physician assistants, and nursing staff for their commitment to the trial. We thank Céline Stocker for designing figure 2. None of those listed received any financial incentives for their contributions. Thermofisher provided procalcitonin kits and provided an unrestricted grant for the organisation of four investigator meetings (2008, 2009, 2013, and 2015). Thermofisher had no involvement in other aspects of the trial.

 **Declaration of interests:** All other authors declare no competing interests.

**REFERENCES**

1 Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: a systematic review. *Lancet Respir Med* 2018; **6**: 223–30.

2 Weiss SL, Fitzgerald JC, Balamuth F, *et al.* Delayed antimicrobial therapy increases mortality and organ dysfunction duration in pediatric sepsis. *Crit Care Med* 2014; **42**: 2409–17.

3 National Collaborating Centre for Women’s and Children’s Health (UK). Antibiotics for Early-Onset Neonatal Infection: Antibiotics for the Prevention and Treatment of Early-Onset Neonatal Infection. London: RCOG Press, 2012 http://www.ncbi.nlm.nih.gov/books/NBK116610/ (accessed July 31, 2019).

4 Shane AL, Sánchez PJ, Stoll BJ. Neonatal sepsis. *Lancet Lond Engl* 2017; **390**: 1770–80.

5 Schulman J, Benitz WE, Profit J, *et al.* Newborn Antibiotic Exposures and Association With Proven Bloodstream Infection. *Pediatrics* 2019; **144**. DOI:10.1542/peds.2019-1105.

6 van Herk W, Stocker M, van Rossum AMC. Recognising early onset neonatal sepsis: an essential step in appropriate antimicrobial use. *J Infect* 2016; **72 Suppl**: S77-82.

7 WHO | Global action plan on AMR. WHO. http://www.who.int/antimicrobial-resistance/global-action-plan/en/ (accessed Jan 18, 2020).

8 Stiemsma LT, Michels KB. The Role of the Microbiome in the Developmental Origins of Health and Disease. *Pediatrics* 2018; **141**. DOI:10.1542/peds.2017-2437.

9 Cantey JB, Baird SD. Ending the Culture of Culture-Negative Sepsis in the Neonatal ICU. *Pediatrics* 2017; **140**. DOI:10.1542/peds.2017-0044.

10 Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Front Pediatr* 2018; **6**: 285.

11 Stocker M, van Herk W, El Helou S, *et al.* Procalcitonin-guided decision making for duration of antibiotic therapy in neonates with suspected early-onset sepsis: a multicentre, randomised controlled trial (NeoPIns). *Lancet Lond Engl* 2017; **390**: 871–81.

12 Stocker M, Hop WCJ, van Rossum AMC. Neonatal Procalcitonin Intervention Study (NeoPInS): Effect of Procalcitonin-guided decision making on duration of antibiotic therapy in suspected neonatal early-onset sepsis: A multi-centre randomized superiority and non-inferiority Intervention Study. *BMC Pediatr* 2010; **10**: 89.

13 DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics* 1988; **44**: 837–45.

14 Eschborn S, Weitkamp J-H. Procalcitonin versus C-reactive protein: review of kinetics and performance for diagnosis of neonatal sepsis. *J Perinatol Off J Calif Perinat Assoc* 2019; **39**: 893–903.

15 Ruan L, Chen G-Y, Liu Z, *et al.* The combination of procalcitonin and C-reactive protein or presepsin alone improves the accuracy of diagnosis of neonatal sepsis: a meta-analysis and systematic review. *Crit Care Lond Engl* 2018; **22**: 316.

16 Ottolini MC, Lundgren K, Mirkinson LJ, Cason S, Ottolini MG. Utility of complete blood count and blood culture screening to diagnose neonatal sepsis in the asymptomatic at risk newborn. *Pediatr Infect Dis J* 2003; **22**: 430–4.

17 Hornik CP, Benjamin DK, Becker KC, *et al.* Use of the complete blood cell count in early-onset neonatal sepsis. *Pediatr Infect Dis J* 2012; **31**: 799–802.

18 Newman TB, Puopolo KM, Wi S, Draper D, Escobar GJ. Interpreting complete blood counts soon after birth in newborns at risk for sepsis. *Pediatrics* 2010; **126**: 903–9.

19 Ur Rehman Durrani N, Rochow N, Alghamdi J, Pelc A, Fusch C, Dutta S. Minimum Duration of Antibiotic Treatment Based on Blood Culture in Rule Out Neonatal Sepsis. *Pediatr Infect Dis J* 2019; **38**: 528–32.

20 Mukherjee A, Davidson L, Anguvaa L, Duffy DA, Kennea N. NICE neonatal early onset sepsis guidance: greater consistency, but more investigations, and greater length of stay. *Arch Dis Child Fetal Neonatal Ed* 2015; **100**: F248-249.

21 Chiesa C, Pellegrini G, Panero A, *et al.* C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. *Clin Chem* 2003; **49**: 60–8.

22 Mjelle AB, Guthe HJT, Reigstad H, Bjørke-Monsen AL, Markestad T. Serum concentrations of C-reactive protein in healthy term-born Norwegian infants 48-72 hours after birth. *Acta Paediatr Oslo Nor 1992* 2019; **108**: 849–54.

23 Lacaze-Masmonteil T, Rosychuk RJ, Robinson JL. Value of a single C-reactive protein measurement at 18 h of age. *Arch Dis Child Fetal Neonatal Ed* 2014; **99**: F76-79.

24 Chiesa C, Panero A, Rossi N, *et al.* Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. *Clin Infect Dis Off Publ Infect Dis Soc Am* 1998; **26**: 664–72.

25 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 Int J Clin Chem* 2011; **412**: 1053–9.

26 Petel D, Winters N, Gore GC, *et al.* Use of C-reactive protein to tailor antibiotic use: a systematic review and meta-analysis. *BMJ Open* 2018; **8**: e022133.

27 Escobar GJ, Puopolo KM, Wi S, *et al.* Stratification of risk of early-onset sepsis in newborns ≥ 34 weeks’ gestation. *Pediatrics* 2014; **133**: 30–6.

28 Achten NB, Klingenberg C, Benitz WE, *et al.* Association of Use of the Neonatal Early-Onset Sepsis Calculator With Reduction in Antibiotic Therapy and Safety: A Systematic Review and Meta-analysis. *JAMA Pediatr* 2019; published online Sept 3. DOI:10.1001/jamapediatrics.2019.2825.

29 Kuzniewicz MW, Puopolo KM, Fischer A, *et al.* A Quantitative, Risk-Based Approach to the Management of Neonatal Early-Onset Sepsis. *JAMA Pediatr* 2017; **171**: 365–71.

**TABLE 1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Stratification into** **4 groups** | **No sepsis** | **Sepsis uncertain** | **Sepsis probable** | **Sepsis proven** |
| **Definitions** | Low to moderate risk of sepsis (risk factors, and/or clinical signs of sepsis, and/or CRP > 10 mg/l or WBC < 5 G/l at start of suspected sepsis), and negative blood cultures, and antibiotic therapy of less than 48 hours without recurrence of infection | Low to moderate risk of sepsis (risk factors, and/or clinical signs of sepsis, and/or CRP > 10 mg/l or WBC < 5 G/l at start of suspected sepsis), and antibiotic therapy of more than 48 hours, and negative blood cultures | High risk of sepsis (risk factors, and clinical signs of sepsis, and CRP > 10 mg/l or WBC < 5 G/l at start of suspected sepsis), and antibiotic therapy of more than 5 days, and negative blood cultures | Positive blood culture, with the exclusion of contaminants |

Legend table 1: Stratification of population into 4 groups according to the following definitions

**TABLE 2**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **all** | **No****sepsis** | **Sepsis uncertain** | **Sepsis****probable** | **Sepsis****proven** |
|  | n = 1678 | n = 553 | n = 952 | n = 147 | n = 26 |
| Sex |  |  |  |  |  |
|  male | 985 (58.7%) | 318 (57.5%) | 572 (60.1%) | 82 (55.8%) | 13 (50.0%) |
|  female | 693 (41.3%) | 235 (42.5%) | 380 (39.9%) | 65 (44.2%) | 13 (50.0%) |
| Gestational age, weeks | 39.6(37.1 – 40.6) | 39.6(37.6 – 40.6) | 39.6(36.6 – 40.9) | 39.6(37.3 – 40.6) | 40.0(39.1 – 41.0) |
| Birth weight, kg | 3.4 (2.9 – 3.8) | 3.3 (2.8 – 3.7) | 3.5 (3.0 – 3.8) | 3.5 (2.9 – 3.8) | 3.4 (3.1 – 3.7) |
| Mode of delivery |  |  |  |  |  |
|  Spontaneous vaginal | 798 (47.6%) | 259 (46.8%) | 452 (47.5%) | 72 (49.0%) | 15 (57.7%) |
|  Vacuum or forceps | 262 (15.6%) | 69 (12.5%) | 177 (18.6%) | 12 (8.2%) | 4 (15.4%) |
|  Primary caesarean section | 140 (8.3%) | 44 (8.0%) | 79 (8.3%) | 15 (10.2%) | 2 (7.7%) |
|  Secondary caesarean section | 475 (28.3%) | 178 (32.2%) | 244 (25.6%) | 48 (32.7%) | 5 (19.2%) |
| Arterial cord pH | 7.23(7.16 – 7.29) | 7.24(7.18 – 7.30) | 7.22(7.15 – 7.28) | 7.22(7.15 – 7.28) | 7.23(7.20 – 7.33) |
| APGAR score |  |  |  |  |  |
|  1 min post partum | 8 (6 – 9) | 8 (7 – 9) | 8 (6 – 9) | 8 (6 – 9) | 9 (7 – 9) |
|  5 min post partum | 9 (8 – 10) | 9 (8 – 10) | 9 (8 – 10) | 9 (8 – 9) | 9 (8 – 10) |
|  10 min post partum | 9 (8 – 10) | 10 (8 – 10) | 9 (8 – 10) | 9 (8 – 10) | 9 (8 – 10) |
| Risk factors |  |  |  |  |  |
|  Group B streptococcus carriage | 240 (14.3%) | 68 (12.3%) | 108 (11.3%) | 57 (38.8%) | 7 (26.9%) |
|  Chorioamnionitis | 324 (19.3%) | 101 (18.3%) | 187 (19.6%) | 32 (21.8%) | 4 (15.4%) |
|  PROM > 18h | 391 (23.3%) | 143 (25.9%) | 187 (19.6%) | 58 (39.5%) | 3 (11.5%) |
|  Gestational age < 37 weeks | 324 (20.5%) | 117 (21.2%) | 186 (19.5%) | 37 (25.2%) | 4 (15.4%) |
| Clinical signs |  |  |  |  |  |
|  Respiratory distress / apnea | 1004 (59.8%) | 262 (47.4%) | 602 (63.2%) | 118 (80.3%) | 22 (84.6%) |
|  Tachycardia or bradycardia | 175 (10.4%) | 45 (8.1%) | 104 (10.9%) | 16 (10.9%) | 10 (38.5%) |
|  Arterial hypotension / poor  perfusion | 151 (9.0%) | 26 (4.7%) | 92 (9.7%) | 22 (15.0%) | 11 (42.3%) |
|  Hypothermia or hyperthermia | 278 (16.6%) | 87 (15.7%) | 150 (15.8%) | 30 (20.4%) | 11 (42.3%) |
|  Seizure / floppy infants /  irritability / lethargy | 162 (9.7%) | 36 (6.5%) | 99 (10.4%) | 20 (13.6%) | 7 (26.9%) |
|  Vomiting / feeding intolerance /  ileus | 114 (6.8%) | 33 (6.0%) | 67 (7.0%) | 13 (8.8%) | 1 (3.9%) |
| Duration of antibiotic therapy, h | 62 (46 – 140) | 36 (28 – 45) | 84 (60 – 153) | 154 (114 – 163) | 320 (211 – 383) |
| Biomarker values within 48 hours |  |  |  |  |  |
|  WBC, n | 4198 | 1349 | 2391 | 386 | 72 |
|  max WBC, G/l | 20.4(15.8 – 25.3) | 20.4(16.0 – 25.6) | 20.7(15.8 – 25.2) | 18.7(14.7 – 25.4) | 15.9(13.4 – 24.1) |
|  CRP, n | 4654 | 1472 | 2659 | 445 | 78 |
|  max CRP, mg/l | 7.6(2.5 – 28.0) | 3.0(1.0– 8.0) | 9.7(3.0 – 33.0) | 41.0(24.8 – 68.0) | 96.6(56.7 – 136) |
|  PCT, n | 2047 | 885 | 960 | 177 | 25 |
|  max PCT, ng/l  | 6.4(2.0 – 19.2) | 2.3(1.0 – 4.7) | 12.0(5.2 – 29.8) | 22.5(7.6 – 50.0) | 70.0(26.4 – 97.5) |
| Blood culture positive | 26 (1.5) |  |  |  | 26 (100) |
|  Group B streptococcus | 20 (1.2) | - | - | - | 20 (76.9) |
|  Escherichia coli | 3 (0.2) | - | - | - | 3 (11.5) |
|  others | 3 (0.2) | - | - | - | 3 (11.5) |

Legend table 2: Baseline characteristics for all patients and according to the group stratification. All values are shown as n (%) or median (IQR)

**Table 3**

|  |  |  |
| --- | --- | --- |
|  | **ROC AUC within 36 hours** | **Cut-off values within 36 hours** |
| **No sepsis versus proven sepsis** |  |  |
|  CRP (n=572) | 0.986 (0.974, 0.998) | 16 mg/l |
|  PCT (n=279) | 0.921 (0.783, 1.000) | 2.8 ng/l |
|  WBC (n=572) | 0.360 (0.238, 0.482) | n/a |
| **No sepsis versus probable/proven sepsis** |  |  |
|  CRP (n=719) | 0.963 (0.950, 0.975) | 13 mg/l |
|  PCT (n=331) | 0.908 (0.853, 0.963) | 1.5 ng/l |
|  WBC (n=718) | 0.435 (0.383, 0.486) | n/a |
| **No sepsis versus uncertain/probable/proven sepsis** |  |  |
|  CRP (n=1649) | 0.749 (0.726, 0.773) | n/a |
|  PCT (n=608) | 0.869 (0.839, 0.897) | n/a |
|  WBC (n=1654) | 0.491 (0.461, 0.520) | n/a |

Legend table 3: AUC of CRP, PCT and WBC given as point estimates with corresponding 95% confidence intervals (lower and upper limit of 95% CI) and cut-off values of maximum CRP and PCT with a sensitivity of 100% discriminating no sepsis versus proven sepsis, and a sensitivity of >95% discriminating no sepsis versus probable/proven sepsis within 36 hours after start of antibiotic therapy. Cut-off values were only calculated if AUC was >0.90: n/a = not applicable.

**LEGENDS FIGURES**

Legend figure 1: Distribution of biomarker measurements within 48 hours after start of antibiotics because of suspected early-onset sepsis. Investigators were asked to collect biomarkers at specific time points after start of antibiotic therapy within a time frame of +/- 6 hours.

Legend figure 2: AUC of CRP, PCT and WBC between start of antibiotics and 18h (green line), 36h (red line), and 48h (blue line), respectively. Purple line = overlap of red and blue line. First row: AUC of CRP comparing no sepsis versus proven sepsis, no sepsis versus proven/probable sepsis and no sepsis versus proven/probable/uncertain sepsis; Second row: AUC of PCT comparing no sepsis versus proven sepsis, no sepsis versus proven/probable sepsis and no sepsis versus proven/probable/uncertain sepsis; Third row: AUC of WBC comparing no sepsis versus proven sepsis, no sepsis versus proven/probable sepsis and no sepsis versus proven/probable/uncertain sepsis