Clinical Infectious Diseases

MAJOR ARTICLE

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

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(See the Editorial Commentary by Weitkamp on pages e391–3.)

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. However, antibiotics may be harmful.

Methods. We performed a secondary analysis of results from the Neonatal Procalcitonin Intervention Study, a prospective, multicenter, randomized, controlled intervention study. The 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 analyzed 1678 neonates with 10 899 biomarker measurements (4654 CRP, 2047 PCT, and 4198 WBC) obtained within the first 48 hours after the start of antibiotic therapy due to suspected EOS. The areas under the curve (AUC) comparing no sepsis vs proven sepsis for maximum values of CRP, PCT, and WBC within 36 hours were 0.986, 0.921, and 0.360, respectively. The AUCs for CRP and PCT increased with extended time frames up to 36 hours, but there was no further difference between start to 36 hours vs start to 48 hours. Cutoff values at 16 mg/L for CRP and 2.8 ng/L for PCT provided a sensitivity of 100% for discriminating no sepsis vs proven sepsis.

Conclusions. Normal serial CRP and PCT measurements within 36 hours 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.

Keywords. neonatal early-onset sepsis; C-reactive protein; procalcitonin; white blood count; negative predictive value.

Sepsis may kill: neonatal sepsis is one of the main causes of 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 nonspecific [3, 4], which drives the massive use of antibiotics for suspected early-onset sepsis (EOS) [5]. The number needed to treat for 1 proven case of EOS in term and late-preterm infants varies in the literature from 40 to more than 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

Received 30 March 2020; editorial decision 20 May 2020; accepted 19 June 2020; published online September 3, 2020.

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Clinical Infectious Diseases® 2021;73(2):e383–90

DOI: 10.1093/cid/ciaa876

life may change the individual microbiome, with possible consequences for individual developmental origins of future health and disease [8].

There is 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 of 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) and showed that procalcitonin (PCT)-guided decision-making reduces the duration of antibiotic treatment significantly, with a low rate of reinfections and no study-related mortality [11]. Nevertheless, overtreatment remains, and controversy around the most suitable marker persists.

Therefore, we conducted a secondary analysis of the NeoPInS cohort, which consisted of 1678 neonates with 10 899 biomarker measurements within the first 48 hours after start of antibiotic therapy because of suspected EOS. We analyzed the diagnostic accuracy of serial measurements of C-reactive protein (CRP), PCT, and white blood count (WBC) to rule out EOS. We aimed to focus on the negative predictive value within different time frames to answer the question of when is it safe to stop antibiotic therapy that was 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 was a secondary analysis of biomarkers in the NeoPInS population [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, multicenter, randomized, controlled intervention study that aimed to reduce the 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 34 weeks' gestation who were suspected of having 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 that used 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 the start of suspected EOS were defined as CRP >10 mg/L and leukocytopenia <5 G/L (<5000 cells/mm^3).

Neonates were randomized to PCT-guided therapy or standard care. Neonates with proven or probable infection were treated with antibiotics for at least 7 days according to local policy at each participating center, independent of randomization. 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 5 to 7 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 analyzed 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 therapy, and other cultures (eg, cerebrospinal fluid cultures) and/or other additional diagnostic tests (eg, radiography) were performed on indication. Follow-up information regarding recurrence of infection, rehospitalization, additional courses of antibiotics, and death was obtained by interviewing the parents during their follow-up visits or by telephone interview at least 1 month after discharge.

Participants and Data Acquisition

The NeoPInS cohort included neonates born after 34 weeks' gestation who received 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 the start of antibiotic treatment were defined as key variables.

Definitions of Infection Groups

For this secondary analysis, we stratified infants into 4 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 1 abnormal finding

in the 3 areas of risk factors, clinical signs, and biomarkers (CRP >10 mg/L or leukocytopenia <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 the start of suspected sepsis and with treatment duration of more than 5 days, but negative blood cultures. In the literature, for infants with sepsis probable, the term "culture-negative sepsis" has been used [10]. Whereas culture-negative sepsis implies that an infection is present, we emphasize that this is unknown. Sepsis uncertain was defined as neonates with a low to moderate risk of sepsis (none to 2 abnormal findings in the 3 areas of risk factors, clinical signs, and biomarkers), antibiotic therapy for more than 48 hours, and a negative blood culture. No sepsis was defined as neonates with a low to moderate risk of sepsis (none to 2 abnormal findings in the 3 areas of risk factors, clinical signs, and biomarkers), antibiotic therapy of less than 48 hours without recurrence of infection, and a negative blood culture. Although 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.

Outcomes

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

Statistical Methods

Data are presented as median (interquartile range [IQR]) for continuous variables and as numbers and frequencies for categorical variables. Primary and secondary outcomes, that is, discrimination of no sepsis vs proven sepsis (or other categories including probable and ambiguous sepsis) by means of WBC, CRP, or PCT criteria were analyzed using receiver operating characteristic (ROC) curves, and corresponding AUCs 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 including 95% confidence intervals. Potential differences between AUCs were analyzed using the test procedure according to DeLong et al [13]. Statistical analyses were conducted with Stata (version 15.2 or later, StataCorp, College Station, TX).

RESULTS

We analyzed 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 vs proven sepsis, no sepsis vs probable/proven sepsis, and no sepsis vs uncertain/probable/proven sepsis for maximum values of CRP, PCT, and WBC within 48 hours are shown in Figure 2. For all comparisons,

Table 1. Stratification of Population Into 4 Groups According to the Definitions Listed

Stratification	Definition
No sepsis	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), negative blood cultures, antibiotic therapy for less than 48 hours without recurrence of infection
Sepsis uncertain	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 for more than 48 hours, and negative blood cultures
Sepsis probable	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 for more than 5 days, and negative blood cultures
Sepsis proven	Positive blood culture, with the exclusion of contaminants

Abbreviations: CRP, C-reactive protein; WBC, white blood count.

Table 2. Baseline Characteristics for All Patients and According to the Group Stratification

Characteristic	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 cesarean section	140 (8.3%)	44 (8.0%)	79 (8.3%)	15 (10.2%)	2 (7.7%)
Secondary cesarean 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 minute post-partum	8 (6–9)	8 (7–9)	8 (6–9)	8 (6–9)	9 (7–9)
5 minute post-partum	9 (8–10)	9 (8–10)	9 (8–10)	9 (8–9)	9 (8–10)
10 minute 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%)
Premature rupture of membranes >18 hours	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, hours	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)	0	0	0	20 (76.9)
Escherichia coli	3 (0.2)	0	0		3 (11.5)
Other	3 (0.2)	0	0	0	3 (11.5)

All values are shown as n (%) or median (interquartile range).

Abbreviations: CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood count.

the AUC for CRP and PCT increased with extended time frames up to 36 hours, whereas there was no difference between start to 36 hours vs start to 48 hours (Figure 2). The AUC for WBC was significantly lower ($P < .001$) than for CRP and PCT for all comparisons and time frames. Determination of cutoff values with a sensitivity of 100% discriminating no sepsis vs proven sepsis within 36 hours was at 16 mg/L for CRP and 2.8 ng/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, 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. In addition, 65.1% of participants with a low or moderate risk of infection had a maximum CRP below the cutoff of 13 mg/L discriminating with a sensitivity of >95% no sepsis vs 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 <13 mg/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

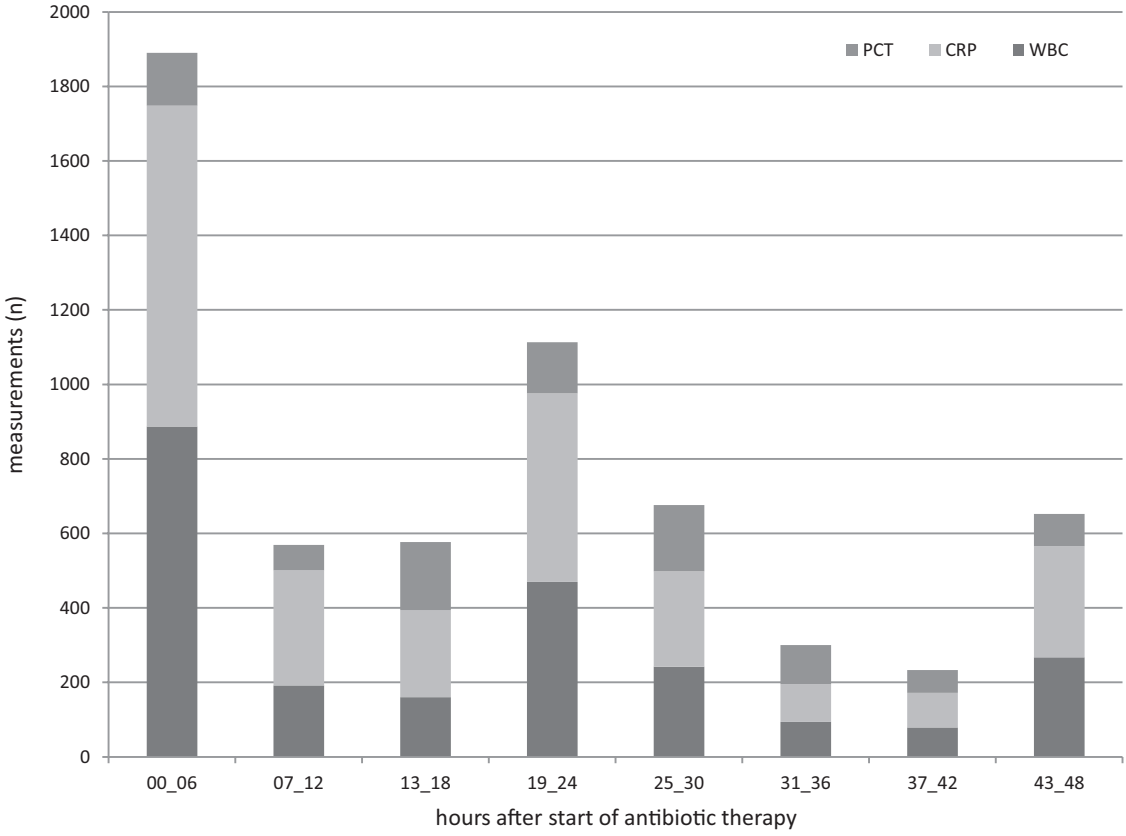

Figure 1. Distribution of biomarker measurements within 48 hours after the start of antibiotics because of suspected early-onset sepsis. Investigators were asked to collect biomarkers at specific time points after the start of antibiotic therapy within a time frame of ±6 hours. Abbreviations: CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood count.

not for WBC. The high negative predictive value of 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 leukocytopenia <5 G/L at the start of antibiotic therapy has a reasonable positive predictive value [16–18].

The question of when is it safe to stop antibiotic treatment that was started for suspected EOS is an important one. By 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

Table 3. Area Under the Curve and Cutoff Values Within 36 Hours

Comparison	Receiver Operating Characteristic Area Under the Curve Within 36 Hours	Cutoff Values Within 36 Hours
No sepsis vs 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 vs 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 vs 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

Cutoff values were only calculated if the area under the curve was >0.90. Area under the curve for C-reactive protein (CRP), procalcitonin (PCT), and white blood count given as point estimates with corresponding 95% confidence intervals (CIs; lower and upper limit of 95% CI) and cutoff values of maximum CRP and PCT with a sensitivity of 100% discriminating no sepsis vs proven sepsis and a sensitivity of >95% discriminating no sepsis vs probable/proven sepsis within 36 hours after start of antibiotic therapy.

Abbreviations: CRP, C-reactive protein; n/a = not applicable; PCT, procalcitonin; WBC, white blood count.

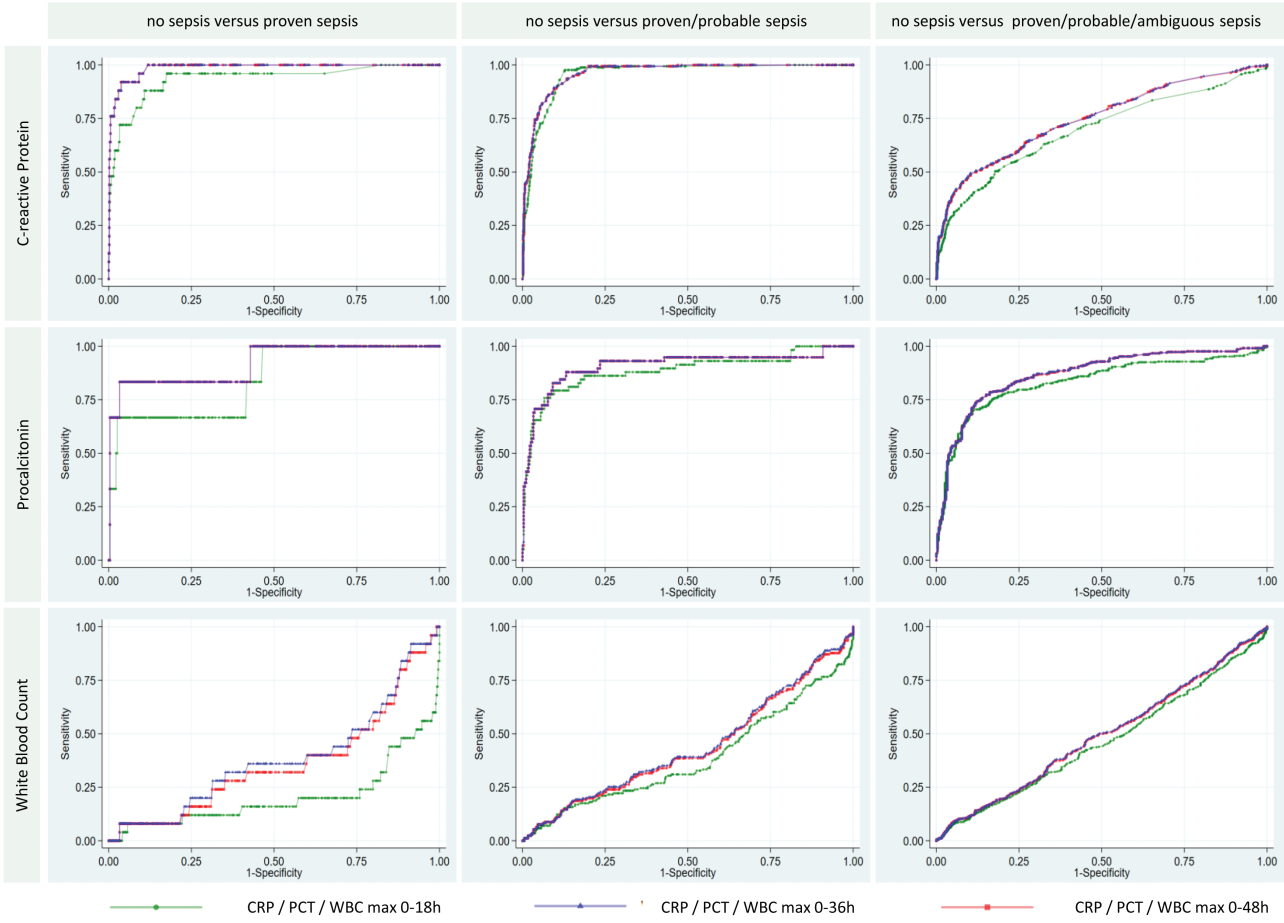

Figure 2. Area under the curve (AUC) for CRP, PCT, and WBC between start of antibiotics and 18 hours (green line), 36 hours (red line), and 48 hours (blue line), respectively. Purple line indicates overlap of red and blue lines. First row: AUC for CRP comparing no sepsis vs proven sepsis, no sepsis vs proven/probable sepsis, and no sepsis vs proven/probable/uncertain sepsis. Second row: AUC for PCT comparing no sepsis vs proven sepsis, no sepsis vs proven/probable sepsis, and no sepsis vs proven/probable/uncertain sepsis. Third row: AUC for WBC comparing no sepsis vs proven sepsis, no sepsis vs proven/probable sepsis, and no sepsis vs proven/probable/uncertain sepsis. Abbreviations: CRP, C-reactive protein; PCT, procalcitonin; WBC, white blood count.

PCT did not increase from 36 to 48 hours. This is an important indication 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 16 mg/L and 2.8 ng/L, respectively, within 36 hours after start of antibiotics, antibiotic treatment can be stopped safely. This time frame is also 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 guidelines recommend considering stopping antibiotic treatment after 36 hours if CRP is reassuring [3]. The NICE guidelines have 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 cutoff value for CRP of 16 mg/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 cutoff value regarding the positive predictive value as up to 20% of newborns have a physiological rise in CRP above 10 mg/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 comparisons of no sepsis vs proven/probable sepsis and no sepsis vs proven/probable/uncertain sepsis. This is important because we really do not know the true infection status of infants in the 2 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. However, 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 of which biomarker is best to rule out EOS within the first 48 hours. Initially, 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 also 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 increased the diagnostic performance of CRP in comparison with PCT. Based on 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 cutoff values (nomogram), we compared the percentage of patients in the PCT group treated for less than 48 hours in the original study with the percentage of patients with a maximum CRP below 13 mg/L without probable or proven sepsis. Close to 50% of PCT-guided neonates were treated for less than 48 hours, and 85% of this group had a CRP below 13 mg/L. Also, 65% of the group with low or moderate risk of infection had a CRP below 13 mg/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.

In addition to 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 our study is that it is a secondary analysis of the NeoPInS cohort, and the study was not designed for this analysis with a potential bias due to unblinded CRP, PCT, and WBC measurements. In addition, there was a small number of proven sepsis cases and we were not able to reasonably analyze positive predictive values. Nevertheless, with more than 10 000 biomarkers, our study is one of the largest biomarker analyses for suspected EOS.

CONCLUSIONS

Normal serial CRP and PCT measurements within 36 hours after the 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 48 hours. Now, it is time for the next step: to stop unnecessary antibiotic therapies within 36 hours.

Notes

Author contributions. Principal investigators: A. v. R. and M. S. Study concept and design: A. v. R., M. S., W. v. H., E. V., and J. v. G.; approved by all authors. Enrollment of patients and data collection: S. e. H., S. D., M. F., F. S., R. v. d. T., J. W., J. J., L. v. d. M., R. M., S. S., E. d. V., A. D., U. Z., L. S., A. d. M., A. H., M. R., M. T., and R. K. (all local investigators). Study supervision: W. v. H., M. S., and A. v. R. Supervision and monitoring data entry and checking database for accuracy: W. v. H. and M. S. Statistical analysis: M. S. and D. L. Analysis and interpretation of data: M. S., W. v. H., A. v. R., D. L., F. P., N. A., and P. H. Obtained funding: A. v. R., M. S., and W. v. H. All authors read, critically revised, and approved the manuscript; approved the final version; and agreed to be accountable for all aspects of the work.

Acknowledgments. The authors 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. Thermo Fisher provided procalcitonin kits and provided an unrestricted grant for the organization of 4 investigator meetings (2008, 2009, 2013, and 2015). Thermo Fisher had no involvement in other aspects of the trial.

Financial support. This work was supported by the Thrasher Foundation (9143), the NutsOhra Foundation (1101-059), and the Sophia Foundation for Scientific Research (681).

Potential conflicts of interest. A. v. R. reports personal fees from Oxford University and Karolinska Institute Stockholm and grants from the Coolsingel Foundation and the Sophia Foundation outside the submitted work. P. H. is a member of the UK National Institute for Health and Care Excellence neonatal infection guideline committee. L. J. S. reports grants from the Children's Hospital Foundation and the National Health and Medical Research Council. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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