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Title: Presepsin as a predictor of positive blood culture among newborns suspected sepsis.

Short title: Presepsin as a predictor neonatal sepsis.

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Abstract

Background: Although the incidence of neonatal sepsis is decreasing, neonatal sepsis remains a severe life-threatening disease. No current biochemical marker can provide perfect diagnostic accuracy for neonatal sepsis.

Objectives: We evaluated the accuracy of presepsin (P-SEP) as a novel biomarker of bacterial infection for neonatal sepsis diagnosis.

Methods: We prospectively studied newborns with sepsis (sepsis group n=13) during the first 30 days after birth and compared them with control preterm newborns (control group n=18). In addition, we evaluated term newborns who exhibited some clinical signs of early onset sepsis (non-sepsis term group n=35).

Results: The P-SEP levels of the sepsis group were significantly higher than the P-SEP levels of the control group (p<0.001) The AUC for P-SEP was 0.868 (95% confidence interval [CI] 0.71-1.00). The P-SEP value of 795 pg/ml was established as the cut-off value with 85% sensitivity and 89% specificity. The positive and negative predictive values were 85% and 89%, respectively. In the non-sepsis term group, P-SEP showed better stability than WBC and CRP for three days after birth.

Conclusions: We are confident that presepsin can better discriminate between infections and non-infectious inflammatory conditions than the currently used biomarkers.

Introduction

Neonatal sepsis is the most common cause of morbidity and mortality during the neonatal period. Neonatal sepsis is classified as either early-onset sepsis (EOS; ≦7 days after birth) or late-onset sepsis (LOS; >7 days after birth). [1] Advances in obstetrical and neonatal care have decreased the incidence of neonatal sepsis, especially EOS. Currently, the incidence of group B *Streptococcus*-specific EOS has declined to 0.3-0.4 cases/1000 live births, and overall the EOS incidence has declined to 0.8-1.0 cases/1000 live births. Nevertheless, EOS remains a severe life-threatening disease with a mortality rate ranging from 1.5% in term infants to almost 40% in very low birth weight infants.[2,3] Early diagnosis and treatment of neonatal sepsis are important to prevent severe complications. However, in this era of multi-resistant microorganisms, it is also important to avoid the unnecessary use of antibiotics in sepsis-negative infants. On the basis of the CDC 2010 guidelines, Escobar et al. have reported that, in a cohort of 7004 infants, 13% of both well-appearing and ill-appearing infants were evaluated for EOS, and 11% were treated empirically with antibiotics, although only 0.04% of the cohort had blood–culture-confirmed infections.[4] Concern has arisen regarding the EOS results of evaluations

and the empirical antibiotic treatments of hundreds of thousands of uninfected newborns annually, which results in maternal/infant separation and significant expenditures. In treating this low-incidence but highly consequential disease, clinicians seek the early identification of infants with EOS, with the goal of identifying those at risk and administering antibiotic treatment to prevent progression to severe disease.

Blood cultures are the gold standard for the diagnosis of sepsis in newborns. However, the long waiting times for results and high false-negative frequencies that are secondary to the small volumes of the blood samples used make it difficult to use blood cultures to make decisions about antibiotic therapies at the beginning of the screening process.[5,6] Hence, antibiotics are often started empirically in infants with perinatal risk factors or clinical signs suggestive of a bacterial infection. Therefore, various biochemical markers are used to aid decision making regarding antibiotic therapy in neonatal sepsis.[7,8] Nevertheless, no current biochemical marker can provide a perfect diagnostic accuracy.

Presepsin (P-SEP), or soluble CD14 subtype, is a truncated variant of soluble CD14, and pathogens stimulate P-SEP shedding from the surfaces of various immune cell types, such as macrophages, monocytes, and neutrophils.[9] Although its function is still unclear, P-SEP is believed to interact with B and T cells to modulate specific immune responses.[10] P-SEP has recently been demonstrated to be a reliable diagnostic and prognostic marker of sepsis in adults.[11] Preliminary reference values of

P-SEP have mainly been evaluated in infants with LOS.[12,13] To the best of our knowledge, few studies have been performed to evaluate the possible role of P-SEP as a marker for EOS. Therefore, we hypothesized that P-SEP might be an accurate biomarker of neonatal sepsis, and presepsin might better discriminate between infections and non-infectious inflammatory conditions than the currently used biomarkers. To test this hypothesis, we performed a prospective study to evaluate changes in the P-SEP serum concentration in newborns with and without possible neonatal sepsis and to assess these changes in the P-SEP serum concentration.

Methods

Study design and population

This study was a prospective study conducted at the neonatal intensive care units (NICUs) of two hospitals: Shinshu University Hospital and Nagano Children's Hospital, Japan. This study ran from September 2014 to December 2015 and was approved by the local ethics committees of these hospitals. Written informed consent was obtained from the parents of each patient included in this study. During this study period, there were 784 patients admitted to the NICU. We assigned patients to three groups: the sepsis group, the control group and the non-sepsis term group. The sepsis group was defined by the

presence of a positive blood culture associated with clinical signs of sepsis during the neonatal period (<30 days after birth). We chose 18 preterm patients to constitute the control group in an arbitrary order and matched the gestational weeks with those of the sepsis group. In the control group, we did not use any antibiotics and there was no clinical sign of sepsis. We compared the sepsis group to the control group. We analyzed the peak levels of P-SEP over the first 3 days after onset or after birth because in some patients we could not evaluate P-SEP on day 0. The non-sepsis term group comprised term neonates who were initially suspected of bacterial infection but whose clinical course and laboratory findings, including negative blood cultures, ruled out the possibility of bacterial infection. Neonates with suspected infection presented with one or more of the following risk factors: maternal factors (e.g., PROM chorioamnionitis, elevated CRP, and intrapartum maternal fever) and neonatal factors (e.g., presence of meconium-stained amniotic fluid, apnea, respiratory problems that required supplemental oxygen, nasal continuous positive airway pressure, and intermittent mandatory ventilation). This definition was based on a previous study that reported elevated C-reactive protein (CRP) values between 11 and 70 mg/l in 16/49 uninfected neonates admitted to the NICU with diagnoses of meconium aspiration pneumonia, anoxic encephalopathy, prolonged rupture of membranes (PROM), respiratory distress syndrome, chorioamnionitis, aspiration pneumonia, and transitory tachypnea[14].

In the non-sepsis term group, we measured the white blood cell (WBC), CRP, and presepsin levels for three days after birth. In all cases, we collected blood cultures before administering antibiotics.

Blood sampling and biomarker measurements

For blood cultures, a blood sample of at least 1 ml was obtained from a peripheral vein. Standard laboratory methods were used to identify the microorganisms that grew in the blood sample cultures. A BD BACTEC (Becton, Dickinson and Company, Tokyo, Japan) was used for the blood cultures. WBC and CRP were measured in serum samples by our central laboratory. WBC was measured using Sysmex XN-9000 (JEOL, Tokyo, Japan). CRP was measured using JCA-BM6070 (JEOL, Tokyo, Japan).

Presepsin measurements

For P-SEP measurements, 100 μl of the blood sample from each patient was collected by heel stick or venous puncture into a tube containing ethylene diamine tetra-acetic acid (EDTA). P-SEP was measured with a rapid chemiluminescent enzyme immunoassay using a PATHFAST immunoanalyzer (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). Each test required 100 μl of whole blood and was completed in 17 minutes.

Statistical analysis

We used SPSS software version 23 (IBM, Tokyo, Japan) for statistical analyses. The variables were assessed using visual (i.e., histogram and probability plots) and analytical methods to determine whether they were normally distributed. Parametric results are expressed as the means±standard deviations. The median values of the nonparametric tests are reported with the interquartile ranges. Statistical analysis was performed with Student's t-tests for parametric continuous variables, and non-normally distributed variables were compared using Mann-Whitney U tests. To evaluate the diagnostic potential of presepsin, receiver-operating characteristic (ROC) curve analysis was performed, including measurements of specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV), and the area under the curve (AUC). All values of $p<0.05$ were considered to indicate a significant difference.

Results

During this study period, there were 13 newborns in the sepsis group, 18 patients in the control group, and 35 newborns in the non-sepsis term group. Table 1 shows the characteristics of the patients in the sepsis group and the control group. Table 2 shows the pathogen of sepsis and the date of onset of the

sepsis group. Figure 1 shows the changes and comparison of the P-SEP levels in the sepsis group and the control group for 3 days after onset or birth. The P-SEP levels of the sepsis group were significantly higher than the levels of the control group during this period. The AUC for P-SEP was 0.868 (95% confidence interval [CI] 0.71-1.00) (Figure 2). The P-SEP value of 795 pg/mL was established as a cut-off value with 85% sensitivity and 89% specificity. The positive and negative predictive values were 85% and 89%, respectively. Table 3 shows the chronological change of the markers in the non-sepsis group. P-SEP did not show significant changes during the 3 days after birth. However, WBC and CRP did show significant changes during the 3 days after birth.

Discussion

In the present study, we demonstrated that P-SEP may be used in the diagnosis of neonatal sepsis. We found that the P-SEP levels were significantly higher in the infants in the sepsis group compared with those in the infants in the control group. Furthermore, P-SEP showed better stability than WBC and CRP for three days after birth in the non-sepsis term group.

Several previous reports have shown the efficacy of P-SEP for the diagnosis of LOS. In 2015, Topucuoglu et al. reported that the P-SEP levels were significantly elevated in preterm infants with

LOS and that the best cut-off value for P-SEP was 800.5 pg/mL, which indicated 67% sensitivity and 100% specificity.[13] In 2015, Poggi et al. reported that the P-SEP levels decreased even on the first day of treatment, whereas the CRP and procalcitonin (PCT) measurements did not differ from the baseline values.[12] In 2017, Montaldo et al. evaluated 32 preterm newborns with EOS and compared them to non-sepsis preterm newborns; the results showed that the AUC for P-SEP was 0.97 and that the best cut-off value was 788 ng/ml, which indicated 93% sensitivity and 100% specificity. In our study, the P-SEP value of 795 pg/mL was established as a cut-off value with 85% sensitivity and 89% specificity.

Recently, several reports have provided the normal value of P-SEP in neonates. In 2012, Mussap et al. evaluated 26 consecutive non-septic preterm newborns with various severe diseases and reported a mean P-SEP blood level for 26 preterm newborns of 643.1 ng/mL with a standard deviation (SD) of 303.8 ng/mL; the median value was 578 ng/mL. They found no correlation between the GA and P-SEP blood levels in neonates born between 26 and 36 weeks.[15] In 2015, Pugni et al. evaluated 684 healthy neonates (484 at term and 200 preterm). In the term infants, the P-SEP median value was 603.5 pg/mL (interquartile range: 466.5–791 pg/mL; 5th and 95th percentiles: 315 and 1178 pg/mL, respectively), and in preterm infants, the P-SEP median value was slightly higher at 620 pg/mL (interquartile range: 503–864 pg/mL; 5th and 95th percentiles: 352 and 1370 pg/mL respectively).[16]

In our study, the P-SEP median value was 501 pg/mL (interquartile range: 391-604 pg/mL) on day 0 in the non-sepsis term group.

A large number of studies have been performed to evaluate the use of the CBC, differential count, and immature-to-total leukocyte (I:T) ratio for the diagnosis of neonatal sepsis. However, Shah et al. described the WBC, ANC, and I:T ratio as having significant limitations in the diagnosis of neonatal sepsis.[1] CRP has been shown to be the best diagnostic marker of neonatal sepsis, with high sensitivity and specificity.[17] However, CRP presents a low sensitivity during the early phases of infection, due to the time needed for release.[18] Interpretation of CRP in the diagnosis of early-onset sepsis may be hindered by several noninfectious conditions that influence the CRP values during the first days after birth.[19] In our study, P-SEP showed better stability than WBC and CRP for three days after birth in the non-sepsis term group.[20]

PCT appears to be more specific than CRP in bacterial infections.[21,22] In neonatal sepsis, PCT concentrations increase after 4 hours due to the proinflammatory actions of bacterial endotoxins, reaching peak levels after 6-8 hours; thus, a rise in PCT occurs earlier than a rise in CRP.[23] PCT has a dynamic cut-off range between 48-72 hours after birth, depending on the clinical conditions and setting. PCT results should be interpreted with caution because significant values exceed 1 μg/l during

the first 3 days after birth, whereas after the third day of life, a cut-off of 0.5 μg/l offers good sensitivity and specificity.[24] To limit the blood sampling frequency, we did not measure PCT.

If our results are confirmed in additional studies, we are confident that P-SEP will be included as a marker that will increase the accuracy of neonatal sepsis screening in infants. The use of P-SEP may also help to decrease false-positive diagnoses and consequent antibiotic overtreatment, which increases the risk of infants developing multidrug-resistant bacterial infections or secondary dysmicrobism.

A limitation of our study is the small size of our population, especially in the sepsis group. EOS is not often seen in developed countries. EOS incidence is estimated to be 0.8-1.0 cases/1000 live births. Therefore, we included patients in the sepsis group from all neonatal ages. Furthermore, we performed the diagnosis of sepsis only on the basis of blood cultures. We cannot account for the possibility of false-positive or false-negative cultures.

In conclusion, we are confident that P-SEP can better discriminate between infections and non-infectious inflammatory conditions than the currently used biomarkers.

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Figure Legends

Figure 1. Changes and comparison of the P-SEP levels of the newborns in the sepsis and the control groups.

*P=0.035; **P=0.010; ***P=0.031 Sepsis group vs Control group

Figure 2. ROC curve of the P-SEP values.

Table 1 Clinical characteristics of the sepsis group and the control group

	Sepsis group (n=13)	Control (n=18)	*p*
Gestational age, weeks	30.3±6.6	31.2±3.4	0.649
Birth weight	1644±1202	1520±605	0.709
Caesarean delivery	8(62)	11(61)	0.981
Apgar 1	5(2-8)	6(5-8)	0.161
Apgar 5	6(4-8)	8(7-8)	0.193
Male/Female	10/3	10/8	0.275
Meconium-stained amniotic fluid	1(8)	1(6)	0.811
PROM	2(15)	3(17)	0.924
Respiratory distress syndrome	9(69)	10(55)	0.440
Mechanical ventilation	7(53)	11(61)	0.690
Mean±SD, n (%), or median and (interquartile range)			
PROM, premature rupture of the membranes			

Table.2 Culture results and date of onset in the sepsis group	
Pathogens	
MRSE	5
GBS	3
K. pneumoniae	1
K. oxytoca	1
E. coli	2
E. faecalis	1
MRSE, methicillin-resistant *Staphylococcus aureus*	
GBS, Group B Streptococcus	
Date of onset	
EOS (before day 7)	7
LOS (after day 7 to day 30)	6

Table 3 Chronological changes of the markers in the non-sepsis term group		
	Non-sepsis term group (n=35)	*p*
P-SEP (pg/mL), [n]		
Day 0	501 (391-604), [35]	0.77
Day 1	431 (361-604), [30]	
Day 2	439 (320-548), [20]	
WBC (/ml), [n]		
Day 0	20,290 (14,920-36,160), [35]	0.01
Day 1	19,185 (14,837-23,852), [34]	
Day 2	15,680 (12,330-18,500), [30]	
CRP (mg/dl), [n]		
Day 0	1.04 (0.16-3.05), [34]	0.04
Day 1	2.26 (0.96-3.49), [34]	
Day 2	1.24 (0.72-1.81), [29]	
Median (interquartile range), [n]		

P-SEP, presepsin

WBC, white blood cell

CRP, C-reactive protein

Figure 1

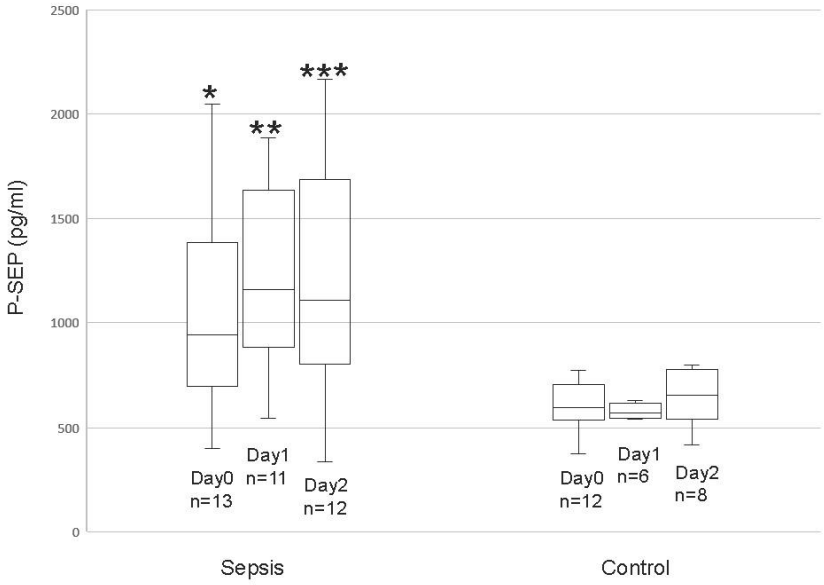

Figure 2