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Original Article

Procalcitonin *vs.* combination of micro-erythrocyte sedimentation rate and C-reactive protein for diagnosing neonatal bacterial sepsis

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Abstract

Background Given the high rates of mortality and morbidity in neonatal sepsis, rapid, easy-to-use, and inexpensive biomarkers with high sensitivity and specificity are needed to diagnose neonatal sepsis. Procalcitonin is often used as a predictor in identifying neonatal sepsis, but C-reactive protein (CRP) and micro-erythrocte sedimentation rate (m-ESR) may also be valid biomarkers of neonatal sepsis.

Objective To compare the accuracy of procalcitonin to the combination of CRP and m-ESR, as well as to find cut-off points for the three tests, in diagnosing bacterial neonatal sepsis.

Methods Subjects were neonates hospitalized from July to October 2016 in Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, with sepsis at clinical presentation and healthy neonates with sepsis risk factors. All subjects underwent complete blood counts, CRP, m-ESR, blood cultures, and procalcitonin examinations.

Results Ninety-four infants were included, of whom 26 had proven sepsis. The combined values of m-ESR and CRP had 85% sensitivity, 59% specificity, and 66% accuracy. A procalcitonin (PCT) cut-off point of 9.7 ng/mL showed 100% sensitivity, 96% specificity, and 97% accuracy level, which were significantly higher than the combined values of m-ESR and CRP.

Conclusion The combined values of m-ESR (13 mm/hour) - CRP (17 mg/dL) and procalcitonin alone (2 ng/mL) are both valid for the diagnosis of bacterial neonatal sepsis, but the accuracy of procalcitonin at 9.7 ng/mL is significantly greater. **[Paediatr Indones. 2017;57:205-10 ; doi: http://dx.doi.org/10.14238/pi57.4.2017.205-10]**.

Keywords: *neonatal sepsis; m-ESR; CRP; procalcitonin; blood culture*

Neonatal sepsis is still the major health issue in some countries, especially in a developing country like Indonesia.[1,2] This condition leads to high rates of morbidity and mortality, especially in preterm and low birth weight babies.[3] Although neonatal intensive care unit (NICU) care has rapidly improved, the rate of mortality in sepsis is 20 to 50%.[1] Factors which influence the risk of infection are generally grouped into three categories: maternal, neonatal, and environmental.[1,2] Definitive diagnosis of neonatal sepsis is based on blood culture. Other supporting examinations to diagnose neonatal sepsis are white blood count (WBC), absolute neutrophil count (ANC), micro-erythrocyte sedimentation rate (m-ESR), and immature neutrophil /total neutrophil (I/T) ratio.[4-6] However, some of these examinations do not have high sensitivity and specificity for diagnosing neonatal sepsis. Additional examinations

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such as C-reactive protein (CRP) and more recently, procalcitonin (PCT), are expected to be more useful in diagnosing neonatal sepsis.[6]

Many studies about procalcitonin have shown that it had high sensitivity as a biomarker for neonatal sepsis.[7] Adib *et al.* reported a procalcitonin cut-off point of 1.1 ng/mL with sensitivity of 70%, specificity 80%, positive predictive value (PPV) 80%, and negative predictive value (NPV) 75%.[8] Also, Sucilathangam et al. reported that a procalcitonin cut-off point of ≥2 ng/mL had high sensitivity.[9] In addition, Chiesa *et al.* found that a 0.5-5 ng/mL procalcitonin cut-off point in neonatal bacterial sepsis had sensitivity 61-85% and specificity 50-97%.[10] Those doing procalcitonin research have yet to reach a consensus on a standard cut-off point for diagnosing bacterial neonatal sepsis.

In neonatal infectious diseases like sepsis and meningitis, CRP levels increase due to the local or systemic inflammatory response.[11] As such, CRP has been used as a parameter to establish a neonatal sepsis diagnosis. However, the studies have varied widely. Ng et al. reported that CRP examination had sensitivity of 84% and specificity of 96%, in 68 very low birth weight infants, as a single marker examination.[12] In contrast to other researchers, Anwer & Mustafa investigated fifty neonates with risk factors in pediatric intensive care unit of Abbasi Shaheed Hospital, Karachi Pakistan, were obtained that the CPR examination could help the establishment of neonatal sepsis diagnosis, CRP had the value of sensitivity of 60% and the specificity of 50%.[13] Another biomarker currently in use to diagnose neonatal sepsis is micro-ESR, an easy and inexpensive test to perform. Sharma *et al.* reported that micro-ESR had sensitivity 68.80%, specificity 76.50%, positive predictive value 57.90%, negative predictive value 83.90%, and accuracy and agreement of 74%.[14]

To improve sensitivity and specificity for diagnosing neonatal sepsis, combinations of biomarkers have been used. Philip *et al.* reported that two or more sepsis biomarkers such as I/T ratio ≥ 0.2, leukocytes < 5000/μL, positive CRP, positive haptoglobin, and mini-ESR ≥ 15/ in the first hour, had sensitivity of 93% and specificity of 88%, in infants with proven sepsis.[15] In addition, Mondal *et al.* used four sepsis biomarkers, namely, m-ESR (> 8 mm/1st hour), I/T ratio (>0.2), morphological changes in neutrophils, and CRP (≥ 6mg/L), and found that any positive two tests had sensitivity 84%, specificity 84%, and positive predictive accuracy 69%. Furthermore, for any three positive tests, sensitivity was 42%, specificity was 88%, and positive predictive accuracy was 95%. If four tests were positive, specificity and positive predictive values were 100%, but sensitivity was only 21%.[16]

Procalcitonin still has the highest sensitivity and specificity, yet it is expensive and has limited availability in Indonesian health centers. Therefore, we aimed to find a fast, easy to perform, and inexpensive screening tool, by combining the values of m-ESR and CRP, in the hopes of obtaining sensitivity and specificity values equivalent to those of procalcitonin.

Methods

This study was carried out in the Neonatal Intensive Care Unit (NICU) and Neonatal Ward of the Department of Pediatrics, Sriwijaya University Medical School/Dr. Mohammad Hoesin Hospital, Palembang, South Sumatera, from July to October 2016. Subjects were newborn infants with clinically diagnosed sepsis and healthy infants who had risk factors of neonatal sepsis, who had not yet received antibotics. Exclusion criteria were children who had perinatal asphyxia, hyaline membrane disease, necrotizing enterocolitis, or lack of parental consent.

Neonatal sepsis was defined as neonates with clinical presentation of sepsis and/or risk factors of neonatal sepsis, proven by blood culture (culture-proven sepsis). Major and minor criteria of suspected neonatal sepsis with the risk factors were as follows: major criteria- premature rupture of membranes > 18 hours, intrapartum fever > 38°C, chorioamnionitis, foul-smelling chorioamniotic fluid, and fetal heart rate > 160 times per minutes; minor criteria- premature rupture of the membranes > 12 hours, intrapartum fever > 37.5°C, low APGAR score, very low birth weight, gestational age < 37 weeks, twins, whitish vaginal discharge, and urinary tract infection.1 We used categories A and B clinical sepsis criteria,2 and the hematologic scoring system by Rodwell et al.17 as shown in **Table 1** and **Table 2**. Four predictors of neonatal sepsis were used, namely, m-ESR, CRP, the combination of m-ESR and CRP, and procalcitonin. The testing machine used, was from *i-Chroma* brand

(Korea) which used immunodetection method for CRP measurement.

Table 1. Hematologic scoring system for predilection of neonatal sepsis using Rodwell criteria[17]

Criteria	Abnormality	Score
Total WBC count	≤ 5,000/μL	1
	≥ 25,000 at birth	1
	≥ 30,000 at 12-24 hours	
	≥ 21,000 at day 2 in ward	
Total PMN count	No mature PMN soon	2
	Increased/decreased	1
Immature PMN count	Increased	1
I/T PMN ratio	Increased	1
I/M PMN ratio	≥ 0.3	1
Degenerative changes in PMN	Toxic granules/cytoplasmic vacuoles	1
Platelet count	≤ 150,000/μL	1

Table 2. Clinical findings in neonates with sepsis

A category	B category
• Respiratory distress (e.g., apnea, respiratory rates > 60 or <30 times per minute, costal retractions, expiratory grunting, central cyanosis)	• Tremor • Lethargy • Somnolence and
• Seizure	• hypoactivity
• Decreased level of	• Irritability
• consciousness	• Vomiting
• Abnormal body tem	• Abdomen distention
• perature	• Seen in the 4th day of live
• Unhygienic delivery room	• Mixed amniotic fluids
• Rapid decrease and dramatically clinical presentation of the neonatal	• Meconium-stained amniotic fluid • Poor sucking reflex

For the diagnostic evaluation of markers for bacterial neonatal sepsis, the sensitivity and specificity for each cut-off point for each marker were recorded. A comparison of the diagnostic accuracy of these markers was made by receiver-operating characteristic (ROC) curve analyses, by calculating the area under curve (AUC). Differences in accuracy were determined by comparing the confidence intervals of their accuracies. If the confidence intervals did not overlap, then the difference was considered to be significant. The estimation analysis with confidence intervals was used to compare the accuracy.

Results

During the study period, 577 neonates were admitted to the NICU and Neonatal Ward of Mohammad Hoesin Hospital. Ninety-four of them showed clinical sepsis or were healthy neonates with risk factors of neonatal sepsis, and were recruited into the study. Twenty-six (27%) neonates had positive blood cultures and were diagnosed to have bacterial neonatal sepsis. *Acinetobacter sp* and *Staphylococcus epidermidis* were the common causes of sepsis. **Table 3** shows the general characteristics of the study subjects. The ratio of males to females was 1.5:1. The majority of the ages of the subjects was < 72 hours (65 neonates, 69%), and at full term gestational age (54.2%). Also, the majority of subjects had weights > 2,500 grams (44 neonates, 46.8%).

As shown in **Table 4**, the combination of m-ESR (15 mm/hour) – CRP (10 mg/dL) had sensitivity of 85%, specificity 59%, and accuracy 66% (95%CI 55 to 75%). We used the reference value of procalcitonin ≥ 2ng/mL to diagnose bacterial neonatal sepsis, and found sensitivity to be 100%, specificity 68%, PPV 54%, NPV 100%, and accuracy value 77% (95%CI 67 to 84%).

In this study, we found different cut-off points of the three neonatal sepsis biomarkers, which we used singly or in combination. The cut-off points were m-ESR >13mm/hour, CRP >17 mg/dL, and procalcitonin 9.7 ng/m. For m-ESR of 13 mm/hour, the sensitivity was 85%, specificity 54%, PPV 42%, NPV 90%, and accuracy 63% (95%CI 52 to 72%) (data not shown). A CRP cut-off of 17mg/dL showed sensitivity of 88%, specificity 59%, PPV 45%, NPV 93%, and accuracy 67% (95%CI 56 to 76%). The combination of m-ESR 13mm/hour - CRP 17 mg/dL cut-off points yielded a sensitivity of 77%, specificity 72%, PPV 51%, NPV 89%, and accuracy 73% (95%CI 63 to 82%). Also, we found that PCT 9.7ng/mL had high sensitivity of 100%, specificity of 96%, and accuracy value of 97% (95%CI 90 to 99%). Hence, PCT was a significantly better biomarker for diagnosing bacterial neonatal sepsis. As shown in **Table 5**, PCT showed a greater accuracy compared with the combination of m-ESR-CRP.

Table 3. General characteristics of subjects

General characteristics	Bacterial neonatal sepsis			Range	
	Positive	Negative	Total	Minimum	Maximum
Age, n					
< 72 hours	17	48	65		
≥ 72 hours	9	20	29	1	10
Gender, n					
Male	15	41	56	-	-
Female	11	27	38		
Birth weight, n					
< 1,500 grams	1	6	7		
1,500-2,500 grams	11	32	43	1,150	4,000
> 2,500 gram	14	30	44		
Gestational age, n					
Pre-term	11	32	43	29 weeks	40 weeks
Full term	15	36	51		

Table 4. Laboratory markers

Laboratory markers	Bacterial neonatal sepsis			Range	
	Positive	Negative	Total	Minimum	Maximum
m-ESR, n					
> 15 mm/hour	18	27	45	2.0	90
≤ 15 mm/hour	8	41	49		
CRP, n					
> 10 mg/L	26	42	69	5.0	275
≤ 10 mg/L	0	26	26		
PCT, n					
≥ 2 ng/mL	26	22	48	0.16	100
< 2 ng/mL	0	46	46		

Table 5. The comparison of four predictors in diagnosing bacterial neonatal sepsis

Cut off points of biomarkers	Sens, %	Spec, %	PPV, %	NPV, %	Accuracy, %	95%CI of accuracy
CRP > 10 mg/dL + m-ESR ≥ 15 mm/hour	85	59	44	77	66	55 to 75
PCT ≥ 2 ng/mL	100	68	54	100	77	67 to 84
PCT ≥ 9.7 ng/mL	100	96	90	100	97	90 to 99
CRP > 17 mg/dL + m-ESR > 13 mm/hour	77	72	51	89	73	62 to 82

Discussion

Neonatal sepsis is a common and catastrophic illness.[18] We aimed to compare the combination of some sepsis biomarkers in order to find an early and accurate means of diagnosis to decrease morbidity for neonatal sepsis. Our study provides insight into the diagnostic value of the combination CRP and m-ESR in neonatal bacterial sepsis. Using m-ESR of 15 mm/hour and CRP 10mg/dL, a fairly high 85% sensitivity value was obtained, but the specificity was only 59%, with PPV 44%, NPV 77%, and accuracy value of 66%. Similarly, Mondal *et al.* used four sepsis biomarkers [m-ESR (>8mm/1st hour), I/T ratio (>0.2), morphological changes in neutrophils, and CRP (≥6mg/L)] and found that by combining two, three, or even four sepsis biomarkers, the sensitivity was 84%, specificity 84%, and PPV was 69%.[16]

Statistical significance was obtained with comparison of the value of accuracy between the combination of value of accuracy of m-ESR was ≥ 15 mm/hour and CRP was > 10 mg/dL with the value of accuracy of procalcitonin by the cut off point was ≥ 2 ng/mL. Yet, if we compared to the procalcitonin with the cut off point was ≥ 9.7 ng/dL, it was obtained that the value of accuracy was not significant or different from the value of accuracy of the procalcitonin.

Using new cut-off points of m-ESR > 13 mm/hour and CRP > 17 mg/dL, we obtained the values of sensitivity and specificity, 77% and 72%, respectively, and a higher value of accuracy of 73% compared to the combined value of m-ESR 15mm/hour and CRP 10 mg/dL, which had an accuracy of 66%. Hence, the new cut-off point was more accurate for diagnosing neonatal sepsis. Philip *et al.* also found that using two or more sepsis biomarkers such as I/T ratio ≥ 0.2, leukocytes < 5,000/μL, positive CRP, positive haptoglobin, mini-ESR ≥ 15 mm in the first hour, resulted in the higher sensitivity and specificity values.[15] A comparison of the new cut-off point of m-ESR and CRP values with the procalcitonin reference value of > 2 ng/mL, revealed similar values in the level of accuracy. Yet, when we used the PCTcut-off of 9.7ng/mL, accuracy dramatically improved. This finding may have been due to procalcitonin value of 9.7 ng/mL having a high value for diagnosing bacterial neonatal sepsis, compared with other markers. Esmat *et al.* also showed that PCT with a 4ng/mL cut-off had sensitivity 100%, specificity 50%, PPV 44.4%, and NPV 100%.[19]

Sensitivity, specificity, and accuracy in diagnosing bacterial neonatal sepsis level is good when we used a PCT cut-off point of 9.7 ng/mL by itself. A previous study has shown that PCT levels were markedly higher in patients with bacterial sepsis than in healthy controls,[20] and PCT was found to be superior to other biomarkers, especially CRP.[21] In a head-to-head comparison with CRP, PCT rose faster (4 hours compared to 6 hours in CRP), peaked faster (8 hours compared to 36-50 hours), normalized faster (48 hours after appropriate therapy compared to 72-96 hours), and was more sensitive and specific to sepsis (92.6% and 97.5%, respectively).[22]

In conclusion, the combination of micro-ESR 13 mm/hour and CRP 17 mg/dL can be used to diagnose bacterial neonatal sepsis, but PCT level of 9.7 ng/mL is a better measurement to diagnose bacterial neonatal sepsis, with sensitivity of 100% and specificity 96%.

Conflict of Interest

None declared.

References

1. Aminullah A, Kosim MS, Yunanto A, Dewi R, Sarosa GI. Sepsis in the newborn. Jakarta IDAI; 2008. p. 70-87.
2. Rohsiswatmo R. Diagnosis controversion in sepsis neonatorum. In: Update in neonatal infection. Jakarta: Pediatrics Department. Faculty of Medicine, University of Indonesia. Cipto Mangunkusumo Hospital; 2005. p. 32-43.
3. Qazi SA, Stoll BJ. Neonatal sepsis: a major global public health challenge. Pediatr Infect Dis J. 2009;28:S1-2.
4. Powel K, Nelson W, Behrman R. Sepsis and shock. In: Nelson textbook of pediatrics. Philadelphia: Saunders; 2000. p. 794-810.
5. Cloherty J, Eichenwald E, Stark A, Puopolo K. Bacterial and fungal infections. In: Manual of neonatal care. Philadelphia: Lippincott William & Wilkins; 2004. p. 287-312.
6. Blommendahl J, Janas M, Laine S, Miettinen A, Ashorn P. Comparison of procalcitonin with CRP and the differential white blood cell count for the diagnosis of culture-proven neonatal sepsis. Scand J Infect Dis. 2002;34:620-2.
7. O'Connor E, Venkatesh B, Lipman J, Mashongonyika C, Hall J. Procalcitonin in critical illness. Crit Care Resusc. 2001;3:236-43.
8. Adib M, Bakhshiani Z, Navaei F, Saheb Fosoul F, Fouladi S, Kazemzadeh H. Procalcitonin: a reliable marker for the diagnostic of neonatal sepsis. Iran J Basic Med Sci. 2012;15:777-82.
9. Sucilathangam G, Amuthavalli K, Velvizhi G, Ashihabegum MA, Jeyamurugan T, Palaniappan N. Early diagnostic markers for neonatal sepsis. J Clin Diag Res. 2012;6:627-31.
10. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M, *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.
11. Pepys MB. C-reactive protein fifty years on. Lancet. 1981;1:653-7.
12. Ng PC, Cheng SH, Chui KM, Fok TF, Wong MY, *et al.* Diagnosis of late onset neonatal sepsis with cytokines,

adhesion molecule, and C-reactive protein in preterm very low birth weight infants. Arch Dis Child Fetal Neonatal Ed. 1997;77:221-7.
13. Anwer SK, Mustafa S. Rapid identification of neonatal sepsis. J Pak Med Assoc. 2000;50:94-8.
14. Sharma A, Kutty CV, Sabharwal U, Rathee S, Mohan M. Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Pediatr. 1993;60:559–63.
15. Philip AG, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics. 1980;65:1036-41.
16. Mondal SK, Nag DR, Bandyopadhyay R, Chakraborty D, Sinha SK. Neonatal sepsis: role of a battery of immunohematological tests in early diagnosis. Int J Appl Basic Med Res. 2012;2:43-7.
17. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988;112:761-7.
18. Küster H, Weiss M, Willeitner AE, Detiefsen S, Jeremias I, Zbojan J, *et al*. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet. 1998;352:1271–7.
19. Esmat MM, Hasan A, Moghazy HM, Sadek AA. Procalcitonin or C-reactive protein or both for diagnosis of neonatal sepsis? J Appl Sci Res. 2012;8:4615-23.
20. Enguix A, Rey C, Concha A, Medina A, Coto D, Dieguez MA. Comparison of procalcitonin with C-reactive protein and serum amyloid for the early diagnosis of bacterial sepsis in critically ill neonates and children. Intensive Care Med. 2001;27:211-5.
21. Sherwin C, Broadbent R, Young S, Worth J, McCaffrey F, Medlicott NJ, *et al.* Utility of interleukin-12 and interleukin-10 in comparison with other cytokines and acute-phase reactants in the diagnosis of neonatal sepsis. Am J Perinatol. 2008;25:629-36.
22. Andreola B, Bressan S, Callegaro S, Liverani A, Plebani M, Da Dalt L. Procalcitonin and C-reactive protein as diagnostic markers of severe bacterial infections in febrile infants and children in the emergency department. Pediatr Infect Dis J. 2007;26:672-7.