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Original Article

A hematologic scoring system and C-reactive protein compared to blood cultures for diagnosing bacterial neonatal sepsis

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Abstract

Background Neonatal sepsis is the leading cause of death after pneumonia. Definitive bacterial sepsis diagnoses are made by blood culture results, which require a lengthy time. C-reactive protein (CRP) levels and a hematologic scoring system by Rodwell *et al.* are rapid tests that may be useful for diagnosing neonatal sepsis.

Objective To determine the diagnostic value of CRP measurement and a hematologic scoring system compared to blood culture as the gold standard for diagnosing neonatal sepsis.

Methods A cross-sectional study was conducted from April to August 2015 in the Neonatology Ward of Haji Adam Malik Hospital, Medan. A total of 43 neonates who were clinically suspected to have sepsis underwent CRP, hematologic scoring, and blood cultures. The IT ratio and procalcitonin indices were also examined. Diagnostic values were analyzed by a 2x2 table.

Results Fourteen percent from all sample had positive bacterial culture. The CRP measurements had a sensitivity of 92.8%, specificity of 62%, positive predictive value (PPV) of 54.1%, negative predictive value (NPV) of 94.7%, positive likelihood ratio (PLR) of 2.44, and negative likelihood ratio (NLR) of 0.11. The hematologic scoring system had a sensitivity of 100%, specificity of 82.7%, PPV of 73.6%, NPV of 100%, PLR of 5.78, and NLR of 0. Procalcitonin and IT ratio show a good value of sensitivity and NPV, respectively.

Conclusion The hematologic scoring system has better specificity than CRP measurement as compared to blood culture. However, both tests have good sensitivity for diagnosing neonatal sepsis.

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Keywords: *C-reactive protein; hematologic scoring system; neonatal sepsis*

Neonatal sepsis is a clinical syndrome in the first 4 weeks of life, with signs of systemic infection and diagnosed by positive blood cultures.[1] Neonatal sepsis is life-threatening, particularly in developing countries, and is the second leading cause of death in neonates after prematurity.[2] Neonates should be protected from bacterial infections to prevent sepsis. The incidence of neonatal sepsis in Indonesia was reported to range from 20.7% to 38.7%.[3]

Etiologies of bacterial neonatal sepsis were identified over a 5-year span with the most common as follows: year 1998: *Klebsiella pneumoniae* (23%); 1999 and 2000: *Staphylococcus aureus* (17% and 6%, respectively); 2001 and 2002: Acinetobacter (6.7% and 20.4%, respectively); and 2003: *Klebsiella*

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pneumoniae (23.4%).[4] At present, neonatal sepsis is classified based on pathophysiology to early-onset sepsis (EOS) and late-onset sepsis (LOS).[5]

A definitive neonatal sepsis diagnosis is established from blood cultures.[1] However, cultures require several days to allow for bacterial growth in culture medium. In central referral hospitals, procalcitonin and CRP examinations can help in diagnosing neonatal sepsis,[6,7] but these examinations cannot be performed in primary health care facilities. As such, several approaches, including a scoring system, may be implemented to simplify the diagnosis of neonatal sepsis.

A US study introduced a hematologic scoring system for diagnosing neonatal sepsis.[8] The scoring system consists of 7 findings, each of which are assigned a score of abnormal white blood cell count, abnormal total neutrophil [polymorphonuclear cells (PMN)] count, elevated immature to total PMN ratio, immature to mature neutrophil (IM) ratio > 3, elevated immature PMN count, platelet count < 150,000/mm^3, and degenerative changes in PMNs.[8,9] C-reactive protein has also been used to diagnose sepsis. The combination of CRP measurement and a hematologic scoring system is expected to be a new modality for rapidly diagnosing neonatal sepsis.

The aim of our study was to to determine the diagnostic value of CRP measurement and a hematologic scoring system compared to blood culture as the gold standard for diagnosing neonatal sepsis.

Methods

This cross-sectional study was done to determine the diagnostic value of CRP measurement and a hematologic scoring system compared to blood cultures, the current gold standard for bacterial neonatal sepsis. This study was held in the Neonatology Unit of, Haji Adam Malik Hospital, Medan, North Sumatera, from April to August 2015. The inclusion criteria were neonates with unstable temperature, lethargy, decreased muscle tone, history of resuscitation, changes in skin color such as jaundice and/or mottled, feeding disturbances, focal infections, metabolic or cardiopulmonary abnormalities, prematurity, history of fetal distress, history of rupture of the membran >18 hours, multiple gestation, premature rupture of the membranes, preterm birth, and/or chorioamnionitis. Exclusion criteria were neonates who had undergone blood culture examination, were diagnosed with sepsis, had received antibiotics prior to the study or whose parents declined participation.

Peripheral blood smears, PMN count, procalcitonin, IT ratio, IM ratio, immature PMN count, and degenerative changes in PMN were measured. Complete blood count and CRP examinations were conducted in the Clinical Pathology Laboratory. Blood cultures were done in the Microbiology Department. Neonates with a hematologic cut-off score of <4 were classified as not having sepsis, and those with a score of ≥ 4 were classified as having sepsis.

We use that cut off point depend on Rodwell's study.[8] Rodwell had formated a number of bacterial infection marker to form a hematologic scoring system which are assigned a score of: abnormal white blood cell count (≤ 5000 /μL or ≥ 25000/μL at birth or ≥ 30000/μL at 12-24 hour or ≥ 21000/μL day 2 onward =1), abnormal total polymorphonuclear cells (PMN)] count (1800-5400 =0, No mature PMN seen= 2, Increase/decrease =1), elevated immature to total PMN ratio (IT ratio > 0.12 =1), immature to mature neutrophil (IM) ratio ≥0.3 (=1), elevated immature PMN count(>600 =1), platelet count < 150,000/mm^3(=1), and degenerative changes in PMNs (toxic granules or cytoplasmic vacuoles =1). CRP examination was done qualitatively with a reference as follows: CRP ≥10 mg/L was considered to have sepsis and CRP <10 mg/L was considered to not have sepsis.

All subjects' parents provided written informed consent. This study was approved by the Health Research Ethics Committee of the University of Sumatera Utara Medical School.

To determine the diagnostic value of CRP and the hematologic scoring system for neonatal sepsis, we used a 2x2 table to obtain sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), and negative likelihood ratio (NLR). The hematologic scoring system was analyzed by receiver-operator characteristic (ROC) curve and measurement of the area under the curve (AUC) to determine the cut-off point.

Results

Forty-three neonates fulfilled the inclusion criteria. The clinical manifestations found in neonates who

participated in this study were: cardiopulmonary abnormalitites (58.1%), metabolic abnormalitites (11.6%), lethargy (7%), changes in skin color (9.3%), temperature changes (9.3%), and focal infections (4.7%). More than half of the neonates were female (55.8%) and had gestational age of <37 weeks (60.5%). The majority of neonates in this study had a chronological age of ≤72 hours (72.1%) and a birth weight of <2,500 grams (51.1%). At the end of the follow up, 14/43 (32.6%) of subjects had positive blood cultures. Positive blood cultures were found in 8/17 of neonates with gestational age of 37-42 weeks, and in 6/26 of neonates with gestational age of <37 weeks. Moreover, positive blood cultures were found in 6/31 of subjects with chronological age <72 hours, and in 8/12 of subjects with chronological age >72 hours. Positive blood cultures were observed in 12/21 of neonates with birth weight of 2,500 - 4,000 grams and in 2/22 of neonates with birth weight of <2,500 grams (**Table 1**).

A 2x2 table analysis revealed that the predictive value of the hematologic scoring system was more accurate than that of CRP measurement for diagnosing neonatal sepsis. The hematologic scoring system had sensitivity of 100% and specificity of 82.7%, while CRP had sensitivity of 92.8% and specificity of 62% (**Table 2**). In addition, the hematologic scoring system had an AUC of 94.6% with excellent statistical significance (**Figure 1**).

In addition to CRP and the hematologic scoring system, we compared procalcitonin and IT ratio to blood cultures as diagnostic modalities for neonatal

Table 1. Demographic characteristics of neonates

Characteristics	Blood culture		P value
	Positive (n=14)	Negative (n=29)	
Gender, n			
Male	7	12	0.837[a]
Female	7	17	
Gestational age, n			
< 37 weeks	6	20	0.191[a]
37-42 weeks	8	9	
Chronological age at admission, n			
≤ 72 hours	6	25	0.005[b]
> 72 hours	8	4	
Birth weight, n			0.002[a]
< 2,500 g	2	20	
2,500-4,000 g	12	9	

[a]Chi-square test, [b]Fisher's exact test

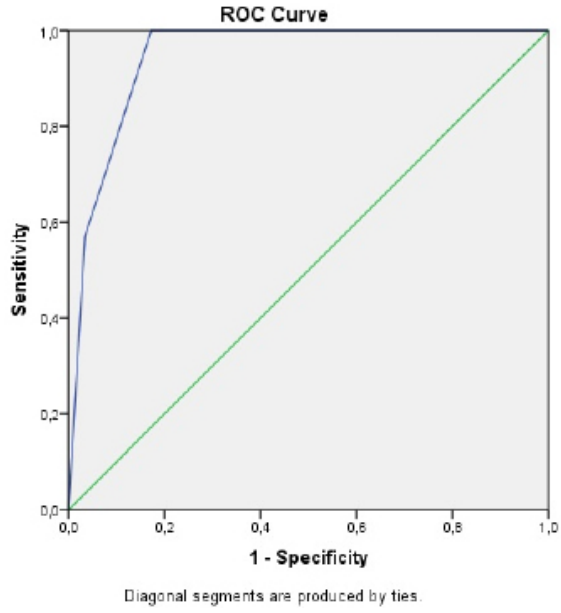

Figure 1. ROC of hematologic scoring system compared to blood cultures

Table 2. Diagnostic values of a hematologic scoring system and CRP compared to blood cultures

Diagnostic parameters	Blood culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
	Positive (n=14)	Negative (n=29)						
Hematologic scoring system*, n								
Positive	14	5	100	82.7	73.6	100	5.78	0
Negative	0	24						
CRP*, n			92.8	62	54.1	94.7	2.44	0.11
Positive	13	11						
Negative	1	18						

*Diagnostic values were analyzed by a 2x2 table

sepsis. The IT ratio had sensitivity of 100% and specificity of 51.7%, while procalcitonin had sensitivity of 100% and specificity of 46.5% (**Table 3**).

Table 3. Diagnostic values of IT ratio and procalcitonin as compared to blood cultures

Diagnostic parameters	Blood culture		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	PLR	NLR
	Positive (n=14)	Negative (n=29)						
IT ratio*, n								
Positive	14	14	100	51.7	50	100	2.07	0
Negative	0	15						
Procalcitonin*, n			100	46.5	33.3	100	1.86	0
Positive	14	28						
Negative	0	1						

*Diagnostic values were analyzed by a 2x2 table

Discussion

Neonatal sepsis is a clinical syndrome in the first 4 weeks of life, with signs of systemic infection and diagnosed by positive blood culture results.[1] In developing countries, neonatal sepsis is the most frequent cause of death after prematurity.[2] A study in Haji Adam Malik Hospital, Medan from 2008 to 2010 found that the mortality rate from neonatal sepsis was 20%.[10] The prevalence of neonatal sepsis in Indonesia was 38.7% in 2005.3 In our study, the prevalence of neonatal sepsis was 33.7%.

The following bacteria were identfied from subjects' blood cultures: 3 *Acinetobacter baumannii*, 3 *Elizabethkingia meningoseptica* , 2 *Klebsiella pneumoniae*, 2 *Staphylococcus haemolyticus*, 1 Kocuria varians, 1 *Bacillus cereus*, 1 *Pseudomonas oryzihabitans*, 1 Salmonella spp , 1 P*asteurella pneumotropica*, and 1 *Flavimonas oryzihabitans*. A previous study also reported the most common etiology of neonatal sepsis to be Gram-negative bacteria.[11]

Bacteria entering the circulation trigger the body's immune response. The cell membrane and wall contain phosphocholine which can activate the complement system. An activated complement system induces granulocyte, phagocyte, and proinflammatory cytokine production.[12] C-reactive protein is an acute phase protein synthesized along with the activation of proinflammatory cytokines. The same thing will happen to granulocyte which is also a component of white blood cell.[12,13] C-reactive protein measurement is an advanced examination commonly used to establish a diagnosis of sepsis. A previous study reported that the diagnostic value of qualitative CRP measurement for neonatal sepsis had a sensitivity of 92.8% and specificity of 62%.[12] Another study involving a large sample size, found that sensitivity and specificity of CRP ranged from 29 to 100% and 6 to 100%, respectively.[14] The wide variability in diagnostic values of CRP may have been influenced by sample characteristics, study design, sample size, inclusion criteria, and differences in CRP cut-off points.[12] C-reactive protein levels increase 24 to 48 hours after clinical manifestations appear.[12] In our study, CRP examination was done according to chronological age of neonates at the time of admission to the Neonatology Unit. Chronological age of subjects ranged from 1 to 240 hours, with most neonates aged 2 hours (18.6%), 4 hours (11.6%), and 24 hours (11.6%). Given that many of our neonates were less than 24 hours old when their CRP was tested, we compared our sensitivity and specificity results to that from another study. A previous study found that the sensitivity of CRP in 24 hours after birth was 79% and the specificity was 78%.[15] The best time to measure CRP is between 24 and 48 hours after the onset of clinical manifestations.[12] Another study suggested measuring CRP after 6 to 12 hours.[16] Newborns' immature immune systems affect the amount of CRP detected in serum.[12]

Gestational age and birth weight also determine CRP level. Low gestational age neonates tend to have lower CRP levels compared to neonates with normal gestational age. A similar finding was observed with birth weight.[17] C-reactive protein levels increase at

a rate of 6% for each additional week of gestational age, and 2.4% for each 100 gram increase in birth weight.[12] We used 10 mg/L as the CRP cut-off point in accordance with previous studies which had ranges from 1.2 to 20 mg/L. The most common used value was 10 mg/L.[12,14,18] A single CRP examination is not considered to be representative due to the physiological changes in CRP level. Chiesa et al. determined cut-off points based on chronological age: 5 mg/L at birth, 14 mg/L at 24 hours, and 9.7 mg/L at 48 hours.[19] The same authors also determined cut-off points for full term and preterm neonates, with the highest levels of 11 mg/L and 13 mg/L, respectively.[20]

Neutropenia, thrombocytopenia, and leukopenia are indicators of severe infection and appear earlier than CRP.[20,21] Physiological changes in blood cells resulting from infection are components of the hematologic scoring system introduced by Rodwell *et al.*[8] this scoring system had sensitivity of 100% and specificity of 82.7% in our study, similar to the sensitivity of 80% and specificity of 90% in a previous study.[22] Leukopenia and neutropenia in neonatal sepsis were clearly observed until the first 3 days of life.[21] We also observed these conditions as the majority of our subjects' blood specimens were examined within 3 days of birth.

The IT ratio and procalcitonin were also measured in this study. The IT ratio had sensitivity of 100% and specificity of 51.7%, while procalcitonin had sensitivity of 100% and specificity of 46.5%. The IT ratio peaks from birth until 6 hours of life, but procalcitonin peaks at 12 hours or later after birth.[13] Vouloumanou et al. reported that procalcitonin had better diagnostic value in cases of late onset sepsis (LOS).[16] The timing of sample collection affected the diagnostic value of IT ratio, but not procalcitonin. Only 27.9% of subjects had procalcitonin levels in accordance with physiological changes.

A limitation of our study was the CRP diagnostic value determination, where variables were measured in a categorical scale. CRP was measured qualitatively, hence, the area under the ROC curve could not be calculated. This limitation influenced the cut-off point and further disrupted the prediction of neonatal sepsis. Furthermore, the non-uniform timing of blood specimen collections might have affected the results. The hematologic scoring system and CRP examinations should be done at 6 to 12 hours after birth, contrary to a previous study in which subjects underwent the examinations based on chronological age at admission.[16] The timing of antibiotic administration also affected the results. Antibiotic administration did not follow physiologic pattern of CRP and white blood cell activity and this might reduce the diagnostic value of CRP measurement.[16,21] In addition, the small sample size may limit the value of study. The NLR from the hematologic scoring system had a value of 0, which was caused by the presence of a cell in the 2x2 table with a value of 0. The strength of association in a comparative test between the hematologic scoring system and blood cultures could not be determined for the same reason.

In conclusion, the hematologic scoring system has better specificity than CRP, as compared to blood culture, for diagnosing neonatal sepsis. Nevertheless, both diagnostic tests have good sensitivity. A similar study with a larger sample size would be useful to confirm the diagnostic value of CRP and the hematologic scoring system.

Conflict of interest

None declared.

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