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The diagnostic accuracy of clinical and blood examination for sepsis in potentially infected neonates

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ABSTRACT

Ari Mulyani, D Setyowireni, Achmad Surjono - ***The diagnostic accuracy of clinical and blood examination for sepsis in potentially infected neonates***

Background: Neonatal sepsis remains a diagnostic challenge due to its nonspesific symptoms. Blood culture examination which is considered to be the gold standard, sometimes it is still a problem because takes time to get the result, expensive and not every health facility is able to perform.
Objective: To evaluate the diagnostic accuracy of clinical symptoms, hematologic findings and C-Reactive Protein (CRP) in neonatal sepsis.
Methods: Samples were taken from potentially infected neonates admitted to the Maternal-Perinatal Unit of Dr. Sardjito Hospital, Yogyakarta, between December 1st, 2000 to March 31st, 2001 using at least one of the criteria: prematurity (<37 weeks gestational of age), very low birth weight infants (<1,500 g), maternal pyrexia during delivery (>38°C or white blood cell count >15,000/μL), premature rupture of the membrane (>24 hours), thick and cloudy amniotic fluid. Clinical symptoms, total white blood cell count, total neutrophil count, platelet count, CRP, and blood culture as gold standard were examined.
Results: Among 99 neonates who were enrolled in this study, the sensitivity, specificity, positive predictive value and negative predictive value of clinical symptoms were 79.3%, 75.7%, 57.5%, and 89.9%, respectively; leukopenia/leukocytosis were 27.6%, 85.7%, 44.4%, and 74.1%, neutropenia/neutrophilia were 41.4%, 71.4%, 37.5%, and 74.6%, thrombocytopenia were 79.3%, 51.8%, 40.4%, and 85.7%, positive CRP were 58.6%, 78.6%, 53.1%, and 82.1%. Parallel test (clinical manifestation, thrombocytopenia, and CRP) increasing sensitivity up to 89.7%. Spesificity, positive predictive value, negative predictive value, and likelihood ratio were 44.3%, 40%, 91.2%, and 1.6, respectively. Serial test (CRP, clinical manifestation, and thrombocytopenia) increasing the spesificity up to 88.6%. Sensitivity, positive predictive value and negative predictive value were 58.6%, 68%, and 83.8%, respectively, likelihood ratio was 5,1.
Conclusion: Clinical sepsis, thrombocytopenia and CRP were sufficiently accurate as diagnostic test for sepsis in potentially infected neonate. Using parallel test increased the sensitivity, where negative finding reveals no sepsis. Serial test increased specificity. There was high probability of having sepsis, if the result was positive.

Key words : Neonatal sepsis - clinical symptoms - hematologic findings - C-reactive protein

ABSTRAK

Ari Mulyani, D Setyowireni, Achmad Surjono - ***Akurasi diagnostik pemeriksaan klinis dan darah untuk sepsis pada neonatus dengan potensial terinfeksi***

Latar Belakang: Diagnosis sepsis neonatorum masih merupakan masalah oleh karena gejalanya yang tidak spesifik. Pemeriksaan biakan darah yang diakui sebagai baku emas, kadang-kadang masih merupakan

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masalah oleh karena memerlukan waktu beberapa hari untuk memperoleh hasil, biaya mahal, dan tidak semua fasilitas kesehatan mampu melakukannya.
Tujuan: Mengetahui ketepatan diagnostik gejala klinis, pemeriksaan hematologis, dan C-Reactive Protein (CRP) pada sepsis neonatorum.
Bahan dan Cara: Sampel diambil dari neonatus dengan potensial terinfeksi yang dirawat di Instalasi Maternal-Perinatal, Rumah Sakit Dr Sadjito, Yogyakarta, antara 1 Desember 2000 sampai dengan 31 Maret 2001 dengan sekurang-kurangnya 1 kriteria : prematuritas (umur kehamilan <37 minggu), bayi berat badan lahir sangat rendah (<1500 gram), ibu demam selama persalinan (>38°C atau jumlah leukosit >15.000/mL), ketuban pecah dini (>24 jam), cairan amnion kental dan keruh. Dilakukan pemeriksaan gejala klinis, jumlah leukosit, jumlah neutrofil, jumlah trombosit, CRP; dan biakan darah sebagai baku emas.
Hasil: Pada 99 neonatus yang diikutkan dalam penelitian ini, sensitivitas dan spesifisitas, nilai ramal positif dan nilai ramal negatif dari gejala klinis berturut-turut adalah : 79,3%, 75,7%, 57,5%, dan 89,9%, untuk leukopenia atau leukositosis adalah 27,6%, 85,7%, 44,4%, dan 74,1%, untuk neutropenia atau neutrofilia adalah 41,4%, 71,4%, 37,5%, dan 74,6%, untuk trombositopenia adalah 58,6%, 78,6%, 53,1%, dan 82,1%. Uji paralel (CRP, klinis sepsis, dan trombositopenia) meningkatkan sensitivitas sampai 89,7%. Spesifisitas, nilai ramal positif, nilai ramal negatif, dan *likelihood ratio* berturut-turut adalah 44,3%, 40%, 91,2%, dan 1,6. Hasil uji negatif hampir dipastikan bayi tidak menderita sepsis. Uji serial (CRP, klinis sepsis, dan trombositopenia) meningkatkan spesifisitas sampai 88,6%. Sensitivitas, nilai ramal positif, nilai ramal negatif, dan *likelihood ratio* berturut-turut adalah 58,6%, 68%, 83,8%, dan 5,1. Apabila dijumpai hasil uji positif maka kemungkinan besar bayi menderita sepsis.
Simpulan: Klinis sepsis, trombositopenia. dan CRP cukup akurat untuk diagnosis sepsis pada neonatus dengan potensial terinfeksi. Uji paralel meningkatkan sensitivitas, hasil negatif dapat menyingkirkan diagnosis sepsis. Uji serial meningkatkan spesifisitas, besar kemungkinan bayi menderita sepsis jika hasil positif.

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INTRODUCTION

One of the most important problem in neonatal care is infection, which is still common in Indonesia, with high morbidity and mortality rates.[1] The major problems are to determine the infected infant to administer appropiate therapy as early as possible, and to discontinue therapy if it is not indicated because the symptoms and sign are usually not specific.[2] Beside the clinical manifestasion, various pattern of hematologic changes is associated with sepsis including total white blood count, total neutrophil count, platelet count and increasing of CRP.

Blood culture examination provides almost 60% detection of neonatal infection cases, whereas obtaining the result requires several days[3] and not every health facility can perform the procedure and the cost is expensive. Although a positive blood culture is generally considered to be the gold standard for diagnosis of septicemia, spurious result from contaminated samples is not infrequent. Thus addition to the blood culture, other laboratory tests are often used in attempt to support a diagnosis of infection.[4] So we need the clinical manifestation, hematologic and CRP examination. The study was conducted to evaluate the diagnostic value of clinical symptoms, white blood cell count, neutrophil count, platelet count and CRP in neonatal sepsis.

METHODS

Babies admitted to the Maternal-Perinatal Unit of Dr.Sardjito Hospital, Yogyakarta during the period of December 1st, 2000 to March 31st, 2001 were included in the study. Any baby who had high risk categories for infection using at least one of the criteria : prematurity (< 37 weeks gestational age), very low birth weight (< 1,500 g), maternal pyrexia during delivery (> 38°C or white blood cell count > 15,000/μL), premature rupture of the membrane (> 24 hours), thick and cloudy amniotic fluid were tested for having infection.[4,5,6] Neonates with congenital anomaly or blood disorder not caused by sepsis were excluded from this study.

All infants were evaluated on clinical manifestations by a pediatrician. The patient was considered clinically sepsis if they met at least 1 sign in 4 out of 6 group categories: (1) general condition (not doing well, poor feeding, fever, hypothermia, sclerema); (2) gastrointestinal system (abdominal distention, vomiting, diarrhea, hepatomegaly); (3) respiratory system (apnea, dyspnea, tachypnea, retraction, flaring, grunting, cyanosis); (4) cardio-

vascular system (tachycardia, bradycardia); (5) central nervous system (irritability, lethargy, tremor, seizure); (6) hematologic system (jaundice, splenomegaly, pallor, petechiae, purpura, and bleeding).[4,7,8,9]

Blood samples for blood examination were taken from vein. White blood cell, neutrophil and platelet count were calculated using standard procedures by Central Laboratory. We defined abnormal white blood cell count if there was leukopenia/leukocytosis (< 7 days old: < 9,000/μL or > 30,000/μL; ≥ 7 days old: < 5,000/μL or > 21,000/μL); neutropenia/neutrophilia (< 7 days old: < 6,000/μL or > 26,000/μL; ≥ 7 days old: < 1,500/μL or > 10,000/μL).[10] Thrombocytopenia was defined as the platelet count < 150,000/μL.[1] CRP measurement was done semi-quantitatively using latex agglutination method, CRP positive > 6 mg/L1. Blood cultures were performed to all of the high risk infants by Central Laboratory and 4-6 days examination was required for the results. Blinding was obtain due to the difference between test and gold standard result. The protocol was approved by the ethic committee of Dr. Sardjito Hospital.

Accuracy of diagnostic test was analysed with sensitivity, specificity, predictive values, and likelihood ratio. Sensitivity, specificity and predictive values of clinical manifestation, white blood cell count, neutrophil count, platelet count and CRP were determined using the usual 2 x 2 tables. Results of blood culture were served as a gold standard.

RESULT

Between December 1st 2000 and March 31st 2001, a total of 278 neonates were admitted to Maternal-Perinatal Unit, Dr. Sardjito Hospital. Of 153 (55%) who met one of the criteria were grouped as potentially infected baby. There were only 99 cases were eligible to study due to uncompletness of examination and exclusion criteria (congenital anomaly or blood disorder not caused by sepsis).

The characteristics of subjects were showed in TABLE 1. Blood cultures were positive in 29 neonates (29.3%). Clinical sepsis were found in 40 neonates (40.4%), leukopenia/leukocytosis were positive in 32 neonates (32.3%). Thrombocytopenia were positive in 57 neonates (57.6%). CRP >6 mg/L were positive in 32 neonates (32.3%).

Sensitivity and specificity of clinical sepsis were 79.3% and 75.7%, respectively (TABLE2). In this way, sensitivity and specificity of thrombocytopenia were high (79.3% and 51.8%, respectively). Sensitivity and specificity of CRP were high (58.6% and 78.6%, respectively). The specificity of leukopenia/leukocytosis was high (85.7%), but the sensitivity was low (27.6%). In this way, the specificity of neutropenia/neutrophilia was high (71.4%) and the sensitivity was low (41.4%)

TABLE 1. - Characteristics of subjects

Variable	n (99)	%
Age		
< 7 days	86	86.9
≥ 7 days	13	13.1
Sex		
Male	60	60.6
Female	39	39.4
Birth weight		
Large BW (4000g)	2	2.0
Normal BW (2500-4000g)	65	65.7
Low BW (1500-2499g)	27	27.3
Very Low BW (<1500g)	5	5.0
Gestational of age		
<37 weeks	21	21.2
37-42 weeks	77	77.8
>42 weeks	1	1.0
Maternal pyrexia/leukocytosis	21	21.2
Premature rupture of the membrane	18	18.1
Thick and cloudy amnion fluid	40	40.4

TABLE 2. - Sensitivity, specificity and predictive values of clinical sepsis in the diagnosis of neonatal sepsis

Indicator	+ Blood culture	- Blood culture	Total
+ Clinical sepsis	23	17	40
- Clinical sepsis	6	53	59
Total	29	70	99

Sensitivity	: 79.3%	(95% CI : 59.7 - 91.3)	
Specificity	: 75.7%	(95% CI : 63.7 -91.3)	
Positive predictive value	: 57.5%	(95% CI : 41.0 - 72.6)	
Negative predictive value	: 89.8%	(95% CI : 78.5 - 95.8)	

TABLE 3. - Sensitivity, specificity, predictive values, and likelihood ratio in diagnosis of neonatal sepsis

Indicator of sepsis	sensitivity %	specificity %	PPV* %	NPV** %	PLR†	NLR‡
Clinically +	79.3	75.7	57.5	89.8		
Leukopenia/leukocytosis	27.6	85.7	44.4	74.1		
Neutropenia/neutrophilia	41.4	71.4	39.5	74.6		
Thrombocytopenia	79.3	51.8	40.4	85.7		
CRP +	58.6	78.6	53.1	82.1		
Parallel test	89.7	44.3	40.0	91.2	1.6	0.23
Serial test	58.6	88.6	68.0	83.8	5.1	0.48

* Positive predictive value
** Negative predictive value
† Positive Likelihood ratio
‡ Negative Likelihood ratio

(TABLE 3). Therefore, clinical manifestation, platelet count and CRP can be considered for neonatal sepsis diagnostic.

Using parallel test (clinical finding, platelet count and CRP) resulted in sensitivity of 89.6%, specificity of 44.2%, positive likelihood ratio (LR) of 1.6, and negative LR of 0.23. Using serial test started from the higher specificity (CRP, clinical manifestation and thrombocytopenia) resulted in sensitivity of 58.6%, specificity of 88.5%, positive LR of 5.1, and negative LR of 0.46. In parallel test, with the prevalence 29.3%, pre test odds 0.41, post test odds 0.65, the post test probability was 0.39. In serial test, pre test odds 0.41, post test odds 2.09, the post test probability was 0.67.

DISCUSSION

The purpose of this study is to compare the diagnostic value of clinical findings, hematologic findings and CRP in neonatal sepsis to blood culture examination as gold standard. Our study showed that sensitivity and specificity of clinical sepsis were 79.3% and 75.7%, respectively. It has been known that total white blood cell count is of limited value in the diagnosis of septicemia of the newborn. Among neonates that were evaluated for suspected sepsis, less than half of those with decreased (<5,000/μL) or elevated (>20,000/μL) cell counts were ultimately identified as being infected[11], while Oski and Naiman[10] defined leukopenia was blood cell count <9,000/μL (<7 days old) or < 5,000/μL (≥7 days old) and leukocytosis was blood cell count >30,000/μL (<7 days old) or >21,000/μL (≥7 days old). Leukopenia/leukocytosis had 27.6% sensitivity and 85.7% specificity, while Kosim *et al*[1] had 16.7% and 66.7 %, respectively. Anwer & Mustafa (2000) found a specificity of 93% but a sensitivity of 14%[13]. Total neutrophil counts were decreased or elevated in only one quarter to one

third of infants with bacteremia, particularly when the counts were obtained early in the course of illness.[12] Neutropenia/neutrophilia had sensitivity and specificity of 41.4% and 71.4%, while Anwer & Mustafa (2000) found over 60% and 50%, respectively[13].

Thrombocytopenia accompanying bacterial infection is thought due to a direct effect of bacteria or bacterial products on platelets and vascular endothelium leading to increased aggregation and adhesion, or by increased platelet destruction caused by immune mechanisms.12,14 Thrombocytopenia (< 150,000/μL) had 79.3% sensitivity and 51.8% specificity, while Kosim *et al* (1993) found 11.1% and 66.7%, respectively[1].

CRP is known to be produced by the fetus and has been found in high concentration in the sera of newborn infants with variety of infection. Thus, the increased CRP level may be used as diagnostics in neonatal infections.[1] In this study CRP with cut off point > 6 mg/L had sensitivity and specificity 58.6% and 78.6%, respectively, while Kosim *et al* (1993) found 83.3% sensitivity and 58.3% specificity[1]. Anwer & Mustafa (2000) found sensitivity of over 60% and specificity of 50%[13].

Result from these analysis showed that both white blood cell count and neutrophil count had low sensitivity; on the other hand clinical manifestations, platelet count and CRP examination had higher sensitivity and specificity. Clinical manifestations, platelet count and CRP were sufficiently accurate as diagnostic test for potentially infected neonates. Parallel test increased sensitivity up to 89.7%. Specificity, positive predictive value, negative predictive value, and likelihood ratio were 44.3%, 40%, 91.2%, and 1.6, respectively. Serial test started from the higher specificity examination (CRP, clinical manifestation and platelet count) increased the specificity up to 88.6%; sensitivity, positive predictive value and negative predictive value were 58.6%, 68%, and 83.8%, respectively, likelihood ratio was 5.1. This test was sufficiently accurate as diagnostic test for sepsis in potentially infected neonates. Parallel test is used in clinical practice. Result of this study is limited to neonates with potentially infected. To generalize the result in large population, studies involving neonates in general are needed.

CONCLUSION

Clinical sepsis, thrombocytopenia and CRP are sufficiently accurate as diagnostic tests for sepsis in potentially infected neonates. Parallel test increases sensitivity, the negative result revealed that it is not sepsis. Serial tests will increase specificity. There is high probability of having sepsis, if the result was positive.

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