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Original Article

Hematological scoring system as an early diagnostic tool for neonatal sepsis

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Abstract

Background Sepsis was the leading cause of death in babies by 30%-50% in developing countries. Early diagnosis of neonatal sepsis is still a difficult problem because of clinical features are not specific. Blood culture is the gold standard, but it takes several days and is expensive. The hematological scoring system (HSS) consists of hematologic parameters (leucocyte count, polymorphonuclear (PMN) cells, degenerative changes, and platelet count) for early diagnosis of neonatal sepsis.

Objective To measure HSS as an early diagnostic tool for neonatal sepsis.

Methods A cross sectional study was conducted in March to June 2013. Samples were collected by consecutive sampling. Fourty neonates suspected sepsis in neonatology unit H. Adam Malik Hospital, Medan, North Sumatera, underwent routine blood count, blood culture, and peripheral blood smear. Each hematologic parameters were analysed using the HSS of Rodwell *et al.* The hematologic parameters were total leucocyte count, total PMN cells, total PMN immature, I:T PMN ratio, I:M PMN ratio, degenerative changes, and platelet count. The total value revealed HSS score. Diagnostic study parameters were calculated.

Results Ten of fourty neonates had sepsis based on blood culture results. The HSS score ≥4 had sensitivity 80%, specificity 90%, with positive predictive value (PPV) 73%, negative predictive value (NPV) 93%, ROC curve showed cut off point 0.902 (95% CI 0.803 to 1.0).

Conclusion Score HSS ≥4 could be used as an early diagnostic tool for neonatal sepsis. **[Paediatr Indones. 2015;55:315-21]**.

Keywords*: neonatal sepsis, hematological scoring system, diagnostic*

Sepsis can develop in pregnancy or during delivery and manifests on the first three-day of life (<72 hours).[1,2] The early diagnosis of sepsis in neonates is still complicated because of the unspecific clinical features.[3-6] Bacterial infection is the major cause of morbidity and mortality in newborns. Sepsis as the cause of death in neonates is about 30%-50% in developing countries,[5,7] and in Indonesia the incidence is about 8.7%-30.29% with mortality rate of 11.56-49.9%.[8,9] Blood culture examination as the gold standard in the diagnosis of sepsis, [4,6,9] takes several days and is high costly.[4,6,9] Hematologic parameter such as leucocyte, C-reactive protein (CRP), procalcitonin, interleucin-6 (IL-6), and clinical manifestations can predict sepsis in neonates.[3,10-13]

The appraisal of seven hematologic parameters with hematological scoring system (HSS) formulated by Rodwell *et al,*[3] can be utilized to establish the

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early diagnosis of sepsis in neonates.[1,4,5] A study in Australia has reported that HSS could be used as a screening tool for sepsis and had been standarized globally.[3] Study in Dhaka has reported that HSS could be utilized to differ an infected baby from a non-infected baby and HSS was significantly related to sepsis.[4] Study in India reported that HSS was a simple, rapid and effective screening tool of sepsis.[5] The objective of our study was to determine whether HSS could be used as an early diagnostic tool for neonatal sepsis.

Methods

We conducted a diagnostic test study to acquire sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of HSS to establish the early diagnosis of sepsis in neonates. This study was performed in neonatology unit of Haji Adam Malik General Hospital, Medan, North Sumatera, since March to June 2013. Suspected sepsis neonates based on risk factors with or without clinical manifestation were included in this study. A blood sample was obtained before the baby was treated with antibiotic or $\leq$ 48 hours after treatment started. All of the subject's parents consented to participate in the study. This study has been approved by Health Ethical Committee from University of Sumatera Utara Medical School.

Complete blood count, blood culture, peripheral blood smear, CRP level, and procalcitonin level were performed to all subjects who fulfilled the inclusion criteria. Laboratory examination was performed by Department of Clinical Pathology, Haji Adam Malik Hospital, Medan. Blood samples were obtained aseptically, and then inserted to the pre-sterilized tube. Blood culture was performed with BACTEC method and bacteries identified with VITEK 2 tool in Microbiology Division of Haji Adam Malik Hospital Medan. Peripheral blood smear that stained with Giemsa was read by the researchers and a trained hematology analyst under binocular microscope with 100 times magnification. Every hematological parameter (total leucocyte count, total PMN cells, total immature PMN cells, I:T PMN ratio, I:M PMN ratio, degenerative change and total platelet count) was identified, analyzed and summed using HSS formulated by Rodwell *et al* (**Table 1**). The appraisal of HSS scoring was performed by researchers.

A positive clinical manifestation characterized with failure to drink, hypothermia or hyperthermia, jaundice/hyperbilirubinemia, gastrointestinal disorder, persistent tachycardia (heart rate >180 x/min), bradicardia (heart rate <80 x/min), poor tissue perfusion (capillary refill time $\geq$3 seconds), tachypneu (respirarion rate >70 x/min), hyperglycemia, hypoglycemia, lethargic, unconsciousness, seizure, groaning. Subject categorized as negative clinical manifestation if none of symptoms above found.

Maternal risk factors of sepsis consisted of early rupture of the membrane, rupture of the membrane > 18 hours, infection in pregnancy (bacterial, parasit, viral, chorioamnionitis), peripartum febrile, urinary tract infection, cloudy and smelly amniotic fluid, multiple pregnancy, and preterm labor. Prematurity, low birth weight, asphyxia neonatorum, required resuscitation, invasive procedures (endotracheal intubation, ventilator, catheterisation, infusion, central venous access) were infant risk factors.

Data analysis was performed by using *SPSS 15*. Diagnostic ability differences of peripheral blood smear was compared with blood culture and were analyzed by Chi-square and Fisher exact test to count sensitivity, specificity, PPV and NPV with confidence interval of 95% and $P < 0.05$. Receiver operating characteristics (ROC) curve was made to determine the best cut-off of diagnostic test.

Results

We enrolled 40 neonates who suffered from neonatal sepsis during March to June 2013. Complete blood count, blood culture examination and peripheral blood smear were done and HSS was identified.

Subjects' characteristics such as gestational age, gender, birth weight, clinical manifestations and risk factors of baby and the mother were demonstrated in **Table 2**. From 40 neonates investigated, 10/40 had positive blood culture results. The gestational age of the majority of subjects was less than 37 weeks with 5/10 neonates were proven sepsis and 17/30 neonates were unproven sepsis. The birth weight of the majority of neonates was approximately 1000-1499 grams in each group (5/10 neonates from the positive blood

culture group and 14/30 neonates from the negative blood culture group). Neonates with positive clinical manifestations were 10/10 from the positive blood culture group and 26/30 from the negative blood culture group. The most common risk factor was found in neonates which 5/10 from the positive blood culture group and 16/30 from the negative blood culture group.

Table 3 demonstrates sensitivity and specificity for each hematological parameter. Total PMN cell count and I:T PMN ratio had a high sensitivity (100%), whereas total leucocyte count and I:M PMN ratio had a high specificity (90%). From those results, the best and rational parameter was I:T PMN ratio which had sensitivity of 100% and specificity of 57% with PPV of 43% and NPV of 100%. The PMN degenerative change was not found in all subjects.

Table 4. demonstrates hematological screening feature in every hematological parameter by using Chi square and Fisher exact to determine the most appropriate cut-off value for clinical importance. Score more than or equal to 4 in HSS had a sensitivity of 80% (95% CI 55% to 100%), a specificity of 90% (95% CI 79% to 100%), PPV of 73% (95% CI 46% to 99%), NPV of 93% (95% CI 84% to 100%) and P=0.001. Those led to score 4 as the best, most

Table 1. Hematological scoring system (HSS)[3]

Variables	Abnormality	Score
Total leucocyte	≤ 5000/µL	1
	≥ 25,000, at birth ≥ 30,000, 12-24 hours ≥ 21,000, day 2 onwards	1
Total PMN cell count	No mature PMN cell seen	2
	Increased/decreased	1
Immature PMN cell count	Increased	1
I:T PMN ratio	Increased	1
I:M PMN ratio	≥ 0.3	1
Degenerative change in PMN	Toxic granules/cytoplasmic vacuoles	1
Platelet count	≤ 150,000/ µL	1

Normal value: total PMN count = 1800-5400/µL, immature PMN count = 600/µL, I:T PMN ratio = 0.12, I:M PMN ratio = ≥ 0.3

Table 2. Demographic data of subjects

Characteristics	Positive blood culture (n= 10)	Negative blood culture (n=30)
Gestational age, n		
Preterm (<37 weeks)	5	17
Aterm (≥37 weeks)	5	13
Gender		
Boy	7	19
Girl	3	11
Birth weight, n		
1000-1499 gram	2	5
1500-2499 gram	5	14
≥ 2500 gram	3	11
Clinical manifestations of sepsis		
Positive	10	26
Negative	0	4
Risk factors of sepsis		
Mother and baby	5	14
Baby	5	16

rational and reliable cut-off value in early diagnosis of neonatal sepsis.

Table 5 shows the comparison of HSS, CRP procalcitonin levels to blood culture results by using Chi square (X^2) and Fisher exact test. The HSS more than or equal to 4 was more significant in early diagnosis of neonatal sepsis compared to CRP and procalcitonin level with P=0.001.

Area under the curve for HSS score of 4 was 0.902 (95%CI 0.803 to 1.0; P = 0.001) with significance of 5%. This results showed that the accuracy of this diagnostic test was excellent. The **Figure 1** demonstrates ROC curve for HSS score of 4 as an early diagnostic tool of neonatal sepsis.

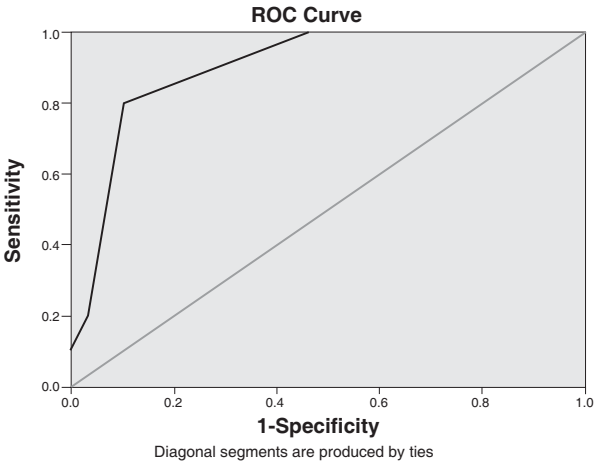

Figure 1. ROC curve for HSS score of 4

Table 3. Hematological parameters of sepsis

Hematological parameters	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total leucocyte count	20	90	40	77
Total PMN cell count	100	20	29	100
Immature PMN cell count	90	50	38	94
I:T PMN ratio	100	57	43	100
I:M PMN ratio	50	90	63	84
Degenerative changes in PMN cells	0	100	0	75
Platelet count	50	57	28	77

PPV: positive predictive value; PMN: polymorphonuclear; I:M: immature to mature ratio; NPV: negative predictive value; I:T : immature to total ratio

Table 4. Hematological scoring system (HSS) of sepsis

HSS Score	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	P value
≥2	100	20	29	100	0.307
≥3	100	53.3	42	100	0.003
≥4	80	90	73	93	0.001
≥5	20	96.7	67	78	0.149
≥6	10	100	100	77	0.250

Table 5. Comparison of HSS score of 4, CRP and procalcitonin levels to blood culture results

Variables	Blood culture results		Total	P value
	Positive	Negative		
HSS score				
≥ 4	8	3	11	0.001
< 4	2	27	29	
CRP level				
Positive	7	13	20	0.144
Negative	3	17	20	
Procalcitonin level				
Positive	10	26	36	0.556
Negative	0	4	4	

CRP level positive ≥ 0.7 mg/dL, procalcitonin positive > 0.05 µg/mL

Disscussion

The mortality rate of neonatal sepsis in Haji Adam Malik Hospital Medan from 2008 until 2010 was 20%.[15] Since 1974, many studies were performed to appraise diagnostic test in several hematological paramater as a tool to help the clinician to establish diagnosis of neonatal sepsis prior obtaining the blood culture results, so that inappropiate management could be avoided, furthermore, mortality and morbidity rate could be lowered.[3,10,16] Hematological parameter such as leucocyte count, platelet count, absolute neutrophil count, I:M PMN ratio, I:T PMN ratio, blood sedimentation rate, CRP level, toxic granules, and cytoplasmic vacuoles in peripheral blood smear can be utilized in early diagnosis of neonatal sepsis.[9-11,17-19]

Our study found that 10 of 40 neonates (25%) were proven sepsis based on positive blood culture results. Seven neonates with proven sepsis (7/10) had low birth weight. We found that bacterial growth consisted of positive Gram bacterial (*Staphylococcus epidermidis, Enterococcus sp*, and *Micrococcus*) in 3/10 neonates and negative Gram bacterial (*E. Coli, Sphingomonas paucimobilis, Achromobacter denitrificans, Salmonella sp, Staphylococcus haemolyticus, Ochrobactrum anthropi,* and *Ralstonia mannitolilytica*) in 7/10 neonates. These results were similar with a study in Medan (2008) which had reported that the major cause of sepsis in neonates was negative Gram bacterial (60%) with mortality rate of 81.1%.[15]

We found that 36 of 40 neonates came with clinical manifestations. Those included instability of temperature (hypothermia or hyperthermia) in 36 neonates (90%), neurological deficit (seizure and lethargy) in 27 neonates (67%), respiratory distress in 22 neonates (55%), cardiovascular disorders (tachycardia or bradycardia) in 16 neonates (40%), hyperglycemia or hypoglycemia in 9 neonates (22.5%), and jaundice or hyperbilirubinemia in 3 neonates (7.5%). Risk factors from mothers and babies were found in all neonates with proven sepsis. Results of this study was similar with the theory declaring that neonates with suspected sepsis showed clinical manifestations and had risk factors from mothers or babies; or had no clinical manifestations but had risk factors from the mother or the baby.[20] Risk factors from mothers included premature or prolonged (>18 hours) rupture of membranes, maternal peripartum fever, foul-smelling or cloudy amnion fluid, and multiple gestation. Risk factors from baby included prematurity, low birth weight, asphyxia neonatorum, required intubation or resuscitation, and invasive procedures.[3,8,20]

Our study reported that CRP level was not a significant tool to establish early diagosis of sepsis, this is in accordance with the theory declaring that CRP is an early inflammatory marker which increase in 4 to 6 hours after infection. This value keeps increasing for 24 hours and it is still consistently high until 2 to 3 days.[13] The CRP examination will be more sensitive to bacterial infection if it is combined with other examination. A study in Germany had reported that the combination of CRP and IL-8 levels were better use in early diagnosis of bacterial infection in newborn, compared to leucocyte count and procalcitonin level.[21]

In our study, a high procalcitonin level was found in 36 neonates (10/10 in proven sepsis vs. 26/30 in unproven sepsis), but this different was not statistically significant. This maybe due to the increasing of procalcitonin level in the first two hours of antigen stimulation. The high procalcitonin level could be found during infection, even before sepsis happened. The increasing procalcitonin value or consistently high value or procalcitonin shows the continuing disease course. The decreasing procalcitonin level reflects disease improvement and declines inflammation reaction. [1,13,14]

We found that 10 neonates with positive blood culture results had a high total PMN count, high immature PMN count, and I:T PMN ratio > 0.12. This results is in accordance with the study in San Francisco that had reported neonates (age below 72 hours) with positive blood culture had a high immature neutrophil count.[22] We used hematological scoring system (HSS) as a diagnostic tool formulated by Rodwell *et al* in 1988 to establish early diagnosis of neonatal sepsis.[3] Hematological parameters in HSS are total leucocyte count, platelet count, total PMN count, immature PMN, I:M PMN ratio, I:T PMN ratio, and degenerative changes in PMN (toxic granules and cytoplasmic vacuoles) in peripheral blood smear. The appraisal to this diagnostic tool is based on several previous studies in Australia,[3] India,[4,5] and Philippines,[10] that found HSS could be used in early diagnosis of neonatal sepsis.[3-5,10]

The HSS assesment consists of 7 hematological parameters with score value from 1 to 8. This study found that I:T PMN ratio had sensitivity of 100% and specificity of 57% with PPV of 43% and NPV of 100%. These results are in accordance with previous studies in Australia and Durham that reported I:T PMN ratio ≥ 0.2 with sensitivity of 100%,[3,23] specificity of 50%, and PPV of 100%.[3,24] The degenerative changes of PMN can be toxic granules or cytoplasmic vacuoles in peripheral blood smear which can be found since the first time of infection and significantly relates to sepsis (bacteremia). The degenerative changes of PMN occurs because of the continuing stimulation of neutrophil production and the rapidly mature neutrophil in bone marrow.[9] The degenerative changes of PMN was not found in all subjects because a granulopoetic system of neonates may not perfectly developed yet.[21]

We found that 8 of 10 neonates with proven sepsis and 3 of 30 neonates with unproven sepsis had an HSS score ≥ 4. The HSS score of 4 had sensitivity of 80% (95%CI 55%-100%) and specificity of 90% (95%CI 79% to 100%) with PPV of 73% (95%CI 46% to 99%) and NPV of 93% (95%CI 84% to 100%). The ROC curve of HSS score of 4 showed area under curve (AUC) value of 0.902 (95%CI 0.803 to 1.0; P = 0.001) with significance of 5%. These results are in agree with a previous study in India which had reported that HSS score of ≥ 4 could be used as a diagnostic tool for early neonatal sepsis with sensitivity of 100%, specificity of 60%, PPV of 26%, NPV of 100%.[4] We found that the best cut off point HSS score was 4, because if the baby came with HSS score of 5 or 6 it showed that the baby came late or with worse condition.

Septic marker examination such as CRP, cytokine, and procalcitonin lvels in the establishment of early diagnosis of neonatal sepsis will give better value if the results are combined each other, but all those examinations are expensive and not available in every health centre.[11,14,25] The utilization of HSS in early diagnosis of neonatal sepsis is more simple, cheaper, and faster examination than other septic markers and available in every health centre. [1,3-5]

The limitation of our study was the possibility of difficulty in blood sampling of neonates for blood culture examination. This was due to that all of our subjects came with clinical manifestations, however positive blood culture results were found only in 10 neonates. In our study as well as in several previous studies, the use of HSS as a diagnostic tool of sepsis was limited to neonates only. Further study is needed to determine the utilization of this tool to other age groups.

We concluded that hematological scoring system (HSS) of ≥ 4 could be used as an early diagnostic tool for neonatal sepsis.

Conflict of interest

None declared.

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