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Original Article

Combination of red cell distribution width and procalcitonin as diagnostic biomarkers of neonatal sepsis in preterm infants

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Abstract

Background Diagnosis of neonatal sepsis is still challenging. Several diagnostics test have been developed to help diagnose of neonatal sepsis, but sometimes it could not be done routinely in limited facilities. Procalcitonin (PCT) and red cell distribution width (RDW) have been reported to have correlations with the risk of developing sepsis.

Objective To evaluate the diagnostic value of combined of PCT and RDW as markers for neonatal sepsis in preterm infants.

Methods A cross sectional study was conducted in the neonatal ward, Dr. Saiful Anwar Hospital, Malang, East Java. The subjects were preterm infants with neonatal sepsis. Blood culture were taken as the gold standard and RDW and PCT levels were assessed as the comparison. All of the test were performed at the begining of the study. The incidence of sepsis was reported as the main outcome. The data obtained were processed and analyzed using the receiver operating characteristic (ROC) method to obtain the area under curve (AUC) value.

Results Fifty-five preterm infants presenting neonatal sepsis were enrolled in this study. The combination of RDW and PCT showed AUC 0.814 (P=0.199) with sensitivity and specificity 78.9% and 80.6%, respectively in diagnosing neonatal sepsis compared to blood culture.

Conclusion Combination of RDW and PCT as markers of sepsis in preterm infants have good sensitivity and specificity. **[Paediatr Indones. 2023;63:29-34-; DOI: https://doi.org/10.14238/pi63.1sup.2022.S29-34**].

Keywords: *neonatal sepsis; procalcitonin; red cell distribution width; diagnosis; preterm*

Neonatal sepsis is a major cause of neonatal morbidity and mortality in developing countries. The *World Health Organization* (WHO) estimates that out of 130 million newborns per year, 4 million die each year, and 36% of them died from infectious diseases. The mortality rate of neonatal sepsis in Indonesia is about 50 - 60%.[1,2] The risk of sepsis in preterm babies with low birth weight is three to ten times higher than in full-term babies with normal birth weight.[3] The incidence of sepsis in preterm infants reached 34.8%, while preterm infant mortality due to sepsis reached 68.8%.[2]

Accurate diagnosis of neonatal sepsis is still a challenge in the medical field. The varied clinical manifestations in neonatal sepsis can cause delay in diagnosis which further increase morbidity and mortality.[4] Currently, the standard for the diagnosis of sepsis is based on a blood culture. However, this technique takes time because the result of blood

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culture analysis requiring 48-72 hours process. In addition, this diagnostic technique is relatively expensive, therefore, another diagnostic method that are practical, fast, and inexpensive to diagnose sepsis is needed.[5]

Sepsis diagnostic markers such as acute phase proteins, cytokines, cell surface antigens, and bacterial genomes have been used to help establish the diagnosis of neonatal sepsis with good sensitivity and specificity. However, those method requires sophisticated and expensive laboratory equipment, utilization of the test in limited facilities is unreachable. Several studies have demonstrated the use of procalcitonin (PCT) as a biomarker that plays a role either in the diagnosis of sepsis (in which PCT levels > 2 ng/mL is associated with the risk of sepsis) or as a prognostic marker of sepsis. Procalcitonin also can be used as a marker to initiate or stop the use of antibiotics with sensitivity and specificity of 87% - 100 %.[6,7]

Sepsis causes several changes in blood cells, both erythrocytes, platelets, and leukocytes. In erythrocytes, infectious conditions can cause changes in red cell distribution width (RDW). In sepsis, infection can trigger inflammatory cytokines and activate the inflammatory cascade.[8] Studies in India found the mean RDW value was significantly higher in neonatal sepsis in term neonates and the cut-off RDW around 17.25-18.55% with a sensitivity of 86-94.55% and a specificity of 87-96.36%.[9,10] Up to today, studies have been carried out on RDW as a prognostic and diagnostic markers for neonatal sepsis, but the application of RDW to diagnose neonatal sepsis in preterm infants has not been studied.

To the best our knowledge, there has not been any study on the combination of PCT and RDW as a prognostic and diagnostic marker for neonatal sepsis in premature infant. Therefore, the aim of our study was to evaluate the role of combination of RDW and PCT as diagnostic biomarkers of neonatal sepsis in preterm infants.

Methods

A cross sectional study was conducted in the neonatology ward of Dr. Saiful Anwar Hospital (RSSA) Malang, East Java, from August 2020. Subjects were taken by consecutive sampling until the minimum number of samples required for the study was met. The inclusion criteria in this study were preterm babies (gestational age of 28-36 weeks), less than 28 days old with suspected sepsis based on SIRS criteria. SIRS was defined as an inflammatory response characterized by the presence of two out of four criterias (one of which must involve an abnormal body temperature or leukocyte count): core body temperature of >38,5°C or <36°C, tachycardia, tachypnea, and leukocytosis or leukopenia or imature neutrophil of >10%.[11] We exclude the subjects with other medical problems with high RDW characteristics that could potentially become confounding factors. History taking, physical examination, and complete blood count tests were done to exclude the possibility of thalassemia, sickle cell disease, megaloblastic anemia and other blood disorders, as well as congenital malformations (digestive system, central nervous system, cardiovascular system), receiving corticosteroid or cytostatic therapy, meconeal aspiration syndrome, or malignancy.

Blood culture were taken as the gold standard and RDW and PCT levels were assessed as the comparison. Blood culture samples were collected early in the study. The sample was examined in the Clinical Pathology Laboratorium on RSSA. Procalcitonin levels in peripheral blood were analyzed using the ELISA technique (*Human Procalcitonin ELISA Kit, Biomatics*, EKU06765), while the RDW value was taken from examination using complete blood examination. Receiver operating characteristic (ROC) curves were made based on the PCT and RDW results, and the combination of both to calculate the area under curve (AUC) and determine the cut off points for neonatal sepsis diagnosis in preterm babies with the best sensitivity and specificity. P value < 0.05 was considered significant.

The study was approved by the Ethical Committee in Health Research of the Dr. Saiful Anwar General Hospital Malang.

Results

Fifty-five preterm infants presenting neonatal sepsis were enrolled in this study. The clinical characteristics of sucjects are shown in **Table 1**. The results showed that there was no difference in gender characteristic

related to the blood culture. However, less birth weight and younger gestational age were significantly prone to have positive blood culture results.

Of the 55 samples of blood cultures obtained, the results were as follow: 36 samples were sterile (65.4%), and 19 samples were positive (34.5%). The etiology of pathogenic bacteria is shown in **Table 2**. The most pathogenic bacteria causing sepsis in our study was Klebsiella pneumonia.

Figure 1 shows the ROC curve on the RDW evaluation, where the diagnostic value was above the 50% line with an AUC value of 0.624 or 62.4% (P=0.135). The AUC value of 62.4% indicating that if the RDW evaluation was used to diagnose neonatal sepsis, it had a power of 62.4% which can be said to be weak to be used as a diagnostic tool (>60%-70%).

The ROC curve for PCT evaluation revealed the diagnostic value was above the 50% line with an AUC value of 0.836 or 83.6% and a P value of 0.000. The AUC value of 83.6% indicating that if the PCT evaluation was used to diagnose neonatal sepsis, it had a power of 83.6% which can be said that the PCT evaluation results were good as a diagnostic tool (>80%-90%) (**Figure 2**). When compared with the RDW evaluation, the PCT result gave a better diagnostic power.

Table 3 shows the cut-of values of RDW and PCT. The cut-off value of the PCT was at the point of > 1.63 with a sensitivity of 78.9% and a specificity of 80.6% (**Table 4**). This means that patients who had a PCT value > 1.63 will be diagnosed as neonatal sepsis.

Discussion

Procalcitonin is the peptide precursor of the hormone calcitonin (CT) which is produced in the C cells of the thyroid gland. Procalcitonin consists of 116 amino acids and has a molecular weight of 13 kDa. Normally, all PCT is broken down in the thyroid into calcitonin. Serum PCT concentrations are very low in healthy individuals.[12] Microbial infection will cause a constitutive release of PCT from all parenchymal tissues and various types of cells throughout the body. Transcriptional expression of mRNA-CT will be increased more uniformly in the setting of sepsis than mRNA of classical cytokines, such as tumor necrosis factor (TNF) and interleukin (IL)-6.[13]

Table 1. Characteristic of subjects

Variables	Negative blood culture n=36	Positive blood culture group n=19	P value
Gender, n			
Male	20	10	0.836
Female	16	9	
Median birth weight (IQR), gram	1,800 (1,549-2,123)	1,300 (1,200-1,400)	0.000
Gestational age, n			
28-31 weeks	5	17	0.000
32-34 weeks	16	2	
35 - <37 weeks	15	0	

Table 2. Distribution of pathogenic bacteria (N=19)

Spesies	n
Klebsiella pneumonia	8
Pseudomonas aeruginosa	4
Acinetobacter baumani	2
Stenotrophomonas maltophilia	1
Bacillus cereus	1
Klebsiella oxytoca	1
Enteroceus faecalis	1
Enterobacter cloacae	1

Table 3. Red distribution witdh and procalcitonin cut off values based on ROC analysis

Variables	AUC value	Cut-off point value	P value
RDW	0.624	> 15.95	0.135
PCT	0.836	> 1.63	0.000

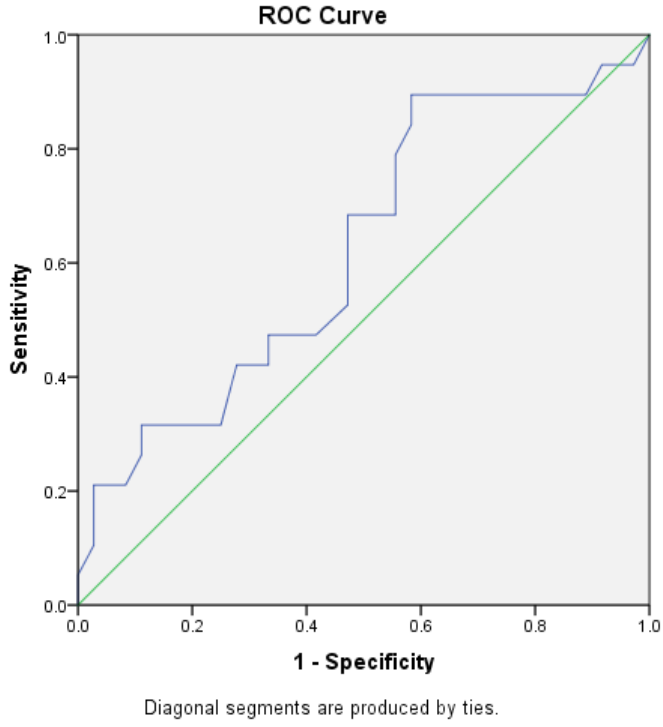

Figure 1. Receiver operating characteristic (ROC) of RDW examination

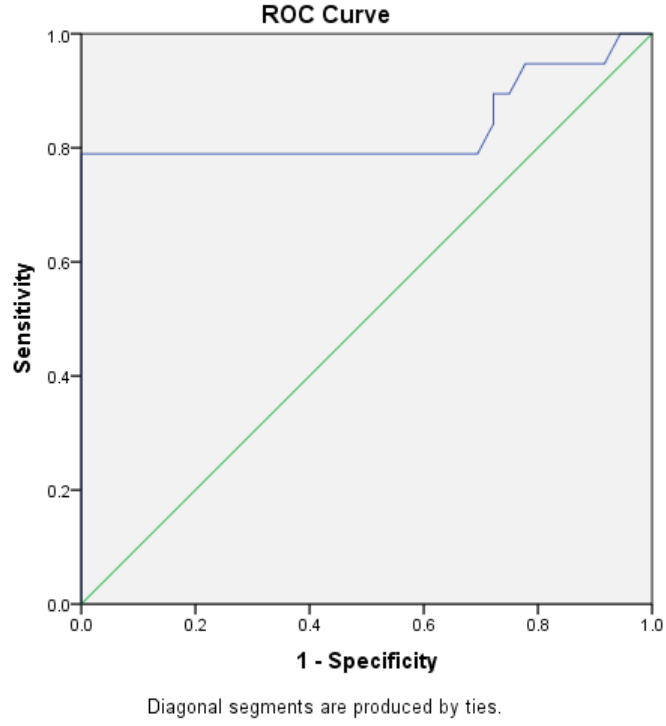

Figure 2. Receiver operating characteristic (ROC) of PCT examination

Table 4. Red distribution width and procalcitonin diagnostic test value

Diagnostic model	AUC value	Sensitivity	Specificity	P value
RDW	0.606	0.684	0.528	0.199
PCT	0.798	0.789	0.806	0.000
RDW+PCT	0.814	0.789	0.806	0.199

The use of PCT for the detection of neonatal sepsis has been widely published and has shown potential sensitivity and specificity values. The analysis from our study showed that PCT levels was a very good predictor of neonatal sepsis with AUC of 0.836 (P=0.000). In our study, infants with clinical sepsis were confirmed by positive blood culture results for a definite diagnosis. The ROC test results showed that PCT had a sensitivity and specificity value as follow: 78.9% and 80.6%, with a cut-off value of 1.63. This results show that PCT is a potential test to detect neonatal sepsis. This is in line with the results of a study which found that the PCT value in infants with suspected neonatal sepsis based on positive blood culture results was 1.0-2.1 ng/mL with sensitivity 78.4% and specificity 80.3% with P<0.01.[14] Another study showed that PCT test has 80% sensitivity and 85.7% specificity with P value = 0.0001 in detecting neonatal sepsis using cut off value 1.1 ng/mL.[15]

Several hypotheses have been proposed regarding the correlation between increased RDW values and the state of infection. Recently, it is suspected that inflammatory cytokines, especially TNF- and IL-1β can inhibit erythropoiesis. The inhibition can be through two different pathways. First, by inhibiting the transcription of the Epo gene in the kidneys and liver, thereby inhibiting the production of Epo. Second, by suppressing erythropoiesis in bone marrow by inhibiting the proliferation of erythroid progenitor cells and proerythroblast maturation by desensitizing cells to Epo. With less Epo produced and desensitization of erythrocyte progenitor cells to Epo thus resulting in fewer mature erythrocytes. Furthermore, it will cause reticulocytes (immature red blood cells) being released into the circulation. Reticulocytes are larger in size than mature erythrocytes, this causes an increase in erythrocyte size variations which affecting the RDW value. So, it can be concluded that the increase in cytokines in inflammatory conditions will trigger anisocytosis.[8]

The results of the ROC test showed that the RDW study had a sensitivity value of 68.4% and a specificity value of 52.8% for a cut-off value of 15.95. This indicating that RDW did not show a significant effect as a marker to detect neonatal sepsis. This is different from a study In India, which aimed to investigate the RDW as a marker of neonatal sepsis in term neonates. The mean of RDW value was significantly higher in neonatal sepsis 21.31 (SD 3.08)% compared to healthy ones 16.23 (SD 1.16)% with P<0.001.[15] In those study, using cut-off value of the RDW 18.55%, the sensitivity and specificity was 94.55% and 96.36% respectively in diagnosing neonatal sepsis. Another study found that the mean of RDW in neonatal sepsis was significantly higher in term of neonates 18.59 (SD 1.28)% compared to healthy neonates 16.21 (SD 1.35)% with P=0.000. Using cut-off value of the RDW 7.25%, the sensitivity and specificity in those study was 86%, and 87%, respectively in diagnosing neonatal sepsis.[16]

The combination of PCT and RDW in diagnosing neonatal sepsis in our study statistically meaningless. It has lower diagnostic value than the PCT only with a sensitivity of 78.9% and a specificity of 80.6% (P=0.000).

In conclusion, no significant correlation is found between RDW and blood culture results in preterm with neonatal sepsis. However, there is significant association between PCT and blood culture results in preterm with neonatal sepsis, with good sensitivity and specificity. The combination of RDW and PCT as a marker of sepsis is not superior compare to PCT only in diagnosis of sepsis in preterm infants. The PCT evaluation is more recommended in diagnosing neonatal sepsis compared to combination of PCT and RDW.

Conflict of interest

None declared.

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