Research Article

Diagnostic Value of Rodwell Hematological Scoring System Compared to Neutrophil Lymphocyte Count Ratio (NLCR) in Diagnosing Early Onset Neonatal Sepsis

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ABSTRACT

Background: Neonatal sepsis is still a major health problem worldwide due to its high morbidity and mortality. Clinical symptomps are nonspecific. Blood culture is a gold standard for diagnosis of neonatal sepsis, although the process requires significant time. Early diagnosis of early onset neonatal sepsis is difficult. A number of novel diagnostic tests are being investigated for establishing the diagnosis of neonatal sepsis.

Objective: To determine the diagnostic value of Rodwell Hematological Scoring System (RHSS) compared to Neutrophil to Lymphocyte Count Ratio (NLCR) in the diagnosis of neonatal sepsis and NLCR cut-off point.

Methodology: Cross-sectional observational analytic study was conducted at Neonatology Division in Dr. Moewardi Hospital from October 2018 to October 2019. Blood samples of neonates fulfilling the inclusion criteria underwent hematological testing for counting Rodwell Hematological Scoring System (RHSS), Neutrophil to Lymphocyte Count Ratio (NLCR) and blood cultures were done. Sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) were calculated.

Results: Fifty neonates were included in this study. 23 neonates (46%) had positive blood cultures. Fourteen neonates (60.9%) of positive blood culture group were males (p value 0.013). Six neonates (26.1%) of positive bood culture group had maternal risk factors. Sensitivity, specificity, PPV, and NPV of RHSS were 73.9%, 100%, 100% and 81.8%, respectively. Sensitivity, specificity, PPV and NPV of NLCR are 65.2%, 70.4%, 65.2% and 70.4%, respectively, with cut-off point 2.495.

Conclusion: Rodwell Hematological Scoring System was superior to NLCR in the diagnosis of early onset neonatal sepsis.

Keywords: Neonatal Sepsis, Rodwell Hematological Scoring System, NLCR, Neonate.

INTRODUCTION

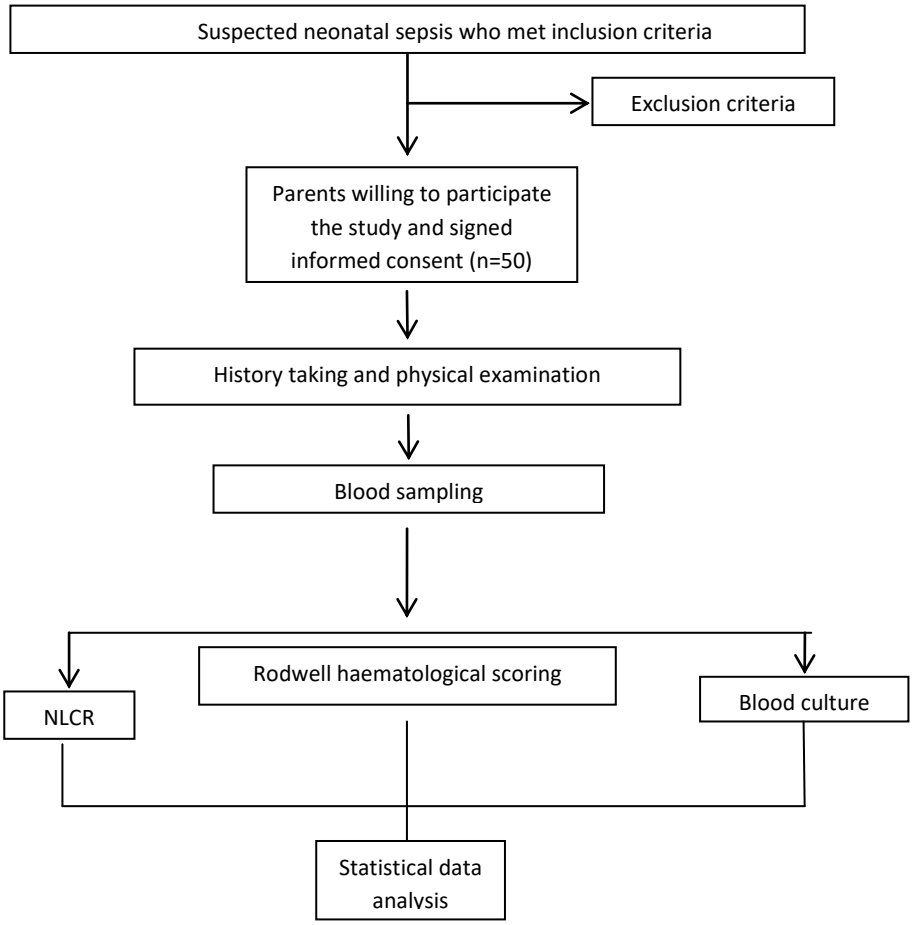
Neonatal sepsis is still a major health problem worldwide, more common in developing countries, especially Indonesia. Neonatal sepsis causes high morbidity and death in infants due to difficulties in diagnosing and case managmint.[1] It cause 2.6 million deaths worldwide every year, 75% of which occur in the first week of life and almost all (99%) occur in middle to lower income countries.[2] In Indonesia, there is no specific data related to the incidence of neonatal sepsis, but Cipto Mangunkusumo Hospital in Jakarta reported that the incidence of neonatal sepsis is quite high, reaching 13.7% with a mortality rate of 14%.[3] Non specific clinical signs and symptoms make problems in the diagnosis of neonatal sepsis. Distinctive symptoms that appear can not be distinguished whether caused by infectious or non-infectious causes, such as hypothermia, respiratory distress, poor feeding, lethargy and other clinical symptoms. Empirical antibiotics are given, if diagnosis is suspected based on clinical and laboratory findings. Delayed management of neonatal sepsis can lead to undesirable outcomes.[4] Blood culture is a gold standard, although it requires 48-72 hours to obtain the result.

In 1988, Rodwell developed a hematological scoring system to help clinicians utilize laboratory findings to establish the diagnosis of neonatal sepsis.[5] This scoring system is based on the immunological processes that occur in neonates as a response to infection.[6–8] The scoring system composed of seven components, those are leucocyte count, immature polymorphonuclear (PMN) count, immature to mature neutrophil ratio (IM ratio), immature to total neutrophil ratio (IT ratio), total PMN count, platelet count and PMN degenerative changes.

NLCR is a reliable marker of inflammation.[9] The immune response to endotoxemia is characterized by an increase in neutrophils and a low number of lymphocytes circulating in the blood.[10] NLCR was previously used as a predictor of the severity of bacteremia and community acquired pneumonia in children; but as a marker of early onset sepsis, the *cut-off point* of the NLCR is still controversial. The aim of this study is to compare the diagnostic value of RHSS and NLCR in diagnosing neonatal sepsis.

METHODS

Study Protocol



We conducted a cross-sectional, observational analytic study at Neonatology Division in Dr. Moewardi Hospital from October 2018 to October 2019. We used a consecutive sampling method. All neonates fulfilling the inclusion criteria were included in the sequential study according to the time of arrival of the patient until the research sample size was completed. All study subjects underwent history taking of neonatal sepsis risk factors, clinical examination and laboratory tests (hematological profile, IT ratio, peripheral blood smear and blood culture).

Inclusion criteria was all neonates born by spontaneous vaginal delivery or Cesarean section who were suspected for neonatal sepsis with an onset less than 72 hours after delivery and whom parents signed the *informed consent* for the study. Exclusion criteria was neonates with major congenital anomalies, required major or minor surgical procedures, gestational age less than 34 weeks, birth weight less than 1000 grams and/or born to immunodeficient mothers. Neonatal sepsis was suspected in presence of maternal and infant septic risk factors. Maternal risk factors were preterm delivery, chorioamnionitis, maternal fever (temperature >38.4°C), maternal urinary tract infection and premature rupture of membranes more than 18 hours. Infant risk factors were perinatal asphyxia and low birth weight.

All blood samples were taken by nurses on duty in the first 5-12 hours after neonates were born. Blood samples were put into the EDTA tubes and sent to the clinical pathology laboratory in Dr. Moewardi Hospital. All blood

samples were analyzed using automatic hemoanalyses machine Siemens Advia 120 Hematology Analyzer. The examination of peripheral blood smear was done manually according to the standard procedure of clinical pathology laboratory in Dr. Moewardi Hospital.

Rodwell hematological scoring system and Neutrophil to Lymphocyte Count Ratio (NLCR) from all study subject were calculated from hematological profile and IT ratio. Assessment using RHSS described as 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/mm3(=1), and degenerative changes in PMNs (toxic granules or cytoplasmic vacuoles =1).[5,11] NLCR score was calculated as neutrophil (%) divided by lymphocyte (%).

Diagnostic values of Rodwell hematologic scoring system and NLCR for neonatal sepsis were taken by making a 2x2 table to obtain sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR) and negative likelihood ratio (NLR). NLCR was analyzed by receiver-operator characteristic (ROC) curve and measurement of the area under the curve (AUC) to determine the cut-off point. This study was approved by Ethical Committee of Dr. Moewardi Hospital.

Statistical Analysis

The data collected was analyzed and presented in the form of narratives, tables and graphs. The basic characteristics of the study subjects consisted of gender, maternal gestational age, birth weight, APGAR at the 5th minute, clinical manifestations of sepsis and risk factors for sepsis. Statistical data was presented as mean, median, standard deviation, minimum and maximum values and percentages. Data analysis was performed using SPSS 23 for Windows. The characteristic data obtained was analyzed by Chi-square test. The diagnostic value of each method was presented in 2x2 table form. Comparison of diagnostic values was analyzed using the ROC (*Receiving-Operating Characteristic*) curve by calculating the area under the curve (AUC). The *cut-off point value for* NLCR was obtained from the ROC (*Receiving-Operating Characteristic*).

RESULT

Fifty neonates were included in this study. Of the 50 subjects, 23 (46%) subjects were proven to have neonatal sepsis, while 27 (54%) subjects had negative blood culture. Twenty one neonates (42%) were male and others were female. The mean gestational age was not different in the two groups (p = 0.968). There was no statistically significant difference in birth weight between the two groups (p = 0.688) as shown in Table 1.

Table1. Demographic characteristics of neonates

Characteristic	Blood culture		p-value
	Negative (n=27)	Positive (n=23)	
Sex[a]			0.013*
Male	7 (25.9%)	14 (60.9%)	
Female	20 (74.1%)	9 (39.1%)	
Maternal Risk Factor[a]			0.121
Yes	2 (7.4%)	6 (26.1%)	
No	25 (92.6%)	17 (73.9%)	
Gestational age[b] (week)	36.70 ±2.03	36.65 ±1.70	0.968
Birth weight[c] (gram)	2341.85 ±720.35	2423.91 ±709.97	0.688
APGAR minute 5[b]	7.56 ±0.85	6.91 ±1.12	0.052

Notes : [a] Chi square test (nominal data),
[b]Mann whitney test (abnormal distribution numeric data),
[c]independent t test (normal distributin numeric data).
* significance if α =5 %

Table2. Laboratory characteristic

	Blood culture		*p-value*
	Negative (n=27)	**Positive** (n=23)	
Leucocyte[c] (cell/mm^3)	12429.63± 4258.55	16958.70 ±3821.43	<0.001*
PMN[c] (cell/mm^3)	7540.00 ±3153.95	11337.04 ±2888.59	<0.001*
IM Ratio[b]	0.16 ±0.06	0.44 ±0.79	<0.001*
IT Ratio[b]	0.14 ±0.04	0.23 ±0.15	<0.001*
Immature PMN [b] (cell/mm^3)	995.63 ±415.20	2671.96 ±1744.93	<0.001*
Thrombocyte[c] (cell/mm^3)	257962.96 ±73109.23	247391.30 ±74372.37	0.615
Eosinophil[b] (%)	1.44 ±1.33	1.07 ±0.97	0.334
Basophil[b] (%)	0.35 ±0.28	0.32 ±0.34	0.616
Neutrophil[c] (%)	59.74 ±12.23	66.93 ±8.59	0.022*
Lymphocyte[b] (%)	29.33 ±10.84	22.46 ±8.34	0.007*

Notes : [a] Chi square test (nominal data),
[b]Mann whitney test (abnormal distribution numeric data),
[c] independent t test (normal distributin numeric data).
* significance if α =5 %

Laboratory characteristics of patients with neonatal sepsis are presented in Table 2. Two groups of different tests were performed with chi square test for nominal data, independent T-test for numerical data with normal data distribution, and Mann Whitney test for numerical data with abnormal data distribution. It shows that group with positive blood cultures have leukocytes, polymorphonuclear values, IM ratios, IT ratios, immature polymorphonuclears, neutrophil counts and lymphocyte counts higher than group with negative blood cultures, with significant p-value (p <0.005). The mean platelet values differed in the 2 groups, but the difference was not significant. The mean value of eosinophils and basophils in the positive culture group was lower than the negative culture group.

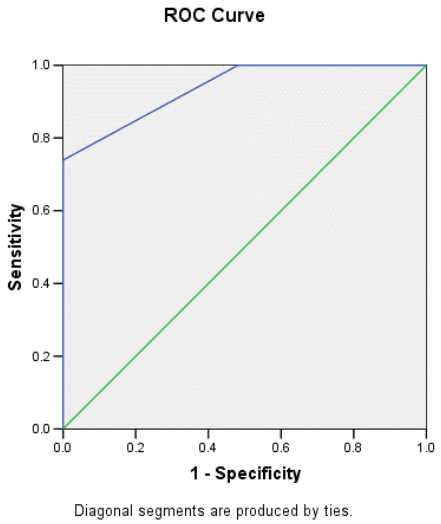


Fig1. Reciever Operating Curve of RHSS

Rodwell scoring as a diagnostic test of neonatal sepsis in this study, with a cut-off point of 4 showing a sensitivity of 0.739; specificity 1.0; positive predictive value 1.0; and a negative estimated value of 0.818 with the area under the 0.937 curve (Figure 1). NLCR as a diagnostic test of neonatal sepsis in this study, with a cutoff of 2.495 showing a sensitivity of 0.652; specificity 0.704; positive predictive value of 0.652; and a negative value of 0.704 with an area under the 0.721 curve (Figure 2). The area value of AUC on the ROC NLCR chart in this

study was 0.721. Compared to the NLCR, Rodwell's hematological scoring has an AUC area on the ROC chart of 0.937 (Table 3).

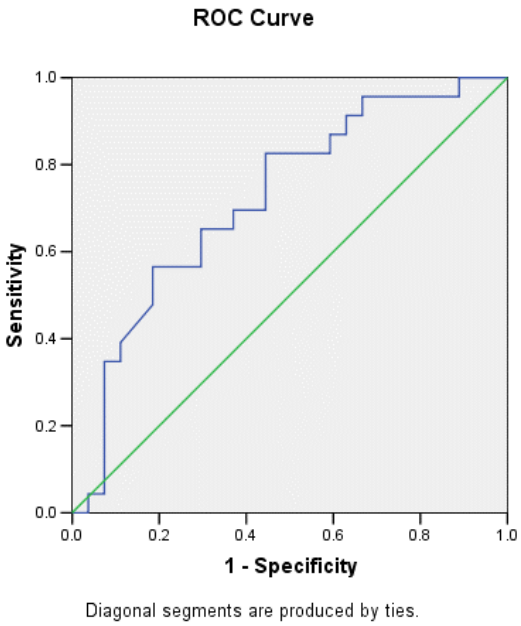


Fig2. Reciever Operating Curve of NLCR

Table3. Comparison of diagnostic value between RHSS and NLCR

Parameters	Diagnostic value						
	Sens	Spes	PPV	NPV	OR	p-value	AUC
RHSS	0.739	1.000	1.000	0.818	N/A	0.007*	0.937
NLCR	0.652	0.704	0.652	0.704	4.4	<0.001*	0.721

DISCUSSION

Neonatal sepsis shows non specific symptoms in each neonate. The clinical signs often overlap with non-infectious causes, so clinician must exclude the possibility of sepsis.[12] Early diagnosis of neonatal sepsis is very important as the course of the disease is progressive, may be very rapid and even can lead to death.[13] Although blood culture is still the gold standard to establish the diagnosis of sepsis, but there are many drawbacks, i.e. it takes 48-72 hours to obtain the results, antibiotics given before blood culture sampling may reduce the yield,[14] results may also be affected by sampling technique. A repeat blood culture examination can be done to improve the test results, but the clinical condition of the neonate may not wait for the results of the next blood culture examination. In view of the difficulty of establishing a diagnosis of neonatal sepsis, many studies have been done to look for various biomarkers that can help clinicians to make a diagnosis.

In this study we evaluated the laboratory profile of neonates with suspicion of early onset sepsis. This study was conducted on 50 neonates with suspicion of early onset sepsis. Twenty three neonates proved to be sepsis by positive blood culture.

We evaluated the RHSS as the diagnostic tool of neonatal sepsis. In our study, we had a sensitivity of 73.9% and a specificity of 100% with the *cut-off value of* 4. This is in line with a previous study conducted by Pramana in Bali in 2016 and Ahlawat, et al. in India in 2017 .[6,7] However, it differs from research conducted by Mousa in Egypt in 2019 where Rodwell's scoring was not superior, as compared to other hematological parameters such as RDW (*Red-cell Distribution Width*) and SNAP II (*Score for Neonatal Acute Physiology II*) with AUC of 0.56.[15]

Total leukocyte counts and total neutrophil counts have long been used as markers of infection, as is the relationship between neutrophilia and pyogen bacterial infections.[16] Inflammatory reactions caused by bacterial infections can increase the number of leukocytes and neutrophil counts. In response to the inflammatory process of endotoxins, there are some serial changes, such as an increase in T lymphocytes. The mechanism of underlying lymphopenia involves the release and distribution of lymphocytes in the lymphatic system and the acceleration of increased lymphocyte apoptosis. This mechanism explains the use of the lymphocyte neutrophil ratio as a marker of the sepsis.[17]

NLCR is a hematological parameter that is calculated from the ratio of neutrophils to lymphocytes in leukocyte differential counts. An increase in the value of NLCR can be caused by an increase in the number of neutrophils or a decrease in the number of lymphocytes, which increase is related to the incidence of inflammation. During the infection, the production of granulocytes by the bone marrow increases along with the production of other blood cells. Increased granulocyte production is shown by increasing the rate of proliferation of hematopetic cells and granulopoetic progenitors which is then followed by the process of differentiation.

In our study, NLCR as a diagnostic test of neonatal sepsis had an AUC of 0.721 at a cut-off of 2.495. NLCR showed sensitivity of 65.2% and specificity of 70.4%. In line with research conducted by Varal & Dogan in 2020 in Turkey, the NLCR can confirm neonatal sepsis with a sensitivity of 68% and a specificity of 82% and AUC of 0.723 at the 1.57 cut-off, while in a study of premature infants with slow onset sepsis, the NLCR gave an AUC of 0.78 at a *cut-off point of* 1.77.[18,19] In another study conducted by Ghrahani in 2019, the NLCR also had a strong correlation with CRP and recommended as an early marker of neonatal sepsis.[20]

RHSS had a superior diagnostic value as compared to NLCR, with AUC 0.937 and 0.721, respectively in this study. This might have happened because there were more components in RHSS. One of Rodwell's Scoring parameters assesses the immaturity of circulating neutrophils due to the systemic infection process in neonates, called I/T ratio, that describes ratio of immature to total neutrophil. It is in line with research by Saboohi et. al, where the $I/T\ ratio \geq 0.2$ can be an early predictor of neonatal sepsis. The I/T ratio has a high specificity and NPV so that it can diagnose early onset neonatal sepsis.[20] While NLCR only depends on neutrophil and lymphocyte values that may increase due to inflammatory processes, it not specific for infectious causes. In this study a significant difference was seen in the immaturity assessment component of the Rodwell scoring between the two groups ($p < 0.001$).

Limitations of The Study

A limitation of our study was that many blood culture results were negative, inconsistent with clinical signs and symptoms in study subjects. More studies with large sample size should be conducted.

CONCLUSION

In summary, we conclude that Rodwell Hematological Scoring System was superior to NLCR in diagnosing early onset neonatal sepsis with 0.937 AUC, sensitivity 73.9%, specificity 100%, while NLCR has 0.721 AUC with cut-off point 2.495, sensitivity 65.2%, and specificity of 70.4%.

CONFLICT OF INTEREST

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