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Original Article

The combination of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio improves accuracy of neonatal sepsis diagnosis

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Abstract

Background Neonatal sepsis remains a challenging issue, due to sophisticated and time consuming tests needed to confirm a diagnosis.

Objective To assess the applicability of neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) as diagnostic markers in neonatal sepsis.

Methods This cross-sectional study was conducted in the Neonatology Ward of Kandou General Hospital, Manado, North Sulawesi. Neonates with suspected sepsis were included by consecutive sampling. We measured NLR and PLR form hematology profiles and differential count. Diagnosis of neonatal sepsis was based on positive blood cultures. A receiver operating characteristic (ROC) curve analysis was done to assess the NLR and PLR cut-off points. Chi-square test was used to analyze the diagnostic value of NLR and PLR.

Results A total of 176 full term neonates with suspected sepsis were included in the study. Of these, 84 (47.7%) subjects were confirmed with neonatal sepsis and 92 (52.3%) were non-sepsis. The mean NLRs were 5.9 (95%CI 2.0 to 13.6) in the sepsis group and 1.6 (95%CI 0.2 to 3.2) in the non-sepsis group. A NLR of 3.0 was determined as the predictive cut-off value of neonatal sepsis (sensitivity 94%, specificity 97.8%, and area under the ROC curve 0.995). NLR can effects on neonatal sepsis diagnosis by 61% . The mean PLRs were 79.9 (95%CI 44.0 to 155.8) in the sepsis group and 44.0 (95%CI 9.7 to 91.8) in the non-sepsis group. A PLR of 60.4 was determined as the predictive cut-off value of neonatal sepsis (sensitivity 86.9%, specificity 87%, area under ROC curve 0.928). PLR can effects on neonatal sepsis diagnosis by 47.5%. When NLR and PLR were combined, they can improve accuracy of neonatal sepsis diagnosis about 72.2%.

Conclusion Neutrophil to lymphocyte ratio (NLR) and ***platelet to*** lymphocyte ratio (PLR) can be used in combination as adjunct diagnostic tests for neonatal sepsis workups. **[Paediatr Indones. 2023;63:213-8; DOI: https://doi.org/10.14238/pi63.4.2023.213-8**].

Keywords: *neonatal sepsis; NLR; PLR; neutrophil; workup; diagnostic*

Neonatal sepsis (NS) is a systemic infection occurring in newborn infants. It plays a significant role in morbidity and mortality among newborns.[1,2] On 2005, neonatal sepsis has caused 1.4 million neonatal deaths, 99% of which were in low and middle-income countries (LMIC).[3] In high-income countries, such as the USA, the incidence of neonatal sepsis varied from one to four infections per 1,000 livebirths, while in developing countries, such as Indonesia, the prevalence of neonatal sepsis was 46.6%.[4,5]

The clinical findings of NS vary and may include non-specific clinical manifestations such as tachypnea, shortness of breath, or lethargy. There has been no recommended definitive method to screen for neonatal sepsis.[2,4,6] There are numerous sepsis biomarkers, but almost all require expensive testing and their effectiveness is limited.[1,6,7] NLR and PLR are sensitive markers of inflammation, and could be useful in LMIC. [8,9]

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The NLR and PLR have been evaluated as biomarkers for sepsis/bacteremia and could be used together or with other biomarkers to increase sensitivity for diagnosis of neonatal sepsis. NLR has been reported to differentiate between inflammation caused by bacteria/sepsis vs. other non-sepsis diseases.[8,9] NLR is thought to be a better biomarker for early sepsis compared to other biomarkers and could also be used to predict outcomes of neonatal sepsis.[9-11] PLR could be used to assess for the inflammation and thrombotic balance due to aggregation and inflammatory processes, thus PLR could also be useful to predict neonatal sepsis.[12] We aimed to assess the applicability of combined NLR and PLR as diagnostic markers in neonatal sepsis.

Methods

A cross-sectional study was conducted in the Neonatology Ward of Prof. Dr. R.D. Kandou General Hospital, Manado, North Sulawesi, from January 2018 to September 2019. The inclusion criteria comprised of all neonates with suspected sepsis, born either vaginally or by caesarean section, with gestational age of 34 to 42 weeks according to ultrasonographic investigations and new Ballard Score.[13] Exclusion criteria were neonates with congenital anomalies, multiple pregnancies, no culture results, and/or incomplete laboratory results.

A total of 176 newborns were included by a consecutive sampling method. Subjects underwent history-taking, physical examination, and laboratory examinations [hematology profiles, differential count, C-reactive protein (CRP) and blood culture].

Neonates were considered to have suspected sepsis from their risk factors if they fulfilled 2 major criteria or 1 major criteria plus 2 or more minor criteria. Major septic risk factors included premature rupture of membranes >18 hours, intrapartum fever (>38°C), chorioamnionitis, greenish and foul-smelling amniotic fluid, and fetal heart rate >160 beats/min. The minor risk factors consisted of premature rupture of membranes >12 hours, intrapartum maternal fever (>37.5°C), low Apgar score (1st minute score < 5 and 5th minute score <7), very low birth weight (<1,500 g), untreated vaginal discharge, and suspected maternal urinary tract infection.[14-16]

This study was approved by the Ethics Committee of Kandou General Hospital, Manado. Subjects' parents provided written, informed consent for particiation. Data were processed with *IBM SPSS Statistics ver. 25.0* software. Descriptive analysis was used to illustrate the characteristics of the data. A receiver operating characteristic (ROC) curve analysis was done to assess the NLR and PLR cut-off points. Chi-square test was used to analyze the diagnostic value of NLR and PLR by calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). The R-squared test was used to analyze the strength of the relationship between NLR or PLR and neonatal sepsis.

Results

There were 176 neonates with suspected sepsis who fulfilled the inclusion criteria (**Figure 1**). Of these, 84 (47.7%) subjects were confirmed with NS and 92 (52.3%) were non-sepsis.

Most subjects had suspected early onset sepsis (<72 hours) [68 (80.9%)] and the rest [16 (19.1%)] had late onset sepsis (≥72 hours). The NS group consisted of 49 males (58.3%). **Table 1** shows the laboratory results of the NS and non-sepsis groups.

Eighty-four blood cultures resulted in positive growth consisting of *Klebsiella pneumonia* (26), *Candida albicans* (10), *Serratia marcescens* (9), *Staphylococcus aureus* (9), *Acinetobacter baumanii* (6), *Staphylococcus epidermidis* (6), *Staphylococcus haemolyticus* (4), *Escherichia coli* (4), *Candida pelliculosa* (2), *Salmonella spp* (2), *Enterobacter aerogenes* (1), *Enterobacter faecium* (1), *Candida tropicalis* (1), *Listeria monocytogenes* (1), *Micrococcus luteus* (1), and *Pseudomonas aeruginosa* (1).

Logistic regression analysis revealed a significant correlation between high NLR and NS (P<0.0001). Furthermore, a very significant correlation was found between NLR with NS incidence in the point biserial result, with rpb=0.8 and P<0.0001. Using a NLR cut-off point of 3.0, the ROC curve analysis showed an AUC of 0.995, with 94% sensitivity, 97.8% specificity, 97.5% PPV, and 94.7% NPV (**Figure 2A**). This cut-off point had an odds ratio (OR) of 711.0 (95%CI 134.2 to 3767.3). The coefficient of determination was 0.6, which means that NLR can effects on neonatal sepsis diagnosis by 60%.

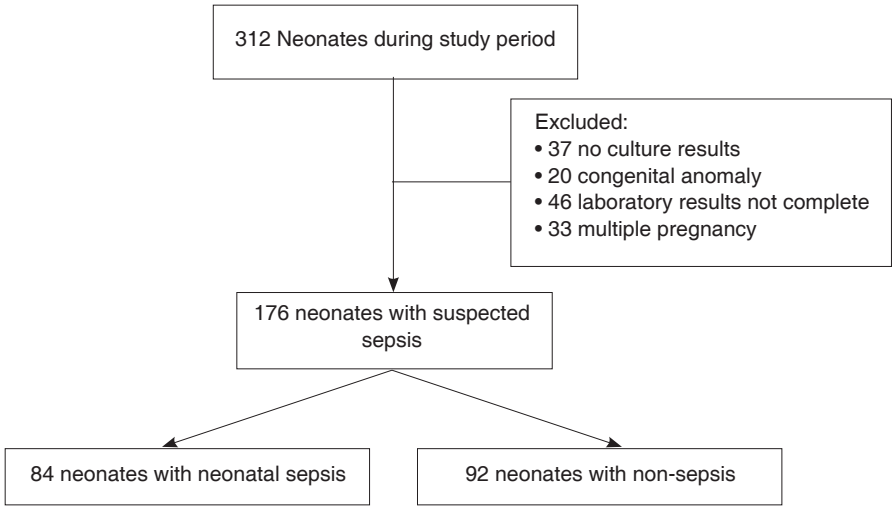

Figure 1. Flow chart of subject inclusion

Table 1. Laboratory characteristics of the study groups (N=176)

Parameters	NS group (n=84)	Non-sepsis group (n=92)	P value
Mean hemoglobin (SD), g/dL	14.3 (3.1)	15.3 (3.0)	0.045
Mean hematocrit(SD), %	41.9 (9.4)	42.35 (9.9)	0.678
Mean WBC (SD), 109/L	23.2 (8.55)	15.8 (5.8)	0.005
Mean platelet (SD), 103/L	233.4 (78.7)	216.6 (91.8)	0.095
Mean lymphocyte (SD)	3.1 (1.3)	5.4 (2.7)	0.005
Mean IT ratio (SD)	0.15 (0.09)	0.05 (0.06)	0.007
Mean CRP (SD), mg/dL	26.3 (21.2)	7.8 (15.85)	0.011
Mean neutrophil (SD)	16.8 (6.8)	7.6 (3.5)	0.002
Mean NLR (SD)	5.9 (2.4)	1.6 (0.7)	0.001
Mean PLR (SD)	79.9 (21.2)	44.0 (16.4)	0.001

CRP=C-reactive protein; IT=immature to total neutrophil; NLR=neutrophil to lymphocyte ratio; PLR=platelet to lymphocyte ratio; WBC=white blood cell

Logistic regression analysis also revealed a significant correlation between high PLR and NS ($P<0.0001$). Furthermore, a very significant correlation was found between PLR with NS incidence in the point biserial result, with $rpb=0.7$ and $P<0.0001$. Using a PLR cut-off point of 60.4, the ROC curve analysis showed an AUC of 0.9, with 86.9% sensitivity, 87% specificity, 85.9% PPV, and 87.9% NPV. This cut-off point had an OR of 44.2 (95%CI 18.4 to 106.4). The coefficient of determination was 0.5, which means that PLR can effects on neonatal sepsis diagnosis by 50%. When combined, the coefficient of determination of NLR and PLR was 0.722, which means that NLR and PLR can improve accuracy of neonatal sepsis diagnosis about 72.2%.

Discussion

Neonatal sepsis is a contributing factor for morbidity and mortality of newborn infants, especially in developing countries. Neonatal sepsis can be categorized into early-onset sepsis (EOS), which happens before 72 hours of life, and late-onset sepsis (LOS), which happens after 72 hours of life.[1,2]

The clinical findings of NS range from subclinical infection to severe manifestations of focal or systemic disease and there is often little difference between sepsis that is caused by infectious pathogens or by other non-infectious pathogens, thus making NS difficult to diagnose.[2,4,6] Diagnosis of NS needs to be done as early as possible in order to start the

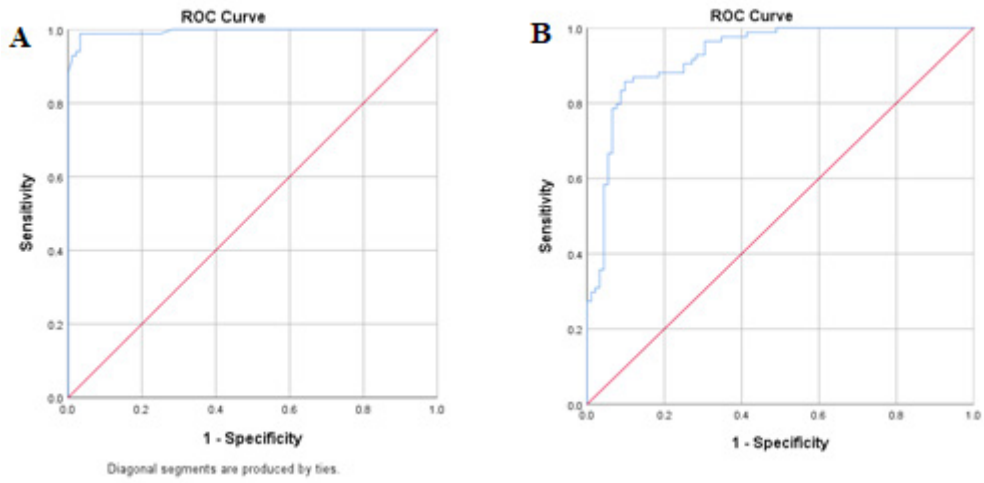

Figure 2. (A) ROC curve predicting sensitivity and specificity of NLR cut-off 3.0 in detecting neonatal sepsis; (B) ROC curve predicting sensitivity and specificity of PLR cut-off 60.4 in detecting neonatal sepsis

appropriate treatment to prevent further morbidity and mortality among infants.[17]

The best methods involve using a combination of maternal risk factors, clinical signs and symptoms, and various laboratory markers that are available.[3] Blood culture, which is the gold standard for neonatal sepsis diagnosis, cannot be obtained for all patients, and the results only come out after 24-48 hours. Moreover, a negative result does not exclude sepsis as the diagnosis.[18] Neonatal sepsis could also be diagnosed by isolating the causative agent from blood or any other sterile body fluid, such as: cerebrospinal fluid (CSF), urine, as well as pleural, joint, and peritoneal fluids, but culture has a low sensitivity and influenced by many factors.[4,17]

The most recent diagnostic criteria for neonatal sepsis was published by the *Pediatric Committee* (PDCO) *of the European Medicines Agency* (EMA) in the *"Expert Meeting on Neonatal and Pediatric Sepsis Consensus 2010 Criteria"*.[18] The diagnosis consists of at least 2 clinical features and 2 laboratory findings. The diagnosis is then confirmed by isolating the causative agent from blood or any other sterile body fluid, such as CSF, urine, or pleural, joint, and peritoneal fluids, or by microscopy or polymerase chain reaction.[4,19,20]

In our study, a statistically significant positive association was found between neutrophil counts, NLR, and PLR in NS. Our results were similar to those a study which compared the diagnostic values of NLR and PLR in detecting early-onset sepsis in neonates with the risk of bacterial infection.[1] Another study also found that NLR had high sensitivity and specificity as a diagnostic marker for early-onset neonatal sepsis.[21] In our study, NLR had a sensitivity of 94% and specificity of 97.8% for diagnosing sepsis.

Neutrophils have a short life-span of only about 24 hours. However, sepsis patients have a prolonged neutrophil lifespan because immature neutrophils are released in the blood.[22] Lymphocytopenia as a sign of lymphocyte apoptosis is a part of the host's normal immune response to stop and control an exaggerated immune response by inducing the release of inflammatory cytokines and interacting with different kinds of bacteria and immune cells, including neutrophils, T-lymphocytes, natural killer (NK) cells, and macrophages, with the aim of stopping further tissue damage. Within the first 24 hours of sepsis, lymphocytopenia occurs because lymphocytes are deployed from the blood circulation to the site of infection.[22-24]

The body immune system works physiologically to increase circulating leukocytes, with increased neutrophil count and decreased lymphocyte count in response to systemic inflammation. Thus, NLR and PLR could be useful markers and predictors of inflammation and thrombosis caused by the inflammatory process itself.[12,25] The NLR and PLR can be easily calculated from complete blood counts which are routinely done in newborns with suspected sepsis.

A previous study found that PLR had a sensitivity

of 88.9% to 91.3% and specificity of 94.7% to 97.6%. They also found that PLR had PPV of 94.3% to 97.4%, and NPV of 88.6% to 91.8% in suspected and proven sepsis diagnosis and that PLR could be used as a parameter to predict NS.[12] Similarly, we noted that PLR had a high sensitivity and specificity in diagnosing NS.

A limitation of our study was we did not exclude other inflammatory processes, thus, possibly influencing our results. The strength of our study was the large sample size, with a total of 176 subjects.

In conclusion, NLR and PLR may be useful as biomarkers for predictors and adjunct diagnostic tests for neonatal sepsis workups. NLR and PLR had high sensitivity and specificity. The combination of NLR and PLR increase the accuracy of NS diagnosis.

Conflict of interest

None declared.

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