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Original Article

Platelet-to-lymphocyte ratio in early onset neonatal sepsis

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Abstract

Objectives Neonatal sepsis is a major contributor to morbidity and mortality worldwide. Although blood culture is the gold standard of sepsis diagnosis, it often lacks sensitivity and diagnostic speed. Platelet-to-lymphocyte ratio (PLR) is a widely available, effective, simple, and affordable marker that can predict early onset neonatal sepsis (EONS).

Objective To assess the correlation between PLR and EONS as well as the diagnostic value of PLR for predicting EONS.

Methods This study included all inpatient neonates with suspected early-onset neonatal sepsis at Dr. R. D. Kandou Hospital, Manado, North Sulawesi, Indonesia. Neonates were categorized into sepsis (confirmed by positive blood culture results) and non-sepsis (negative blood culture results) groups. Bivariate analysis, including the chi-square test for categorical data and independent t-test for numerical data, was performed to identify any significant associations between the platelet-to-lymphocyte ratio (PLR) and EONS. The sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve were calculated to determine the optimal PLR cut-off point to predict EONS.

Results In this study, we investigated the relationship between PLR and early-onset neonatal sepsis (EONS) in 176 neonates with suspected EONS. Blood cultures confirmed sepsis in 84 neonates (47.7%), with Klebsiella pneumoniae being the most common causative organism. We found a significant positive correlation between PLR and EONS ($p<0.001$), and a PLR cut-off point of 61.806 was identified to predict EONS with high sensitivity (90.2%) and specificity (85.7%)

Conclusion Our study demonstrates a strong positive correlation between PLR and EONS, and a PLR cut-off point of 61.806 can be used as a valuable marker for predicting EONS in neonates with suspected sepsis. These findings could aid in the early identification and treatment of neonates with sepsis, ultimately improving patient outcomes. **[Paediatr Indones. 2023;63:202-7; DOI: https://doi.org/10.14238/pi63.3.2023.202-7**].

Keywords: *early-onset neonatal sepsis; platelet to lymphocyte ratio; sepsis predictor*

Neonatal sepsis remains a major cause of morbidity and mortality.[1,2] At least 1 million neonatal deaths per year are caused by infections.[3] Infant mortality rate is used as a national health indicator. *The 2012 Indonesian Health Demographic Survey*, reported 32 deaths per 1,000 live births.[4,5] The *United Nations International Children's Emergency Fund* (UNICEF) reported 14 deaths per 1000 live births in neonates in Indonesia.[6] In 2009, the *World Health Organization* (WHO) reported 3.3 million neonatal sepsis deaths out of a total of 6 million infant deaths.[7]

Early diagnosis of neonatal sepsis is of utmost importance in managing the disease and defining the prognosis. A late diagnosis increases the mortality risk and worsens prognosis.[4] Blood culture has been the gold standard of sepsis diagnosis, yet it has intrinsic liabilities in test sensitivity and diagnostic speed. Delayed treatment worsens clinical outcomes and can cause multiple complications, including death. On the

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other hand, overtreatment increases antibiotic usage and duration of hospitalization, thereby increasing cost. For these reasons, there is a need for other diagnostic approaches to increase the effectiveness of a sepsis diagnosis.[8]

Platelet-to-lymphocyte ratio (PLR) is a widely available, effective, simple, and affordable marker that can be performed even in limited-resource settings.[9-11] Several studies reported that PLR was associated with increased morbidity and mortality in patients with severe sepsis and septic shock.[12-14] Therefore, we aimed to evaluate the possible correlation between PLR and early onset neonatal sepsis in order to assess the diagnostic value of PLR.

Methods

This observational analytic study with cross-sectional design took place in the neonatal intensive care unit (NICU) of the Department of Child Health, Universitas Sam Ratulangi /Prof. Dr. R. D. Kandou Hospital, Manado, North Sulawesi, from May to September 2019. The study was conducted under the approval of the Research Ethics Committee of Dr. R. D. Kandou Hospital.

We included all inpatient neonates initially suspected of having early onset neonatal sepsis (EONS) in the NICU. Suspected EONS was indicated by the presence of two major or one major and two minor risk factor criteria for neonatal sepsis according to the Indonesian Pediatric Society that were present in the first 72 hours after birth. Major criteria included premature rupture of membranes of >18 hours, maternal intrapartum fever of >38°C, chorioamnionitis, sustained fetal heart rate of >160 bpm, and foul-smelling amniotic fluid. Minor criteria included premature rupture of membranes of >12 hours, maternal intrapartum fever of >37.5°C, low APGAR scores, very low birth weight of <1,500 grams, untreated maternal leukorrhea, and untreated maternal urinary tract infection.[4,15]

Inclusion criteria for this study were all neonates with suspected EONS born through any mode of delivery. Neonates with congenital birth defects needing surgical repair, birth trauma, or congenital heart disease were excluded from the study. To determine the appropriate sample size, we used a formula for correlational studies and obtained a minimum required sample size of 92 subjects. Subjects were included by consecutive sampling. All neonates with suspected EONS were enrolled into the study. After blood culture results were available, subjects were categorized into sepsis and non-sepsis groups.

Neonatal sepsis was defined as the presence of four out of six clinical profiles (abnormalities in the respiratory, cardiovascular, metabolic, neurologic, gastrointestinal, or hematologic system)[15-17] and two positive hematologic profile parameters [Hb <15 g/dL, white blood cell count (WBC) >25,000/mm^3 or <5,000/mm^3, platelet count <100,000/mm^3, C-reactive protein (CRP) >6 mg/dL, or immature-to-total neutrophil (I/T) ratio >0.2][18] accompanied by a positive blood culture. Patients who did not meet those criteria were allocated to the non-sepsis group.

The main outcome of our study was the occurrence of culture-proven sepsis, and the independent variable was PLR. PLR was calculated by dividing absolute platelet count by absolute lymphocyte count. We also collected clinical and laboratory data such as complete blood count parameters, CRP, and neutrophil-to-lymphocyte ratio (NLR).

In our study, we recorded the occurrence of sepsis as a dichotomous variable (sepsis or non-sepsis) and calculated the mean and standard deviation of the PLR, a numeric variable. To assess the association between PLR and EONS, we conducted bivariate analyses and receiver-operator characteristics (ROC) curve analysis to determine the optimal cut-off point of PLR for predicting EONS. All statistical analyses were done using *SPSS version 25.0* (*IBM*, Armonk, NY). Results with P values <0.05 were considered to be statistically significant.

Results

We included a total of 176 neonates with suspected early-onset neonatal sepsis (EONS) in this study. Of these, 84 neonates were confirmed to have sepsis (47.7%) based on blood culture results, while the other 92 neonates were categorized as non-sepsis group (52.3%). **Table 1** presents the characteristics of subjects with and without sepsis. There appears to be no significant differences in age, sex, hematocrit, and

Table 1. Subject characteristics based on diagnosis

Variables	Non sepsis (n=92)	Sepsis (n=84)
Mean age (SD), days	1.91 (2.55)	1.57 (3.22)
Sex, n(%)		
Male	51 (55.4)	49 (58.3)
Female	41 (44.6)	35 (41.7)
CRP level, n (%)		
<6 µg/mL	65 (70.7)	25 (30.5)
6 µg/mL	9 (9.8)	2 (2.4)
12 µg/mL	3 (3.3)	1 (1.2)
24 µg/mL	4 (4.3)	17 (20.7)
48 µg/mL	11 (12.0)	37 (45.1)
Mean hemoglogin (SD), g/dL	15.35 (3.03)	14.34 (3.07)
Mean hematocrits (SD), %	42.78 (9.04)	41.87 (9.42)
Mean leukocytes (SD), 10^3/µL	15.77 (5.80)	23.16 (8.55)
Mean lymphocytes (SD), 10^3/µL	5.42 (2.73)	3.08 (1.27)
Mean neutrophils (SD), 10^3/µL	7.62 (3.51)	16.80 (6.82)
Mean platelets (SD), 10^3/µL	216.60 (91.80)	233.40 (78.67)
Mean NLR (SD)	1.60 (0.66)	5.86 (2.38)
Mean PLR (SD)	43.98 (16.39)	79.88 (21.18)
Mean I/T ratio (SD)	0.05 (0.06)	0.15 (0.09)

NLR=neutrophil-to-lymphocyte ratio; PLR= platelet-to-lymphocyte ratio

platelets between the two groups, while lymphocyte counts tended to be lower in the sepsis group. On the other hand, leukocyte counts, PLR, NLR, and IT-ratio appear to be higher in the sepsis group compared to the non-sepsis group.

Among the neonates with sepsis, the most commonly isolated microorganisms in the blood cultures were *Klebsiella pneumoniae* (29.76%), *Candida albicans* (11.9%), and *Serratia marcescens* (10.71%), as shown in **Table 2**. Using the ROC curve (**Figure 1**), we determined that a PLR cut-off point of 61.806 can predict EONS with a sensitivity of 90.2%, specificity of 85.7%, and AUC of 0.928 (95%CI 0.89 to 0.965, P<0.001).

Discussion

In our study, slightly more males than females had confirmed sepsis. This finding is consistent with those of previous studies in Indonesia.[19-21] The male predominance is thought to be due to genes on the X chromosome which affect host susceptibility through their actions on the thymus gland and immunoglobulin synthesis.[22]

In this study, blood culture results confirmed bacteria as the causative agent in the majority of positive cultures. *Klebsiella pneumoniae* was the most common microorganism found, consistent with two previous studies.[23,24] Interestingly, a small percentage of positive cultures yielded fungi as the pathogen. Although blood culture remains the gold standard in diagnosing neonatal sepsis, a negative result cannot completely rule out the presence of sepsis, and a positive culture may be the result of contamination. Moreover, the long waiting period of 3 to 5 days for blood culture results poses an additional challenge in using it as a diagnostic tool for neonatal sepsis.[17,25]

Platelets and lymphocytes are important components of the immune system that provide the first line of defense against infection. In sepsis, activated platelets secrete proteins such as cytokines, chemokines, coagulation mediators, and antimicrobial peptides. Platelets also play a role in the secretion of the neutrophil extracellular trap, platelets do not directly release NETs, but they can contribute to the process indirectly. Platelets can activate neutrophils, which leads to the release of NETs. Additionally, platelets can enhance the formation and stability of NETs by promoting the aggregation of neutrophils

Table 2. Distribution of microorganisms found in EONS patients' blood culture

Microorganism, n(%)	N=135
Acinetobacter baumanii	5 (3.7)
Acinetobacter junii	2 (1.5)
Candida albicans	14 (10.4)
Candida pelliculosa	2 (1.5)
Candida tropicalis	1 (0.7)
Elizabeth meningoseptica	1 (0.7)
Enterobacter aerogenes	8 (5.9)
Enterobacter cloacae complex	1 (0.7)
Enterococcus faecium	1 (0.7)
Escherichia coli	6 (4.4)
Klebsiella pneumoniae	57 (42.2)
Listeria monocytogenes	1 (0.7)
Micrococcus luteus	1 (0.7)
Methicillin-resistant staphylococcus aureus	1 (0.7)
Pseudomonas aeruginosa	2 (1.5)
Salmonella sp	1 (0.7)
Serratia marcescens	9 (6.7)
Staphylococcus aureus	9 (6.7)
Staphylococcus epidermidis	7 (5.2)
Staphylococcus haemolyticus	4 (3.0)
Staphylococcus hominis	1 (0.7)
Staphylococcus saprophyticus	1 (0.7)

ESBL+: extended-spectrum beta-lactamase-positive

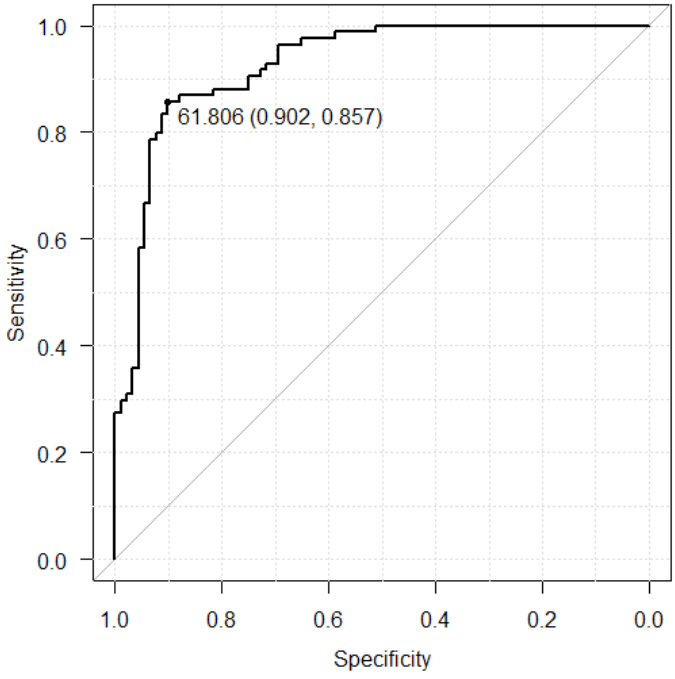

Figure 1. ROC curve to determine the optimal PLR

around the site of infection. Neutrophil extracellular traps (NETs) are web-like structures composed of DNA, histones, and antimicrobial peptides that are released by neutrophils in response to infection and inflammation. NETs can trap and kill bacteria and other pathogens, thereby playing an important role in the innate immune response.[26,27]

As infection progresses, interactions between antigen-presenting cells and lymphocytes build up, and lymphocytes move from blood vessels to the infection site, leading to lymphocytopenia. In sepsis patients, lymphocyte apoptosis may also be found.[28,29] Decreased lymphocyte count is the foundation of for using PLR to predict the diagnosis of sepsis in adults.[30] Studies on the use of PLR to predict neonatal sepsis are still rare.

We found a significant positive association between PLR and EONS. Can et al. found that subjects with EONS had significantly higher neutrophil count, axillary temperature, neutrophil-to-lymphocyte ratio, PLR, CRP, and procalcitonin levels.[31] In addition, Arcagok et al. found that neonates with confirmed and suspected EONS had higher PLR compared to the control group. The same study reported that PLR had an area under the curve (AUC) of 0.89 to 0.93 for cut-off points between 39.5 and 57.7, with a sensitivity of 88.9% to 91.3% sensitivity and specificity of 94.7% to 97.6%, to distinguish between suspected and confirmed EONS.[32]

Our study had several limitations, including not differentiating PLR cut-off points between term and preterm neonates. Preterm infants are known to have higher baseline levels of inflammatory markers, including platelets and neutrophils, which may influence the PLR.

We conclude that a PLR value of >61.81 has a sensitivity of 90.2% and a specificity of 85.7% for diagnosing EONS. Therefore, PLR has potential as a marker for diagnosing EONS, although further research is needed to determine the optimal cut-off point for term and preterm neonates.

Conflict of interest

None declared.

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References

1. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, *et al.* Global, regional, and national causes of child mortality. Lancet. 2012;380:1251-61. DOI: https://doi.org/10.1016/S0140-6736(12)60560-1.
2. Mathur NB. Neonatal mortality and infections: global perspective. In: Mathur NB, editor. Neonatal sepsis. New Delhi: Elsevier; 2010. p. 8-9.
3. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, Basani DG, *et al*. Global, regional and national causes of child mortality in 2008: a systemic analysis. Lancet. 2010;375:1969-87. DOI: https://doi.org/10.1016/S0140-6736(10)60549-1.
4. Aminullah A. Sepsis pada bayi baru lahir. In: Kosim MS, Yunanto A, Dewi R, Sarosa GI, Usman A, editors. Buku Ajar Neonatologi. Edisi 1. Jakarta: Badan Penerbit IDAI; 2012. p.170-87.
5. Badan Pusat Statistik (BPS), Badan Koordinasi Keluarga Berencana Nasional (BKKBN), Kementerian Kesehatan Republik Indonesia, ICF International. Indonesian National Demographic and Health Survey 2012. [cited 2020 February 10]. Available from: https://dhsprogram.com/pubs/pdf/fr275/fr275.pdf.
6. UNICEF. Maternal and newborn health disparities in Indonesia. [cited 2020 March 20]. Available from: file:///D:/Users/Anna/Downloads/country%20profile_IDN-1.pdf.
7. World Health Organization. The Global Health Observatory: Explore a world of health data. [cited 2020 January 15]. Available from: https://www.who.int/data/gho.
8. McGowan KL, Foster JA, Coffin SE. Outpatient pediatric blood cultures time to positivity. Pediatrics. 2000;106:251-5. DOI: https://doi.org/10.1542/peds.106.2.251.
9. Balta S, Ozturk C. The platelet-lymphocyte ratio: a simple, inexpensive, and rapid prognostic marker for cardiovascular events. Platelets. 2015;26:680-1. DOI: https://doi.org/10.3109/09537104.2014.979340.
10. Yazar FM, Bakacak M, Emre A, Urfalıoglu A, Serin S, Cengiz E, *et al.* Predictive role of neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios for diagnosis of acute appendicitis during pregnancy. Kaohsiung J Med Sci. 2015;31:591-6. DOI: https://doi.org/10.1016/j.

kjms.2015.10.005.

11. Yavuzcan A, Cağlar M, Ustün Y, Dilbaz S, Ozdemir I, Yildiz E, *et al*. Mean platelet volume, neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in severe preeclampsia. Ginekol Pol. 2014;85:197-203. PMID: 24783431
12. Biyikli E, Kaymaz AE, Kavalci C. Effect of platelet–lymphocyte ratio and lactate levels obtained on mortality with sepsis and septic shock. Am J Emerg Med. 2018;36:647-50. DOI: https://doi.org/10.1016/j.ajem.2017.12.010.
13. Yildiz A, Yigit A, Benli AR. The prognostic role of platelet to lymphocyte ratio and mean platelet volume in critically ill patients. Eur Rev Med Pharmacol Sci. 2018;22:2246-52. DOI: https://doi.org/10.26355/eurrev_201804_14811.
14. Kartal O, Kartal AT. Value of neutrophil to lymphocyte and platelet to lymphocyte ratios in pneumonia. Bratisl Lek Listy. 2017;118:513-6. DOI: https://doi.org/10.4149/BLL_2017_099.
15. Tripathi S, Malik GK. Neonatal sepsis: past, present and future; a review article. Int J Med. 2010;5:45-54. DOI: https://doi.org/ 10.4314/ijmu.v5i2.56163.
16. Polin RA, The Committee on Fetus and Newborn, Papile L, Baley JE, Bhutani VK, Carlo WA, *et al.* Management of neonates with suspected or proven early onset bacterial sepsis. Pediatrics. 2012;129:1006-15. DOI: https://doi.org/10.1542/peds.2012-0541.
17. Shah BA, Padbury JF. Neonatal sepsis: an old problem with new insights. Virulence. 2014;5:170-8. DOI: https://doi.org/10.4161/viru.26906.
18. Haque KN. Definitions of bloodstream infection in the newborn. Pediatr Crit Care Med. 2005;6:45-9. DOI: https://doi.org/10.1097/01.PCC.0000161946.73305.0A.
19. Juniatiningsih A, Aminullah A, Firmansyah A. Micro-organism profile of neonatal sepsis in Child Health Department of Ciptomangunkusumo Hospital Jakarta. Sari Pediatri. 2008;10:60-5. DOI: https://doi.org/10.14238/sp10.1.2008.60-5.
20. Putra PJ. Incidences and factors related to neonatal sepsis in Sanglah Hospital, Denpasar. Sari Pediatri. 2012;14:205-10. DOI: https://doi.org/10.14238/sp14.3.2012.205-10.
21. Kardana IM. Incidence and factors associated with mortality of neonatal sepsis. Paediatr Indones. 2011;51:144-8. DOI: https://doi.org/10.14238/pi51.3.2011.144-8.
22. Lawn JE, Wilczynska-Ketende K, Cousens SN. Estimating the causes 4 million neonatal deaths in the year 2000. Int J Epidemiol. 2006;35:706-18. DOI: https://doi.org/ 10.1093/ije/dyl043.
23. Patel D, Nimbalkar A, Sethi A, Kungwani A, Nimbalkar S. Blood culture isolates in neonatal sepsis and their sensitivity in Anand District of India. Indian J Pediatr. 2014;81:785-90. DOI: https://doi.org/10.1007/s12098-013-1314-2.
24. Kayange N, Kamugisha E, Mwizamholya DL, Jeremiah S, Mshana SE. Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania. BMC Pediatr. 2010;10:39. DOI: https://doi.org/10.1186/1471-2431-10-39.
25. Goldstein B, Giroir B, Randolph A, International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med. 2005;6:2-8. DOI: https://doi.org/10.1097/01.PCC.0000149131.72248.E6.
26. de Stoppelaar SF, van 't Veer C, van der Poll T. The role of platelets in sepsis. Thromb Haemost. 2014;112:666-77. DOI: https://doi.org/10.1160/TH14-02-0126.
27. Greco E, Lupia E, Bosco O, Vizio B, Montrucchio G. Platelets and multi-organ failure in sepsis. Int J Mol Sci. 2017;18:2200. DOI: https://doi.org/10.3390/ijms18102200.
28. Shubin NJ, Monaghan SF, Heffernan DS, Chung CS, Ayala A. B and T lymphocyte attenuator expression on CD4+ T-cells associates with sepsis and subsequent infections in ICU patients. Crit Care. 2013;17:R276. DOI: https://doi.org/10.1186/cc13131.
29. Hotchkiss RS, Monneret G, Payen D. Sepsis induced immunosuppression: from cellular dysfunctions to immunotherapy. Nat Rev Immunol. 2013;13:862-74. DOI: https://doi.org/10.1038/nri3552.
30. Kutlucan L, Kutlucan A, Basaran B, Dagli M, Basturk A, Kozanhan B, *et al*. The predictive effect of initial complete blood count of intensive care unit patients on mortality, length of hospitalization, and nosocomial infections. Eur Rev Med Pharmacol Sci. 2016;20:1467-73. PMID: 27160116.
31. Can E, Hamilcıkan S, Can C. The value of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio for detecting early-onset neonatal sepsis. J Pediatr Hematol Oncol. 2018;40:e229-32. DOI: https://doi.org/10.1097/MPH.0000000000001059.
32. Arcagok BC, Karabulut B. Platelet to lymphocyte ratio in neonates: a predictor of early onset neonatal sepsis. Mediterr J Hematol Infect Dis. 2019;11:e2019055. DOI: https://doi.org/10.4084/MJHID.2019.055.