Profile of Immature to Total Neutrophil (IT) Ratio and Platelet to Lymphocyte Ratio as Initial Predictive Value of Early Onset Neonatal Sepsis at Sanjiwani Hospital, Gianyar, Bali

Made Nindya Prahasari Wismawan, and Romy Windiyanto

**ABSTRACT**

**Introduction:** Early-onset neonatal sepsis (EOS) is a serious health problem manifested as systemic inflammation in the neonates that occurred within 72 hours of life. Diagnosis is challenging as the signs and symptoms of neonatal sepsis are not specific. Blood culture as the gold standard has its limitation, therefore we aim to evaluate the role of immature-to-total neutrophil ratio (IT ratio) and platelet-to-lymphocyte ratio (PLR) as a biomarker for early prediction of EOS.

**Materials and Method:** This is a cross-sectional study which included patients aged within 28 days (neonates) with clinical suspicion of early-onset neonatal sepsis in Sanjiwani Hospital, Gianyar, Bali from January to December 2022. The primary outcome of the study was the diagnostic performance of IT ratio and PLR.

**Results:** 131 patients were included in this study, in which 55.7% were diagnosed with EOS. IT ratio with the cut-off value of 0.2 has sensitivity and specificity of 98.6% and 96.9%, respectively, with OR 2023.5 (178.92–22884.5; p < 0.001). No significant difference was seen in PLR between suspected and proven EOS.

**Conclusion:** IT ratio could be considered a promising biomarker as an early predictor of EOS, while the utility of PLR remains uncertain.

**Keywords:** Early-onset neonatal sepsis, IT ratio, PLR, Predictor.

I. INTRODUCTION

Neonatal sepsis is a serious global health problem which manifested as a systemic inflammatory response during 28 days of life. Neonatal sepsis can be classified as early-onset neonatal sepsis, which occurred within the first 72 hours of life, while late-onset neonatal sepsis occurred after 72 hours of life [1]. World Health Organization (WHO) reported that sepsis was attributed to 15% of global neonatal mortality, in which the number was even higher in developing countries [2]. The incidence of early-onset neonatal sepsis (EOS) in Indonesia varies among tertiary referral hospitals, as much as 8.76–30.29% [3].

The prognosis of neonatal sepsis primarily depends on time to diagnosis and treatment. However, diagnosis is challenging as the signs and symptoms of neonatal sepsis are not specific. Blood culture has been the gold standard for the diagnosis of neonatal sepsis. However, it is associated with a longer time needed for diagnosis (around 24 to 72 hours, maybe longer in limited resources settings), higher cost, and a high probability of false negative results [4], [5]. False-negative results in blood culture may lead to a poorer prognosis as it may result in delayed antibiotic administration. Therefore, a more effective biomarker for diagnosis has been sought to aid clinicians in the diagnosis of neonatal sepsis.

It is hypothesized that during sepsis, there was an increase in the neutrophil count with a decrease in lymphocyte count as a result of the inflammatory response to pathogen infection. There was also an increase in platelet count, which occurred as a consequence of the marked increase in platelet destruction with subsequent stimulation of bone marrow to release young, large-sized platelet in the peripheral circulation. Regarding this, multiple biomarkers have been reported as an early predictor of neonatal sepsis, including leucocyte count, procalcitonin (PCT), immature-to-total neutrophil ratio (IT ratio), platelet count, and C-reactive protein (CRP). However, PCT, or CRP has been reported to be not specific with a high probability of false positive results [6]. On the other hand, complete blood count can be difficult to interpret as the value varies with age (days) and gestational age [4]. Previous studies also reported the use of platelet-to-lymphocyte ratio (PLR). There was no accepted cut-off that may predict neonatal sepsis in the biomarkers. Thus, this study aimed to evaluate the role of IT ratio and PLR as a biomarker for early prediction of EOS.
II. METHODS

A. Study Design and Patients

This cross-sectional study included neonates (patients aged within 28 days) with a clinical suspicion of early-onset neonatal sepsis at Sanjivani Hospital in Gianyar, Bali from January to December 2022. Symptoms associated with EOS included hyperthermia, hypothermia, respiratory distress, apnea, cyanosis, icterus, lethargy, irritability, and anorexia. Neonates with hematological disorders, congenital anomalies, autoimmune diseases, immunodeficiencies, or incomplete hematological examinations in their medical records were excluded from the study. Data collected from patient medical records included gender, gestational age, mode of delivery, birth weight, presence of asphyxia, symptoms associated with neonatal sepsis, and hematological parameters such as neutrophil, lymphocyte, and platelet counts. The primary outcome was the diagnostic performance of the immature-to-total neutrophil ratio (IT ratio) and the platelet-to-lymphocyte ratio (PLR) as early predictors of EOS. This study has received ethical clearance by the authorized Health Research Ethics Committee with reference number: 14/PEPK/I/2023.

B. Statistical Analysis

The statistical analysis was carried out using version 26.0 of the SPSS software for Mac (SPSS Inc., Chicago, IL, USA). The data was displayed in the form of tables and figures. Continuous variables were shown as mean ± SD or median (minimum, maximum) depending on the normality of the data. Categorical variables were shown as frequencies and proportions (percentages). Continuous variables were analyzed using either an independent t-test or a Mann-Whitney U test. The diagnostic performance between biomarkers (IT ratio and PLR) and the diagnosis of EOS was evaluated using binary logistic regression, followed by a receiver operating characteristic (ROC) analysis to determine the area under the curve (AUC) and the optimal cut-off. The odds ratio (OR) with a 95% confidence interval (CI) for diagnosis was obtained using a chi-square analysis with crosstabulation from each cut-off. A p-value of less than 0.05 was considered to be statistically significant.

III. RESULTS

131 patients were included in this study, of which 73 patients (55.7%) were diagnosed with EOS. Patients are dominated by males (50.4%), normal birth weight (52.7%), mode of delivery using cesarean section (64.9%), and term gestational age (61.1%). The majority of patients were born with moderate asphyxia (51.9%) and symptoms of respiratory distress (36.6%). Subject characteristics based on diagnosis are shown in Table I. No significant difference was seen between the groups.

This study evaluated the predictive role of IT ratio and PLR in the diagnosis of EOS. A significant difference was observed in the IT ratio between proven and suspected EOS. No significant difference was seen in PLR. The association between the IT ratio and PLR to EOS can be shown in Table II.

![ROC Curve](image-url)  
Fig. 1. ROC curve in IT ratio for EOS diagnosis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total n = 131</th>
<th>Proven EOS n = 73</th>
<th>Suspected EOS n = 58</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IT ratio</td>
<td>(0.2, 0.34)</td>
<td>(0.25, 0.34)</td>
<td>(0.13, 0.2)</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PLR</td>
<td>(81.5, 77.7)</td>
<td>(81.5, 77.7)</td>
<td>(79.56, 945.45)</td>
<td>0.739</td>
</tr>
</tbody>
</table>

*p value <0.05 was statistically significant.

ROC analysis was performed to determine the optimal cut-off in IT ratio and PLR for EOS diagnosis. ROC curve in IT ratio and PLR was seen in Fig. 1 and Fig. 2, respectively. AUC for IT ratio was 0.990 (excellent) with a cut-off value of 0.2. With the given cut-off, the sensitivity and specificity were 98.6% and 96.9%, respectively. AUC for PLR was 0.51 (unsatisfactory) with a cut-off value of 76.99 (sensitivity and specificity were 60.3% and 50%, respectively). The performance of the IT ratio and PLR in EOS diagnosis is shown in Table III.

![Table III](image-url)
Early-onset neonatal sepsis (EOS) is a systemic infection that presents as bacteremia/meningitis in neonates (age within 28 days) within the first 48–72 hours of life. EOS is associated with significant mortality and morbidity in neonates, making early diagnosis and prompt treatment essential. Several risk factors are associated with EOS, including prematurity, low birth weight, the presence of congenital anomalies, instrument-assisted delivery, and a low APGAR score (asphyxia) [1]. Diagnosing EOS can be difficult as there are no specific signs or symptoms. Blood culture is currently considered the gold standard for diagnosing sepsis. However, it has several drawbacks, including a longer time required for diagnosis (around 24 to 72 hours, possibly longer in resource-limited settings), higher cost, and a high probability of false diagnosis (around 24 to 72 hours, possibly longer in resource-limited settings). Moreover, the success rate of blood culture depends on the volume of blood collected during sample collection, which can be challenging to achieve in the neonate population [4], [6].

An effective marker as an early predictor is needed to aid clinical in the diagnosis of EOS. Multiple biomarkers have been reported, including CRP, procalcitonin, and leukocyte count. CRP is a sensitive inflammatory marker, but it is not specific for sepsis as it can also increase in non-infective inflammation. Moreover, the onset in CRP increases relatively late (10–12 hours). Procalcitonin has an earlier onset (3–4 hours), but the value usually normalizes within 24 hours [6]. Therefore, the use of modified parameters such as immature-to-total neutrophil (IT) ratio and platelet-to-lymphocyte (PLR) ratio has been widely discussed.

In this study, IT ratio with a cut-off value of 0.2 can be used as an early predictor in EOS, with 98.6% sensitivity and 96.6% specificity. Saboohi et al. [7] showed that IT ratio can be used as an early indicator of EOS, in which IT ratio >0.2 was associated with sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 76.47%, 83.82%, 54.16%, and 93.44%, respectively. Other studies also reported the use of IT ratio in neonatal sepsis. A study conducted in Indonesia showed that IT ratio with a similar cut-off can be used for neonatal sepsis diagnosis with bacterial etiology with sensitivity, specificity, PPV, and NPV of 88.46%, 81.84%, 82.14%, and 88%, respectively [8].

An increase in IT ratio during sepsis is associated with left shift leukocyte production towards immature neutrophil. In neonates, neutrophil acts as the first-line defense system for bacterial invasion. Bacterial infection leads to granulocyte production in bone marrow, with subsequent increase in precursor cell proliferation, decrease in neutrophil storage time, and increase in neutrophil release from bone marrow reserve. As a result, a higher IT ratio can be seen as a consequence of a surge in the number of immature (band) neutrophil in circulation. However, a substantial increase in neutrophil production does not necessarily improve neonates immune system during sepsis, as immature neutrophil has lower proinflammatory protein concentration due to incomplete or absence of gelatin and/or secretory granules, which results in a less effective immune response to infection [9], [10].

It needs to be highlighted that the innate immune system in neonates has not fully developed, in which previous study reported that initiation of neutrophil response towards bacteria required a longer time (3–4 hours) compared to adults (30–90 minutes). Neutrophil in neonates also lack functional capabilities compared to those found in adults, in which there was a decrease in the capacity of bacterial recognition, adhesion to vascular endothelial, chemotaxis, phagocytosis, induction of cell death, and apoptosis [11].

However, utilization of IT ratio as an early predictor in EOS needs to be done with caution, as multiple factors can affect the neutrophil level in blood, including perinatal hypoxia (asphyxia), maternal hypertension, stress during the delivery process, and prolonged oxytocin induction. Another study reported the use of IT ratio in neonatal sepsis. A pooled analysis showed that IT ratio ≥0.2 is a good indicator of sepsis, with sensitivity and specificity of 76.4% and 82.1%, respectively [12]. Murphy et al. also showed that a normal IT ratio (>0.2) in two separate laboratory examinations with sterile blood culture has NPV of 100% in the diagnosis of EOS [13].

Platelet has also been widely studied as a diagnostic marker for EOS. Several parameters have been reported, including platelet distribution width (PDW), mean platelet volume (MPV), platelet count, and platelet-to-lymphocyte ratio (PLR) [14]. The use of parameters based on complete blood count is associated with lower cost, faster turn-around time (TAT), and more accessibility. Also, a complete blood count is performed as early protocol of every hospitalized patient, thereby eliminating the need for additional expense for diagnosis [15].

Our study failed to show a significant association between PLR in diagnosis of EOS. However, many studies have reported the use of PLR. A meta-analysis conducted by Bai et al. [16] reported a pooled sensitivity of 87% (95% CI 76–89%) and specificity of 80% (95% CI 78–100%), despite

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![ROC Curve](image.png)

**Fig. 2.** ROC curve in PLR for EOS diagnosis.

| Table III: Performance of IT ratio and PLR in EOS diagnosis |
|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|
| Parameter       | Cut-off (IT) | Sensitivity (%) | Specificity (%) | OR   | CI 95%     | p-value          |
| IT ratio        | 0.2          | 98.6            | 96.6            | 2023.5 | 178.92–22884.5 | <0.001*          |
| PLR             | 76.99        | 60.3            | 50              | 1.52  | 0.76–3.04   | 0.289            |

*p value <0.05 was statistically significant.

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IV. DISCUSSION

Early-onset neonatal sepsis (EOS) is a systemic infection that presents as bacteremia/meningitis in neonates (age within 28 days) within the first 48–72 hours of life. EOS is associated with significant mortality and morbidity in neonates, making early diagnosis and prompt treatment essential. Several risk factors are associated with EOS, including prematurity, low birth weight, the presence of congenital anomalies, instrument-assisted delivery, and a low APGAR score (asphyxia) [1]. Diagnosing EOS can be difficult as there are no specific signs or symptoms. Blood culture is currently considered the gold standard for diagnosing sepsis. However, it has several drawbacks, including a longer time required for diagnosis (around 24 to 72 hours, possibly longer in resource-limited settings), higher cost, and a high probability of false-negative results [4], [5]. Additionally, the success rate of blood culture depends on the volume of blood collected during sample collection, which can be challenging to achieve in the neonate population [4], [6].

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significant heterogeneity regarding different gold standard and cut-off used between studies. Another study conducted by Can, Hamilcikan, and Cen [17] reported a sensitivity and specificity of 97.4% and 100%, respectively in a cut-off value of 94.05. The lower cut-off was reported in multiple studies, including Rompis et al. [18], which showed a sensitivity and specificity of 90.2% and 85.7% with cut-off value of 61.806 and Arcagok and Karabulut [6] which showed a sensitivity and specificity of 91.3% and 97.6% with cut-off value of 57.7 in diagnosing EOS. Regarding the varying cut-off value and diagnostic accuracy, more studies with larger populations are needed for the validation of PLR as an early predictor of EOS.

The increase in PLR in EOS can be explained by the role of platelets in the pathophysiology of neonatal sepsis. Following pathogen (e.g., bacteria) invasion, the coagulation system near the infection area is activated, which results in the development of thrombus in local capillaries. Formation and subsequent breakdown of thrombus lead to a decrease in fibrinolytic and fibrinogenic substances, which induces the production of young platelets in the bone marrow. Some proinflammatory cytokines (e.g., IL-6 and IL-3) that increase during sepsis also has a direct and indirect effect on platelet level in circulation. For instance, IL-6 has a role in the conversion of megakaryocytes into platelets. Together with IL-6, IL-3 promotes an increase in megakaryocyte production. Platelets are associated with sepsis-induced coagulopathy that improves platelet adhesion and aggregation, which were facilitated by expression of P-selectin. Platelets also have a toll-like receptor (TLR), a receptor that may recognize different pathogen-associated molecular patterns (PAMP). Interaction between TLR and its ligand may directly cause platelet activation [19],[20].

During sepsis, there is also an increase in the production of thrombopoietin. However, excess in thrombopoietin stimulation paradoxically resulted in the release of large-sized, immature platelets. In prolonged sepsis, thrombocytopenia can be seen as neonatal bone marrow could not compensate with rapid depletion of megakaryocyte reserve and its precursor [14].

It should be noted that the response to infection in neonates primarily depends on the innate immune system. Lymphocyte-related cytokine production as a response to bacterial infection is lower in neonates than those in adults. This may be attributable to the fact that the spleen, being an important organ for lymphocyte development and pathogen clearance, has not fully developed. A significant decrease in lymphocytes is also attributable to apoptosis during sepsis [21],[22].

However, there were several limitations regarding this study. Cross-sectional studies are inherently limited in their ability to establish causality, and the findings can only describe associations between variables at a specific point in time. Moreover, the sample size may not be large enough to detect subtle differences or be representative of a larger neonatal population. As the study was done in a single center, the generalizability of the study's findings to other populations or regions may also be limited since practices, population genetics, and environmental factors could vary between hospitals and regions. Further studies may be needed with a larger population and a more suitable study design. The use of multiple parameters together as predictors of the diagnosis of EOS can be useful in the clinical setting prior to a definitive diagnosis with blood culture to find the etiology of sepsis.

To conclude this research, Early onset neonatal sepsis (EOS) is a health threat to newborn infants. This study shows that the immature to total neutrophil (IT) ratio has good predictive value for diagnosing EOS, while Platelet to Lymphocyte (PLR) ratio is not significant as an early predictor. However, further research and consideration are needed before the IT ratio can be adopted as a standard diagnostic tool.

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This study has not received funding from any sources.

**CONFLICT OF INTEREST**

Authors declare that they do not have any conflict of interest.

**REFERENCES**


