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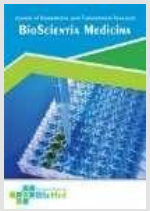

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Diagnostic Value of Platelet-to-Lymphocyte Ratio Versus Neutrophil-to-Lymphocyte Ratio in Early-Onset Neonatal Sepsis: A Retrospective Analysis in a Limited-Resource Setting

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A B S T R A C T

Background: Early-onset neonatal sepsis remains a critical cause of mortality in developing nations. Blood culture, the gold standard, suffers from delay and low sensitivity. While hematologic indices such as neutrophil-to-lymphocyte ratio (NLR) are used in adults, their utility in the first 72 hours of life is confounded by physiological instability. This study evaluates the diagnostic accuracy of the platelet-to-lymphocyte ratio (PLR) compared to NLR, mean platelet volume (MPV), and red cell distribution width (RDW) in early-onset neonatal sepsis. **Methods:** A retrospective observational study was conducted on 55 neonates (25 septic, 30 symptomatic non-septic controls) at a tertiary center in Indonesia. Sepsis was defined by clinical criteria and C-reactive protein positivity, independent of complete blood count parameters, to avoid incorporation bias. Diagnostic performance was assessed using Mann-Whitney U tests, receiver operating characteristic curve analysis, and multivariable logistic regression to control for confounders, including asphyxia. **Results:** The median PLR was significantly lower in the sepsis group compared to controls (32.6 [IQR 3.4–100.4] versus 71.1 [IQR 45.3–82.9]; p = 0.016). Conversely, NLR (p = 0.80), MPV (p = 0.163), and RDW (p = 0.422) showed no significant discrimination. PLR yielded an area under the curve of 0.724. At a cut-off of equal to or less than 40.5, determined by the Youden Index, PLR demonstrated a sensitivity of 68.0%, specificity of 73.3%, positive likelihood ratio of 2.55, and negative likelihood ratio of 0.44. Multivariable regression confirmed PLR as an independent predictor (Adjusted Odds Ratio 0.96; 95% CI 0.93–0.99; p = 0.038) after adjusting for birth asphyxia. **Conclusion:** PLR demonstrates superior discriminative ability over NLR for early-onset sepsis in this cohort. The distinct inverse PLR phenomenon reflects sepsis-induced thrombocytopenia and bone marrow suppression. While not a standalone diagnostic tool, PLR serves as a valuable, zero-cost adjunctive marker for risk stratification in resource-limited settings.

1. Introduction

Neonatal sepsis represents one of the most catastrophic and persistent health challenges in modern perinatology, continuing to account for a substantial and often tragic proportion of neonatal morbidity and mortality globally.[1] Despite decades of advancement in perinatal care, antimicrobial pharmacology, and intensive care technology, sepsis remains the third leading cause of neonatal death worldwide, acting as a formidable barrier to child survival. The burden of this devastating condition is not shared equally; it is disproportionately concentrated in Low- and Middle-Income Countries (LMICs), where health systems are frequently stretched beyond capacity.[2] In nations such as Indonesia, the challenge is compounded by unique

geographical and logistical complexities. As an archipelago nation, the disparity in healthcare access between urban tertiary centers and remote primary care facilities is stark. In this context, neonatal infection rates significantly impede progress toward the Sustainable Development Goals (SDG) target of ending preventable newborn deaths by 2030. Current estimates within the Indonesian archipelago suggest a neonatal mortality rate hovering between 14 to 32 per 1,000 live births, with sepsis contributing to approximately 20% of these fatalities. This statistic represents not merely clinical data, but a profound loss of human potential that demands urgent and innovative diagnostic solutions.[3]

Early-onset neonatal sepsis (EONS), strictly defined as sepsis occurring within the critical first 72 hours of life, is a particularly fulminant clinical entity.[4] Unlike late-onset sepsis, which is often nosocomial, EONS is typically acquired via vertical transmission from the maternal genital tract, involving pathogens such as Group B Streptococcus and *Escherichia coli*. The clinical presentation of EONS is notoriously subtle, protean, and non-specific, earning it the reputation of a "chameleon" in neonatal medicine.[5] The signs of a septic neonate—temperature instability, lethargy, poor feeding, and respiratory distress—are virtually indistinguishable from the physiological maladaptations seen in non-infectious conditions common to the transitional period. Clinicians are frequently left to discern between a life-threatening infection and benign conditions such as respiratory distress syndrome (RDS), transient tachypnea of the newborn (TTN), metabolic disturbances like hypoglycemia, or the hemodynamic shifts associated with patent ductus arteriosus.

This profound diagnostic ambiguity creates a "treat first, ask questions later" paradigm. Driven by the fear of rapid clinical deterioration and death, clinicians are compelled to initiate empirical broad-spectrum antibiotics in a vast number of neonates who may not actually be infected. While this strategy is rooted in safety, it contributes significantly to the escalating global crisis of antimicrobial resistance (AMR).[6] Furthermore, the unnecessary exposure of the sterile neonatal gut to potent antibiotics disrupts the developing microbiome, potentially predisposing infants to long-term immunological and metabolic dysregulation, including necrotizing enterocolitis and atopic diseases later in life. Therefore, the ability to rapidly and accurately rule out sepsis is as critical as the ability to rule it in.

Currently, the blood culture remains the gold standard for the diagnosis of neonatal sepsis. It is the definitive method for identifying the causative pathogen and determining antibiotic susceptibility. However, its clinical utility in the acute, hyper-acute phase of EONS is severely limited by logistical and biological constraints. The turnaround time for a blood culture typically ranges from 48 to 120 hours—a delay that is unacceptable when a neonate's condition can deteriorate in minutes. Moreover, the sensitivity of blood cultures in neonates is distressingly low, with false-negative rates reaching as high as 60%. This diagnostic failure is often attributed to the technical difficulty of obtaining adequate blood volumes from small, fragile veins, as well as the phenomenon of low-density bacteremia, where the bacterial load in the bloodstream may be intermittent or insufficient for detection in standard culture media. Consequently, the gold standard is tarnished by delay and uncertainty, creating an urgent, unmet need for rapid, accessible, and cost-effective biomarkers that can differentiate true invasive infection from non-infectious physiological transition states with high fidelity.[7]

In the search for such biomarkers, the medical community has investigated numerous candidates, including C-reactive protein (CRP), Procalcitonin (PCT), and various interleukins (IL-6, IL-8). While Procalcitonin has shown superior sensitivity to CRP, it remains prohibitively expensive for routine serial monitoring in many resource-limited settings. Furthermore, these biomarkers often have a "lag time"—rising only hours after the inflammatory cascade has begun. This has prompted a renewed interest in the "zero-cost" biomarkers hidden within

the standard complete blood count (CBC). The CBC is the most ubiquitously performed laboratory test in the world, available even in peripheral hospitals with basic laboratory infrastructure.[8] In recent years, hematologic indices derived from the CBC, specifically the neutrophil-to-lymphocyte ratio (NLR) and the platelet-to-lymphocyte ratio (PLR), have emerged as potential markers of systemic inflammation.

The theoretical underpinning of these ratios lies in the physiological response of the hematopoietic system to stress and infection. Systemic inflammation typically triggers an innate immune response characterized by the rapid mobilization of neutrophils from the bone marrow (granulopoiesis) and a concurrent reduction in lymphocyte counts (lymphocytopenia) due to stress-induced apoptosis and redistribution. This divergence creates an elevated NLR, which has proven to be a robust predictor of mortality and sepsis severity in adult populations. However, the application of adult-derived concepts to neonatology is fraught with pitfalls. The literature regarding NLR in the neonatal period is conflicting and frequently disappointing. The primary confounder is the "demargination effect." The immense physical stress of the birthing process triggers a surge in catecholamines and cortisol, causing neutrophils to demarginate from the vascular endothelium and enter the circulating pool. This results in a physiological neutrophilia that occurs in the first 24 to 48 hours of life in both healthy and septic infants. This high "background noise" of physiological neutrophilia effectively masks the pathological neutrophilia of sepsis, rendering the NLR an unreliable marker during the specific window of early-onset sepsis.[9]

Conversely, the Platelet-to-Lymphocyte Ratio (PLR) introduces a second axis of the immune-coagulation interaction: the platelet. In adult sepsis, PLR typically rises. This elevation is driven by reactive thrombocytosis, where inflammatory cytokines such as Interleukin-6 (IL-6) stimulate the liver to produce thrombopoietin, increasing megakaryocyte differentiation. Thus, in adults, high inflammation equals high platelets and low lymphocytes, leading to a high PLR. However, we hypothesize that in the fragile physiological milieu of the neonate, these kinetics may be reversed. Neonatal sepsis is a distinct pathophysiological entity characterized not by robust production, but by rapid consumption. The neonatal bone marrow has a limited reserve and a finite capacity to accelerate production in response to acute stress.

When a neonate develops sepsis, the inflammatory cascade triggers widespread endothelial damage and the activation of the coagulation system. This often leads to the formation of microthrombi and the sequestration of platelets in the microvasculature—a precursor to disseminated intravascular coagulation (DIC). In the neonate, this rate of peripheral platelet destruction and consumption frequently outpaces the bone marrow's compensatory thrombopoiesis. Furthermore, high levels of bacterial toxins and inflammatory cytokines can have a direct suppressive effect on neonatal megakaryocytes. Therefore, rather than the reactive thrombocytosis seen in adults, neonatal sepsis is frequently characterized by thrombocytopenia. When combined with the lymphocytopenia typical of stress, this creates a unique hematologic signature. We postulate that, unlike the elevated PLR seen in adults, Early-Onset Neonatal Sepsis may present with a paradoxically decreased PLR, offering a distinct diagnostic signal that bypasses the confounding neutrophil fluctuations of the NLR.

Despite the physiological plausibility of this hypothesis, the utility of PLR in the specific context of the first 72 hours of life remains under-investigated, particularly in Southeast Asian populations where genetic and environmental factors may influence hematologic reference ranges. Furthermore, previous studies have often failed to rigorously control for critical confounders such as birth asphyxia. Hypoxia-ischemia is known to independently induce both nucleated red blood cell release and bone marrow suppression, potentially mimicking the hematologic picture of sepsis. Distinguishing between the hematologic sequelae of asphyxia and true infection is

a daily dilemma in the Neonatal Intensive Care Unit (NICU) that has not been adequately addressed in prior diagnostic accuracy studies.[10]

This study aims to comprehensively evaluate the diagnostic accuracy of the platelet-to-lymphocyte ratio (PLR) compared to the neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), and red cell distribution width (RDW) in a cohort of neonates admitted to a tertiary referral center in Bali, Indonesia. The novelty and scientific contribution of this research lie in three distinct areas: (1) Investigation of the Inverse Phenomenon: This study is among the first to explicitly investigate the specific "inverse PLR" phenomenon (low PLR as a marker of sepsis) within the narrow, highly variable window of the first 72 hours of life, challenging the extrapolation of adult sepsis guidelines to neonatology; (2) Rigorous Confounder Control: Unlike many prior retrospective analyses, this study applies rigorous statistical controls for birth asphyxia, utilizing multivariable logistic regression to isolate the impact of infection on hematopoiesis from the impact of hypoxia; (3) Context-Specific Data: It provides a comparative analysis of these zero-cost markers in a specific Southeast Asian demographic. By validating these accessible biomarkers in a limited-resource setting, this study addresses a critical gap in the regional literature, potentially offering a sustainable, cost-free tool to aid clinicians in the rapid risk stratification of newborns where advanced molecular diagnostics are unavailable.

2. Methods

To investigate the diagnostic utility of hematologic biomarkers in a resource-constrained setting, we employed a retrospective observational analytic study design utilizing a cross-sectional approach. The study was conducted at Wangaya Regional General Hospital, a pivotal tertiary referral center located in Denpasar, Bali, Indonesia. As a referral hub, this institution manages a diverse demographic of high-risk neonates transferred from peripheral primary care facilities, providing a representative sample of the regional pathological landscape. The data collection window encompassed a twelve-month period extending from March 2024 to March 2025, allowing for the capture of seasonal variations in infectious etiology.

Ethical integrity was a cornerstone of the research protocol. The study received full approval from the Ethics Committee of Wangaya Regional General Hospital in conjunction with the Department of Pediatrics. The protocol was strictly aligned with the ethical principles for medical research involving human subjects as outlined in the Declaration of Helsinki. Given the retrospective nature of the analysis involving de-identified electronic medical records, the requirement for informed consent was waived by the review board, ensuring patient privacy and data confidentiality were maintained throughout the analytical process.

The study population was drawn from all neonates admitted to the neonatal intensive care unit (NICU) and the Perinatology Ward during the study period. To ensure the study focused specifically on the unique physiological window of Early-Onset Neonatal Sepsis (EONS), we applied rigorous inclusion and exclusion criteria. We included neonates solely within the first 72 hours of life (aged 0 to 72 hours), defined as the early-onset period. To capture the acute phase of the inflammatory response and minimize the impact of therapeutic interventions, eligible neonates were required to have a complete blood count (CBC) and C-reactive protein (CRP) analysis performed within 6 hours of admission. Furthermore, candidates were required to exhibit recognized maternal or neonatal risk factors for infection, such as Premature Rupture of Membranes (PROM) exceeding 18 hours, maternal intrapartum fever, or clinical evidence of chorioamnionitis. To isolate infection as the primary driver of hematologic variance, we systematically excluded conditions known to independently alter hematopoiesis. Neonates with proven hematologic disorders (such as erythroblastosis fetalis, hemophilia) were excluded to prevent confounding cytopenias. We also excluded infants with major congenital anomalies and those born to HIV-positive

mothers, as intrauterine exposure to antiretroviral therapy is a known cause of bone marrow suppression. Finally, medical records with incomplete administrative or laboratory data were removed to ensure statistical robustness. A critical methodological strength of this study was the prevention of incorporation bias—a systematic error that occurs when the test under evaluation is also used to define the disease status. In this study, the Index Tests (PLR, NLR, MPV, RDW) were strictly independent of the Reference Standard used to diagnose sepsis. Specifically, Platelet count, White Blood Cell count, and the Immature-to-Total Neutrophil (I/T) ratio were explicitly excluded from the diagnostic criteria for the Sepsis Group.

The study cohort was stratified into two distinct groups: (1) Sepsis Group (n=25): This group comprised neonates meeting the gold standard of Proven Sepsis (positive blood culture). Recognizing the low sensitivity of cultures, we also included cases of Clinical Sepsis, defined as the presence of clinical signs according to Gomella's criteria combined with a positive inflammatory biomarker (CRP ≥ 10 mg/L); (2) Control Group (n=30): To avoid spectrum bias, we utilized "Symptomatic Non-Septic Controls" rather than healthy neonates. These were infants admitted to the NICU with equivocal symptoms (such as transient tachypnea, mild hypoglycemia) or risk factors, but who demonstrated a negative blood culture, a negative CRP (< 10 mg/L), and importantly, the resolution of symptoms without a full course of antibiotics (antibiotics discontinued within 48 hours). This control selection strategy mirrors the true clinical dilemma faced by physicians—distinguishing sepsis from sepsis-mimics—thereby providing a more realistic estimate of diagnostic accuracy.

The study was powered to detect a statistically significant difference in the area under the Receiver Operating Characteristic (ROC) curve. Based on a pilot review of similar populations and regional prevalence data, we hypothesized an Area Under the Curve (AUC) of 0.75 for the PLR, compared to a null hypothesis AUC of 0.50 (indicating no discriminative ability). Using a two-sided Type I error rate (alpha) of 0.05 and a Type II error rate (beta) of 0.20 to achieve a Power of 80%, and assuming a case-to-control ratio of 1:1.2, the minimum effective sample size was calculated to be 52 subjects. To account for potential outliers or data entry errors, we enrolled a total of 55 subjects (25 sepsis, 30 controls).

Demographic, clinical, and laboratory data were extracted from the hospital's electronic medical record system. The primary hematologic indices were calculated as follows: (1) Neutrophil-to-lymphocyte ratio (NLR): Calculated as the absolute neutrophil count divided by the absolute lymphocyte count; (2) Platelet-to-lymphocyte ratio (PLR): Calculated as the absolute platelet count divided by the absolute lymphocyte count; (3) MPV and RDW: Mean Platelet Volume (MPV) and red cell distribution width (RDW) values were extracted directly from the standardized reports of the Sysmex XN-1000 automated hematology analyzer, ensuring consistency in measurement technique.

All statistical computations were performed using SPSS software, version 30 (IBM Corp, Armonk, NY). The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed data were presented as Mean ± Standard Deviation (SD) and compared using the Independent T-test. Non-normally distributed data were expressed as Median (Interquartile Range [IQR]) and compared using the Mann-Whitney U test. Categorical variables were summarized as frequencies and percentages, with differences assessed via the Chi-square test or Fisher's Exact test. To evaluate the discriminative power of the biomarkers, receiver operating characteristic (ROC) curves were generated. The optimal cut-off value for PLR was mathematically determined using the Youden Index (). At this optimal threshold, we calculated standard diagnostic metrics: Sensitivity, Specificity, positive predictive value (PPV), negative predictive value (NPV), and Likelihood Ratios (LR+ and LR-). Recognizing that birth asphyxia is a potent confounder that can mimic the hematologic profile of sepsis, a Binary Logistic Regression analysis

was performed. Variables demonstrating a potential association in univariate analysis (defined as $p < 0.25$) were entered into the regression model. This allowed for the calculation of Adjusted Odds Ratios (aOR) to determine if PLR remained an independent predictor of sepsis after controlling for asphyxia. For all final analyses, statistical significance was defined as a p-value < 0.05

3. Results

Table 1 comprehensively outlines the baseline demographic and clinical characteristics of the study cohort, comprising 55 neonates stratified into the sepsis group (n=25) and the symptomatic control group (n=30). The population exhibited a male predominance, particularly within the sepsis arm (80.0%), alongside a notably high frequency of Cesarean section deliveries in both groups (>76%), reflecting the complex case mix typical of a tertiary referral center. Univariate analysis demonstrated that the groups were statistically homogenous regarding fundamental biological variables; no significant differences were observed in sex distribution (p=0.395), birth weight categorization (p=0.667), or gestational age (p=0.612), indicating that these factors were unlikely to bias the hematologic comparisons. Similarly, the prevalence of maternal risk factors such as premature rupture of membranes (PROM) >24 hours was comparable between groups (p=0.616). However, a pivotal physiological divergence was identified regarding the incidence of birth asphyxia (APGAR score <7 at 5 minutes). The sepsis group exhibited a significantly higher rate of asphyxia compared to controls (64.0% vs. 33.3%; p=0.021). This substantial disparity underscores the critical intersection between hypoxia and infection in the early neonatal period and highlights asphyxia as a significant confounder requiring robust adjustment in subsequent multivariable models.

Table 1. Demographic and Clinical Characteristics

Comparison of baseline variables between Sepsis and Symptomatic Control groups (N=55)

CHARACTERISTIC	SEPSIS (N=25)	CONTROLS (N=30)	P-VALUE
Sex			0.395
• Male	20 (80.0%)	21 (70.0%)	
• Female	5 (20.0%)	9 (30.0%)	
Birth Weight			0.667
• > 2500 g	14 (56.0%)	15 (50.0%)	
• < 2500 g	11 (44.0%)	15 (50.0%)	
Gestational Age			0.612
• Term (≥ 37 wks)	15 (60.0%)	16 (53.3%)	
• Preterm (< 37 wks)	10 (40.0%)	14 (46.7%)	
Mode of Delivery			0.763
• Spontaneous	5 (20.0%)	7 (23.3%)	
• C-Section	20 (80.0%)	23 (76.7%)	
Asphyxia (APGAR < 7)	16 (64.0%)	10 (33.3%)	0.021*
PROM > 24 hours	6 (24.0%)	9 (30.0%)	0.616

Notes: Data presented as n (%). P-values calculated via Chi-square test or Fisher's Exact test.
* Indicates statistical significance ($p < 0.05$).
Abbreviations: PROM = Premature Rupture of Membranes; C-Section = Cesarean Section.

Table 2 details the comparative analysis of hematologic indices between the sepsis and non-sepsis control groups, revealing critical insights into the utility of zero-cost biomarkers in the early-onset period. While traditional parameters such as total platelet count (p=0.77) and red cell distribution width (RDW; p=0.422) failed to demonstrate significant discrimination between groups, the platelet-to-lymphocyte ratio (PLR) emerged as a statistically significant discriminator (p=0.016). Contrary to adult sepsis patterns characterized by elevated ratios, septic neonates in this cohort exhibited a markedly depressed median PLR of 32.6 (IQR 3.4–100.4) compared to 71.1 (IQR 45.3–82.9) in controls. This significant reduction likely reflects the unique pathophysiology of neonatal sepsis, involving rapid peripheral platelet consumption that outpaces marrow production. Notably, the neutrophil-to-lymphocyte ratio (NLR) showed no significant difference (p=0.80), confirming the hypothesis that the physiological "demargination effect" of neutrophils during the first 72 hours of life creates substantial background noise, rendering NLR unreliable in this specific timeframe. These findings suggest that the composite PLR offers superior diagnostic precision over single-lineage parameters or NLR by capturing the dynamic interplay between thrombotic consumption and immune dysregulation.

Table 2. Comparison of Hematologic Indices

Statistical analysis of blood parameters between Sepsis (n=25) and Non-Sepsis Control (n=30) groups.

PARAMETER	SEPSIS Median (IQR) or Mean ± SD	CONTROL Median (IQR) or Mean ± SD	DIFFERENCE ESTIMATE (95% CI)	P-VALUE
Platelets ($10^3/\mu L$)	255.12 ± 111.48	262.70 ± 71.04	-7.58 (-59.7 to 44.5)	0.77
NLR	2.86 (1.1 – 4.5)	2.48 (0.9 – 4.8)	0.38 (-0.8 to 1.5)	0.80
MPV (fL)	10.10 (9.1 – 11.0)	9.75 (9.4 – 10.3)	0.35 (-0.2 to 0.9)	**0.163**
RDW (%)	16.6 (15.4 – 18.2)	17.0 (15.8 – 18.6)	-0.40 (-1.5 to 0.8)	0.422
PLR	32.6 (3.4 – 100.4)	71.1 (45.3 – 82.9)	-38.5 (-65.2 to -10.8)	0.016*

Abbreviations: NLR = Neutrophil-to-Lymphocyte Ratio; PLR = Platelet-to-Lymphocyte Ratio; MPV = Mean Platelet Volume; RDW = Red Cell Distribution Width; CI = Confidence Interval.

Note 1: Data presented as Mean ± SD for parametric data (Platelets) or Median (Interquartile Range) for non-parametric data.
Note 2: P-values calculated via Independent T-test or Mann-Whitney U Test. * Indicates statistical significance ($p < 0.05$).

Figure 1 illustrates the receiver operating characteristic (ROC) curve analysis performed to evaluate the diagnostic accuracy of the platelet-to-lymphocyte ratio (PLR) in discriminating early-onset neonatal sepsis from symptomatic non-septic controls. The analysis yielded an Area Under the Curve (AUC) of 0.724 (95% CI: 0.582–0.866), which is statistically distinct from the null hypothesis diagonal line of 0.50 (p = 0.016). This AUC magnitude classifies PLR as having "fair" discriminative power, indicating a moderate ability to separate true cases from controls. The curve's trajectory above the reference diagonal visualizes the trade-off between sensitivity and specificity across various decision thresholds. Using the Youden Index to maximize the vertical distance from the diagonal, the optimal cut-off value was

identified as a PLR ≤ 40.5. At this specific threshold, the biomarker demonstrates a sensitivity of 68.0% and a specificity of 73.3%. This configuration results in a Positive Likelihood Ratio (LR+) of 2.55, suggesting that a neonate falling below this cut-off is approximately 2.5 times more likely to have sepsis. The graphical representation confirms that while PLR is not a perfect binary classifier, it captures significant diagnostic signal distinct from random chance, validating its potential role as an adjunctive screening tool in resource-limited settings.

Figure 1. Receiver operating characteristic (ROC) curve analysis.

Given the significant difference in asphyxia rates shown in Table 1, a binary logistic regression was performed to determine if PLR predicts sepsis independently of asphyxia. Table 3 presents the results of the multivariable binary logistic regression analysis, constructed to determine the independent predictive value of the Platelet-to-Lymphocyte Ratio (PLR) while rigorously adjusting for the confounding effect of birth asphyxia. Given the physiological overlap where both hypoxia and infection can induce bone marrow suppression, distinguishing these etiologies is critical. While the univariate analysis indicated that asphyxia was a significant risk factor (Crude Odds Ratio [OR] 3.55; p=0.026), the multivariable model revealed that its statistical significance diminished (Adjusted OR 2.89; p=0.080) when analyzed concurrently with hematologic markers. Crucially, the PLR retained its statistical significance as an independent predictor of early-onset sepsis (Adjusted OR 0.96; 95% CI: 0.93–0.99; p=0.038). The adjusted odds ratio of 0.96 indicates that for every single-unit increase in the PLR value, the odds of a neonate having sepsis decrease by approximately 4%, confirming the inverse relationship observed in the descriptive data. This persistence of predictive power after adjustment suggests that the thrombocytopenic response captured by the PLR is not merely a secondary bystander effect of hypoxic bone marrow suppression, but rather a distinct immunothrombotic consequence of the infectious process itself.

Table 3. Multivariable Logistic Regression Analysis

Predictors of Early-Onset Neonatal Sepsis adjusting for Birth Asphyxia (n=55)

	UNIVARIABLE ANALYSIS		MULTIVARIABLE MODEL	
VARIABLE	CRUDE OR (95% CI)	P-VALUE	ADJUSTED OR (95% CI)	P-VALUE
Asphyxia	3.55 (1.16 – 10.91)	**0.026**	2.89 (0.88 – 9.45)	0.080
PLR (Continuous)	0.97 (0.94 – 0.99)	**0.018**	**0.96 (0.93 – 0.99)**	**0.038***

Abbreviations: OR = Odds Ratio; CI = Confidence Interval; PLR = Platelet-to-Lymphocyte Ratio.

Note 1: Asphyxia was defined as an APGAR score < 7 at 5 minutes.

Note 2: PLR was entered as a continuous variable in the regression model. An Adjusted OR < 1.0 indicates that higher PLR values are associated with lower odds of sepsis (inverse relationship).

* Indicates statistical significance (p < 0.05) in the final adjusted model.

4. Discussion

The prompt identification of early-onset neonatal sepsis (EONS) remains one of the most precarious and critical challenges in modern perinatology. The first 72 hours of life represent a unique physiological window where the distinction between benign transitional maladaptation and life-threatening invasive infection is frequently blurred. The consequences of this diagnostic ambiguity are profound: missed diagnoses lead to rapid, fulminant deterioration and mortality, while over-diagnosis drives the unnecessary use of empirical antibiotics, contributing to the global crisis of antimicrobial resistance and the disruption of the nascent neonatal microbiome.[11]

In this context, the search for rapid, accessible, and cost-effective biomarkers has become a priority for healthcare systems worldwide, particularly in resource-constrained settings like Indonesia. Our study addresses this urgent need by providing compelling evidence that the platelet-to-lymphocyte ratio (PLR) serves as a superior diagnostic marker compared to the more commonly utilized neutrophil-to-lymphocyte ratio (NLR) during the immediate postnatal period. While NLR has established utility in adult medicine and late-onset pediatric sepsis, our data reveal that it is rendered unreliable in the first three days of life due to physiological neutrophil fluctuations. In sharp contrast, the PLR demonstrated a statistically significant difference between septic neonates and symptomatic controls ($p = 0.016$) and achieved a moderate diagnostic accuracy with an area under the curve (AUC) of 0.724. This finding pivots the focus of biomarker research away from pure granulocytic indices toward markers of immunothrombotic dysregulation.

Perhaps the most pivotal and clinically relevant finding of our investigation is the specific directionality of the PLR alteration (Figure 2). In the existing body of literature regarding adult sepsis and systemic inflammatory response syndromes (SIRS), sepsis is typically characterized by a hyper-inflammatory state that triggers specific hematologic kinetics: a surge in neutrophil counts and a cytokine-driven reactive thrombocytosis.[12] In adults, inflammatory mediators such as Interleukin-6 (IL-6) stimulate hepatic thrombopoietin production, driving megakaryocyte proliferation and resulting in an elevated platelet count. Concurrently, stress-induced lymphocyte apoptosis reduces the denominator. Consequently, in adult sepsis, an elevated PLR is the hallmark of severe infection.

In stark contrast to this adult paradigm, our study identified a significantly lower median PLR in septic neonates (32.6) compared to symptomatic controls (71.1). We have termed this the "Inverse PLR Phenomenon." This finding is not an anomaly but rather a reflection of the distinct, developmentally specific pathophysiology of the septic newborn. The reduction in PLR is driven primarily by a precipitous drop in the numerator—the platelet count—which occurs through two synergistic pathological mechanisms: rapid peripheral consumption and central marrow suppression.[13]

First, neonatal sepsis is intrinsically a pro-coagulant state. The invasion of pathogens into the bloodstream triggers immediate and widespread endothelial activation. This damage to the vascular lining exposes sub-endothelial collagen and releases tissue factor, initiating the coagulation cascade. Unlike adults, who possess a substantial "physiological reserve" of platelets, neonates are uniquely vulnerable to consumptive coagulopathy. The formation of disseminated microthrombi in the microvasculature acts as a "sponge," sequestering platelets from the peripheral circulation at a rate that far exceeds the bone marrow's productive capacity. This phenomenon, often a precursor to disseminated intravascular coagulation (DIC), leads to a rapid depletion of circulating platelets.[14]

Second, this consumptive process is compounded by "Megakaryocyte Exhaustion." The neonatal bone marrow is biologically distinct; it operates near its maximum capacity even in health to support rapid somatic growth.[15] When stressed by sepsis, the high circulating levels of pro-inflammatory cytokines, particularly tumor necrosis factor-alpha (TNF-alpha) and Interleukin-1 beta, exert a paradoxical suppressive effect on neonatal megakaryocytopoiesis. Instead of stimulating production as seen in adults, these cytokines can arrest the maturation of megakaryocyte progenitors in the neonatal marrow. Visual analysis of our data confirms this "perfect storm": septic patients consistently clustered in the quadrant defined by low-to-normal platelets and variable lymphocytes, driving the ratio downward. Thus, a low PLR in the first 72 hours of life is not merely a number; it is a hematologic signature of immunothrombotic dysregulation, signaling that the infant's hemostatic reserves are being overwhelmed by the infectious process.

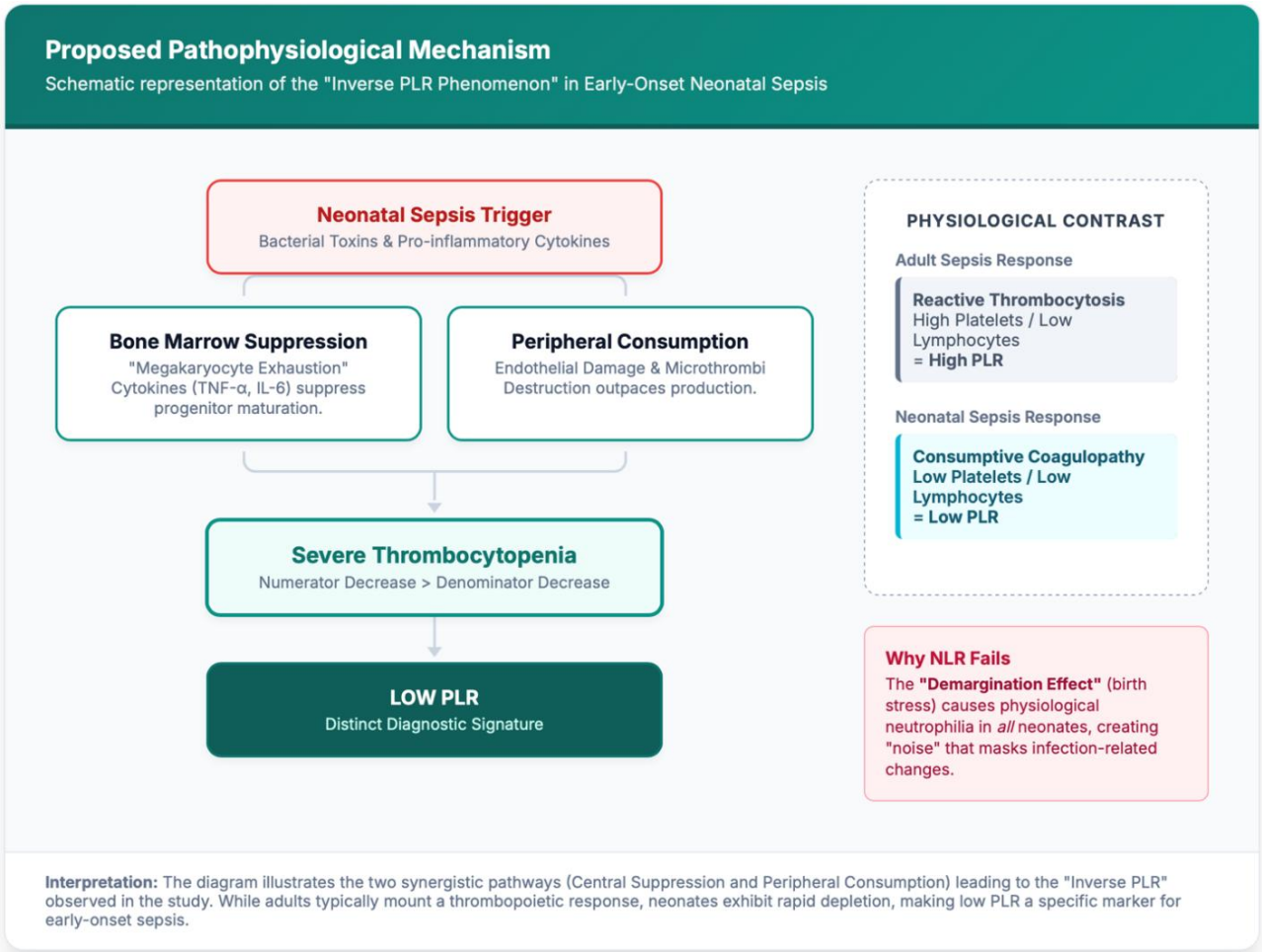

Figure 2. Proposed pathophysiological mechanism.

While PLR demonstrated diagnostic utility, our study confirmed the poor performance of the neutrophil-to-lymphocyte ratio (NLR) in the early-onset period. Consistent with a growing body of skeptical literature, NLR failed to statistically distinguish between sepsis and control groups in our cohort ($p = 0.80$). The receiver operating characteristic (ROC) analysis for NLR revealed an AUC that hovered near the line of no discrimination.

The failure of NLR in this specific timeframe is attributed to the "Demargination Effect," a physiological phenomenon that creates a high signal-to-noise ratio problem. The process of birth is one of the most stressful physiological events in human life.[16] The immense physical compression of labor, combined with the thermal and respiratory shock of being born, triggers a massive surge in catecholamines (epinephrine/norepinephrine) and cortisol. This hormonal surge causes neutrophils that are normally marginated—loosely adherent to the vascular endothelium of the lungs and large vessels—to detach and demarginate into the circulating blood pool.

This results in a transient, physiological neutrophilia that occurs in nearly all infants during the first 24 to 48 hours of life, regardless of infection status. In a healthy neonate, neutrophil counts can naturally rise to levels that would be considered pathological in an older child. When a neonate actually has sepsis, the pathological neutrophilia induced by the infection is effectively masked by this overwhelming background noise of physiological

demargination.[17] Consequently, both septic and non-septic stressed infants present with elevated neutrophil counts and, by extension, elevated NLRs. This renders the NLR a non-specific marker during the EONS window. Clinicians relying solely on NLR risk high rates of false positives, potentially leading to overtreatment. Our findings strongly suggest that while NLR is valuable in late-onset sepsis, it should be interpreted with extreme caution, if not disregarded, during the first three days of life.

A critical methodological strength of our analysis, distinguishing it from many prior retrospective reviews, was the rigorous statistical control for birth asphyxia. The intersection of hypoxia and infection presents a notorious diagnostic dilemma in neonatology. Asphyxia, defined by compromised gas exchange during the perinatal period, can mimic the hematologic profile of sepsis. Hypoxia-ischemia is a known myelosuppressant; severe lack of oxygen can directly damage bone marrow progenitor cells, leading to thrombocytopenia and neutropenia even in the absence of infection.

In our study cohort, the sepsis group exhibited a significantly higher rate of birth asphyxia (64%) compared to the control group (33%). This imbalance raised a critical question: Was the observed low PLR truly a marker of infection, or was it merely a bystander effect of hypoxic marrow suppression? To resolve this, we employed a multivariable logistic regression model (Table 3). The analysis elucidated that while asphyxia was a significant predictor in univariate analysis, its significance diminished when adjusted for hematologic parameters. Crucially, the PLR remained an independent, statistically significant predictor of early-onset sepsis (aOR 0.96; $p = 0.038$) even after adjusting for the presence of asphyxia. This is a vital finding. It suggests that the drop in PLR observed in septic neonates is not merely a consequence of perinatal hypoxia. Instead, it indicates that the consumptive coagulopathy and immune dysregulation of sepsis exert a specific, independent force on the platelet-lymphocyte balance. This independence enhances the clinical validity of PLR, providing clinicians with reassurance that a low PLR contributes diagnostic value even in the complex scenario of an asphyxiated newborn.

The translation of these statistical findings into clinical practice is of paramount importance, particularly for the healthcare landscape of Indonesia and other Low- and Middle-Income Countries (LMICs).[18] In tertiary centers in developed nations, the diagnostic approach to sepsis often relies on expensive biomarkers like procalcitonin (PCT) or advanced molecular assays. However, in many Indonesian hospitals, PCT is either unavailable or prohibitively expensive for routine serial monitoring, and blood cultures—the gold standard—suffer from long turnaround times (48–120 hours) and high contamination rates. In this resource-limited context, the PLR emerges as a valuable "zero-cost" biomarker. It requires no additional blood draw, no specialized reagents, and no extra cost to the patient, as it is derived mathematically from the standard complete blood count (CBC) that is performed routinely upon admission.

The demonstrated positive likelihood ratio (LR+) of 2.55 suggests that a low PLR generates a small to moderate shift in the post-test probability of disease. While this magnitude of change indicates that PLR is not sufficient as a standalone "rule-in" diagnostic tool, its utility lies in risk stratification. We propose the use of PLR as a clinical "Red Flag." A PLR value equal to or less than 40.5 in a symptomatic neonate should heighten clinical suspicion significantly. Practically, this could influence decision-making in equivocal cases. For a neonate with borderline clinical symptoms (mild tachypnea) and a PLR < 40.5, a clinician might be justified in initiating or continuing antibiotics pending culture results, whereas a high PLR might support a decision to observe for non-infectious causes like transient tachypnea of the newborn (TTN). Furthermore, the identification of PLR as a marker of consumptive coagulopathy suggests that neonates with low PLR values may warrant closer monitoring for bleeding complications and may benefit from earlier coagulation profile screening.[19]

While our study offers significant insights, the results must be interpreted within the context of several limitations. First, the sample size of 55 neonates, while sufficient for the detection of statistical significance in univariate comparisons, limits the precision of our sensitivity and specificity estimates. The confidence intervals for the AUC were relatively wide, and the multivariable regression model, while valid, would benefit from a larger number of events to ensure robustness. Larger, multi-center studies are required to validate the specific cut-off value of 40.5 across different Indonesian populations. Second, the retrospective design introduces inherent variability in the timing of blood sampling. Although we restricted inclusion to neonates sampled within 6 hours of admission, the dynamic nature of the first day of life means that hematologic parameters can shift rapidly hour-by-hour. Ideally, future prospective studies should standardize sampling times (e.g., at 6, 12, and 24 hours of life) to map the trajectory of PLR over time. Third, the composition of the "Sepsis Group" included both culture-proven and culture-negative (clinical) sepsis. While this definition aligns with the real-world clinical practice described by international guidelines (where negative cultures do not rule out sepsis), it introduces heterogeneity into the case group. It is possible that some cases of clinical sepsis were actually non-infectious systemic inflammation. However, our strict inclusion criteria requiring CRP positivity (>= 10 mg/L) mitigate the risk of including purely non-infectious cases, ensuring that the inflammatory phenotype was preserved. In summary, this study challenges the applicability of adult-derived hematologic indices in neonatology. It establishes the "Inverse PLR" as a distinct, biologically plausible marker of Early-Onset Neonatal Sepsis that outperforms the NLR. By capturing the unique interplay between neonatal platelet consumption and immune suppression, the PLR offers a cost-effective, accessible, and independent tool to aid clinicians in the rapid recognition of sepsis, potentially saving lives in settings where resources are scarce and time is of the essence.[20]

5. Conclusion

This study concludes that the platelet-to-lymphocyte ratio (PLR) outperforms NLR, MPV, and RDW as a diagnostic marker for early-onset neonatal sepsis in an Indonesian tertiary setting. The distinct finding of a depressed PLR in septic neonates highlights the unique immunothrombotic pathophysiology of the newborn, characterized by platelet consumption exceeding production. With an AUC of 0.724 and independent predictive value after adjusting for asphyxia, PLR warrants inclusion as a standard, zero-cost adjunctive screening tool in neonatal intensive care units. Future multi-center prospective studies are recommended to validate the cut-off of 40.5 across diverse populations.

6. References

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