Indonesian Journal of Global Health Research
Volume 8 Number 5, October 2026
e-ISSN 2715-1972; p-ISSN 2714-9749
http://jurnal.globalhealthsciencegroup.com/index.php/IJGHR

THE ASSOCIATION OF NLR AND PLR WITH HARD CALLUS FORMATION IN SINGLE CLOSED LONG BONE FRACTURES

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ABSTRACT
Closed long bone fractures require a complex healing process involving inflammation and callus formation. The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) have been proposed as potential biomarkers in the fracture healing process. To analyze the correlation between NLR, PLR, and hard callus formation in patients with a single closed long bone fracture. This retrospective cohort study involved 42 patients treated at RSUP H. Adam Malik Medan from January 2023 to December 2025. Patients were divided into a hard callus group (n=27) and a non-hard callus group (n=15) based on six-month radiological evaluation. Baseline neutrophil, lymphocyte, and platelet counts, NLR, PLR, and radiological findings were collected from medical records. Radiological findings were assessed by comparing the initial radiographs with those obtained at the six-month follow-up to evaluate callus formation. No significant differences in hematological parameters were found between the two groups ($p > 0.05$). Neither NLR ($p = 0.694$) nor PLR ($p = 0.581$) showed a significant association with hard callus formation. Logistic regression and ROC analysis also demonstrated low predictive ability for both NLR (AUC 0.537) and PLR (AUC 0.448). NLR and PLR are not associated with hard callus formation and have limited predictive value for bone healing in closed long bone fractures.

Keywords: closed fracture; hard callus; long bone; NLR; PLR

How to cite (in APA style)
Rahayu, M. P., Nasution, M. R., & Ketaren, A. S. P. (2026). The The Association of NLR and PLR with Hard Callus Formation in Single Closed Long Bone Fractures. *Indonesian Journal of Global Health Research*, *8*(5), 265–272. https://doi.org/10.37287/ijghr.v8i5.2182.

INTRODUCTION

A fracture is defined as a disruption in bone continuity caused by excessive force, applied either directly or indirectly (Blom et al., 2018). Traffic accidents remain the leading cause of fractures worldwide, with the World Health Organization (WHO) reporting approximately 1.19 million fatalities in 2021 (World Health Organization, 2023). In Indonesia, 139.258 traffic accident cases were recorded in 2022, resulting in 28.131 fatalities (Kementrian Kesehatan Republik Indonesia, 2023). This high accident rate significantly contributes to the rising incidence of long bone fractures, which demand long-term management and rehabilitation. In closed fractures, the bone fragments do not penetrate the skin, thereby isolating the injury from the external environment (Rastu et al., 2021). The bone healing process involves inflammatory, reparative, and remodeling phases, all of which are influenced by blood supply, mechanical stability, immunological status, and systemic inflammation (ElHawary et al., 2021). Excessive inflammation can impair bone regeneration and elevate the risk of delayed union or non-union (Newman et al., 2021). Consequently, easily accessible and cost-effective biomarkers are needed to evaluate the inflammatory response and predict the bone healing process.

The neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) are hematological parameters derived from routine complete blood counts that are widely utilized as systemic inflammatory markers. NLR and PLR are considered highly relevant because they reflect the equilibrium between the inflammatory response and immune regulation. Following a fracture, neutrophils clear tissue debris and produce inflammatory mediators, while lymphocytes assist in controlling inflammation and supporting the regenerative phase (Chen et al., 2022). Several studies indicate that elevated NLR and PLR levels correlate with poorer prognoses in trauma and fracture patients. A study conducted in Makassar reported that higher NLR values were associated with a worse prognosis in patients with open tibial fractures (Johan et al., 2024). In China, a post-trauma PLR value of < 145 was associated with an increased risk of bone non-union (Liu & Chien, 2023). Furthermore, research in Turkey demonstrated that an NLR value of NLR ≥6,45 and a PLR value of ≥172,2 hold potential as biomarkers for assessing the severity of proximal femur fractures (İzzet Korkmaz et al., 2022).

However, findings regarding the role of NLR and PLR in fracture healing remain inconsistent. Some studies have reported no significant differences in NLR and PLR values between fracture patients with normal bone mass and those with reduced bone mass ($p > 0.05$), suggesting that these ratios cannot yet be reliably used to evaluate post-fracture bone conditions (Yao et al., 2023). Moreover, the majority of prior studies have focused primarily on open fractures and trauma severity. Research exploring the correlation of NLR and PLR with hard callus formation in closed long bone fractures remains limited, particularly in Indonesia. Therefore, this study was conducted to analyze the correlation between neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) values with hard callus formation in patients with closed long bone fractures treated at RSUP H. Adam Malik Medan.

METHOD

This study utilized a retrospective cohort design conducted at RSUP H. Adam Malik Medan, in collaboration with the Department of Clinical Pathology and the Department of Orthopaedics, Faculty of Medicine, Universitas Sumatera Utara, from January 2023 to December 2025. Sampling was performed using a consecutive sampling method, including all patients who met the study criteria based on medical record data. A total of 42 subjects were enrolled in this study. The characteristics of the subjects are presented in tabular form and described according to each study group. Collected data included neutrophil count, lymphocyte count, platelet count, NLR value, PLR value, and the results of the 6th-month radiological evaluation to determine hard callus formation. Subjects were then classified into the hard callus group and the non-hard callus group based on the radiological findings.

The inclusion criteria for this study comprised patients aged 18–55 years with a diagnosis of a single closed long bone fracture confirmed by an orthopaedic specialist, with no history of blood transfusion or comorbidities such as diabetes mellitus, cancer, and hematological disorders. The exclusion criteria included patients with multiple fractures, neglected fractures, febrile conditions, and incomplete medical records. Data analysis was performed using SPSS software. Differences in NLR and PLR values between the groups were analyzed using the Independent T-test for normally distributed data or the Mann–Whitney U test for non-normally distributed data. The determination of cut-off values and the evaluation of the diagnostic performance of the parameters were conducted using receiver operating characteristic (ROC) analysis to obtain the area under the curve (AUC) value with a 95% confidence interval (95% CI). A p-value < 0.05 was considered statistically significant.

This study received ethical approval from the Health Research Ethics Committee of Universitas Sumatera Utara under number 1203/KEPK/USU/2025. The study also obtained research clearance from the Human Resources, Education, and Research Division of RSUP H. Adam Malik Medan

under number DP.04.03/D.XXVIII.2.2.3/1880/2025, as well as permission for medical record data retrieval under number DP.04.03/D.XXVIII.2.2.3/1893/2025.

RESULT

A total of 42 patients with closed long bone fractures were divided into two groups: the hard callus formation group consisting of 27 patients and the non-hard callus formation group consisting of 15 patients.

Table 1.

Demographic and Laboratory Characteristics of Patients with Hard Callus Formation in Single Closed Long Bone Fractures

Variabel	*Hard Callus*		*p value*
	Yes (n=27)	No (n=15)	
Gender (%)			
• Male	18 (66,7%)	9 (60,0%)	0.666
• Female	9 (33,3%)	6 (40,0%)	
Age (years)	40 (21 – 55)*	36 (21 – 53)*	0.627
Fracture Location (%)			
• Radius	3 (11,1%)	5 (33,3%)	0.418
• Tibia	7 (25,9%)	2 (13,3%)	
• Femur	9 (33,3%)	5 (33,3%)	
• Humerus	7 (25,9%)	3 (20,0%)	
• Ulna	1 (3,7%)	0 (0,0%)	
Fracture Grade (%)			
• Grade 0	3 (11,1%)	0 (0,0%)	0.579
• Grade 1	14 (51,9%)	8 (53,3%)	
• Grade 2	9 (33,3%)	6 (40,0%)	
• Grade 3	1 (3,7%)	1 (6,7%)	
Duration of Hospitalization (days)	4 (2 – 10)*	5 (2 – 9)*	0.759
Neutrophils l (10^3/ μL)	6,28 (2,83 – 29,72)*	6,35 (3,32 – 14,87)*	1.000
Lymphocytes (10^3/ μL)	2,05 (0,09 – 5,59)*	2,00 (0,69 – 4,91)*	0.948
Platelets (10^3/ μL)	272,3±61,72**	288±80,91**	0.486
NLR	3,131 (1,164 – 36,444)*	2,556 (1,058 – 12,855)*	0.694
PLR	124,137 (62,254 – 2.877,780)*	144,910 (50,102 – 549,275)*	0.581

Data are presented as median (min–max), mean ± SD, Significance set at p < 0.005$*

Based on the analysis of 42 patients with closed long bone fractures, no significant differences were found between the hard callus and non-hard callus groups regarding subject characteristics, including gender, age, fracture location, fracture grade, and length of hospital stay ($p > 0.05$). Hematological parameters, including neutrophil count, lymphocyte count, platelet count, neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR), also showed no statistically significant differences between the two groups. These results indicate that NLR and PLR are not significantly associated with hard callus formation in patients with closed long bone fractures.

Table 2.

The Association of NLR and PLR with Hard Callus Formation in Single Closed Long Bone Fractures

Variables	Outcome	*p value*	OR	95% CI
NLR	*Hard Callus*	0.618	0.968	0.854 - 1.098
PLR		0.640	0.999	0.997 – 1.002

Significant at p < 0.005

Consistent with the absence of significant differences in these values, binary logistic regression analysis demonstrated that neither NLR ($p = 0.618$; OR = 0.968$) nor PLR ($p = 0.640$; OR = 0.999) had a significant association with hard callus formation.

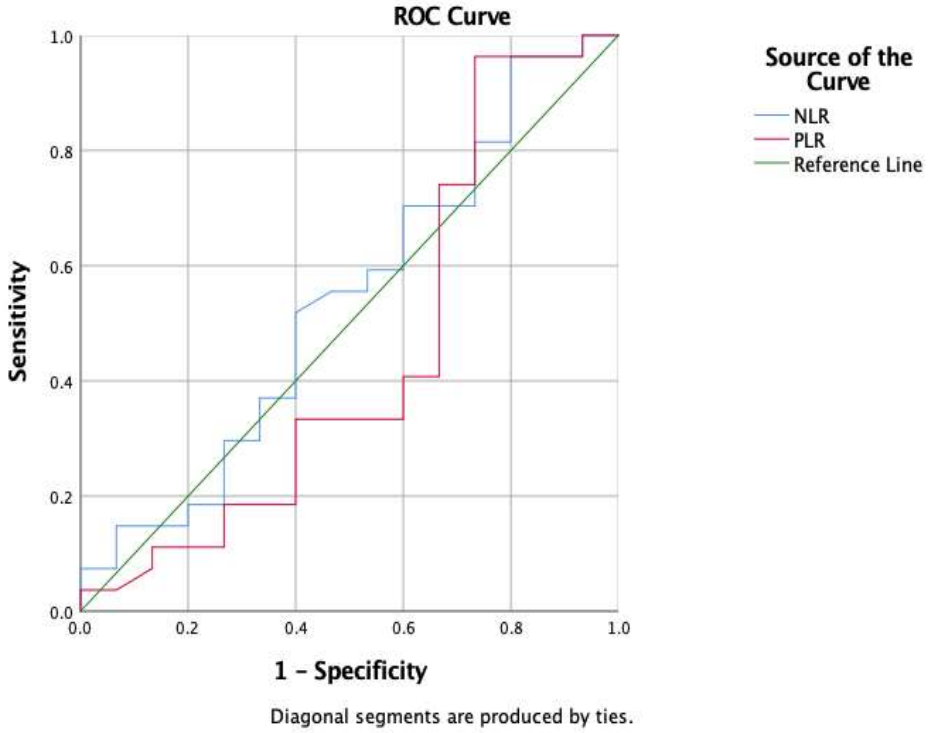

Figure 1. Comparative ROC Curve Analysis of NLR and PLR for Hard Callus Formation in Single Closed Long Bone Fractures

ROC analysis demonstrated that both NLR (AUC = 0.537; p = 0.694) and PLR (AUC = 0.448; p = 0.581$) have low discriminative ability in predicting hard callus formation in patients with closed long bone fractures. The optimal cut-off value of 1.326 for NLR and 76.775 for PLR showed high sensitivity but very low specificity, indicating that both parameters are inadequate for accurately differentiating between patients who achieve and those who do not achieve hard callus formation. Overall, NLR and PLR demonstrate no significant predictive value for hard callus formation in this study.

DISCUSSION

This study divided 42 subjects into a group with hard callus formation (n = 27) and a group without hard callus formation (n = 15). Based on age, no statistically significant difference was found between the groups that did and did not form a hard callus (p = 0.627). Physiologically, the process of osteogenesis tends to decline with advancing age due to reduced osteoblast activity, decreased bone mineral density, and alterations in bone microarchitecture (Blom et al., 2018; ElHawary et al., 2021). This discrepancy is likely attributable to the age distribution of the subjects, which was dominated by young to middle-aged adults who clinically still possess adequate bone regenerative capacity to support consistent hard callus formation (ElHawary et al., 2021). No significant association was found between the fracture location and hard callus formation (p = 0.418). Although the distribution of fracture locations varied between both groups, literature indicates that long bones with thick cortices and robust periosteum, such as the femur, tend to have a dominant periosteal blood supply and superior mechanical stability, which support the consistency of callus formation (Wolters Kluwer Health, Lippincott Williams, 2019). Regarding treatment outcomes, there was no significant difference in the length of hospital stay between the two groups, with a median of 4 days compared to 5 days (p = 0.759). This finding aligns with a study by Stina Ek et al., which reported that a short duration of hospitalization (2–4 days) yielded clinical outcomes equivalent to a longer stay (9–12 days) (Ek et al., 2023).

The results of routine hematological analysis revealed no significant differences in neutrophil counts (p = 1.000), lymphocyte counts (p = 0.948), or platelet counts (p = 0.486) between the

groups with and without hard callus. This finding differs from a study by Xu et al., which suggested that elevated neutrophil levels in trauma patients are associated with poorer outcomes, such as prolonged hospitalization and even mortality, reflecting a more severe inflammatory response in patients with a worse prognosis (Xu et al., 2022). However, the results of this study are consistent with research by Vijayakumar et al., which reported no significant differences in absolute neutrophil counts (ANC) among three bone sarcoma groups categorized by clinical outcome (Vijayakumar et al., 2024). Neutrophils cannot serve as a sole predictor of bone healing because their absolute count does not reflect the cell heterogeneity and functional status that dictate the reparative process, including NET (neutrophil extracellular trap) activity, ROS (reactive oxygen species) production, and the equilibrium between pro-inflammatory and reparative neutrophil phenotypes that influence callus formation (Renò et al., 2025).

The non-significant lymphocyte findings also align with a study by Templeton et al. (2014), which demonstrated that absolute lymphocyte counts do not predict clinical outcomes post-fracture and thus cannot be used as a standalone marker, despite a one-year mortality rate reaching 27.1% (Templeton et al., 2014). Theoretically, lymphocytes are not the primary drivers of bone healing; rather, their role is regulatory through immune signaling pathways involving mediators like RANKL and IFN-γ. Conversely, the process of callus formation is predominantly governed by the coordinated actions of neutrophils, macrophages, and osteoprogenitor cells. Consequently, evaluating peripheral lymphocytes alone is not sensitive enough to predict successful callus formation (Molitoris et al., 2024). Furthermore, the non-significant platelet results are consistent with a study by Yang et al. (2023), which reported that a platelet count <200x103 was associated with wound prognosis in diabetic ulcer patients (Yang et al., 2023). Additionally, Bacevich et al. noted that the effects of platelets on bone remodeling are only observable with the administration of platelet-rich plasma (PRP) concentrates, which contain multifold higher platelet concentrations. Therefore, platelet counts within the normal physiological range are not expected to exert a significant influence on callus formation (Bacevich et al., 2024).

Based on the differential analysis, no significant difference in NLR values was found between the two groups ($p = 0.694$). The analysis was subsequently extended using binary logistic regression to evaluate the relationship between NLR and hard callus formation in closed long bone fractures. The results demonstrated that NLR had no significant association with hard callus formation ($p = 0.618$; OR = 0.968; 95% CI: 0.854–1.098). An OR approaching 1 combined with a wide confidence interval indicates the absence of a meaningful association between NLR and hard callus formation, suggesting that NLR is not a robust predictor of closed fracture healing. This is in line with a study by Yao (2024), which reported that among fracture patients, NLR values did not differ significantly between groups with normal bone mass and low bone mass ($p > 0.05$) (Jingyu et al., 2024). Conversely, a differing perspective was presented by Korkmaz et al., who stated that NLR values were statistically significant when comparing patients with stable fractures (median 4.7; IQR: 3.2–7.1) to those with unstable fractures (median 7.2; IQR: 5.1–11.4) ($p = 0.001$), where NLR reflects a stronger inflammatory response associated with severe clinical conditions (Z. Korkmaz et al., 2022). Theoretically, NLR is an indicator of systemic inflammation that reflects alterations in neutrophils and lymphocytes due to bodily stress and inflammatory responses. However, because the bone healing process is predominantly determined by the local inflammatory response within the fracture hematoma, changes in systemic inflammatory parameters, including NLR, do not always parallel the local dynamics of osteogenesis (ElHawary et al., 2021; Steppe et al., 2023).

Based on the differential analysis, PLR values between the groups with and without hard callus formation showed no significant difference ($p = 0.581$). Binary logistic regression analysis further demonstrated that PLR was not associated with hard callus formation ($p = 0.640$; OR= 0.999; 95% CI: 0.997–1.002). An OR approaching 1 combined with a narrow confidence interval indicates that changes in PLR do not exert a significant influence on the likelihood of hard callus formation. A

study on fracture patients by Yao (2024) also reinforces this finding, reporting that PLR did not differ significantly between groups with normal bone mass and bone loss, thereby suggesting that PLR is insensitive to alterations in bone condition following trauma ($p > 0.05$) (Jingyu et al., 2024). Although studies correlates PLR with the bone remodeling process in fracture patients remain limited, several studies in trauma patients have demonstrated clinical associations. One study reported that a low baseline PLR value during the early management of severe trauma patients was associated with increased mortality ($p = 0.007$; OR= 0.994; 95% CI: 0.990–0.998) (Lee, 2020). Overall, these findings indicate that data regarding NLR and PLR within the context of bone healing remain heterogeneous and limited; thus, these research outcomes must be interpreted with caution.

This study has several limitations, including its retrospective design, which could not account for all potential confounding factors. Additionally, NLR and PLR measurements were performed only once, thereby failing to capture the longitudinal dynamics of inflammation. The evaluation of hard callus formation was also limited to conventional radiography. Furthermore, the small sample size restricted to closed long bone fractures limits the generalizability of these findings. Moreover, as markers of systemic inflammation, NLR and PLR lack specificity for the process of osteogenesis, which likely explains the absence of a consistent association with bone remodeling. Therefore, future studies should consider utilizing a prospective design with serial measurements, alongside more bone-specific biomarkers such as bone-specific alkaline phosphatase or osteocalcin.

CONCLUSION

Based on the findings of this study on patients with closed long bone fractures, no significant association was found between either the neutrophil-to-lymphocyte ratio (NLR) or the platelet-to-lymphocyte ratio (PLR) and hard callus formation. Neutrophil, lymphocyte, and platelet counts, along with NLR and PLR values, demonstrated no significant differences between the groups with and without hard callus formation. Furthermore, both logistic regression and ROC analyses indicated low predictive value regarding the bone healing process.

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