RESEARCH ARTICLE

pISSN: 0126-074X | eISSN: 2338-6223
https://doi.org/10.15395/mkb.v57.4161
Majalah Kedokteran Bandung. 2025;57(4):336–343

Majalah Kedokteran Bandung (MKB)

Received: November 4, 2024
Accepted: December 8, 2025
Available online: December 31, 2025

Correlation of Leukocytes, Neutrophil-To-Lymphocyte Ratio, C-Reactive Protein, and Serum Lactate with Amputation Incidence in Diabetic Foot Ulcer Patients

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Abstract

Diabetic foot ulcers are a major cause of approximately 85% of amputations in non-trauma patients. One of the contributing factors is the presence of extensive gangrenous infections. Effective management of diabetic foot infections to prevent amputation requires knowledge of various factors such as ulcer location, depth, neuropathy, vasculopathy, infection, and the presence of infection, which can be identified through inflammatory markers. This retrospective cohort study aimed to evaluate the correlation between leukocyte count, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), and serum lactate levels with the incidence of amputation in DFU patients. All DFU patients admitted to Dr. Hasan Sadikin General Hospital, Bandung, from January 1 to December 31, 2023, who met the inclusion criteria were included using purposive sampling. A total of 47 patients with diabetic foot ulcers were included in this study. The mean, median, and range of leukocytes were 18,532, 16,015, and 55,184, respectively, with higher values observed in amputation cases. NLR values were also higher in amputation cases, with a mean of 9.54, median of 6.00, and range of 47.32. Similarly, CRP levels were elevated in amputation cases, with a mean of 9.56, median of 6.75, and range of 26.16. There is a significant correlation between leukocytes, NLR, CRP, and serum lactate with the incidence of amputation in diabetic foot ulcer patients.

Keywords: Amputation, C-reactive protein, leukocytes, neutrophil-to-lymphocyte ratio, serum lactate

Introduction

Diabetes Mellitus (DM) is a chronic condition with a rising global prevalence, leading to significant morbidity and mortality.[2] One of its most severe and common complications is diabetic foot ulcer (DFU), which is primarily caused by peripheral neuropathy and peripheral artery disease (PAD).[3] In Indonesia, infection is a common factor exacerbating DFU cases, with studies showing high rates of sepsis and gram-negative infections among patients.[5,6] Inadequate management of DFU significantly increase the mortality risk and often result in amputation, which underscores the urgent need for improved diagnostic and treatment strategies.

Previous studies have identified infection and PAD as significant contributors to DFU complications. Research in Indonesia has highlighted sepsis and gram-negative microorganisms as primary factors worsening DFUs, with infection rates exceeding 68% in some hospitals.[5,6] A review published in 2022 reported that PAD was present to 50–70% of DFU.[7] Additionally, comprehensive assessment of DFU characteristics, such as ulcer location, depth, neuropathy, vasculopathy status, and inflammatory markers, has been explored to guide interventions.[8,9] However, specific biomarkers that predict the risk of amputation remain underutilized, particularly in Indonesian healthcare settings.

Inflammatory and metabolic biomarkers such as leukocyte count, neutrophil-to-lymphocyte ratio (NLR), C-reactive protein (CRP), and serum lactate levels have shown potential value in reflecting infection severity, systemic inflammation, and tissue hypoperfusion. These

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biomarkers are routinely available, relatively inexpensive, and easily integrated into clinical workflows.[10] Despite their practicality, evidence regarding their combined predictive value for amputation risk in DFU patients is still insufficient, especially within local healthcare settings.

The objective of this study was to determine the relationship between leukocyte count, NLR, CRP, and serum lactate levels and the incidence of amputation among DFU patients at Dr. Hasan Sadikin General Hospital. This study highlights the novelty of evaluating commonly available biomarkers as predictors of amputation risk, aiming to strengthen early risk stratification and support preventive strategies to improve clinical outcomes in DFU management.

Methods

This observational analytical study used a retrospective cohort design. Purposive sampling was carried out to include all DFU patients aged 18 years or older who were hospitalized at Dr. Hasan Sadikin General Hospital, Bandung, from January 1 to December 31, 2023, and had complete medical record documentation of leukocyte count, NLR, CRP, and serum lactate levels. The exclusion criterion was incomplete patient records. Secondary data were obtained by the researchers from the medical records of DFU patients treated at Dr. Hasan Sadikin Hospital, Bandung, who had received clinical evaluations and treatments aligning with study requirements. Both independent and dependent variables were examined in this study. The independent variables were leukocyte count, NLR, CRP, and serum lactate levels, while the dependent variable was amputation occurrence in DFU patients. Operational definitions were established to clarify each variable. Diabetic foot ulcer was defined as a full-thickness skin lesion distal to the ankle in diabetic patients, classified using the PEDIS system. Amputation was described as the total loss of a body part in the transverse anatomical plane of the lower extremity, further categorized as major (above the ankle) or minor (below the ankle). Leukocyte count represented the white blood cell components recorded in the medical records (in 10^3/mL). NLR was calculated by dividing the neutrophil count by the lymphocyte count, CRP was noted as a protein produced in response to inflammation (measured in mg/L), and serum lactate as a byproduct of anaerobic glucose metabolism (measured in mg/dL). Additional variables included gender (male or female) and age (in years).

Data were extracted using a standardized abstraction form. Statistical analyses were conducted using SPSS version 22.0. The Kolmogorov-Smirnov test was used to assess the normality of numerical variables, with a p-value greater than 0.05 indicating a normal distribution. Statistical analysis was performed to examine the relationship between leukocyte count, NLR, CRP, and serum lactate levels with amputation outcomes. ANOVA tests were applied to normally distributed data, while the Kruskal-Wallis test was used for non-normal distributions. Statistical significance was set at $p<0.05$, and Pearson or spearman correlation tests were applied to evaluate the strength of associations. Ethical approval was obtained from the Health Research Ethics Committee of Dr. Hasan Sadikin General Hospital (DP.04.03/D. XIV.6.5/223/2024).

Results

A total of 47 subjects met the inclusion criteria without meeting exclusion criteria, as detailed in Table 1. The data analysis indicated a distribution of sample characteristics based on age, gender, diabetes duration, neuropathy, PAD and ABI, sepsis, organ failure, HbA1C levels, length of hospital stays, and amputation occurrence. The mean ages of patients without amputation, those who underwent minor amputation, and those who underwent major amputation were 58, 55, and 57 years, respectively. Males were more commonly associated with major amputation cases, while females were more prevalent among cases without amputation. Among patients with a diabetes duration of less than 5 years, 7 did not undergo amputation, while 10 underwent major amputation. Of those with diabetes for more than 5 years, 12 did not undergo amputation, 6 underwent minor amputation, and 12 underwent major amputation. Neuropathy was found to be more prevalent among amputated patients, with 6 cases in minor amputations and 22 cases in major amputations (Table 1).

In this study, 37 patients experienced PAD, with 20 undergoing major amputation, 5 undergoing minor amputation, and 12 not requiring amputation. The average ABI values for non-amputated patients, minor amputations, and major amputations were 0.94, 0.88, and 0.81, respectively. A total of 33 patients developed

Table 1 Demographic Characteristics of the Study Population

Characteristic	No Amputation (n=19)	Minor Amputation (n=6)	Major Amputation (n=22)
Age			
Average	58	55	57
Median	58	60	57
Range	33–84	34–66	37-90
Gender			
Male	7 (36.8%)	2 (66.7%)	14 (63.6%)
Female	12 (63.2%)	4 (33.3%)	8 (36.4%)
Diabetes Duration			
<5 years	7 (36.8%)	0 (0%)	10 (45.5%)
>5 years	12 (63.2%)	6 (100%)	12 (54.5%)
Neuropathy			
No	10 (52.6%)	2 (66.7%)	9 (41%)
Yes	9 (47.4%)	4 (33.3%)	13 (59%)
PAD			
No	7 (36.8%)	1 (16.67%)	2 (9%)
Yes	12 (63.2%)	5 (83.33%)	20 (91%)
ABI			
Average	0.941	0.88	0.81
Median	1	0.98	0.83
Range	0.6-1.6	0.7-1	0.4-1.3
Sepsis			
No	10 (52.6%)	0	4 (18.18%)
Yes	9 (47.4%)	6 (100%)	18 (81.82%)
Organ Failure			
No	14 (73.68%)	5 (83.33%)	14 (63.63%)
Yes	5 (26.32%)	1 (16.67%)	8 (36.37%)
HbA1C Level			
Average	6.34	6.41	6.27
Median	6.4	6.3	6.4
Range	4.6-7.6	4.7-8.4	5.1-8.8
Length of Stay			
Average	5.88	6.09	6.38
Median	5	6	6
Range	3-9	4-11	3-12

sepsis; of these, 18 underwent major amputation, 6 underwent minor amputation, and 9 did not require amputation. Organ failure was observed in 14 patients, of whom 8 underwent major amputation, 1 underwent minor amputation, and 5 did not undergo amputation. The mean HbA1C values for the sample were 6.34, 6.41, and 6.27 in the non-amputated, minor amputation, and

Table 2 Relationship and Correlation of Leukocytes, NLR, Serum Lactate, and CRP With Amputation

Inflammatory Marker	No Amputation (n=19)	Minor Amputation (n=6)	Major Amputation (n=22)	r	p-value
Leukocytes				0.447**	<0.001
Average	13,658	17,577	23,434		
Median	12,340	24,454	54,330		
Minimum	3,960	1,976	2,830		
Maximum	39,550	26,430	57,160		
NLR				0.292**	<0.009
Average	6	13	12		
Median	5	12	9		
Minimum	1	4	1		
Maximum	18	23	48		
Serum Lactate				0.309**	<0.006
Mean	2	4	4		
Median	2	2	3		
Minimum	1	1	1		
Maximum	9	15	13		
CRP				0.319**	<0.004
Mean	7	10	12		
Median	5	10	12		
Minimum	0	3	0		
Maximum	24	24	26		

*CRP: c-reactive protein; NLR: neutrophil-to-lymphocyte ratio

major amputation groups, respectively. Length of stay averaged 5.88 days for patients without amputation, 6.09 days for patients with minor amputations, and 6.38 days for those with major amputations.

Higher leukocyte levels were observed in patients who underwent amputation compared with those who did not, with the highest values identified in the major amputation group. Correlation analysis demonstrated a statistically

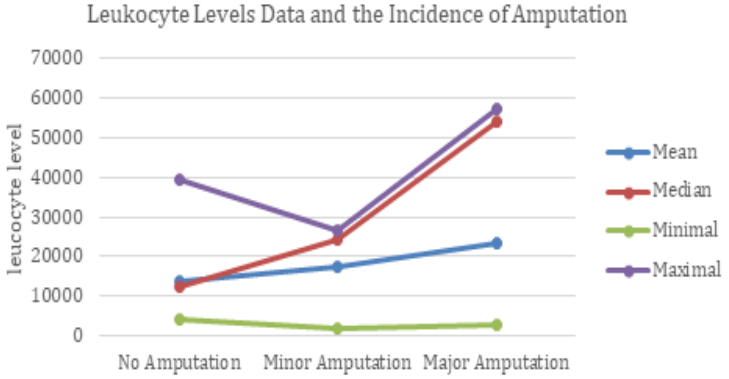

Figure 1 Leukocyte Levels and the Incidence of Amputation

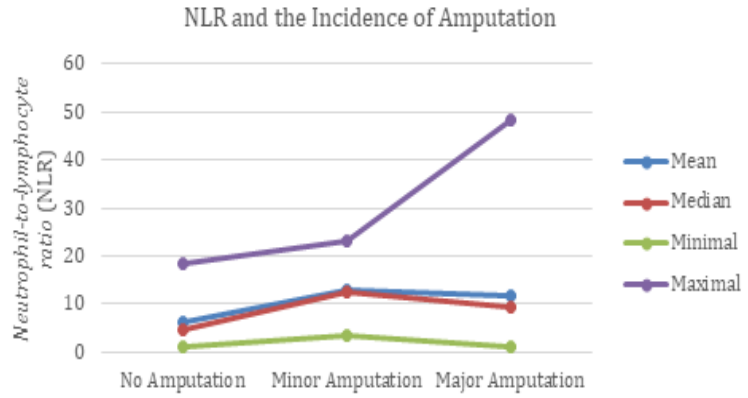

Figure 2 Neutrophil-to-Lymphocyte Ratio (NLR) and the Incidence of Amputation

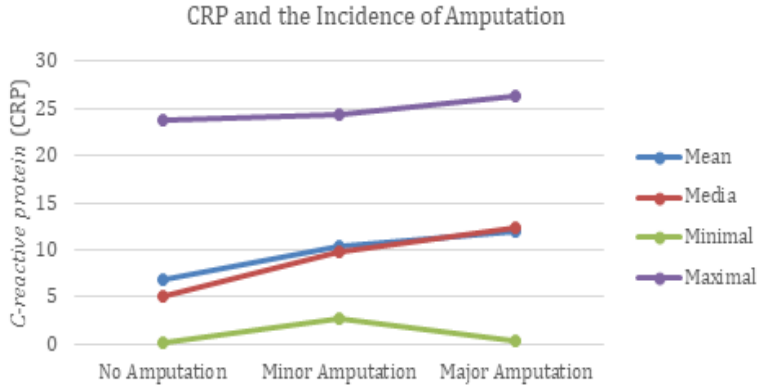

Figure 3 C-Reactive Protein (CRP) Levels and the Incidence of Amputation

significant positive association between leukocyte count and amputation severity. These findings indicate an increasing inflammatory response corresponding to the extent of tissue loss (Table 2, Figure 1).

Elevated neutrophil-to-lymphocyte ratio values were observed in amputated cases compared with non-amputated cases. A significant positive correlation was identified between neutrophil-to-lymphocyte ratio and

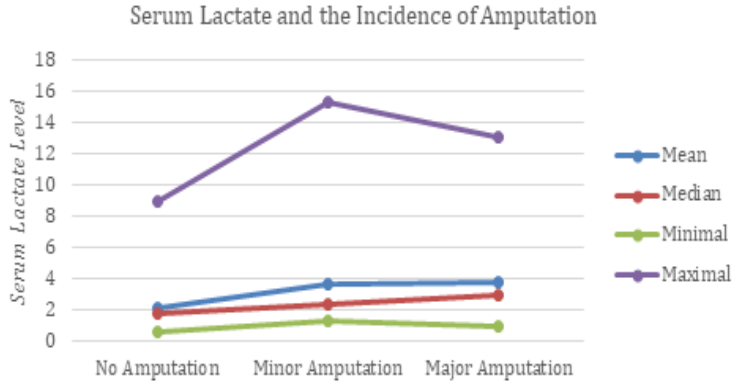

Figure 4 Serum Lactate Levels and the Incidence of Amputation

amputation severity, supporting its association with worsening clinical outcomes (Table 2, Figure 2).

C-reactive protein levels were higher among patients who underwent amputation, with increasing values corresponding to greater amputation severity. Correlation analysis confirmed a significant positive association between C-reactive protein levels and amputation status, reflecting heightened systemic inflammation in more severe cases (Table 2, Figure 3).

Serum lactate levels were elevated in patients undergoing amputation compared with those without amputation. Higher serum lactate concentrations were associated with increasing amputation severity, and correlation analysis demonstrated a significant positive relationship between serum lactate levels and amputation outcomes (Table 2, Figure 4).

Discussion

Lower extremity amputation represented one of the most feared complications for diabetic patients. Both minor and major amputations served as surgical procedures intended to remove damaged tissue and optimize outpatient rehabilitation for patients with diabetic foot infections. Major lower extremity amputation was defined as an amputation above the ankle joint, while minor amputation preserved the ankle joint. In this study, the incidence rate of amputation was 28 cases (55.3%), comprising 22 cases of major amputation and 6 of minor amputation. This result aligned with previous studies showing that major amputation was more common than minor.[11,12] The higher frequency of major amputation may be related to delayed presentation, extensive infection, and widespread gangrene, which are frequently reported in settings with a high prevalence of diabetes-related complications.[12] Nonetheless, this finding remained uncertain, as other factors could influence the incidence of amputation.

Elevated leukocyte counts were more frequently observed among patients who underwent either minor or major amputation. This finding corresponded with research conducted in Malaysia, which indicated that leukocytosis was present in up to 66.6% of amputated patients.[13] These results also demonstrated a significant association between leukocytosis and amputation incidence (p=0.001). Leukocyte levels in the blood could reflect the severity of infection in diabetic foot ulcers, potentially increasing amputation rates.[14] However, other studies with univariate analysis suggested that leukocytosis did not significantly affect amputation rates.

The neutrophil-to-lymphocyte ratio (NLR) showed a significant association with amputation incidence, with higher mean values observed in amputated patients compared with non-amputated cases. Elevated NLR reflects an imbalance between inflammatory activation and immune regulation, indicating more severe infection and tissue injury. This result is consistent with previous findings demonstrating that higher NLR values are associated with poorer healing outcomes and increased amputation risk in DFU patients. [15–20] Variations in proposed NLR cutoff values across studies suggest the need for population-specific thresholds and further validation.

C-reactive protein (CRP) levels were also higher among patients undergoing both minor and major amputations. This observation aligns with previous studies reporting a strong association between elevated CRP levels and DFU severity and infection.[21,22] As an acute-phase reactant, CRP reflects systemic inflammation and bacterial burden, which may contribute to progressive tissue destruction and the need for surgical intervention. Differences in reported CRP levels among studies may result from heterogeneity in infection grading, timing of laboratory measurements, and patient characteristics.

The mean serum lactate level was found to be 3.04 mmol/L. In cases of amputation, the mean levels were 3.62 mmol/L for minor amputations and 3.74 mmol/L for major amputations, which were higher than the mean serum lactate level of 2.16 mmol/L in non-amputated cases. A study conducted by Reitz et al. found that subjects with a mean serum lactate level of 4.6 mmol/L faced a higher risk of amputation compared to a mean level of 3.1 mmol/L, with a significant p-value of <0.001.[22] Serum lactate served as a sensitive but nonspecific indicator of metabolic stress, produced through anaerobic glycolysis. Serum lactate levels increased during hypoxia, stress, and critical illness. Many bacteria associated with infected wounds used fermentation to generate energy, a metabolic process that increased wound organic acid levels, including lactate.[22] A study by Loffler et al. indicated significantly higher serum lactate levels in DFU patients with infections compared to those without (p=0.001).[23]

The retrospective cohort design limited

control over exposure timing and restricted assessment of temporal causality to information available in medical records. The small sample size may reduce generalizability, and confounding factors such as glycemic control, comorbidities, and diabetes duration were not accounted for. Additionally, the anatomical classification of amputations did not consider functional outcomes or quality of life.

In conclusion, elevated leukocyte count, NLR, CRP, and serum lactate levels are significantly associated with the incidence of minor and major amputations in patients with diabetic foot ulcers. These biomarkers can serve as practical tools for assessing amputation risk and guiding clinical decisions. Early identification and targeted management of patients with elevated biomarker levels could improve outcomes and reduce the burden of amputation among DFU patients.

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