BMC Endocrine Disorders

RESEARCH

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Neutrophil-lymphocyte ratio (NLR); an accurate inflammatory marker to predict diabetic foot ulcer amputation: a matched case-control study

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Abstract

Background Diabetic foot ulcer (DFU) is a well-known complication of diabetes. The main therapeutic options for treating DFU include surgical debridement. However, conditions such as sensory loss and insufficient blood supply can lead to lower extremity amputations. Inflammatory biomarkers, including the neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR), have shown promise in predicting the development of diabetes complications.

Methods This study included 126 individuals with known DFUs who underwent amputation or debridement surgery during hospitalization between January 2017 and December 2022. The participants were divided into two groups, each containing 63 patients, based on the treatment they received. Analyses were conducted via univariate and multivariate regression models. The linearity of the relationship between each inflammatory index and the risk of amputation was further examined via restricted cubic spline (RCS) curves with four knots.

Results Categorical regression analysis showed an elevated risk of amputation in patients with an NLR greater than 6.08, with an OR of 13.090 (95% CI: 5.143–33.320, $P<0.001$), compared with those with an NLR less than 6.08. Additionally, patients with a PLR greater than 210 demonstrated a similarly elevated risk of amputation with an OR of 2.31 (95% CI: 1.066–4.669, $P=0.033$); however, those with lymphocyte–white blood cell ratio (LWR) levels of greater than 0.1265 exhibited reduced likelihood of having amputation (OR: 0.092 (95% CI: 0.038–0.226, $P<0.001$)).

Conclusions This study supports that NLR, PLR and LWR may have value as a predictive marker for amputation in patients with DFUs.

Keywords Diabetic foot ulcer, Neutrophil–lymphocyte ratio, Platelet–lymphocyte ratio, Lymphocyte–white blood cell ratio

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Introduction

Diabetes, a metabolic disorder characterized by chronic hyperglycemia, affects an estimated 9.3% of adults globally [1]. Its prevalence is projected to rise to 10.9% by 2045, with a steep increase of 150% between 2000 and 2035 expected in South Asian countries [2, 3]. Chronic hyperglycemia induces systematic inflammatory reactions that can lead to several consequences, such as micro- and macrovascular complications, peripheral neuropathy, and diabetic foot ulcer (DFU) [4–6]. Among these, DFU poses a significant challenge to both patients and healthcare systems [7, 8].

DFU causes can be categorized into neuropathic, ischemic, and neuro-ischemic causes. Moreover, peripheral arterial disease (PAD) occurs in approximately 50% of patients with DFUs [7]. Among individuals with diabetes, DFU is among the most common causes of hospitalization and can result in disability and death [9, 10]. The global lifetime risk of developing a DFU in patients with diabetes is estimated to be 15–25%, with approximately 18.6 million people affected annually [11–13]. A multidisciplinary approach is warranted to decrease undesirable outcomes such as amputation and death [14]. The main therapeutic options for DFU include surgical debridement, relieving weight-bearing pressure on the ulcers, and treating foot infections [1]. However, conditions such as sensory loss, insufficient blood supply due to arterial impairment, and dysfunctions in foot biomechanics can lead to lower extremity amputations (LEAs) [15, 16]. The surgical debridement procedure includes the excision of necrotic and infected tissues. LEA might be an option in patients who have PAD and severe progressing infection and in patients with insufficiently controlled diabetes followed by chronic ischemia who have unsuccessful angioplasty surgery [17, 18].

Recent research has focused on inflammatory biomarkers as potential predictors of DFU outcomes. Defects in glucose metabolism augment reactive oxygen species production, leading to lymphocyte dysfunction and an accelerated apoptosis cascade [2]. Despite this impairment, lymphocytes play a crucial modulatory role in the inflammatory response by inducing interleukin-10 expression, which could contribute to tissue repair processes [19]. The neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) are inflammatory biomarkers that can predict mortality risk in populations with cardiovascular disease (CVD) and cancer [20–22]. Moreover, these ratios have shown promise in predicting the development of diabetes complications [23].

The mechanisms underlying the predictive value of these biomarkers are multifaceted. Elevated PLRs are associated with increased secretion of mediators associated with atherosclerosis, consequently increasing the risk of thrombosis and inflammation due to increased platelet activity. Similarly, an elevated NLR may contribute to endothelial impairment and dysfunction through increased neutrophil activity [2]. Notably, the NLR can be used as an independent predictor of wound healing [24]. Postoperative values of the PLR and NLR can be considered reliable predictors of mortality in patients with DFU who undergo LEA [2].

Another biomarker of interest is the lymphocyte–white blood cell ratio (LWR), which reflects systemic inflammatory reactions. A decreased LWR can be considered either an impaired or enhanced immune response [25]. Mean platelet volume (MPV) represents the average platelet volume and activity. Larger platelets, which are typically younger and more active [26], may play a considerable role in diabetes complications. Diabetes can induce hyperactivity of platelets through various mechanisms, such as high blood sugar, dyslipidemia, insulin resistance, and oxidative and inflammatory states [27]. Increased platelet activity induces the development of diabetes-related vascular complications, including DFU. The platelet volume is influenced by the pathogenic factors that lead to DFUs. A high MPV indicates more potent platelet release, leading to more thrombogenic conditions that enhance DFU development and subsequent amputation risk [28].

LEA occurs ten times more frequently in patients with diabetes than in individuals without diabetes [29]. Moreover, the risk of mortality in the population with diabetes who have experienced LEA is two to three times greater than that in those without LEA [4]. The economic burden is equally substantial, with a total of $41 billion, accounting for approximately 1,6% of all medical healthcare expenditures, attributed to individuals with diabetes and lower extremity wounds in the United States Medicare program in 2012 [30]. Given the significant clinical and economic impacts of DFUs and their complications, there is a pressing need for accessible and simple parameters to assess the severity of DFUs and predict outcomes. The NLR and PLR are systemic inflammation markers that can be acquired from routine blood tests, such as complete blood count (CBC), and can be applied in clinical practice. The present study aims to compare the NLR, PLR, LWR, and MPV in a population with DFU who underwent LEA or experienced surgical debridement, potentially identifying valuable predictors of amputation risk in this group.

Method

Study design

Retrospective data were obtained from 126 individuals with known DFUs referred to a university hospital from 2017 to 2022. Participants were identified from the hospital's electronic medical records using the ICD-10 diagnosis code E11.621 (DFU). Additional verification was

performed through manual review of clinical charts. Patients were initially selected on an alternating basis depending on whether they underwent amputation or debridement during hospitalization. Following this selection, adjustments were made for age and sex to minimize confounding effects. The two groups were adjusted for age and sex to ensure comparability but were not strictly matched in a 1:1 manner, and each contained 63 patients. This study included individuals with known DFUs who were hospitalized due to complications such as severe infection, gangrene, ischemia, or failure of outpatient wound management. Some patients required urgent surgical intervention due to disease progression. All patients underwent either LEA, which could be categorized as either major (above the ankle) or minor (below the ankle) amputation, or debridement during hospitalization. The participants were older than 18 years. Diabetes was diagnosed based on criteria outlined by the American Diabetes Association (ADA) [31]. DFU is described as a defect that involves all layers of skin and needs at least 2 weeks to heal [32]. DFU diagnosis was based on clinical features (edema, erythema, pain, tenderness, wound discharge, warmth, and foul odor). The size, location, depth, and color were determined via physical examination. While some of these signs suggest infection, this study included both infected and noninfected DFUs. The presence of infection was determined on the basis of additional clinical and microbiological assessments.

The decision to perform amputation or debridement was based on the severity of the ulcer, presence of infection, vascular status, and overall prognosis.

The surgical debridement procedure included the excision of necrotic and infected tissues along with the daily use of cotton gauze moistened with saline. Debridement of the involved tissue was continued until bleeding was obtained from a healthy base.

After identifying patients admitted to hospital with DFUs in the study-period, the following exclusion criteria were applied; the presence of malignancy, transfusion history within past three months, end stage renal disease (ESRD), autoimmune disease, pregnancy, hematologic disorders, simultaneous infection in other organ systems, and consumption of antibiotics or glucocorticoids within the past four weeks before admission. (Fig. 1)

This study was conducted in accordance with the principles of the Helsinki Declaration. The hospital's ethics committee approved the study protocol. (Ethic code: IR.TUMS.IKHC.REC.1399.507). All patients had previously provided general consent for their medical data to be used for research purposes upon hospital admission.

Data collection and laboratory analysis

Patient data were collected from medical records at initial admission. The data included demographic characteristics (age, sex, smoking history), duration of hospitalization, and type of therapeutic intervention (debridement or amputation). Clinical parameters such as fasting blood sugar (FBS) and hemoglobin A1c (HbA1c) were also documented.

As part of routine clinical practice, anthropometric measurements, including blood pressure, temperature, and pulse rate, were recorded at the time of admission by hospital staff. These data were retrospectively extracted from medical records. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured via a standard mercury sphygmomanometer after 10 min of rest in a seated position. The pulse rate was recorded by counting the number of beats for 60 s, and the temperature was measured using a calibrated thermometer held under the tongue for 30 s to 1 min. All laboratory measurements were also retrospectively collected from patient records. The Quetelet formula was utilized to assess body mass index (BMI; kilograms per square meter).

Approximately 10 ml of fresh blood sample was obtained from patients following ten to twelve hours of overnight fasting and was assessed via kits certified by the central reference laboratory. Whole blood was used for the estimation of FBS, HbA1c, and CBC. HbA1c levels were measured with high-performance liquid chromatography (DS5 Pink Kit; Drew, Marseille, France). To calculate the FBS content, calorimetry methods were carried out along with the glucose oxidase test. To measure CBC, fresh blood was collected in 1.5 mg/ml tubes containing ethylenediamine tetraacetic acid (EDTA), and an automatic cell counter machine (XT-1800i model, Sysmex, Japan) was used. Lymphocyte and neutrophil counts were estimated via total white blood cells (WBC) count and differential percentages and were reported as both total count and percentage. The PLR was estimated by dividing the platelet count by the lymphocyte count, whereas the NLR was measured by dividing the neutrophil count by the lymphocyte count. The MPV was calculated as the ratio of the plateletcrit (PCT) to the total platelet count. The ratio of the lymphocyte count to the total WBC count was presented as the LWR.

Statistical analyses

All the statistical analyses were carried out via R software (version 4.2.3, R Foundation for Statistical Computing, Vienna, Austria) and IBM SPSS software version 24.0 (SPSS Inc., Chicago, Illinois, USA). P values less than 0.05 were considered statistically significant. To assess the normal distribution of the study population, Shapiro–Wilk tests, p-p tests, plots, and histograms were applied. Continuous variables were expressed as the median (First quartile, third quartile) (Q1, Q3) for variables without a normal distribution and means ± standard deviations (SD) for variables with a normal distribution. The

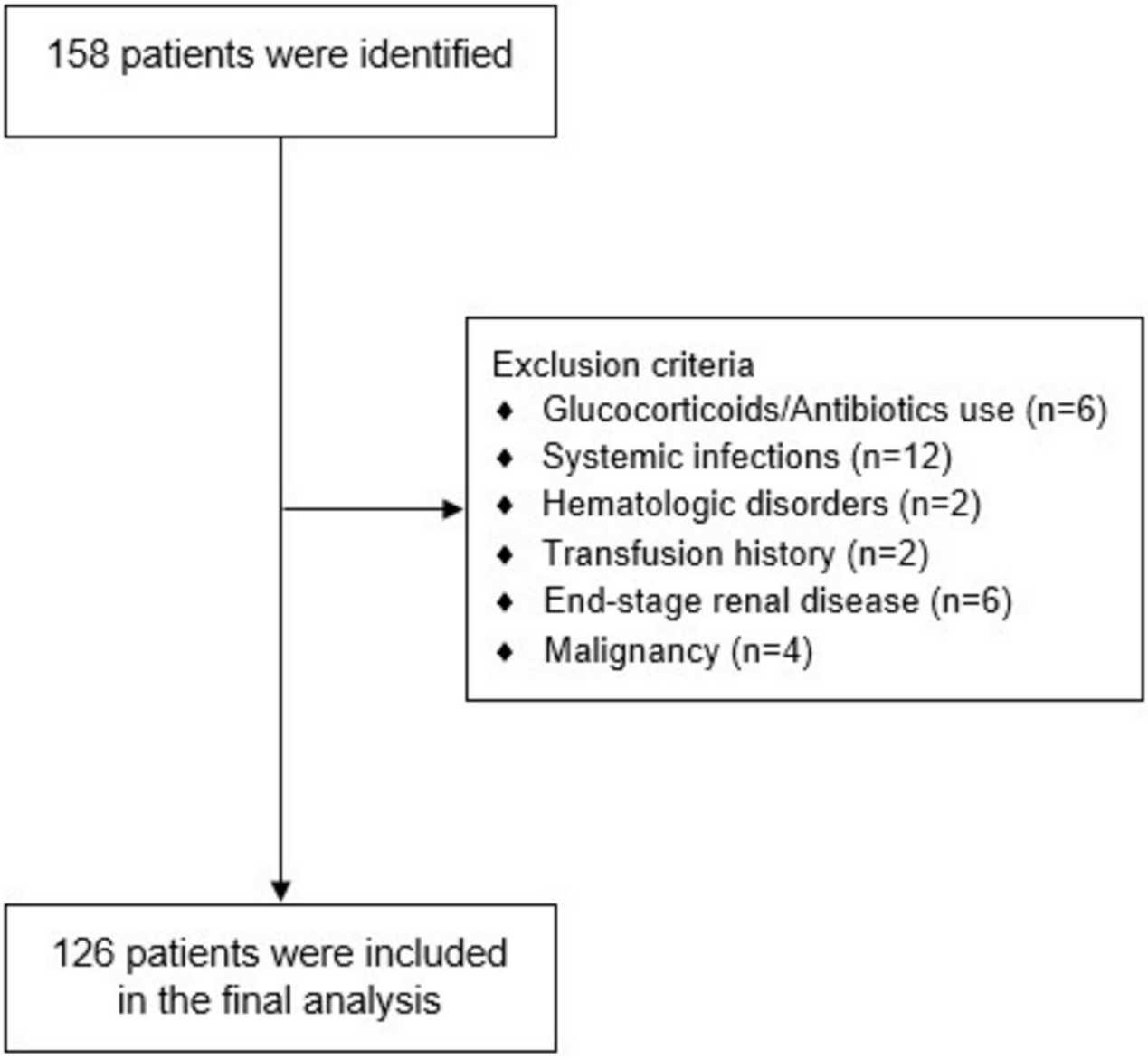

Fig. 1 Flowchart representation of the patient selection process (PRISMA flowchart)

normally distributed variables were compared within the two studied groups via the t-test, and for variables without a normal distribution, the Whitney U test was applied. Categorical variables were presented as frequencies or percentages, and to evaluate their relationships, the chi-square test was applied. Violin plots were also designed to show the distributions of the NLR, PLR, MPV, and LWR variables between the two study groups, including those planned for amputation and those planned for debridement. Additionally, receiver operating characteristic (ROC) curves were employed to assess the predictive capability of the NLR, PLR, MPV, and LWR for amputation. The maximum Youden index was applied to identify the optimal threshold for each plot. Binary conditional logistic regression was employed to evaluate the odds ratios (ORs) and 95% confidence intervals (CIs) of the inflammatory indices for amputation plans in individuals with DFUs. Inflammatory values within a normal range were considered a reference, with an odds ratio of 1.00. Analyses were conducted via univariate and multivariate regression models adjusted for gender, age, diabetes medications, BMI, duration of diabetes, and smoking status. The association between each inflammatory index and amputation was further examined via restricted cubic spline (RCS) model with four knots.

Results

Baseline characteristics

A total of 126 patients were identified for the current study, with women constituting 38.9% of the total population. Patients with extensive necrosis, critical limb ischemia, or severe, uncontrolled infection were more likely to undergo amputation. In contrast, patients with viable tissue, adequate blood supply, and a controlled infection were considered for debridement. The final decision was made by the attending surgical team on the basis of the patients' clinical judgment and condition.

Table 1 Baseline characteristics of the studied participants

Variable		Amputation group (*n* = 63)	Debridement group (*n* = 63)	*P* value
Age (years)		60.81 ± 11.21	57.05 ± 11.90	0.850
Female (%)		20(31.7%)	29(46%)	0.920
Duration of admission (days)		12(8–18)	9(7–15)	0.045
BMI (kg/m^2)		26.51 ± 0.74	26.30 ± 0.63	0.089
Duration of diabetes (years)		11.04 ± 1.53	10.58 ± 1.25	0.067
Pulse Rate (Beats per minute)		90(85–98)	90(80–100)	0.758
Temperature (°C)		37.3 (37–38)	37.5 (36.9–38)	0.895
Blood pressure (mmHg)	**SBP**	129.5 (110-142.50)	130 (110–140)	0.849
	DBP	74 (68–85)	75 (70–80)	0.792
FBS (mg/dL)		258 (168.75-348.25)	252 (165–351)	0.923
HbA1c (%)		9.6 (7.8-11.55)	9.5 (7.5–10.8)	0.774
WBC (cells/μL)		14,000 (10800–18700)	11,800 (8300–15300)	0.004
Neutrophil (%)		88.34%	77.44%	< 0.001
Neutrophil (Absolute)(cells/μL)		12368.5 (8586–16652)	9138.8 (5592-11735.1)	< 0.001
Lymphocyte (%)		8.63%	15.3%	< 0.001
Lymphocyte (Absolute) (cells/μL)		1208.4 (1027.2-1564.2)	1805.4 (1421.9-2318.8)	< 0.001
Platelet (10^3/μL)		327 (249–455)	321 (241–426)	0.563
MPV (fL)		9.3 (8.7–10.2)	9.45 (8.7–10.3)	0.776
NLR		9.41 (6.41–16.52)	5.53 (3.45–6.50)	< 0.001
PLR		245.37 (143.91–354.60)	175.90 (135.29-233.77)	0.008
LWR		0.0910 (0.0530–0.1260)	0.1430 (0.1200–0.2100)	< 0.001

Data are presented as the means ± SDs or medians (Q1, Q3) for continuous variables or as numbers (%) for categorical variables

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBS: fasting blood sugar; HbA1c: hemoglobin A1c; WBC: white blood cells; MPV: mean platelet volume; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; LWR: lymphocyte-to-WBC ratio; SD: standard deviation; Q1: first quartile; Q3: third quartile

The mean (± SD) age of the patients was 60.81 ± 11.21 years in the amputation group and 57.05 ± 11.90 years in the debridement group. The duration of admission was a median (Q1, Q3) of 12, (8, 18) days in patients who were amputated and 9 (7, 15) days in the debridement group ($p = 0.015$). Approximately 79.3% of patients received oral anti-diabetic drugs (OADs), followed by 7.9% OADs plus insulin and 12.8% insulin alone. There were no significant differences in medication use between the debridement and amputation groups. About 39% of patients in the amputation group had a history of smoking, which was not significantly higher than the 36.5% reported in the debridement group. The median levels of FBS and HbA1c were slightly greater in patients who were candidates for amputation than in those in the other group, the difference was, however, not statistically significant ($p = 0.923$ and $p = 0.774$, respectively). No significant differences were found in the values of SBP, DBP, temperature, or pulse rate between the two groups ($p = 0.849$, $p = 0.792$, $p = 0.895$, and $p = 0.758$, respectively). The baseline characteristics and laboratory findings of the patients in each category are presented in Table 1.

The laboratory findings in the amputation group, including WBC, neutrophil, lymphocyte, and platelet counts, had median (Q1, Q3) values of 14,000 (10800, 18700), 12368.5 (8586, 16652), 1208.4 (1027.2, 1564.2), and 327 (249–455) $\times 10^3$/μL, respectively. In comparison, the debridement group presented the following median (Q1, Q3) values: WBC: 11,800 (8300, 15300), neutrophil count: 9138.8 (5592, 11735.1), lymphocyte count: 1805.4 (1421.9, 2318.8), and platelet count: 321 (241–426) $\times 10^3$/μL. Significant differences in the WBC ($P = 0.004$), neutrophil ($p < 0.001$), and lymphocyte ($p < 0.001$) counts were detected between the debridement and amputation groups, whereas the platelet counts did not significantly differ between the two groups ($p = 0.563$).

Systemic inflammatory response markers, including the NLR, PLR, LWR, and MPV, were compared between the two groups, revealing significant differences in the NLR ($P < 0.001$) and PLR ($P = 0.008$), which were substantially greater in patients who underwent amputation. However, LWR ($p < 0.001$) was significantly higher in those with a debridement plan compared to the other group.

Violin plots were generated to visualize the distributions of the NLR (A), PLR (B), LWR (C), and MPV (D) in the two studied groups. The results indicated that the median (Q1, Q3) NLR was 9.41 (6.41, 16.52) in the amputation group and 5.53 (3.45, 6.50) in those with debridement plan (Fig. 2A). As shown in Fig. 2B and C, the median (Q1, Q3) PLR and LWR values were 245.37 (143.91, 354.60) and 0.091 (0.053, 0.126), respectively, in

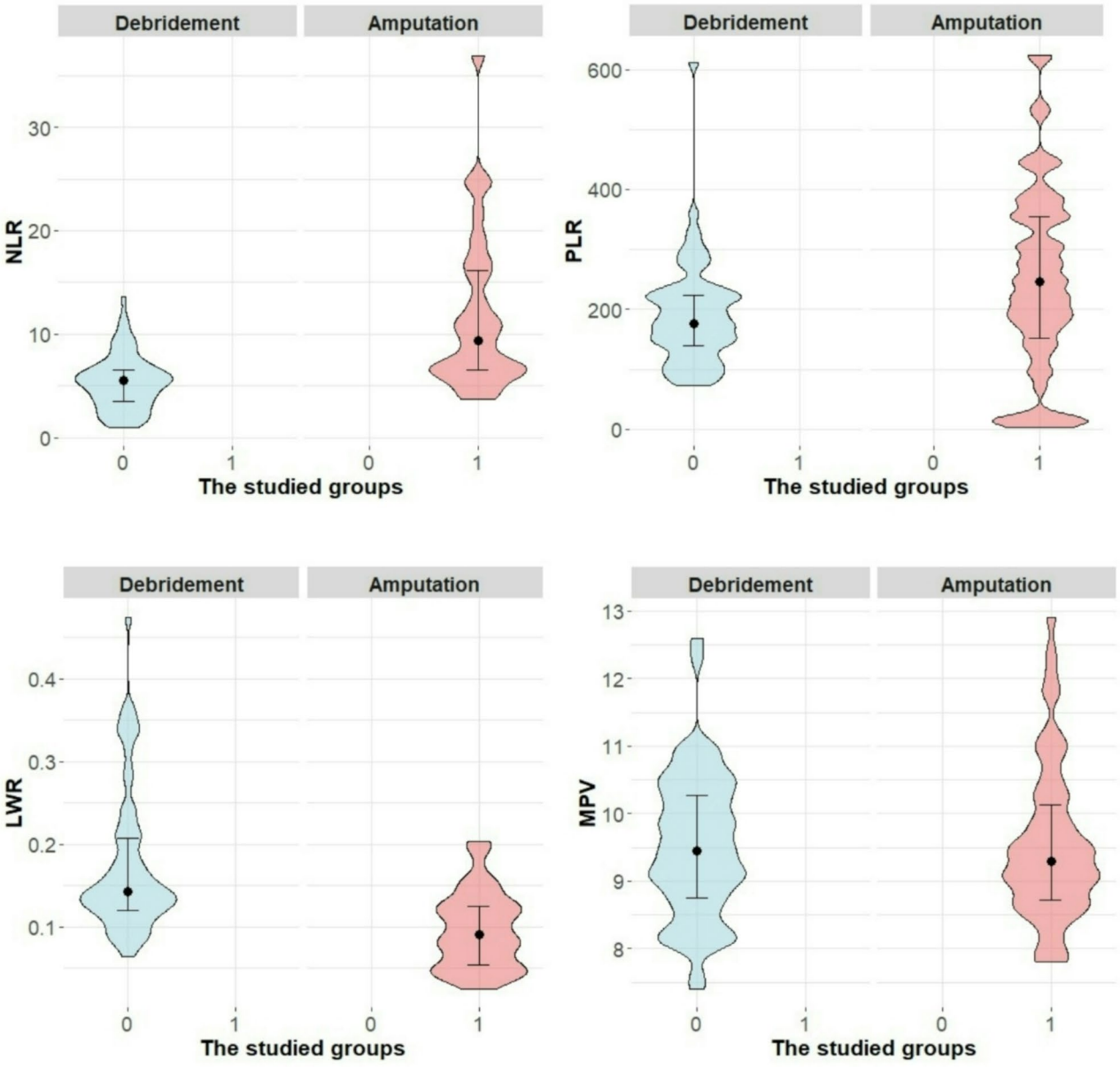

Fig. 2 Violin plots of the NLR, PLR, LWR, and MPV in the amputation group versus the debridement group. Violin plots were constructed to represent the distributions of the NLR (A), PLR (B), LWR (C), and MPV (D) in the amputation and debridement groups. The median (Q1, Q3) NLR was 9.41 (6.41, 16.52) in the amputation category and 5.53 (3.45, 6.50) in the debridement category (Fig. 2A). The group that underwent debridement had significantly lower PLRs but higher LWRs, with median (Q1, Q3) values of 175.90 (135.29, 233.77) and 0.1430 (0.1200, 0.2100), respectively (Fig. 2B and C). The median (Q1, Q3) MPV was 9.3 (8.7, 10.2) for patients who underwent amputation, whereas it was 9.45 (8.7, 10.3) for those who were treated by debridement (Fig. 2D). NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; LWR: lymphocyte-to-WBC ratio; MPV: mean platelet volume; Q1: first quartile; Q3: third quartile

the amputation group, whereas the debridement group had lower median (Q1, Q3) PLR [175.90 (135.29, 233.77)] and higher LWR values [0.143 (0.120, 0.210)]. Figure 2D shows the median (Q1, Q3) value of the MPV, which was 9.3 (8.7, 10.2) in the amputation group and 9.45 (8.7, 10.3) in the debridement group.

ROC curve analysis was performed to assess the diagnostic accuracy of the NLR, LWR, PLR, and MPV for predicting the need for amputation in patients with DFUs. (Fig. 3-Table 2) The area under the curve (AUC) values were 0.822 for NLR, 0.812 for LWR, 0.637 for PLR, and 0.515 for MPV. Among these systemic inflammatory response markers, the NLR demonstrated the highest discriminatory power for the need for amputation, with an optimal cutoff value of 6.08, a sensitivity of 82.5%, and a specificity of 70%. The optimal cutoff values for the other markers were as follows: LWR = 0.126 (sensitivity = 77.7%, specificity = 73%), PLR = 242.44 (sensitivity = 50.7%,

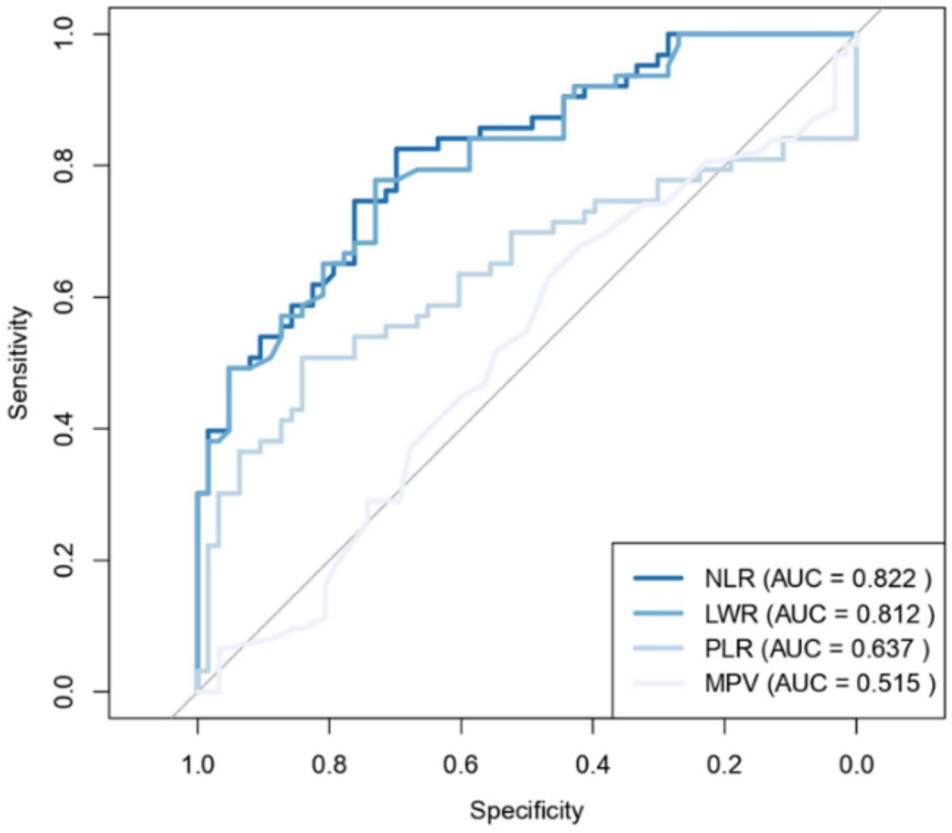

Fig. 3 ROC curves of the NLR, PLR, LWR, and MPV in predicting the need for amputation in patients with DFU

Table 2 ROC curve analysis of systemic inflammatory response markers for detecting the need for amputation in patients with DFU

	AUC	Specificity	Sensitivity	Cutoff
NLR	0.822	%70	%82.5	6.08
LWR	0.812	%73	%77.7	0.126
PLR	0.637	%84	%50.7	242.44
MPV	0.515	%50	%67.7	9.75

ROC: receiver operating characteristic; DFU: diabetic foot ulcer; NLR: neutrophil-to-lymphocyte ratio; LWR: lymphocyte-to-WBC ratio; PLR: platelet-to-lymphocyte ratio; MPV: mean platelet volume; AUC: area under the curve

specificity = 84%), and MPV = 9.75 (sensitivity = 67.7%, specificity = 50%).

The AUC values for the markers were as follows: NLR (0.822), LWR (0.812), PLR (0.637), and MPV (0.515). The NLR showed the strongest discriminatory ability for amputation necessity, with an optimal cutoff of 6.08, yielding a sensitivity of 82.5% and specificity of 70%. The optimal cutoff values for the other markers were 0.126 for the LWR, with a sensitivity of 77.7% and a specificity of 73%, 242.44 for the PLR, with a sensitivity of 50.7% and a specificity of 84%, and 9.75 for the MPV, with a sensitivity of 67.7% and a specificity of 50%.

ROC: receiver operating characteristic; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; LWR: lymphocyte-to-WBC ratio; MPV: mean platelet volume; DFU: diabetic foot ulcer; AUC: area under the curve.

Logistic regression analysis revealed a significant association between the odds of amputation and certain systemic inflammatory response markers, specifically the NLR and PLR. The unadjusted analysis revealed a substantial relationship between the NLR (OR: 1.436, 95% [CI]: 1.231–1.675, $P \leq 0.001$) and the PLR (OR: 1.004, 95% CI: 1.001–1.007, $P = 0.015$), with increased odds of amputation compared with debridement. This association remained significant after adjusting for potential confounding factors, including age, sex, diabetes medications, BMI, duration of diabetes, and smoking, with ORs of 1.509 (95% CI: 1.257–1.811, $P < 0.001$) and 1.004 (95% CI: 1.001–1.007, $P = 0.017$), respectively.

Categorical regression analysis further highlighted the elevated odds of amputation in patients with an NLR greater than 6.08, with an OR of 13.090 (95% CI: 5.143–33.320, $P < 0.001$), compared with those with an NLR less than 6.08. Additionally, patients with a PLR greater than 210 had significantly higher odds of amputation (OR: 2.231, 95% CI: 1.066–4.669, $P = 0.033$), whereas those with an LWR greater than 0.1265 had significantly

lower odds (OR: 0.092, 95% CI: 0.038–0.226, $P<0.001$) (Table 3).

The RCS models revealed that an NLR higher than 6.08 (A), a PLR greater than 210 (B), and an LWR less than 0.1265 (C) were associated with an increased likelihood of requiring amputation in patients with DFUs (Fig. 4). Each RCS model incorporated four knots, which were determined on the basis of the distribution of the associated variable. The reference values for these curves were established as follows: NLR = 6.08; PLR = 210; LWR = 0.1256; and MPV = 9.8.

Discussion

This study demonstrated that patients who underwent amputation had significantly higher NLR and PLR values and lower LWR values compared to those who underwent debridement. These findings suggest that systemic inflammatory markers may be associated with the severity of DFUs and the likelihood of requiring amputation. However, the MPV was not significantly associated with DFU outcomes in the current study.

Table 3 Association of systemic inflammatory response markers with the odds of amputation

		OR	95% CI	*P* value
Unadjusted				
MPV		1.020	(0.740–1.407)	0.902
NLR		1.436	(1.231–1.675)	< 0.001
PLR		1.004	(1.001–1.007)	0.015
LWR		0.148	(0.062–0.304)	< 0.001
Adjusted*				
MPV		1.053	(0.757–1.464)	0.760
NLR		1.509	(1.257–1.811)	< 0.001
PLR		1.004	(1.001–1.007)	0.017
LWR		0.151	(0.063–0.310)	< 0.001
Cutoffs		**OR**	**95% CI**	**P value**
MPV in category	MPV < 9.8	1.00 (reference)	-	-
	MPV ≥ 9.8	0.704	(0.330–1.502)	0.364
NLR in category	NLR < 6.08	1.00 (reference)	-	-
	NLR ≥ 6.08	13.090	(5.143—33.320)	< 0.001
PLR in category	PLR < 210	1.00 (reference)	-	-
	PLR ≥ 210	2.231	(1.066–4.669)	0.033
LWR in category	LWR < 0.1265	1.00 (reference)	-	-
	LWR ≥ 0.1265	0.092	(0.038–0.226)	< 0.001

* Adjusted for age, sex, diabetes medications, body mass index, duration of diabetes, and smoking status

OR: odds ratio; CI: confidence interval; MPV: mean platelet volume; NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; LWR: lymphocyte-to-WBC ratio

The significant association between the NLR and the need for amputation in DFUs is consistent with previous research highlighting the crucial role of inflammation in diabetic foot complications [33–40]. Yüce et al. reported that a high preoperative NLR was significantly associated with increased 1-year mortality in patients who underwent amputation due to diabetic foot complications [38]. Similarly, Xu et al. reported that higher NLR values were significantly correlated with worse prognoses, including higher rates of major amputations and mortality in patients with DFU [35]. Moreover, Zhu et al. identified the NLR as an independent risk factor for diabetic ulcer-related amputation, indicating that patients with higher NLR values had a lower amputation-free survival rate [40]. Demirdal and Sen also highlighted that the NLR, along with other inflammatory markers, could predict the need for amputation in diabetic foot infections (DFIs) [33]. The NLR demonstrated the highest AUC (0.822) in the ROC analysis for the prediction of amputation in the current study. This robust association underscores the potential of the NLR as a valuable prognostic tool in DFUs. These findings align with those of Vatankhah et al., who reported an association between increased NLR values and having a greater likelihood of nonhealing ulcers [24].

Logistic regression analysis further confirmed the significance of the NLR, revealing that patients with an NLR ≥ 6.08 had substantially greater odds of requiring amputation (OR: 13.090, 95% CI: 5.143–33.320; $P<0.001$). This association persisted after adjusting for potential confounding factors, underscoring the potential predictive ability of the NLR for amputation. An elevated NLR reflects an increased neutrophil count and/or a decreased lymphocyte count, indicating an ongoing inflammatory response. Neutrophils are key players in the acute inflammatory response, releasing enzymes and reactive oxygen species (ROS) that can exacerbate tissue damage. Conversely, lymphocytes are crucial for the adaptive immune response, and their reduction may signify impaired immune function [33, 36, 41]. In DFUs, chronic inflammation and infections are common. Elevated NLR values have been reported to be associated with more severe infections and poorer wound healing outcomes, which could be explained by the role of neutrophils in both combating infection as well as causing collateral tissue damage through the release of proteolytic enzymes and oxidative agents [24, 42]. Patients with diabetes often suffer from microvascular complications, which can be exacerbated by systemic inflammation. An elevated NLR was also shown to have a link with poorer outcomes in patients with PAD, a common comorbidity in patients with DFU, which can lead to critical limb ischemia and necessitate amputation [33, 43].

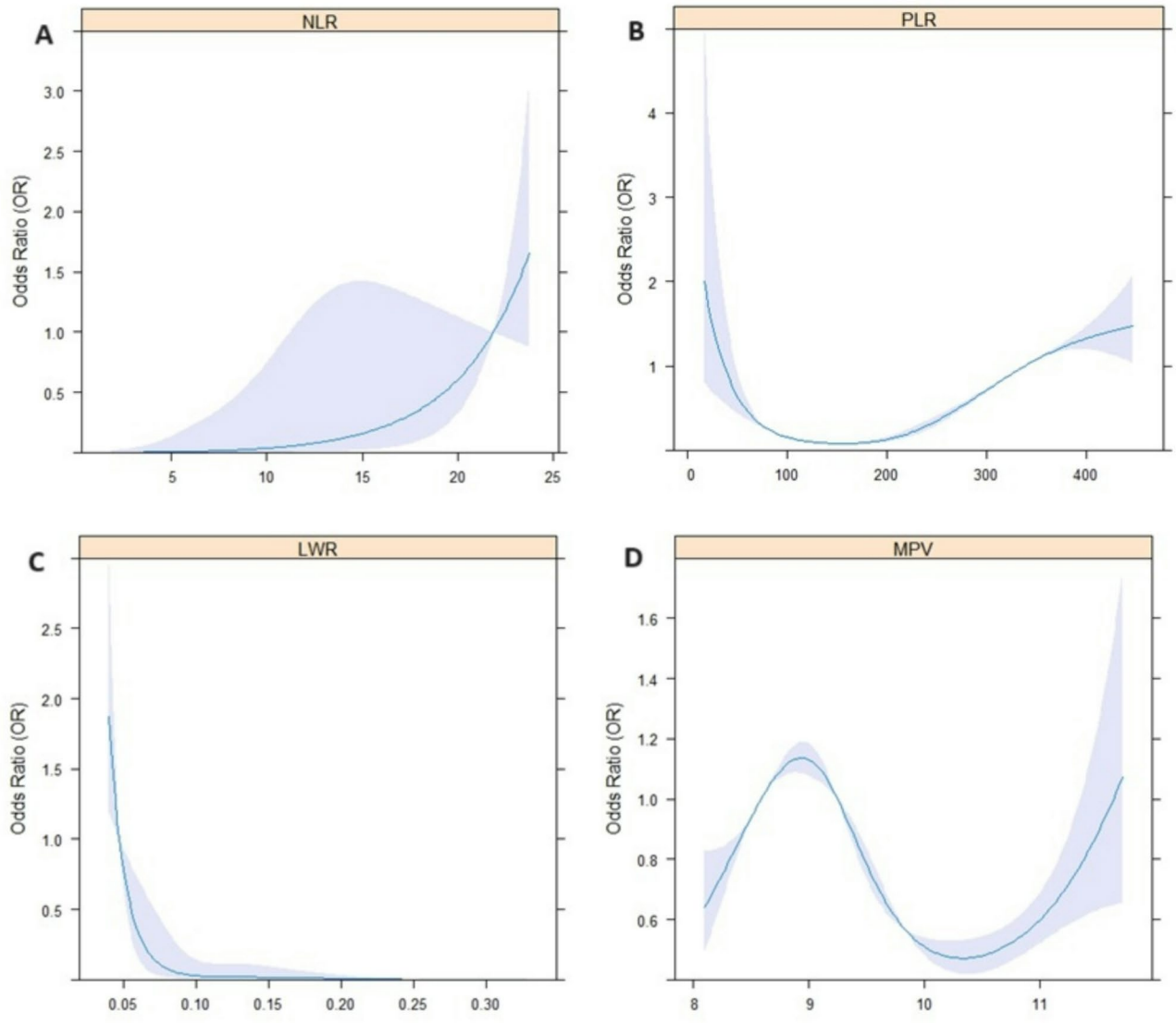

Fig. 4 Associations between the NLR, PLR, LWR and MPV and amputation in the treatment of DFU. The RCS models were used to investigate the associations between the NLR (A), PLR (B), LWR (C) and MPV (D) and the likelihood of amputation as the treatment of DFUs. Each RCS consisted of four knots on the basis of the distribution of the associated variable. The reference values for the aforementioned curves were as follows: NLR = 6.08; PLR = 210; LWR = 0.1256; and MPV = 9.8. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; LWR: lymphocyte-to-WBC ratio; MPV: mean platelet volume; DFU: diabetic foot ulcer; RCS: restricted cubic spline

In the current study, PLR was significantly associated with an increased likelihood of amputation, which was similar to previous studies [33, 36, 44–46]. Demirdal and Sen reported that the PLR, along with the NLR and lymphocyte-to-monocyte ratio (LMR), could serve as predictors for amputation in those with DFIs. Specifically, the PLR was significantly greater in patients who required amputation than in those who needed only debridement or drainage [33]. Xu et al. also identified the PLR as an independent risk factor for amputation in patients with DFUs. They highlighted the PLR, along with C-reactive protein (CRP) and the NLR, as key markers that can aid in predicting the risk of amputation [36]. Aydın et al. further supported these findings by showing that higher PLR values were associated with an increased risk of amputation in patients with DFU. Their study demonstrated that the PLR, along with other inflammatory markers, could serve as a cost-effective and readily available tool for assessing the risk of amputation [45].

Platelets play crucial roles in the inflammatory response and tissue repair processes, and alterations in the PLR may reflect systemic inflammation and vascular dysfunction, both of which are implicated in diabetic foot complications [47, 48]. An elevated PLR reflects a heightened inflammatory state and an altered immune response. Platelets play a role in inflammation and thrombosis,

whereas lymphocytes are crucial for immune regulation. An increased PLR indicates a greater platelet count relative to the lymphocyte count, suggesting an imbalance favoring inflammation and a pro-thrombotic state [33, 36, 49]. In patients with diabetes, chronic hyperglycemia leads to endothelial dysfunction and microvascular complications. Elevated PLR is associated with worse microvascular health, which can exacerbate tissue ischemia and impair wound healing, increasing the risk of infection and subsequent amputation [40, 50]. In the current study, ROC analysis revealed an AUC of 0.637 for the predictive ability of PLR for amputation, further supporting its potential as a prognostic marker.

In the current study, the LWR showed promising results, with an AUC of 0.812 and an optimal cutoff value of 0.1265 (sensitivity of 77.7%, specificity of 73%) for the prediction of amputation. The inverse relationship between LWR and odds of amputation, as illustrated by the RCS model, suggested that lower LWR values were associated with a greater probability of amputation. This finding was consistent with the concept that lymphopenia, often observed in severe inflammatory states, may be indicative of poor outcomes in patients with DFU [51]. Demirdal and Sen reported that lower levels of LMR were associated with amputation risk in DFIs [33]. The pathophysiological mechanisms include increased oxidative stress, impaired angiogenesis, and excessive M1 macrophage polarization, which are all detrimental to wound healing. Seraphim et al. also reported that a lack of lymphocytes impairs macrophage polarization and angiogenesis, further complicating the healing process in diabetic wounds [52].

Despite the controversy regarding the relationship between the MPV and DFU outcome [53–56],, no significant association between MPV levels and the likelihood of amputation was found in the current analysis. Moon et al. demonstrated that elevated platelet counts were a significant risk factor for major amputation in patients with diabetic forefoot ulcers [55]. Furthermore, a review by Korniluk et al. discussed the role of the MPV as a biomarker in various inflammatory conditions, including diabetes. An increased MPV was reported to be associated with increased platelet reactivity and proinflammatory states [54]. MPV, a measure of platelet size and a proposed marker of platelet activation and function, demonstrated the lowest AUC (0.515) among the inflammatory markers in this study. Studies have shown that a low MPV is associated with a greater risk of critical limb ischemia (CLI) in patients with PAD, which is a common comorbidity in patients with diabetes [57]. Furthermore, patients with diabetes often exhibit increased platelet reactivity and a larger MPV, which correlates with poor glycemic control and increased cardiovascular risk. This heightened platelet activity can exacerbate microvascular and macrovascular complications, contributing to the pathogenesis of DFUs and increasing the likelihood of amputation [58]. The discrepancy with some earlier studies might be due to differences in study populations or glycemic control status, which can influence MPV levels [59].

This study had some limitations. Because of the design of the study, it was not possible to examine causal relationships. This study encountered gaps in the data for some variables, such as CRP and estimated glomerular filtration rate (eGFR) levels, and other medical treatments, because this information was not uniformly accessible in all patient records. Incorporating these tests might have improved the scientific significance of the study. Moreover, although the sample size of 126 patients offers useful insights, conducting larger prospective studies that include control groups would be advantageous to further confirm these results and investigate their applicability to various populations.

Conclusion

In conclusion, the current findings support the potential role of NLR, PLR, and LWR as predictive markers for amputation risk in patients with DFU. However, additional prospective studies and large-scale epidemiological investigations are necessary to validate these findings before they can be integrated into routine clinical decision-making.

Abbreviations

DFU	Diabetic foot ulcer
PAD	Peripheral arterial disease
LEA	Lower extremity amputation
NLR	Neutrophil–lymphocyte ratio
PLR	Platelet–lymphocyte ratio
LWR	Lymphocyte–white blood cell ratio
MPV	Mean platelet volume
CVD	Cardiovascular disease
CBC	Complete blood count
ADA	American diabetes association
ESRD	End stage renal disease
FBS	Fasting blood sugar
HbA1c	Hemoglobin A1c
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
WBC	White blood cells
PCT	Plateletcrit
Q1	First quartile
Q3	Third quartile
SD	Standard deviations
ROC	Receiver operating characteristic
OR	Odds ratio
CI	Confidence interval
RCS	Restricted cubic spline
OAD	Oral anti-diabetic drug
AUC	Area under the curve
DFI	Diabetic foot infection
ROS	Reactive oxygen species
LMR	Lymphocyte-to-monocyte ratio
CRP	C-reactive protein
CLI	Critical limb ischemia
eGFR	Estimated glomerular filtration rate

Acknowledgements
The authors wish to thank the patients for their participation and kind cooperation.

Author contributions
FA and SK and SR: Conception and design of the study. AY and FM and MD and SR: Acquisition of data. FA and FM: Analysis of data. SAA and SHS and PN and MA: Drafting the article. MN and AE and SK: Critical revision of the article. All authors read and approved the final manuscript.

Funding
The authors did not receive support from any organization for the submitted work.

Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was performed in accordance with the principles of the Declaration of Helsinki. Informed consent was obtained from all participants or their legal guardians. Approval was granted by the Research Ethics Committee of Tehran University of Medical Sciences. (Ethic code: IR.TUMS.IKHC.REC.1399.507).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

Received: 2 January 2025 / Accepted: 17 April 2025
Published online: 27 April 2025

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