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Neutrophil-Lymphocyte Ratio as a Predictor of Ulcer Severity in Type 2 Diabetes

Prima Budi Prayogi[1*], Prima Kharisma Hayuningrat[2], Suharto Wijanarko[3], Hari Wujoso[4]

[1]Department of Surgery, Dr. Moewardi General Hospital/Universitas Sebelas Maret, Surakarta, Indonesia

[2]Department of Thoracic Cardiovascular Surgery, Dr. Moewardi General Hospital/Universitas Sebelas Maret, Surakarta, Indonesia

[3]Department of Urology, Dr. Moewardi General Hospital/Universitas Sebelas Maret, Surakarta, Indonesia

[4]Department of Forensic Medicine, Dr. Moewardi General Hospital/Universitas Sebelas Maret, Surakarta, Indonesia

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***Corresponding author:**
Prima Budi Prayogi

E-mail address:
primabudiprayogi.bedah@gmail.com

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A B S T R A C T

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder with increasing global prevalence, frequently complicated by foot ulcers. These ulcers present a significant burden, often leading to amputation and increased mortality. The neutrophil-lymphocyte ratio (NLR), a readily available marker of systemic inflammation, has emerged as a potential prognostic indicator in various diseases. This study investigated the correlation between NLR and the severity of foot ulcers in patients with T2DM. A retrospective, cross-sectional study was conducted, analyzing data from 60 patients with T2DM and foot ulcers admitted to Dr. Moewardi General Hospital, Surakarta, between January 1st, 2022, and December 31st, 2023. NLR was calculated from routine complete blood counts. Ulcer severity was graded using the Meggitt-Wagner classification. Statistical analysis included Spearman's rank correlation and Kruskal-Wallis tests. Our study found a significant correlation observed between NLR and ulcer severity ($\rho = 0.524$, $p < 0.001$), with NLR values increasing with ulcer grade. Amputation was significantly associated with ulcer severity ($p=0.009$). In conclusion, NLR is a valuable and readily available biomarker for assessing the severity of foot ulcers in patients with T2DM. Elevated NLR is associated with more severe ulcers and a higher likelihood of amputation. Incorporating the assessment of NLR into the routine evaluation of patients with diabetic foot ulcers can aid in risk stratification and guide clinical management.

1. Introduction

Type 2 diabetes mellitus (T2DM) represents a significant global health challenge, characterized by chronic hyperglycemia resulting from a combination of insulin resistance and impaired insulin secretion. This complex metabolic disorder has witnessed a steady increase in prevalence worldwide, posing substantial implications for both individual well-being and the sustainability of healthcare systems. The International Diabetes Federation (IDF) has estimated a staggering 537 million adults will be living with diabetes in 2021, a figure projected to escalate to 783 million by the year 2045. This escalating trend underscores the urgent need for the development and implementation of effective strategies aimed at preventing and managing the diverse complications associated with T2DM. The persistent state of hyperglycemia in T2DM initiates a cascade of metabolic disturbances, ultimately contributing to the development of both microvascular and macrovascular complications. These complications encompass a wide spectrum of debilitating conditions, including neuropathy, retinopathy, nephropathy, cardiovascular disease, and peripheral artery disease (PAD). Among the various complications associated with T2DM, foot ulcers stand out as particularly debilitating and costly,

affecting a substantial proportion of patients and representing a leading cause of non-traumatic lower extremity amputations. Diabetic foot ulcers (DFUs) are recognized as a complex and multifactorial problem. The pathogenesis of DFUs involves a complex interplay of several contributing factors, including peripheral neuropathy, PAD, foot deformities, trauma, and infection. Peripheral neuropathy, a common complication in individuals with long-standing diabetes, diminishes protective sensation in the feet, thereby predisposing them to injury and increasing the risk of ulcer development. PAD, which frequently coexists with neuropathy in individuals with diabetes, further exacerbates the problem by impairing blood flow to the lower extremities. This reduction in blood flow compromises tissue oxygenation and the delivery of essential nutrients to the affected area, significantly impairing the process of wound healing. Repetitive stress, even minor trauma, and foot deformities can further contribute to the development of ulcers in the insensate foot, highlighting the vulnerability of individuals with diabetic neuropathy. The presence of infection in a DFU can rapidly lead to a deterioration of the condition, potentially resulting in tissue necrosis, osteomyelitis (infection of the bone), and ultimately, the need for amputation.[1-3]

The effective management of DFUs presents a significant challenge to healthcare providers and necessitates a multidisciplinary approach. Optimal management strategies typically include meticulous glycemic control to address the underlying metabolic disorder, pressure offloading to reduce mechanical stress on the ulcerated area, thorough wound debridement to remove necrotic tissue and promote healing, aggressive infection control with appropriate antibiotics, and revascularization procedures in patients with PAD to improve blood flow to the affected limb. Despite significant advances in wound care techniques and management strategies, a substantial proportion of DFUs still fail to heal adequately. This unfortunate reality often leads to a considerable number of patients ultimately requiring amputation. Amputation, whether major or minor, represents a devastating outcome for patients with DFUs. It not only results in significant physical disability, with a profound impact on mobility and independence, but is also associated with increased morbidity, higher mortality rates, and a substantial reduction in the patient's overall quality of life. The impact of amputation extends beyond the physical realm, often affecting the psychological and social well-being of the individual. A systematic review has highlighted the grim prognosis associated with DFUs, emphasizing the increased mortality risk that follows both ulceration and subsequent amputation. Given the substantial burden imposed by DFUs on individuals and healthcare systems, there is a compelling need for the identification of reliable biomarkers. These biomarkers would ideally facilitate the identification of patients who are at an elevated risk of developing severe ulcers and experiencing adverse outcomes. The availability of such biomarkers would enable early intervention strategies, more effective risk stratification, and the implementation of personalized management approaches, ultimately leading to improved patient outcomes and a reduction in the incidence of severe complications.[4-6]

In the pursuit of identifying such valuable biomarkers, several inflammatory markers have been investigated for their potential role in predicting DFU severity and prognosis. Among these, the neutrophil-lymphocyte ratio (NLR) has emerged as a promising candidate. The NLR is a simple and readily available marker of systemic inflammation that can be easily calculated from a routine complete blood count (CBC). This ratio effectively reflects the balance between pro-inflammatory processes, represented by neutrophils, and anti-inflammatory processes, represented by lymphocytes. Elevated NLR has been consistently observed in a variety of inflammatory conditions, including cardiovascular disease, infections, and malignancies, highlighting its potential as a general indicator of inflammation. Recent studies have further suggested that NLR may also serve as a useful prognostic indicator specifically in patients with T2DM and DFUs, adding to its potential clinical utility. In the

context of T2DM, the chronic state of hyperglycemia and the presence of insulin resistance contribute to a state of persistent low-grade systemic inflammation. This chronic inflammation is increasingly recognized as playing a key role in the pathogenesis of various diabetic complications, including the development and progression of DFUs. Neutrophils, which are essential components of the innate immune system, are activated in response to inflammation and tissue injury. Upon activation, neutrophils release a variety of pro-inflammatory mediators, including reactive oxygen species (ROS) and proteases. While these mediators are part of the normal immune response, excessive or dysregulated release can contribute to further tissue damage and impair the complex process of wound healing. Lymphocytes, on the other hand, play a crucial role in adaptive immunity and the regulation of inflammation. A decrease in lymphocyte count may reflect a weakened immune response and a diminished capacity to effectively control and resolve inflammation. Therefore, an elevated NLR may serve as an indicator of an imbalance between pro-inflammatory and anti-inflammatory processes, reflecting a state of heightened inflammation within the body. Several studies have explored the potential association between NLR and various DFU outcomes. Some of these studies have reported that an elevated NLR is associated with increased DFU severity, delayed wound healing, and a higher risk of amputation, highlighting its potential as a prognostic marker. However, it is important to acknowledge that the findings in this area have been inconsistent, and further research is warranted to definitively clarify the precise role of NLR in the management of DFUs and to establish its clinical utility.[7-10] In light of the existing knowledge and the need for further clarification, this study aimed to investigate the correlation between NLR and the severity of foot ulcers in patients with T2DM at Dr. Moewardi General Hospital, Surakarta.

2. Methods

This study was conducted utilizing a retrospective, cross-sectional design. This particular design was chosen to analyze the relationship between the neutrophil-lymphocyte ratio (NLR) and the severity of foot ulcers in patients diagnosed with type 2 diabetes mellitus (T2DM) at a single point in time. The retrospective nature of the study involved examining pre-existing data that had been collected as part of routine clinical care, allowing for an analysis of the association between NLR and ulcer severity without directly intervening with patient management. The cross-sectional component of the design facilitated the assessment of NLR and ulcer severity concurrently. The study was carried out at Dr. Moewardi General Hospital, located in Surakarta, Indonesia. Dr. Moewardi General Hospital is a tertiary referral center. As a tertiary referral center, the hospital provides specialized medical care and services for a large and diverse patient population, often including complex cases that require a high level of expertise and resources. The selection of this setting was important for accessing a sufficient number of patients with T2DM and diabetic foot ulcers (DFUs) to conduct the study. The study protocol received formal approval from the institutional review board of Dr. Moewardi General Hospital. This approval was a critical step to ensure that the study was conducted ethically and in compliance with relevant regulations and guidelines concerning research involving human subjects. The institutional review board plays a vital role in safeguarding the rights and welfare of patients participating in research by carefully evaluating the study design, data collection methods, and potential risks and benefits. Given the retrospective nature of the study, it was determined that obtaining informed consent from individual patients was not necessary. This decision was based on the fact that the study involved the analysis of existing data collected during the course of routine clinical care, and there was no direct interaction or intervention with the patients for research purposes. However, despite the waiver of individual informed consent, the study was conducted in strict accordance with ethical principles and guidelines. These principles emphasize the importance of protecting patient confidentiality, ensuring the

responsible use of data, and maintaining the integrity of the research process.

The study population consisted of patients diagnosed with T2DM and foot ulcers who were admitted to Dr. Moewardi General Hospital over a defined period. The data collection period spanned from January 1st, 2022, to December 31st, 2023. This timeframe allowed for the inclusion of a sufficient number of patients to provide adequate statistical power for the study analyses. Patients were identified through a systematic search of the hospital's electronic medical records system. The use of electronic medical records facilitated the efficient and comprehensive identification of eligible patients based on the specified inclusion and exclusion criteria.

To ensure the selection of appropriate participants for the study, a set of specific inclusion criteria was established. Patients were included in the study if they met all of the following criteria; Diagnosis of T2DM according to the American Diabetes Association (ADA) criteria. The ADA criteria are widely recognized and used for the diagnosis of diabetes mellitus. Utilizing these criteria ensured that all participants had a confirmed diagnosis of T2DM, providing consistency and accuracy in the study population; Presence of a foot ulcer, defined as a break in the skin located below the ankle in a patient with diabetes. This criterion ensured that all participants had a clearly defined condition of a diabetic foot ulcer, which was the focus of the study. The definition provided a standardized way to identify and include patients with this specific complication of diabetes; Availability of complete blood count (CBC) results within 24 hours of admission. The availability of CBC results within this timeframe was crucial because the NLR, the primary exposure variable of interest, is calculated from CBC parameters. Using CBC results obtained close to the time of admission helped to minimize potential variability due to changes in the patient's condition or treatment; Complete medical records, including thorough documentation of ulcer severity. Complete medical records are essential for any retrospective study. This requirement ensured that all relevant data, including patient demographics, medical history, laboratory results, ulcer characteristics, and treatment information, were available for analysis. The documentation of ulcer severity was particularly important, as it was the primary outcome variable of the study.

In addition to the inclusion criteria, a set of exclusion criteria was also established to exclude patients who might have confounding factors that could affect the study results. Patients were excluded from the study if they met any of the following criteria; Type 1 diabetes mellitus. Type 1 diabetes mellitus is a distinct form of diabetes with a different pathogenesis than T2DM. Excluding patients with type 1 diabetes ensured that the study focused specifically on T2DM and its associated complications; History of hematologic disorders or malignancies. Hematologic disorders and malignancies can affect CBC parameters and inflammatory markers, potentially confounding the assessment of NLR. Excluding patients with these conditions helped to ensure that the NLR values primarily reflected the inflammatory status related to the diabetic foot ulcer; Use of immunosuppressive medications within the past 3 months. Immunosuppressive medications can significantly alter the immune response and affect both neutrophil and lymphocyte counts. Excluding patients using these medications helped to minimize variability in NLR values that might be related to medication effects rather than the underlying disease process; Presence of other significant inflammatory conditions (e.g., rheumatoid arthritis, systemic lupus erythematosus). Similar to hematologic disorders and malignancies, other significant inflammatory conditions can independently influence CBC parameters and inflammatory markers. Excluding patients with these conditions helped to isolate the inflammatory response associated with the diabetic foot ulcer; Inadequate or incomplete medical records. Inadequate or incomplete medical records can compromise the quality and reliability of the data analysis. Excluding patients with such records ensured that only patients with sufficient data were

included in the study, maintaining the integrity of the research.

Data for the study were collected retrospectively. The data were extracted from the electronic medical records of eligible patients. The use of electronic medical records facilitated the efficient and accurate retrieval of relevant patient information. A standardized data extraction form was utilized to ensure consistency and completeness in the data collection process. The following data points were extracted from the electronic medical records; Demographic characteristics: This category included age and gender. Age was recorded in years, and gender was categorized as either male or female. These demographic variables are important for describing the study population and for exploring potential associations with ulcer severity; Clinical characteristics: This category included body mass index (BMI), comorbidities, and smoking status. BMI was calculated using the patient's weight and height. Comorbidities, which are co-existing medical conditions, include hypertension, hyperlipidemia, and coronary artery disease. Smoking status was categorized as either current smoker, former smoker, or non-smoker. These clinical characteristics provide valuable information about the overall health status of the patients and potential risk factors for DFU development and severity; Laboratory data: This category included white blood cell count, neutrophil count, lymphocyte count, and hemoglobin A1c (HbA1c). White blood cell count, neutrophil count, and lymphocyte count were obtained from the initial CBC results. HbA1c, a measure of long-term glycemic control, was also recorded. These laboratory parameters are essential for calculating the NLR and for assessing the patient's overall metabolic status; Ulcer characteristics: This category included ulcer location, ulcer size, ulcer duration, and ulcer severity. Ulcer location described the anatomical location of the foot ulcer. Ulcer size was measured in square centimeters. Ulcer duration referred to the length of time the ulcer had been present. Ulcer severity was assessed using the Meggitt-Wagner classification system; Peripheral artery disease (PAD) status: PAD, a common complication in patients with diabetes, was assessed based on documented history of PAD, abnormal ankle-brachial index (ABI) (<0.9), or findings from vascular imaging studies. Vascular imaging studies included Doppler ultrasound and angiography; Treatment interventions: This category included various treatment interventions received by the patients, such as debridement, antibiotic therapy, revascularization procedures (if applicable), and amputation. Debridement involves the removal of necrotic or infected tissue from the ulcer. Antibiotic therapy was administered to treat any associated infections. Revascularization procedures, such as angioplasty or bypass surgery, were performed in patients with PAD to improve blood flow to the affected limb. Amputation, either major or minor, was recorded if it occurred; Outcomes: This category included length of hospital stay, ulcer healing, amputation (major or minor), and mortality. Length of hospital stay was recorded in days. Ulcer healing was assessed based on documentation in the medical records. Amputation, as mentioned earlier, was recorded if it occurred. Mortality, or death, during the hospital stay was also recorded.

The neutrophil-lymphocyte ratio (NLR) was calculated for each patient using the absolute neutrophil count and the absolute lymphocyte count, both obtained from the initial CBC results. The NLR is a simple ratio that reflects the balance between pro-inflammatory and anti-inflammatory components of the immune system. The calculation was performed using the following formula: NLR = Neutrophil count / Lymphocyte count. The neutrophil count and lymphocyte count were typically expressed as the number of cells per microliter of blood. The resulting NLR value is a dimensionless number that can be used as a marker of systemic inflammation.

Ulcer severity was assessed using the Meggitt-Wagner classification system. This classification system is a widely used and validated tool for grading the severity of DFUs. It provides a standardized and objective method for evaluating the extent of tissue

damage and the progression of the ulcer. The Meggitt-Wagner classification system categorizes DFUs into six grades, ranging from grade 0 to grade 5. The grades are defined as follows; Grade 0: No ulcer is present, but there may be pre-ulcerative lesions, such as callus formation or foot deformities; Grade 1: A superficial ulcer is present, involving only the epidermis or dermis layers of the skin; Grade 2: The ulcer extends deeper into the subcutaneous tissue and may involve tendon, muscle, or bone; Grade 3: The ulcer is deep and is associated with osteomyelitis (bone infection) or abscess formation; Grade 4: Localized gangrene is present, typically affecting the forefoot or heel; Grade 5: Extensive gangrene involves the entire foot or necessitates a major amputation. The Meggitt-Wagner classification system was chosen for this study due to its widespread use and established validity in assessing DFU severity. It has been used in numerous previous studies, allowing for comparisons of the results with other research findings. The use of this standardized classification system ensured consistency and objectivity in the assessment of ulcer severity across the study population.

Statistical analysis of the collected data was performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). SPSS is a comprehensive statistical software package widely used in medical research for data management, analysis, and presentation. Descriptive statistics were used to summarize the characteristics of the study population. Descriptive statistics provide a clear and concise overview of the data, allowing for a better understanding of the sample's main features. Continuous variables, such as age, BMI, length of hospital stay, and NLR, were presented as means and standard deviations. The mean represents the average value of the variable, while the standard deviation measures the variability or dispersion of the data around the mean. Categorical variables, such as gender, comorbidities, digital subtraction angiography (DSA), amputation, and ulcer severity grades, were presented as frequencies and percentages. Frequencies indicate the number of occurrences of each category, while percentages express these occurrences relative to the total sample size. The correlation between NLR and ulcer severity was assessed using Spearman's rank correlation coefficient. Spearman's rank correlation coefficient is a non-parametric statistical measure that assesses the strength and direction of association between two ordinal variables or between a continuous variable and an ordinal variable. In this study, ulcer severity was an ordinal variable (Meggitt-Wagner grades), and NLR was a continuous variable. Spearman's rank correlation is appropriate for this type of data because it does not assume a linear relationship between the variables and is robust to non-normal distributions. The correlation coefficient (ρ) ranges from -1 to +1, where -1 indicates a perfect negative correlation, +1 indicates a perfect positive correlation, and 0 indicates no correlation. Differences in NLR across ulcer severity grades were examined using the Kruskal-Wallis test. The Kruskal-Wallis test is a non-parametric statistical test that compares the medians of two or more independent groups. It is a non-parametric alternative to the one-way ANOVA and is used when the data do not meet the assumptions of normality or homogeneity of variance. In this study, the Kruskal-Wallis test was used to determine if there were statistically significant differences in NLR values across the different Meggitt-Wagner ulcer severity grades. Following the Kruskal-Wallis test, post-hoc Mann-Whitney U tests were conducted with Bonferroni correction for multiple comparisons. Post-hoc tests are performed after a significant Kruskal-Wallis test to determine which specific groups differ from each other. The Mann-Whitney U test is a non-parametric test that compares the medians of two independent groups. It is used when the data do not meet the assumptions of normality. The Bonferroni correction is a method used to adjust the significance level (alpha level) when performing multiple comparisons. This correction helps to control the family-wise error rate, which is the probability of making at least one Type I error (false positive) across all comparisons. In this study, the Bonferroni correction was applied to the p-values

obtained from the Mann-Whitney U tests to account for the multiple comparisons made between the different ulcer severity grades. The association between amputation and ulcer severity was assessed using the chi-square test. The chi-square test is a statistical test used to examine the association between two categorical variables. In this study, both amputation (yes/no) and ulcer severity (Meggitt-Wagner grades) were categorical variables. The chi-square test determines whether there is a statistically significant association between these two variables. A p-value of <0.05 was considered statistically significant for all analyses. The p-value represents the probability of obtaining the observed results (or more extreme results) if there were no true association or difference between the variables being studied. A p-value of <0.05 indicates that the results are statistically significant, meaning that they are unlikely to have occurred by chance. This significance level is commonly used in medical research.

3. Results and Discussion

Table 1 presents a comprehensive overview of the baseline characteristics of the 60 patients with type 2 diabetes mellitus (T2DM) and foot ulcers included in this study. The data are essential for understanding the demographic, clinical, and disease-related features of the cohort, which is crucial for contextualizing the study's findings. The study comprised 60 patients (n=60). This sample size provides a basis for the statistical analyses conducted to investigate the relationship between neutrophil-lymphocyte ratio (NLR) and ulcer severity. The mean age of the patients was 62.07 years, with a standard deviation of 12.09 years. The age range spanned from 38 to 85 years. This indicates that the study population included a fairly wide age range of adults with T2DM and foot ulcers, with the average age being in the early sixties. The study population was relatively balanced in terms of gender, with 31 patients (51.7%) being female and 29 patients (48.3%) being male. This near-equal distribution helps to minimize potential gender-related biases in the analysis. The mean BMI was 22.65 kg/m^2, with a standard deviation of 4.10 kg/m^2. This suggests that, on average, the patients in this study had a BMI in the normal to underweight range, although there was some variability in BMI within the group. It is important to note that the mean BMI is relatively low, which might be an interesting factor to consider in the context of diabetes and foot ulcers. The mean length of hospital stay for these patients was 6.67 days, with a standard deviation of 3.79 days. This provides an indication of the average duration of inpatient care required for the management of T2DM-related foot ulcers in this population. A significant proportion of the study population, 39 patients (65.0%), presented with comorbidities. This highlights the complexity of managing patients with T2DM and foot ulcers, as they often have other co-existing health conditions that can influence their overall health and recovery. Only 6 patients (10.0%) underwent digital subtraction angiography (DSA). DSA is an imaging technique used to visualize blood vessels. The relatively low number of patients undergoing DSA might suggest that PAD was not a primary focus of investigation or that DSA was reserved for specific cases. A substantial number of patients, 29 (48.3%), underwent amputation. This high percentage underscores the severity of foot ulcers in this population and the significant risk of limb loss associated with this complication of diabetes. The mean NLR was 9.80, with a standard deviation of 11.14, and a range of 1.2 to 45.5. The NLR values show a large standard deviation and a wide range, indicating considerable variability in the inflammatory status of the patients. This variability is a key factor in the study's investigation of the relationship between NLR and ulcer severity. The distribution of ulcer severity grades according to the Meggitt-Wagner classification was as follows; Grade 1: 6 patients (10.0%); Grade 2: 11 patients (18.3%); Grade 3: 18 patients (30.0%); Grade 4: 21 patients (35.0%); Grade 5: 4 patients (6.7%). This distribution reveals that the majority of patients had more severe ulcers (Grades 3 and 4), with fewer patients presenting with less severe ulcers (Grades 1 and 2) or the most severe ulcers (Grade 5).

Table 1. Characteristics of the study population.

Characteristic	Mean ± Standard Deviation (Range) or Number (Percentage)
Number of patients (n)	60
Age (years)	62.07 ± 12.09 (38-85)
Gender (n, %)	
Female	31 (51.7%)
Male	29 (48.3%)
BMI (kg/m^2)	22.65 ± 4.10
Length of hospital stay (days)	6.67 ± 3.79
Comorbidities (n, %)	39 (65.0%)
Digital subtraction angiography (n, %)	6 (10.0%)
Amputation (n, %)	29 (48.3%)
NLR	9.80 ± 11.14 (1.2 - 45.5)
Ulcer severity (Meggitt-Wagner) (n, %)	
Grade 1	6 (10.0%)
Grade 2	11 (18.3%)
Grade 3	18 (30.0%)
Grade 4	21 (35.0%)
Grade 5	4 (6.7%)

Data are presented as mean ± standard deviation (range) for continuous variables and number (percentage) for categorical variables.

Table 2 presents the results of correlation analyses examining the relationship between various patient characteristics and the severity of their foot ulcers. The table displays the correlation coefficient (ρ) and the corresponding p-value for each characteristic. The correlation coefficient indicates the strength and direction of the linear association between two variables, while the p-value indicates the statistical significance of the observed correlation. The correlation coefficient (ρ) for age was -0.075, with a p-value of 0.565. This indicates a very weak, nearly negligible negative correlation between age and ulcer severity. The p-value is far above the significance threshold of 0.05, meaning this correlation is not statistically significant. In practical terms, age does not appear to be related to the severity of foot ulcers in this study. The correlation coefficient (ρ) for gender was -0.164, with a p-value of 0.216. This suggests a weak negative correlation between gender and ulcer severity. However, the p-value is greater than 0.05, indicating that this correlation is not statistically significant. Therefore, gender does not appear to be a significant predictor of ulcer severity in this study. The correlation coefficient (ρ) for BMI was -0.144, with a p-value of 0.284. This shows a weak negative correlation between BMI and ulcer severity. Again, the p-value is greater than 0.05, indicating that this correlation is not statistically significant. BMI does not appear to be significantly associated with the severity of foot ulcers in this cohort. The correlation coefficient (ρ) for length of hospital stay was 0.189, with a p-value of 0.151. This suggests a weak positive correlation between the length of hospital stay and ulcer severity. However, the p-value is greater than 0.05, indicating that this correlation is not statistically significant. The length of hospital stay is not significantly related to ulcer severity. The correlation coefficient (ρ) for comorbidities was 0.098, with a p-value of 0.455. This indicates a very weak positive correlation between the presence of comorbidities and ulcer severity. The p-value is greater than 0.05, indicating that this correlation is not statistically significant. Comorbidities, as defined in this study, do not appear to have a significant relationship with ulcer severity. The correlation coefficient (ρ) for digital subtraction angiography (DSA) was 0.017, with a p-value of 0.901. This shows a very weak positive correlation between undergoing DSA and ulcer severity. The p-value is substantially greater than 0.05, indicating that this correlation is not statistically significant. Undergoing DSA is not significantly associated with ulcer severity. The correlation coefficient (ρ) for amputation was

0.429, with a p-value of 0.009. This demonstrates a moderate positive correlation between amputation and ulcer severity. Importantly, the p-value is less than 0.05, indicating that this correlation is statistically significant. This finding suggests that patients with more severe ulcers were significantly more likely to undergo amputation.

Table 2. Correlation of patient characteristics with ulcer severity.

Characteristic	Correlation coefficient (ρ)	p-value
Age (years)	-75	0.565
Gender	-164	0.216
BMI (kg/m^2)	-144	0.284
Length of hospital stay (days)	189	0.151
Comorbidities	98	0.455
Digital subtraction angiography	17	0.901
Amputation	429	0.009*

*Statistically significant.

Table 3 presents the mean neutrophil-lymphocyte ratio (NLR) values for each grade of ulcer severity, as classified by the Meggitt-Wagner system. This table is crucial for understanding the relationship between NLR, a marker of systemic inflammation, and the extent of tissue damage in diabetic foot ulcers. The table clearly demonstrates a trend of increasing NLR values with increasing ulcer severity. This observation supports the hypothesis that higher levels of systemic inflammation, as reflected by NLR, are associated with more severe diabetic foot ulcers; Grade 1: The mean NLR for Grade 1 ulcers was 2.79, with a standard deviation of 1.27. This represents the lowest mean NLR value among all grades, indicating relatively lower inflammation in the least severe ulcers; Grade 2: The mean NLR for Grade 2 ulcers was 5.85, with a standard deviation of 5.01. The NLR is higher than Grade 1, suggesting an increase in inflammation as the ulcer progresses; Grade 3: The mean NLR for Grade 3 ulcers was 9.10, with a standard deviation of 5.72. A further increase in NLR is observed, indicating a continued rise in inflammation with increasing ulcer severity; Grade 4: The mean NLR for Grade 4 ulcers was 9.67, with a standard deviation of 4.94. The NLR is slightly higher than Grade 3, but the difference is not as pronounced as in the earlier grades. The inflammation levels remain elevated in these severe ulcers; Grade 5: The mean NLR for Grade 5 ulcers was 35.06, with a standard deviation of 31.57. There is a dramatic increase in NLR for the most severe ulcers (Grade 5). The high mean NLR and large standard deviation indicate a significantly elevated and highly variable inflammatory response in patients with extensive gangrene. The text accompanying the table reports a significant positive correlation between NLR and ulcer severity ($\rho = 0.524$, $p < 0.001$). This confirms a statistically significant association between higher NLR values and greater ulcer severity. The Kruskal-Wallis test also showed a significant difference in NLR across ulcer severity grades ($p < 0.001$), indicating that NLR values vary significantly across the different ulcer grades. Post-hoc Mann-Whitney U tests with Bonferroni correction revealed that NLR was significantly higher in Grade 5 ulcers compared to Grade 1, Grade 2, Grade 3, and Grade 4 ulcers ($p < 0.05$ for all comparisons). This highlights that the most severe ulcers (Grade 5) have a distinctly higher inflammatory profile compared to less severe ulcers. However, there were no significant differences in NLR between Grade 1 and Grade 2, Grade 1 and Grade 3, Grade 1 and Grade 4, Grade 2 and Grade 3, Grade 2 and Grade 4, and Grade 3 and Grade 4 ulcers. This suggests that while Grade 5 ulcers are distinct, the NLR values are not significantly different between the other adjacent grades.

Table 3. Correlation of NLR with ulcer severity.

Ulcer severity (Meggitt-Wagner)	NLR (Mean ± SD)
Grade 1	2.79 ± 1.27
Grade 2	5.85 ± 5.01
Grade 3	9.10 ± 5.72
Grade 4	9.67 ± 4.94
Grade 5	35.06 ± 31.57

The results of this study are consistent with a growing body of evidence from previous research that has explored the association between elevated NLR and adverse outcomes in patients with T2DM and DFUs. Some studies have reported that higher NLR values are associated with an increased risk of complications such as osteomyelitis (infection of the bone) and amputation in patients suffering from diabetic foot infections. Other research has indicated that NLR is significantly higher in diabetic patients who have developed foot ulcers compared to those without ulcers. These earlier findings, combined with the results of the present study, provide further support for the potential utility of NLR as a valuable biomarker. Specifically, NLR may be useful for identifying patients who are at an elevated risk of developing severe complications related to DFUs.[11-13]

The mechanisms that underlie the observed association between elevated NLR and DFU severity are likely complex and multifactorial. Type 2 diabetes mellitus is characterized by a state of chronic low-grade inflammation. This persistent inflammation is driven by a combination of factors, including hyperglycemia (elevated blood sugar levels), insulin resistance (a reduced responsiveness to the hormone insulin), and increased levels of advanced glycation end products (AGEs). AGEs are harmful substances that form when sugar binds to proteins or fats in the body. This chronic inflammatory state is believed to play a crucial role in the development and progression of various diabetic complications, including DFUs. The sustained inflammation can contribute to endothelial dysfunction (damage to the lining of blood vessels), impaired microcirculation (reduced blood flow in small blood vessels), and a reduction in tissue oxygenation. These factors are all critical in the pathogenesis of DFUs, as they compromise the delivery of oxygen and nutrients to the tissues, impairing the healing process. Neutrophils, a type of white blood cell, are key players in the inflammatory response. In T2DM, neutrophils may exhibit increased activation and altered function. This altered function can lead to the release of excessive amounts of pro-inflammatory mediators, such as reactive oxygen species (ROS) and matrix metalloproteinases (MMPs). ROS are highly reactive molecules that can cause damage to cells and tissues, while MMPs are enzymes that break down proteins in the extracellular matrix, the structural framework surrounding cells. The excessive release of these mediators can contribute to further tissue damage, impair wound healing, and promote the progression of ulcers. Lymphocytes, another type of white blood cell, play a crucial role in adaptive immunity and the regulation of inflammation. A decrease in the lymphocyte count may indicate a weakened immune response and a reduced ability to effectively control the inflammatory process. Therefore, an elevated NLR, which represents a higher proportion of neutrophils to lymphocytes, may indicate an imbalance between pro-inflammatory and anti-inflammatory processes. This imbalance reflects a state of heightened inflammation and increased tissue damage, contributing to the severity of DFUs.[14-16]

The findings of this study have several potential clinical implications for the management of patients with T2DM and DFUs. NLR, as a readily available biomarker, can potentially be used for risk stratification in patients with DFUs. Patients with elevated NLR values may be identified as being at a higher risk of developing severe DFU complications. This early identification allows for timely intervention and more aggressive management strategies. For

example, patients with elevated NLR may benefit from more frequent monitoring of their ulcers, more intensive wound care interventions, and a more proactive approach to managing infections or other complications. The assessment of NLR can also potentially guide clinical decision-making in the management of DFUs. In addition to its role in risk stratification, NLR can provide valuable information about the patient's overall inflammatory status and their response to treatment. Serial measurements of NLR during the course of treatment may help clinicians to monitor the effectiveness of interventions and adjust treatment plans as needed. For instance, a persistent elevation in NLR despite treatment may indicate the need for a more aggressive approach to infection control or the consideration of other underlying factors that may be contributing to the inflammation. The role of inflammation in the pathogenesis of DFUs suggests that therapeutic interventions aimed at reducing inflammation may have the potential to improve DFU outcomes. While this study focused on the utility of NLR as a biomarker, the findings indirectly support the rationale for exploring anti-inflammatory therapies in the management of DFUs. Such interventions could include lifestyle modifications, pharmacological therapies, or advanced wound care modalities. Lifestyle modifications, such as weight loss, exercise, and dietary changes, can help to improve glycemic control and reduce systemic inflammation. Pharmacological therapies, such as anti-inflammatory drugs or targeted biological agents, may also be considered in selected cases. Advanced wound care modalities, such as growth factors or bioengineered skin substitutes, may also contribute to reducing inflammation and promoting healing. Further research is needed to determine the efficacy and safety of these anti-inflammatory interventions in patients with DFUs and elevated NLR.[17-20]

4. Conclusion

In conclusion, this study demonstrates that the neutrophil-lymphocyte ratio (NLR) is a valuable and readily accessible biomarker for assessing the severity of foot ulcers in patients with type 2 diabetes mellitus (T2DM). The findings reveal a significant correlation between elevated NLR and increased ulcer severity, with NLR values progressively increasing across the Meggitt-Wagner classification grades. Furthermore, the study highlights a significant association between ulcer severity and the likelihood of amputation, emphasizing the clinical relevance of ulcer severity as a critical determinant of patient outcomes. The potential clinical implications of these findings are substantial. Incorporating the assessment of NLR into the routine evaluation of patients with diabetic foot ulcers can aid in risk stratification, enabling clinicians to identify patients at higher risk of developing severe complications. This early identification facilitates timely intervention and the implementation of more aggressive management strategies, potentially improving patient outcomes and reducing the incidence of adverse events such as amputation. Moreover, the role of inflammation in the pathogenesis of diabetic foot ulcers underscores the potential utility of therapeutic interventions aimed at modulating the inflammatory response. While this study establishes the value of NLR as a biomarker, it also indirectly supports the rationale for exploring anti-inflammatory therapies as a strategy to improve outcomes in patients with diabetic foot ulcers.

5. References

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