RESEARCH ARTICLE

Predictive value of neutrophil-lymphocyte ratio in assessing the outcomes of diabetic foot ulcer

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Abstract

Objective: To assess the correlation of neutrophil-lymphocyte ratio with clinical outcomes in diabetic foot ulcer cases.

Method: The retrospective, cross-sectional, single-centre study was conducted at Section of Vascular Surgery, Aga Khan University Hospital, Karachi (Pakistan), and comprised data from January 2019 to January 2022 of diabetic foot inpatients of either gender aged at least 18 years. Hospital complications and outcomes were noted along with other data on a self-designed proforma. Diagnostic performance of neutrophil-lymphocyte ratio and its correlation with qualitative variables were assessed. Data was analysed using SPSS 26.

Results: Of the 130 patients with mean age 60.13+/-11.75 years, 98(75.4%) were males. Surgical intervention was needed in 118(90.8%) cases, with below-knee amputation being the most frequent procedure 38(32.3%). Neutrophil-lymphocyte ratio >4 was significantly associated with a higher grade of diabetic foot ulcer, major amputation, and postoperative complications (p<0.0001) as well as with in-hospital (p=0.008) and 1-year mortality (p=0.01).

Conclusion: Neutrophil-lymphocyte ratio could be potentially used as a sensitive and economically feasible biomarker for evaluating the prognosis of patients presenting with diabetic foot ulcers.

Key Words: Diabetic foot, Outcomes, Complications, Surgery.

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Introduction

Diabetes is major health concern affecting over 425 million people annually.[1] The prevalence of diabetes in Pakistan was estimated to be around 26.3% by the National Diabetes Survey of Pakistan 2016-17.[2] Diabetes leads to numerous complications of which a frequent one seen in healthcare centres in Pakistan is diabetic foot ulcer (DFU). Studies in Pakistan have indicated that the prevalence of DFUs ranges 4-14%.[3,4] The major risk factors for amputation in such patients include poor glycaemic control, increasing age, prolonged length of hospital stay (LOS), referral delays, non-palpable popliteal artery, wound infection, and poor foot hygiene practices.[5,6] DFUs can impose a significant economic burden on patients, especially in low- and middle-income countries (LMICs).[7] The severity of DFUs worsens due to sepsis, which is commonly seen in diabetic patients. Development of sepsis leads to a coagulopathic state as a result of endothelial dysfunction, increasing the risk of thrombotic vascular occlusion. The vasopressors used for the treatment of sepsis have also been shown to compromise blood flow to the limbs because of vasoconstriction, and, hence, further amplify the risk of amputation.[8]

Neutrophil-lymphocyte ratio (NLR) has been widely demonstrated as a useful prognostic marker in multiple inflammatory processes, such as cancer and cardiovascular disease (CVD). It has also proven to be a significant marker for diagnosis and prognosis of sepsis as well as a prognostic indicator post-amputation in patients with critical limb ischaemia (CLI).[9]

To our knowledge, no loco-regional study is available regarding the association of NLR with DFUs. The current study was planned to fill the gap by assessing the correlation of NLR with clinical outcomes in DFU cases.

Materials and Methods

The retrospective, cross-sectional, single-centre study was conducted at Section of Vascular Surgery, Department of Surgery, Aga Khan University Hospital, Karachi (Pakistan), and comprised data from January 2019 to January 2022. After approval from the institutional ethics review committee, of the Aga Khan University, (No-2022-7982-23089) the sample size was calculated using Epi-Info[10]

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with 95% confidence interval (CI), 5% margin of error and the prevalence of all-cause mortality due to DFUs 24.6%. The sample was raised using non-probability consecutive sampling technique.

All participants aged at least 18 years admitted with diabetic foot as their primary complaint were included. Those who lacked sufficient laboratory workup or failed to keep follow-up appointments, patients suffering from prior inflammatory conditions, such as infection, malignancy, and immunocompromised patients were excluded.

The blood samples had been collected from the patients at the time of hospital admission. Diabetic foot was defined as per the International Working Group on the Diabetic Foot (IWGDF) criteria which described the condition as infection, ulceration or destruction of tissues of the foot associated with neuropathy and/or peripheral artery disease in the lower extremity of a person with (a history of) diabetes mellitus.[11]

Grading of diabetic foot was done as follows: 1 = uninfected, with no systemic or local symptoms or signs of infection; 2 = mild infection, with no systemic manifestations, involving only the skin or subcutaneous tissue (not the deeper tissues), and no erythema, if present, extending >2cm around the wound; 3 = moderate infection, with no systemic manifestations, and involving erythema extending ≥2cm from the wound margin, and/or tissue deeper than skin and subcutaneous tissues (e.g. tendon, muscle, joint, bone,); and 4 = severe infection, with associated systemic manifestations of the systemic inflammatory response syndrome (SIRS), as manifested by ≥2 of the 4 conditions, including temperature >38°C or <36°C, heart rate (HR) >90 beats/minute, respiratory rate (RR) >20 breaths/minute or partial pressure of carbon dioxide (PaCO2) <4.3kPa, and white blood cell (WBC) count >12,000/mm^3 or <4000/mm^3, or >10% immature (band) forms.[11]

Data was collected on a specially-designed 3-part proforma. The first part was related to socio-demographic characteristics of patients along with their co-morbidities and aetiology. The second part comprised laboratory workup, including glycated haemoglobin (HBA1c) levels, NLR, platelet and creatinine levels of patients on arrival at the emergency department or ward bed. The third part was regarding the classification of diabetic foot, type of amputation required, in-hospital complications and mortality. Index surgical procedure was taken for analysis. Decision for debridement was made clinically by the attending vascular surgeon. Patient outcomes were assessed using in-hospital and one-year mortality.

Data was analysed using SPSS 26. Quantitative variables were reported as mean ± standard deviation (SD), while qualitative variables were reported as frequencies and percentages. Bivariate analysis of qualitative variables was done using chi-square test. The correlation of qualitative variables with NLR was assessed using t-test. The diagnostic performance of NLR was analysed using the receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was calculated to determine an optimum cut-off point for NLR values associated with adverse diabetic foot outcomes. Since the institution where the study was conducted used NLR 4 as the cut-off point, hence, its sensitivity and specificity at that level was assessed. $P<0.05$ was considered significant.

Results

Of the 200 DFU patients, 130(65%) were included. The overall mean age was 60.13+/-11.75 years, and 98(75.4%) of the subjects were males. The majority of them were admitted electively 75(57.6%), hypertension (HTN) was the most prevalent co-morbidity 84(64.6%), most patients relied solely on insulin for diabetic control 97(74.6%), IWGDF grades 2, 3 and 4 were found in 37(28.5%) patients each. Surgical intervention was needed in 118(90.8%) cases, with below-knee amputation being the most

Table-1: Socio-demographic characteristics.

Variables	N (%)
GENDER	
Male	98(75.4)
Female	32(24.6)
CO-MORBIDS	
Hypertension	84(64.6)
Ischaemic heart disease	41(31.5)
Chronic kidney disease	20(15.4)
DIABETIC FOOT GRADING	
1	19(14.6)
2	37(28.5)
3	37(28.5)
4	37(28.5)
MANAGEMENT	
Surgical	118(90.8)
Conservative	12(9.2)
TYPE OF AMPUTATION	
Major (Above knee and below knee)	42(35.3)
Minor (debridement, ray, transmetatarsal)	77(64.7)
In-hospital complications	27(20.8)
Re-amputation	49(41.5)
OUTCOME	
Discharged	122(93.8)
30-day mortality	8(6.1)
1-year mortality	16(12.3)

Table-2: Correlation of socio-demographics, intervention and outcomes with neutrophil-lymphocyte ratio (NLR).

Variables	NLR (mean+/-SD)	p-value
GENDER		
Male	10.5+/-9.3	<0.0001*
Female	4.6+/-2.8	
CO-MORBIDS		
Hypertension	10.3+/-9.9	0.002*
Ischaemic heart disease	9.9+/-11.0	0.06
Chronic kidney disease	13.5+/-11.3	0.002*
DIABETIC FOOT GRADING		
1	7.5+/-6.1	<0.0001
2	5.4+/-4.1	
3	6.8+/-6.2	
4	15.7+/-11.2	
MANAGEMENT		
Surgical	9.5+/-8.8	0.1
Conservative	5.5+/-5.8	
DIABETIC FOOT GRADING		
Major (Above knee and below knee)	13.1+/-10.9	<0.0001*
Minor (debridement, ray, transmetatarsal)	7.3+/-6.4	
In-hospital complications	18.2+/-11.9	<0.0001*
Re-amputation	10.9+/-8.4	0.06
OUTCOME		
Discharged	8.5+/-8.2	0.008*
30-day mortality	16.9+/-10.5	
1-year mortality	13.5+/-7.29	0.01*

*= p-value<0.05. SD: Standard deviation.

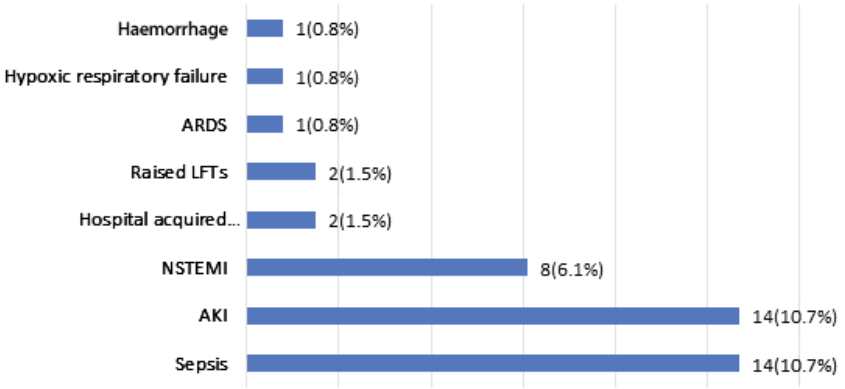

Figure-1: Post-operative complications.

frequent procedure 38(32.3%). Among the subjects, 30-day mortality was 8(6.1%), while 1-year mortality was 16(12.3%) (Table 1).

Mean NLR was 9.10+/-8.64, mean WBC count was 14.01+/-6.05 x103cells per microliter, mean platelets were 376.8+/-150.7x103 cells per microliter, mean HbA1c level was 9.06+/-2.3% and mean creatinine level was 1.8+/-1.5mg/dl.

Most patients presented with ischaemic ulcer 58(44.6%), followed by infected DFU 56(43.1%), gangrenous ulcer, necrotising infection 10(7.7%) and osteomyelitis 6(4.6%).

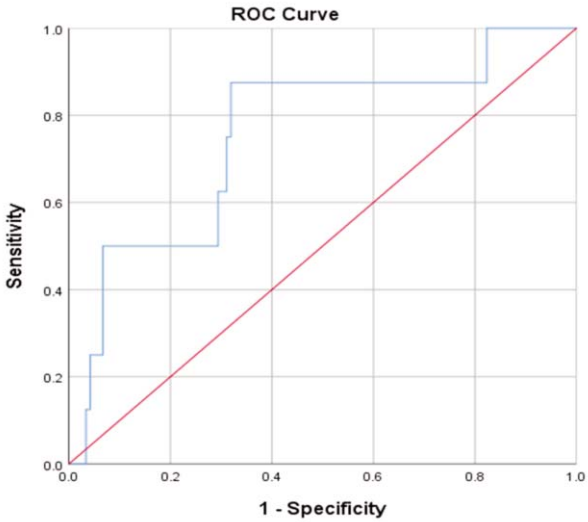

Figure-2: Receiver operating characteristic (ROC) curve.

The median LOS was 4 days (interquartile range [IQR]: 3 days); 5 days (IQR: 4 days) for major, and 3 days (IQR: 2.5 days) for minor amputations. Post-operative complications occurred in 27(20.8%) patients. Acute kidney injury (AKI) and sepsis were the most common post-operative complications (Figure 1).

Higher NLR values were noted in males, as well as hypertensive and chronic kidney disease (CKD) patients (p<0.05). Higher NLR values were associated with higher DFU grade, major amputation, in-hospital mortality and one-year mortality (p<0.05). No significant association was found between NLR and the need for re-amputation (Table 2).

ROC curve demonstrated AUC value 0.755 (p=0.016). NLR cut-off of 4 showed 87.5% sensitivity and 63.9% specificity in predicting in-hospital mortality in patients with DFU (Figure 2).

Discussion

DFU is a major cause of hospitalisation among diabetic patients. In an LMIC setting, there is a high incidence of DFU due to poor glycaemic control and lack of foot hygiene practices. The 5-year mortality rate of DFUs has been as high as 24.6% with CKD, cardiovascular events, and sepsis being the major causes leading to mortality.[11] According to a meta-analysis of 14,201 patients with DFU

in Pakistan, the pooled DFU prevalence among diabetics was 12.16% with the highest cases in the Punjab province.[12]

The current study showed a mean NLR value of 9.1 on admission which is higher than what has been seen in other similar studies where NLR values ranged 5.5-8.46.[13, 14] This leads to the need for surgical intervention in the majority of patients. The average frequency of amputations in patients with diabetic ulcers in Pakistan ranges 22-28%.[15] However, among the current patients, 118(90.8%) required surgical intervention, which is quite high. The current study included only inpatients as patients requiring surgical interventions are admitted as per the institutional practice. Patients who can be potentially treatable conservatively with intravenous (IV) antibiotics are mostly treated as outpatients.

The current patients also had a high HbA1c count with a mean of 9.06% whereas recent studies in Pakistan showed the levels ranging 5.62-8.56%.[16]

Neutrophils are inflammatory cells which release superoxide ions that damage vascular endothelium. On the other hand, lymphocytes are responsible for regulating neutrophil activity. Hence, an increase in NLR reflects uncontrolled neutrophil activity secondary to superimposed infection or other causes, leading to endothelial dysfunction and poor outcomes.[17]

AKI and sepsis were found to be the most common post-operative complications amongst the current patients (10.7%). The reason for sepsis in spite of surgical intervention maybe inadequate surgery, suggesting that patients with higher NLR had late intervention, other sources of sepsis, or inadequate debridement in order to prevent the development of sepsis.

A higher NLR value reflects a greater systemic inflammatory response and was found to be associated with higher grade of DFU, major amputation, post-operative complications, in-hospital mortality as well as 1-year mortality in the current study ($p<0.05$). Similar results have been shown in other studies.[18-20]

There is no established departmental protocol for DFU management at the hospital where the study was conducted. Culture and sensitivity tests were sent for all the specimen, but the relevant data was not available, which was a limitation. Also missing was data related to the type of antibiotics used. There was also a selection bias in the study as only inpatients needing surgical management for DFU were included.

The current study also did not include other circulating biomarkers, like neutrophils or leukocytes in isolation, C-reactive protein (CP) and albumin. These parameters may have predictive value in DFU outcomes and can be good areas for further research. CRP, erythrocyte sedimentation rate (ESR) and procalcitonin (PCT) have also shown to be significant biomarkers for predicting DFU severity in several studies.[21,22] However due to the economic feasibility of NLR, it can potentially be the preferred biomarker for predicting DFU severity.[22]

As type 2 diabetes is seen in adult population and the presence of diabetes in individuals aged <18 years is usually due to autoimmune causes, hence the current study only included patients aged at least 18 years.

The wound, ischaemia, and foot infection (WIFI) classification is the recommended one for DFUs,[23] but the current study lacked the data for ischaemic component as the toe-brachial pressure measurement facility was not available at the study site. As such, the IWGDF classification was used.

Also part of the study's limitations were single-centre data and limited sample size. Also, As the data was collected from medical records, patients' knowledge regarding DFU and hygiene practices could not be assessed. Besides, peripheral vascular disease was not assessed using non-invasive methods, such as the ankle brachial pressure index. Prospective studies are required to compare NLR's sensitivity compared to biomarkers, such as CRP, ESR and PCT in similar settings.

Conclusion

NLR was found to be a highly sensitive biomarker that could serve as an independent predictor of grade, amputation, postoperative complication and mortality due to DFU. NLR could prove to be an economically feasible test in tertiary care centres in LMICs.

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Conflict of Interest: None.

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Authors' Contribution:
ZR: Concept, design, writing, revision, final approval and agreement to be accountable for all aspects of the work.
HS: Data collection, analysis, interpretation, writing, revision and final approval.
FB: Data collection, writing, revision and final approval.