The Neutrophil Lymphocyte Ratio as a Predictor of Major Amputation Among Patients with Diabetic Foot Ulcer Admitted at a Tertiary Government Hospital: A Retrospective Cohort Study

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Abstract

Introduction. There is significantly increased morbidity among patients who undergo major amputation because of diabetic foot ulcers (DFU). Various risk factors contribute to this outcome with endothelial dysfunction potentially linked to poor wound healing. The Neutrophil Lymphocyte Ratio (NLR) may indicate endothelial dysfunction.

Objectives. This study aimed to evaluate the accuracy and utility of NLR as a predictor of major amputation in patients admitted for DFU, whose white blood cell counts (WBCs) were elevated or normal. Major amputation is defined as amputation above the tibiotalar joint

Methodology. This retrospective cohort analysis included patients admitted at East Avenue Medical Center for DFU. The primary endpoint was major amputation of the lower extremities. Data were analyzed using Receiver Operating Characteristic (ROC) analysis and logistic regression.

Results. The study included 280 DFU patients, predominantly male (62.86%) with a mean age of 57.01±10.72 years. Elevated WBC was found in 176 patients (62.86%) while 104 had normal WBC (37.14%). Major amputation of the lower extremity was performed on 112 patients (40%), with 81 having elevated WBC and 31 normal WBC. The median NLR in patients with elevated WBC undergoing major amputation was 12.86 (IQR: 2.36-95) compared to 5.71 (IQR: 1.91-31.67) in those who did not, indicating NLR as an independent predictor of major amputation (Adjusted OR 1.23; 95% CI 1.14-1.34; p<0.001). ROC analysis showed an AUC of 0.8234 with an optimal cutoff of 9.33 (72.8% sensitivity, 86.3% specificity). Other predictive variables included University of Texas Stage 3D (Adjusted OR 8.20; 95% CI 2.52-26.75; p<0.001), Wagner Grade 4 (Adjusted OR 4.6; 95% CI 1.28-16.55; p=0.019), and severe infection (Adjusted OR 2.91; 95% CI 1.22-6.93; p=0.016). For patients with normal WBC, median NLR was 18 (IQR: 1.54-45.5) in those who had major amputation versus 3.13 (IQR: 1.14-12.29) in those who did not undergo major amputation. ROC analysis showed an AUC of 0.9068 with an optimal cutoff of 6.92 (87.1% sensitivity, 98.6% specificity). NLR was also an independent predictor in these subjects (Adjusted OR 1.48; 95% CI 1.14-1.92; p=0.003) alongside smoking history (Adjusted OR 9.14; 95% CI 1.26-66.56; p=0.029) and UT3D (Adjusted OR 17.38; 95% CI 2.21-136.59; p=0.007).

Conclusion. Forty percent of DFU patients had major amputations. NLR independently predicted major amputation in DFU patients with both elevated and normal WBC.

Keywords. Diabetes Mellitus, Diabetic Foot Ulcer, Neutrophil Lymphocyte Ratio, Amputation

Introduction

Diabetic foot ulcers are one of the major complications of diabetes mellitus which may result in amputation. It causes significant morbidity in the population with a global prevalence of 6.3%.[1] The possible outcomes of diabetic foot ulcers are: (1) Healed diabetic foot ulcer described as full epithelialization of the ulcer, (2) Major amputations defined as an above ankle amputation,(3) Minor amputation defined as below ankle amputation or (4) A persistent non- healing ulcer. Major amputations occur in 11% of patients suffering from diabetic foot ulcer[2]. Risk factors associated with diabetic foot ulcers

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include peripheral vascular disease, neuropathy, diabetes mellitus with a duration of greater than 10 years, insulin use, retinopathy, nephropathy, age >45, cerebrovascular disease, poor glycemic control, coronary artery disease, male gender, smoking, and hypertension[3]. Other variables that were previously found to be associated with major amputation include history of previous amputation, smoking, Wagner 5, higher Hba1c, low hemoglobin, and higher WBC count[4]. The University of Texas classification has also been evaluated in predicting outcome of diabetic foot ulcers and it was found that the higher the University of Texas classification grade the more frequent the amputations are and has been found to be a better predictor of outcome than the Wagner Classification [5]. The Wagner classification of diabetic foot classifies diabetic foot on the extent of the ulcer with grade 0 having no ulcer but at risk for developing an ulcer, Grade 1 a superficial ulcer, Grade 2 having deep full thickness ulcer, Grade 3 having deep abscess or osteomyelitis, Grade 4 having partial gangrene of the forefoot and Grade 5 presence of extensive gangrene [6]. The University of Texas Classification is used to describe the depth of ulcer and presence of infection and the presence of ischemia. It is graded from 0 to 3 which indicates the depth of ulcer and A to D describes presence of infection and/or ischemia. Grade 0 indicates no skin break Grade 1 indicates superficial ulcer Grade 2 indicates the wound penetrates the tendon or capsule and Grade 3 penetrates the bone or joint. It is further categorized into stages based on presence or absence of ischemia and/or infection. Stage A indicates no infection or ischemia, Stage B indicates presence of infection, Stage C indicates presence of ischemia and Stage D indicates presence of both infection and ischemia.[7]

Inflammation has been associated with changes in circulating white blood cells particularly neutrophilia with relative lymphocytopenia[8]. The ratio of neutrophil to lymphocyte (NLR) has been implicated in various conditions including severe inflammation and has been found to be a predictor of poor outcomes in malignancy. Indeed it has been shown to be of prognostic value in patients who had ST segment myocardial infarction[9] and mortality in patients with coronary artery disease[10]. It also moderately identifies early sepsis in ICU patients[11]. It has also been shown to predict mortality among patients with influenza and chronic lower respiratory disease and chronic kidney disease.[12]

Neutrophil Lymphocyte ratio has been associated with multiple microvascular complications of diabetes mellitus including retinopathy, neuropathy, and nephropathy[13] and endothelial dysfunction. Endothelial dysfunction may be involved in the pathogenesis of poor wound healing. Improvement of endothelial dysfunction has been shown in vitro to have a positive role in diabetic wound healing.[14]

Neutrophil lymphocyte ratio has been shown to predict outcome in diabetic foot ulcers in a study in Turkey where the level of less than 4.3 has been shown to predict full recovery.[15] However, in this study all subjects in had normal WBC levels

Significance of the Study

The study evaluates a cheap readily available biomarker for prognosticating outcomes of diabetic foot ulcer patients

Research Qustion

Does Neutrophil Lymphocyte Ratio predict outcomes in patients admitted for diabetic foot ulcer?

General Objective

To determine the association between neutrophil lymphocyte ratio and major amputation among patients admitted for diabetic foot ulcer at tertiary government hospital from 2016 to 2019

Specific Objectives

1. To determine the neutrophil lymphocyte ratio level among major amputations in patients with diabetic foot ulcers.
2. To determine the accuracy of Neutrophil Lymphocyte ratio as a predictor of major amputation in admitted patients with diabetic foot ulcers
3. To determine the association of Neutrophil Lymphocyte ratio and major amputation among patients with diabetic foot ulcers with normal White Blood Cell Count(WBC) and those with elevated WBC

Methodology

This paper was written following the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

Study Design

It is a retrospective cohort study.

Study Setting and Time Period

This study was conducted in a tertiary government hospital in Quezon city. The medical records of patients who were admitted from January 1, 2016, to December 31, 2019, were retrieved and reviewed

Study Population

Initially all patients previously admitted at the East Avenue Medical Center for Diabetic foot ulcer from

January 2016 to December 2019 were included in the study.

Inclusion criteria for those who were included in the study were:

1. Patients previously admitted whose records contained the following data: history of previous amputation or no history of previous admission, smoking history, Wagner classification, University of Texas Classification, Hba1c, and CBC. The presence of infection and its severity were also evaluated for accuracy by the primary investigator using on the Definition of diabetic foot ulcer infection by the International Working Group on the Diabetic Foot (IWGDF) and based on the physical examination description in the patients' records

The exclusion criteria were

1. Patients with foot ulcers who were not diabetic
2. Patients on immunosuppressive therapy
3. Patients with current or previous malignancy
4. Patients with known hematologic disorders.
5. Previously admitted patients whose charts had incomplete data.
6. Patients with concomitant or other foci of infection
7. Patients with concomitant autoimmune vasculitis
8. Patients with concomitant acute vascular insult on admission (myocardial infarction, stroke)
9. Patients who were unable to complete in hospital management and were discharged against medical advice
10. Patients who expired prior to completing the therapeutic plan.

Sample Size

Minimum sample size requirement was computed using G*Power version 3.1. A minimum sample size of 211 is needed to achieve 80% power with 5% level of significance in a logistic regression analysis to detect an odds ratio with small to medium effect size (OR=2.00) in a population expected to have amputation of 13%[16], and an R-squared of about 30% for the different covariates.

Operation Definition

1. Complete Blood Count is a laboratory test using blood extracted and placed in EDTA tubes and analyzed using hematology automated counter
2. Hba1c is a laboratory test that determines the average blood sugar level for the past 3 months. It is measured using the immunoassay method using Abbott Architect Plus c4000. The Hba1c has verified linearity with NGSP standards from 4.0-15% with tolerance of ± 0.14%
3. Serum Creatinine was measured by enzymatic method using the Abbott Architect Machine
4. Presence of infection is determined by the definition of IWGDF Guidelines if there is presence of 2 of the following
    a. Erythema >0.5 cm around the wound
    b. Local tenderness or pain
    c. Warmth
    d. Discharge
    e. Absence of any other causes such as gout, trauma, osteoarthropathy, fracture, thrombosis and venous stasis
5. Ulcer is defined as presence of break in the skin
6. Gangrene is defined as devitalized, necrotic tissue.
7. Major amputation is defined as an amputation above the tibiotalar joint including but not limited to below knee amputation and above knee amputation
8. Minor amputation is defined by amputation below the tibiotalar joint and can include Ray amputation, disarticulation Syme amputation

Data Collection

A census of patients admitted for diabetic foot ulcer from 2016-2019 was obtained from the East Avenue Medical center records section. All the patient records were retrieved and counterchecked with the census. The author selected the records of patients based on the inclusion and exclusion criteria outlined in the study. The neutrophil lymphocyte ratio (NLR) was computed as the absolute neutrophil count and the absolute lymphocyte count based on the CBC upon admission which was obtained and processed at the East Avenue Medical Center laboratory. The eGFR was computed using the CKD Epi formula[17]

The following demographic data were collected from the patient records

1. Age
2. sex
3. history of previous amputation
4. smoking history
5. Wagner classification
6. University of Texas Classification
7. Hba1c
8. WBC
9. Creatinine
10. eGFR
11. Neutrophil
12. Lymphocyte
13. Hemoglobin
14. Outcome

The outcome of the patient was classified as major amputation or minor amputation (those with below ankle amputation, healed, persistent non healing ulcer). The Wagner classification and University of Texas Classification were evaluated for accuracy by the primary investigator based on the foot ulcer description on the physical exam noted in the patient records as well as ancillary procedures done to the patient including but not limited to foot radiography and Arterial-Venous duplex scan results done on the subjects. Inherent to the retrospective design is a probability of bias since the assurance of data quality was not identified ahead of time. Efforts were made to address this bias by counterchecking classifications of the wound with actual physical exam description if it matched the wound classification and ancillaries done and correction was made if errors were noted.

The data collection included the patient's name and hospital number and date of birth. However, this

information was only available to the primary investigator. The Data collection form was a password protected Excel file in the primary investigators personal laptop and was kept for 36 months. The patient's identity was changed to a randomly generated Subject ID number, and this data was used for the statistical analysis. The primary investigator strictly complied with the Data Privacy Act (2012) and the National Ethical Guidelines for Health and Health Related Research (2017). Approval was obtained from the Institutional Ethics Review Board.

Data Analysis

Descriptive statistics was used to summarize the general and clinical characteristics of the participants. Frequency and proportion were used for categorical variables. Shapiro-Wilk test was used to determine the normality distribution of continuous variables. Continuous quantitative data that met the normality assumption were summarized using mean and standard deviation (SD), while those that do not were described using median and range.

A Receiver Operating Characteristic (ROC) curve analysis was performed and the optimal cutoff point of NLR in predicting major amputation was determined based on the Youden's index. Separate cutoff levels were obtained by WBC classification (normal and elevated) and two-by-two tables were constructed.

Evaluation for interaction/effect measure modification was done for NLR with Elevated WBC was determined by LR test that model without interaction is nested in the model with interaction.

Kruskal Wallis test was used to identify if there is significant difference of NLR between subjects with no infection, moderate infection and severe infection, and Post-hoc Dunn Test was used to identify differences between each group.

Logistic regression was used to determine the predictors of major amputation in admitted patients with diabetic foot ulcers. Variables associated with the outcome were identified using stepwise logistic regression. Crude and adjusted odds ratios and its corresponding 95% confidence intervals were reported. Missing variables were neither replaced nor estimated. Null hypothesis was rejected at 0.05α-level of significance. STATA 17.0 (StataCorp SE, College Station, TX, USA) was used for data analysis.

Data Analysis

A total of 330 patient records were reviewed and 50 were excluded leaving a total of 280 participants in the analysis. (Fig1)

Table 1 lists the demographic and clinical profile of the subjects included in the study. The mean age of patients was 57.01 (±10.72), the majority were male comprising 176 of 280 subjects (62.86%) 17 of 280 (6.07%) had history of previous amputation done and 59 of 280 (21%) had a history of tobacco use. The most common Wagner Grade was 4 comprising 172 of 280 (61.43%) of the subjects. The most frequent University of Texas classification is 3D comprising 156 of 280 (55.71%) of the subjects. One hundred and twelve out of 280 (40%) had major amputation

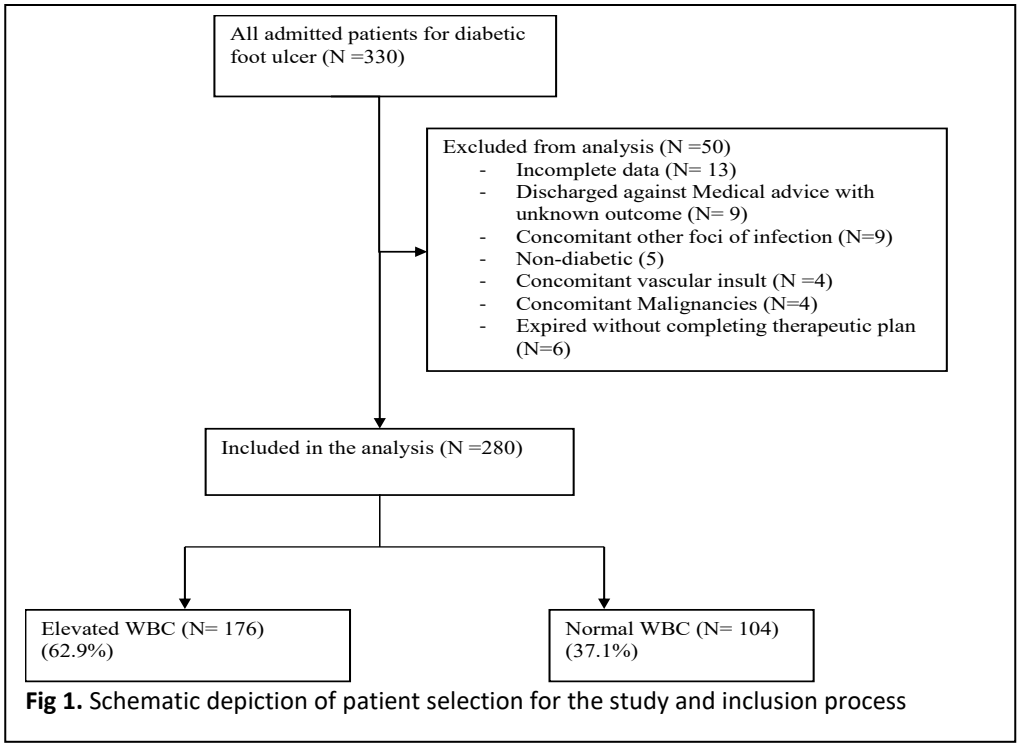


Fig 1. Schematic depiction of patient selection for the study and inclusion process

Table 1. Demographic and clinical profiles of the patients (n= 280)

Profile	Mean (SD); Median (IQR); Frequency (%)
Age, years	57.01 (10.72)
Sex	
Male	176 (62.86%)
Female	104 (37.14%)
Clinical findings	
History of previous amputation	17 (6.07%)
Smoking history	59 (21.07%)
Wagner classification	
Grade 0	0
Grade 1	12 (4.29%)
Grade 2	33 (11.79%)
Grade 3	49 (17.50%)
Grade 4	172 (61.43%)
Grade 5	14 (5.00%)
University of Texas Classification	
1A	0
1B	3 (1.07%)
1C	0
1D	1 (0.36%)
2A	2 (0.71%)
2B	36 (12.86%)
2C	8 (2.86%)
2D	12 (4.29%)
3B	61 (21.79%)
3C	1 (0.36%)
3D	156 (55.71%)
Presence of chronic kidney disease	163 (58.21%)
Presence of infection	
No infection	11 (3.93%)
Moderate infection	180 (64.29%)
Severe infection	89 (31.79%)
Laboratory findings	
Elevated WBC count	176 (62.86%)
Neutrophil to lymphocyte ratio (NLR)	6.11 (1.14-95)
Hemoglobin, g/L	105 (31)
HbA1c, %	9.13 (4.2)
Creatinine, mg/dL	102.35 (43.4-1367)
eGFR, mL/min/1.73m^2	65.55 (3.2-123)
Outcome	
Major amputation	112 (40.00%)

Table 2 lists the clinical and demographic profile of the subjects as stratified based on the level of WBC. On stratification of subjects based on their WBC, 176 had elevated WBC and 104 had normal WBC levels. Eighty one (46%) of the patients with elevated WBC underwent major amputation and 31(29.81%) of patients with normal WBC underwent major amputation. Among patients with normal WBC, those who underwent major amputation were older with a mean age of 64.29(±11.69) compared to those who did not with a mean age of 53.36 (±9.01). Men predominantly comprised the subjects who underwent major amputation (90.32%), while women comprised 9.68% of the subjects. The most frequent Wagner grade in subjects who underwent major amputation was Grade 4 (83.87%) as was those who did not undergo major amputation at 38.36%. The most frequent University of Texas classification was 3D in those that underwent major amputation (90.32%), while University of Texas 3B was the most frequent University of Texas Classification among subjects who did not undergo major amputation (41.10%). The median NLR for those that underwent major amputation was higher at 18 (IQR: 1.54-45.5) compared to those that did not undergo major amputation at 3.13 (IQR:1.14-12.29) Majority of the subjects who underwent major amputation had chronic kidney disease (83.87%), while less than half among the subject who did not undergo major amputation had chronic kidney disease (43.84%). Most of the patients who did not undergo major amputation had moderate infection (83.56%) while more than half of those who underwent major amputation had severe infection (51.61%).

Among patients with elevated WBC counts, the mean age between those who had major amputation and did not undergo major amputation were 57.86 years (±10.08) and 56.71 years (±10.97) respectively. More men comprised the subjects who had major amputation (60.49%). The most common Wagner Grade in the subjects with elevated WBC was Wagner 4 accounting for 79.01% of the subjects who underwent major amputation and 56.84% of those who did not undergo major amputation. The most frequent University of Texas classification was 3D accounting to 86.42 % of the cases who underwent major amputation as well as those that did not undergo major amputation (44.21%) Median NLR was higher in those subjects who had major amputation at 12.86(IQR: 2.36-95) compared to those that did not 5.71(IQR: 1.91-31.67). More patients who underwent major amputation had severe infection than those who did not at 58.02% and 23.16% respectively.

Table 2. Demographic and clinical profile of the patients stratified to WBC count.

Profile	Elevated WBC cohort		Normal WBC cohort	
	n = 176		n = 104	
	Major amputation	No major amputation	Major amputation	No major amputation
	n = 81	n = 95	n = 31	n = 73
	Mean (SD); Median (IQR); Frequency (%)		*Mean (SD); Median (IQR); Frequency (%)*	
Age, years	57.86 (10.08)	56.71 (10.97)	64.29 (11.69)	53.36 (9.01)
Sex				
Male	49 (60.49%)	60 (63.16%)	28 (90.32%)	39 (53.42%)
Female	32 (39.51%)	35 (36.84%)	3 (9.68%)	34 (46.58%)
Clinical findings				
History of previous amputation	7 (8.64%)	4 (4.21%)	3 (9.68%)	3 (4.11%)
Smoking history	7 (8.64%)	4 (4.21%)	28 (90.32%)	20 (27.40%)
Wagner classification				
Grade 0	0	0	0	0
Grade 1	0	6 (6.32%)	1 (3.23%)	5 (6.85%)
Grade 2	1 (1.23%)	14 (14.74%)	1 (3.23%)	17 (23.29%)
Grade 3	5 (6.17%)	21 (22.11%)	0	23 (31.51%)
Grade 4	64 (79.01%)	54 (56.84%)	26 (83.87%)	28 (38.36%)
Grade 5	11 (13.58%)	0	3 (9.68%)	0
1A	0	0	0	0
1B	0	3 (3.16%)	0	0
1C	0	0	0	0

1D	1 (1.23%)	0	0	0
2A	0	0	0	2 (2.74%)
2B	1 (1.23%)	18 (18.95%)	0	17 (23.29%)
2C	0	2 (2.11%)	1 (3.23%)	5 (6.85%)
2D	1 (1.23%)	8 (8.42%)	1 (3.23%)	2 (2.74%)
3B	8 (9.88%)	22 (23.16%)	1 (3.23%)	30 (41.10%)
3C	0	0	0	1 (1.37%)
3D	70 (86.42%)	42 (44.21%)	28 (90.32%)	16 (21.92%)
Presence of chronic kidney disease	50 (61.73%)	55 (57.89%)	26 (83.87%)	32 (43.84%)
Presence of infection				
No infection	0	2 (2.11%)	1(3.23%)	8(10.96%)
Moderate infection	34 (41.98%)	71 (74.74%)	14 (45.16%)	61 (83.56%)
Severe infection	47 (58.02%)	22 (23.16%)	16 (51.61%)	4 (5.48%)
Laboratory findings				
Neutrophil to lymphocyte ratio (NLR)	12.86 (2.36-95)	5.71 (1.91-31.67)	18 (1.54-45.5)	3.13 (1.14-12.29)
Hemoglobin, g/L	99 (23)	105 (34)	93 (19)	120 (24)
HbA1c, %	9.45 (4.2)	8.7 (4.2)	14 (5.7-18.6)	8.4 (5-20.5)
Creatinine, µmol/L	123.1 (43.4-912.9)	103.6 (44.5-1000)	279.45 (206.14)	122.83 (167.84)
eGFR, mL/min/1.73m^2	54.2 (5.4-115.1)	68 (3.4-123)	29.1 (29.6)	80.4 (36.9)

An ROC was constructed (Fig 2) and it showed that NLR is an excellent predictor of major amputation among patients with normal WBC count with an Area Under the Curve (AUC) of 0.9068 and for patients with elevated WBC with an AUC of 0.8234. The ROC analysis showed that the optimal NLR cut off point for major amputation was 9.33 for those with elevated WBC, with sensitivity of 72.8% and specificity of 86.3% and a cutoff point of 6.92 among patients with normal WBC with a sensitivity of 87.1% and specificity of 98.6% (Table 3)

Kruskal Wallis test was done showing significant difference of NLRs between the three groups of no infection moderate infection and severe infection (H(2) = 52.421, p <0.001) Post-hoc Dunn Test showed severe infection has significantly higher NLR (median: 12.43 IQR: 5.85-22.25), than moderate infection (median: 4.93, IQR:3.11-8.6) (p = 0.008) and no significant difference between no infection(median: 2.916, IQR: 1.87-6.56) vs moderate infection (p =0.166).

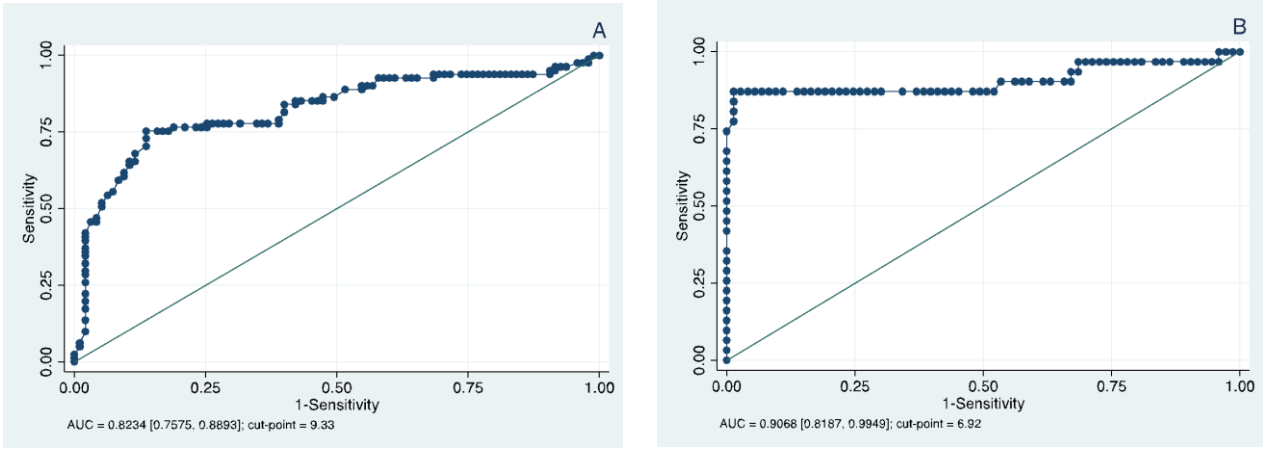


Figure 2: Receiver operating characteristic curve of NLR showing that it is a good predictor of major amputation among: A – patients with elevated WBC count (AUC = 0.8234); B – normal WBC count (AUC = 0.9068).

Table 3: Neutrophil Lymphocyte Ratio and major Amputation outcomes

NLR*	Major amputation	No major amputation	Sensitivity	Specificity
	Frequency (%)			
Among elevated WBC				
≥9.33	59 (72.84%)	13 (13.68%)	72.8%	86.3%
<9.33	22 (27.16%)	82 (86.32%)		
Among normal WBC				
≥6.92	27 (87.10%)	1 (1.37%)		
<6.92	4 912.90%)	72 (98.63%)	87.1%	98.6%

Effect modification among variables was also evaluated with its relationship with major amputation, it was identified that elevated WBC is a significant effect measure modifier to the relationship of NLR with major amputation. There was no sufficient evidence to show that University of Texas classification are significant effect modifiers with the relationship of Wagner Grade with major amputation and between each other with major amputation (table 4)

Table 4: Effect-measure modification among the variables of interest in its relationship with major amputation.

Interactions of Interest	LR test p-value
NLR with Elevated WBC	0.0075
Wagner grade with University of Texas classification	0.5818

On univariate logistic regression analysis, the following variables were found were found to be predictors of major amputation among patients with elevated WBC: Neutrophil Lymphocyte Ratio, Wagner classification ≥ grade 4, University of Texas 3D, presence of severe infection and creatinine level. On multivariable logistic regression model, Neutrophil Lymphocyte ratio University of Texas classification 3D, presence of severe infection, and Wagner Grade 4 remained significant predictors of major amputation (Table 5)

Among patients with normal WBC, the predictors of major amputation seen on univariate logistic regression analysis are: Neutrophil Lymphocyte Ratio, Age, smoking history, Wagner Grade ≥ 4, University of Texas classification 3D, chronic kidney disease, severe infection, higher HbA1c and higher serum creatinine. The characteristics that were associated with lower odds of undergoing major amputation are female sex, higher Hemoglobin level and higher eGFR. On multivariable analysis only Neutrophil Lymphocyte Ratio, smoking history and University of Texas Classification 3D remained significant predictors. (Table 6)

Table 5. Predictors of major amputation among patients with elevated WBC count.

Factors	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Neutrophil to lymphocyte ratio (NLR)	1.24	1.15, 1.34	<0.001	1.23	1.14, 1.34	<0.001
Age	1.01	0.98, 1.04	0.467			
Female sex	1.12	0.61, 2.06	0.717			
History of previous amputation	2.15	0.61, 7.63	0.235			
Smoking history	2.15	0.61, 7.63	0.235			
Wagner classification ≥ Grade 4	9.49	3.76, 23.94	<0.001	4.60	1.28, 16.55	0.019
University of Texas classification 3D	8.03	3.78, 17.06	<0.001	8.20	2.52, 26.75	<0.001
Presence of chronic kidney disease	1.17	0.64, 2.15	0.605			
Presence of severe infection	4.09	2.16, 7.75	<0.001	2.91	1.22, 6.93	0.016
Hemoglobin[a]	0.99	0.98, 1.00	0.076			
HbA1c[b]	1.06	0.95, 1.18	0.304			
Creatinine[c]	1.00	1.00, 1.00	0.026			
eGFR[d]	0.99	0.98, 1.00	0.053			

a. Hemoglobin in g/L b. Hba1c in % c. creatinine in μmol/L d eGFR in ml/min/m^2

Table 6: Predictors of major amputation among patients with normal WBC count

Factors	Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Neutrophil to lymphocyte ratio (NLR)	1.6	1.30, 1.97	<0.001	1.48	1.14, 1.92	0.003
Age	1.12	1.06, 1.18	<0.001			
Female sex	0.12	0.03, 0.44	0.001			
History of previous amputation	2.5	0.48, 13.14	0.279			
Smoking history	24.73	6.76, 90.48	<0.001	9.14	1.26, 66.56	0.029
Wagner classification ≥ Grade 4	23.3	5.16, 105.34	<0.001			
University of Texas classification 3D	33.25	8.94, 123.66	<0.001	17.38	2.21, 136.59	0.007
Presence of chronic kidney disease	6.66	2.30, 19.29	<0.001			
Presence of severe infection	16.51	5.22, 52.22	<0.001			
Hemoglobin[a]	0.96	0.94, 0.99	0.002			
HbA1c[b]	1.53	1.29, 1.81	<0.001			
Creatinine[c]	1.01	1.00, 1.01	0.002			
eGFR[d]	0.95	0.93, 0.97	<0.001			

Data Analysis

The rates of major amputation were higher in this study as compared to the study presented by Felipe et al[2] at 11% in the outpatient patient diabetic foot ulcer clinic. This was expected as patients in the outpatient setting have less severe ulcers (66.3% had below Wagner stage 4) compared to the inpatient population who had Wagner stage 4 or higher (66.43%).

The data in our study indicates that NLR, even after adjusting for other covariates was found to be associated with major amputation. This was congruent with the findings of Gonzales-Fajardo et.al.[18] that NLR is associated with increased risk of amputation among patients with chronic critical limb ischemia and the findings of Altay et.al[19] that higher NLR level is associated with more severe infection and need for revascularization and prolonged hospital stay and was corollary to the findings of Vatankhah et. al.[16] that lower NLR is predictive of wound healing of diabetic foot ulcers. This is also consistent with the findings of Demildar and Sen [20] that NLR was elevated (with a cut off value of >8.2)in patients who required amputation compared to those who had medical treatment and debridement. The NLR cut off identified in our study was 9.33 and 6.92 for subjects with elevated WBC and normal WBC respectively which was lower compared to the findings of Demildar and Sen[20], but was higher than that of the previously proposed cut-off of 5.2 for patients with chronic critical limb ischemia. Also in the same study, the odds ratio of amputation was not identified, and it was not adjusted for other possible confounders affecting amputation outcomes. Our study indicates that the odds of major amputation increases by 1.6 times for every unit increase of NLR for patients with normal WBC and 1.24 times for patients with elevated WBC.

The elevated NLR could be a marker of endothelial dysfunction which leads to worse outcomes in diabetic foot ulcers and the endothelial dysfunction and damage are caused by the inflammatory mediators released by neutrophils (Vatankhah etal., 2017)[16]. Lymphocytes induce modulatory effects on inflammation and there is increased lymphocyte apoptosis during inflammation (de Jager et.al, 2010). [21] Additionally, the reduced lymphocyte counts may be due to oxidative stress damage caused by hyperglycemia on lymphocytes (Otton et.al., 2004).[22]

Other predictors of major amputation for subjects with elevated WBC count found in our study included University of Texas 3D, which was consistent with the study of Vera-Cruz et.al [23]. as well as presence of severe infection as amputation may have been used as a source control measure to prevent life threatening infection.

In subjects with normal WBC other identified predictors of major amputations include smoking history consistent with the study of Liu et.al. [24].

Of note HbA1c was noted to be only correlated to major amputation in subjects with normal WBC but not in those with elevated WBC. This correlation however disappears in the fully adjusted model which is congruent with the study of Fasseha et.al.[25] where HbA1c was not found to be associated with wound healing among patients with diabetic foot ulcer. A possible reason for this is that the HbA1c determined at the time of evaluation was not reflective of the real glycemic control of the patients since the mean hemoglobin of the subjects was low at 105 g/L.

Hemoglobin level was not found to be associated with major amputation in the fully adjusted model in both groups of subjects with normal and elevated WBC in

contrast to the study of Gezawa et.al.[26] However in the study of Gezawa the type of amputation performed was not identified. These differences may be attributable to differences in the population where more of their subjects had more severe diabetic foot ulcers wherein 16.1% had Wagner Grade 5 compared to our studies population that only 5% had a Wagner grade 5.

Our study indicates that the Neutrophil Lymphocyte ratio may be a good predictor for major amputation in patients with diabetic foot ulcer and can be used in prognosticating outcomes of patients upon admission and warrants immediate referral to orthopedic surgery. The results of this study may lack generalizability given the set-up in the institution where limb sparing strategies are preferred and patients stay for prolonged periods of time receiving intravenous antibiotics and frequent sharp debridement.

The limitations of this study are its retrospective design and differences in physicians' documentation of the foot ulcer that limit its accuracy. Furthermore, the stability of NLR was not assessed over time. We recommend for future research, a prospective study design where all the foot ulcers are evaluated by a single person. Moreover, a larger sample size might better evaluate the association of NLR with major amputation outcomes.

Conclusion

Neutrophil Lymphocyte ratio is a good predictor of major amputation among subjects admitted for diabetic foot ulcer. Neutrophil lymphocyte ratio maintained a significant association with major amputation even after controlling for covariates in subjects with normal and elevated WBC. The optimal cutoff point was 9.33 in subjects with elevated WBC with a good accuracy with an AUC of 0.8234 and a cutoff value of 6.92 for subjects with normal WBC with an excellent accuracy with an AUC of 0.9068. Other significant predictors found for major amputations were smoking history and University of Texas classification of 3D for subjects with normal WBC and presence of severe infection among subjects with elevated WBC.

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