

RESEARCH

Evaluation of osteomyelitis in diabetic foot ulcers with exposed bone

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Abstract

Purpose Exposed bone and periosteal damage promote the adherence of pathogens to bone matrix components. Damage to the periosteum adversely affects bone perfusion and creates a more suitable environment for pathogens. The purpose of this study was to determine the prevalence of osteomyelitis among patients with diabetic foot ulcers with bone exposure and to identify the role of simple serological markers in the diagnosis of osteomyelitis in this patient group.

Methods Patients who underwent amputation and debridement due to diabetes-related foot wound were included in the study. The pathology results were evaluated according to presence of osteomyelitis. C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), complete blood count (CBC), platelet/lymphocyte ratio, neutrophil/lymphocyte ratio (NLR) and microbial growth were analysed.

Results Patients with ESR of 79mm/h or lower had a 3.046-fold higher risk of osteomyelitis. The risk of osteomyelitis was 2.901-fold higher at lymphocyte percentages of 12.3% or higher. Patients with a neutrophil percentage of 78% or lower had a 3.010-fold higher risk of osteomyelitis. Patients with NLR of 6.02 or lower had a 2.901-fold risk of osteomyelitis. When ESR was evaluated with neutrophil percentage, lymphocyte percentage and NLR, the sensitivity was calculated as 86.76% for osteomyelitis.

Conclusion We believe that the immune response caused by bone exposure to the environment is different than the immune response caused by soft tissue infection in the classical diabetic foot. The combined evaluation of multiple diagnostic parameters increases the sensitivity of osteomyelitis diagnosis.

Keywords osteomyelitis, diabetic foot ulcers

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Introduction

Diabetic osteomyelitis generally occurs as a result of an open chronic wound of the foot becoming infected and extending to the bone. Approximately 50–60% of severe foot infections are complicated by osteomyelitis^{1,2}. Furthermore, the prevalence of osteomyelitis can be as high as 66% in existing infected foot wounds³.

Chronic wounds carry high risk for osteomyelitis. Weight distribution on the feet is disrupted due to orthopaedic deformities, leading to non-healing wounds and further increasing the risk of osteomyelitis⁴⁻⁷. Ulcers larger than 2cm² are reported to have 56% sensitivity and 92% specificity for the diagnosis of osteomyelitis. The incidence of underlying osteomyelitis in deep ulcers (>3mm) compared to superficial ulcers is 33% versus 82%^{1,8}.

Three independent risk factors have been identified for the development of diabetic osteomyelitis – wounds that penetrate to the bone or joint, history of previous lower limb wound, and recurrent or multiple wounds. Among these factors, the relative risk of osteomyelitis was calculated as 23.1 for wounds penetrating to the bone or joint⁹. Exposed bone and periosteal damage promote the adherence of pathogens to bone matrix components. Damage to the periosteum adversely affects bone perfusion and creates a more suitable environment for pathogens.

The probe-to-bone test, which determines the relationship between the bone and the external environment, is another criterion used in the diagnosis of osteomyelitis. It has been reported that this test has 66–87% sensitivity and 85–91% specificity in the diagnosis of osteomyelitis^{3,10,11}. However,

although the probe-to-bone test is the most useful clinical examination, its reliability may be affected by factors such as the performing clinician's technique and experience, the ulcer's location, and its aetiology¹²⁻¹⁴.

According to the International Working Group on the Diabetic Foot (IWGDF) Infection Guideline 2019¹⁵, in a person with diabetes and suspected osteomyelitis of the foot, advanced diagnostic imaging of the foot is not recommended if simple and accessible methods such as plain X-ray and clinical and laboratory findings are compatible with osteomyelitis; advanced imaging methods are recommended only in doubtful cases¹⁵. However, characteristic changes may not occur until approximately 2 weeks after the development of infection, and this should be taken into account to avoid a premature diagnosis¹¹.

Although advanced imaging modalities such as MRI, radiolabelled white blood cell (WBC) single-photon emission computed tomography/computed tomography (SPECT/CT), and 18F-FDG positron emission tomography/CT (PET/CT) can also be used in the diagnosis of osteomyelitis, the gold standard test for osteomyelitis is histopathological or microbiological studies of a bone biopsy or aspiration of pus from the bone. However, biopsy is an invasive procedure that often requires anaesthesia, and obtaining results can take days¹⁶.

Many recent studies have investigated the use of serological markers such as C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), platelet/lymphocyte ratio, and neutrophil/lymphocyte ratio (NLR) in the diagnosis and treatment follow-up of osteomyelitis¹⁷⁻¹⁹. These parameters can be measured from patient blood and serum samples at a much lower cost compared to other methods or can be easily calculated from measured parameters.

Most previous studies using these parameters have focused on the relationship between diabetic foot infection and osteomyelitis. The aim of this study at Sivas Cumhuriyet University Plastic Reconstructive and Aesthetic Surgery Clinic was to determine the role of NLR, platelet/lymphocyte ratio, CRP and ESR as biomarkers in the diagnosis of osteomyelitis in patients with bone exposure associated with a diabetes-related ulcer.

Methods

Patients who underwent surgery for diabetes-related foot wounds were retrospectively screened from the electronic records system. The pathology results of amputation and bone biopsy materials obtained from patients who had a diabetic foot ulcer with exposed bone or positive probe-to-bone test between 1 January 2006 and 30 April 2020 were evaluated.

Patients who underwent amputation and debridement for a diabetes-related foot wound under operating room conditions were included in the study. Bone biopsy was performed

in patients with bone exposure by using Rounger forceps to obtain bone fragments depending on the size of the bone being examined. Amputation materials were sent for histopathological examination after the surgical procedure. Tissue biopsy for microbiological examination was obtained under sterile conditions during the operation. Tissue biopsy cultures were performed to assess bacterial growth in the wounds. However, the tissue cultures contained soft tissue and/or bone because the bone and/or soft tissue were sent together. Therefore, tissue separation could not be made in cultures and only histopathological analysis was used for the diagnosis of osteomyelitis.

Based on the histopathologic results, the patients were divided into the osteomyelitis group and non-osteomyelitis group. Patients without osteomyelitis were also classified based on the presence of pathologic changes in the bone marrow such as bone marrow inflammation and/or oedema.

Analysis of CRP level, ESR, complete blood count (CBC) values, and microbial growth in wound site cultures were done prior to amputation and biopsy. From the CBC results, WBC, platelet, neutrophil, lymphocyte, monocyte and basophil counts and percentages were used to calculate platelet/lymphocyte ratio and NLR. Parameters that showed a statistically significant difference between the groups in initial comparisons were further analysed to determine cut-off, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR) and negative LR.

Patients who did not have histopathological evaluation of bone tissue, whose histopathological evaluation could not be performed due to insufficient material, or whose tissue culture, CBC, ESR or CRP results could not be obtained were excluded from the study. Other exclusion criteria were having undergone amputation and debridement because of ischaemic conditions or Buerger disease and history of haematological disease.

Statistical analyses of the data were performed using NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) software. Descriptive statistical methods (mean, standard deviation, median, frequency, percentage, minimum, maximum) were used to summarise the data. Normal distribution of quantitative data was tested using the Shapiro-Wilk test and graphical methods. For pairwise comparisons of numerical data, the Student's t-test was used for normally distributed variables and the Mann-Whitney U test for non-normally distributed variables. Pearson's chi-square test was used for comparisons of qualitative data. Receiver operating characteristic (ROC) curve analysis and diagnostic screening tests were used to determine optimal cut-off points for the differentiation of patients with and without osteomyelitis; p value <0.05 was considered statistically significant.

The measures of diagnostic accuracy used were:

- Sensitivity: Ability of the test to identify patients who have osteomyelitis.
- Specificity: Ability of the test to identify patients who do not have osteomyelitis.
- PPV: Probability that a patient with a positive result is truly positive (osteomyelitis).
- NPV: Probability that a patient with a negative result is truly negative (non-osteomyelitis).
- Positive LR: sensitivity / (1 – specificity)
- Negative LR: (1 – sensitivity) / specificity

Results

Patients who were operated for a diabetes-related foot wound at Sivas Cumhuriyet University Plastic Reconstructive and Aesthetic Surgery Clinic between 1 January 2006 and 30 April 2020 were identified from hospital records. Of these, data for all analysed parameters were obtained for 136 patients (15.4% [n=21] women; 84.6% men [n=115]) with exposed bone or positive probe-to-bone test. The patients ranged in age from 38–92 years, with a mean age of 65.74±10.03 years. The patients’ descriptive characteristics are shown in Table 1.

Table 1. Distribution of the patients’ descriptive characteristics

Descriptive characteristic	n (%)
Age (years)	
Min–max (median)	38–92 (66)
Mean±SD	65.74±10.03
Sex	
Female	21 (15.4)
Male	115 (84.6)
Osteomyelitis	
Present	68 (50.0)
Absent	68 (50.0)
Bone marrow changes without osteomyelitis (n=68)	
Present	52 (76.5)
Absent	16 (23.5)

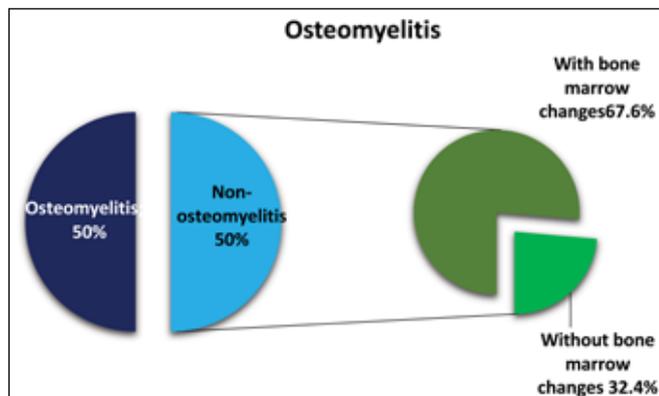


Figure 1. Distribution of osteomyelitis status

Table 2. Distribution of bone marrow changes

Bone marrow changes	n (%)
Oedema	14 (20.6)
Inflammation	28 (41)
Inflammation and oedema	2 (3)
Fibrosis and sklerosis	2 (3)
Total	46 (67.6)

Osteomyelitis was present in 50.0% (n=68) of the patients and absent in 50.0% (n=68). Bone marrow changes were observed in 67.6% (n=46) of patients without osteomyelitis (Figure 1). Bone marrow changes detected in the patients are shown in Table 2.

Osteomyelitis was reported as acute in 53% (n=36) and chronic in 47% (n=32) of patients in the osteomyelitis group. Diabetic microangiopathy was detected on histopathological examination in 44.1% (n=60) of the patients.

Biopsy culture was negative for 27 patients (19.9%). By group, cultures were negative in 13 patients in the non-osteomyelitis group (19.1%) and 14 patients in the osteomyelitis group (20.5%). Biopsy cultures were positive in the remaining 109 patients (80.1%), 55 of whom were in the non-osteomyelitis group (80.9%) and 54 of whom were in the osteomyelitis group (79.5%). The positive cultures yielded a single organism in 49 patients (36%), two organisms in 47 patients (34.5%), three organisms in six patients (4.5%), and four organisms in one patient (0.7%). In total, 171 microbial agents were isolated, including 93 (54.3%) gram-negative bacteria, 76 (44.4%) gram-positive bacteria, and fungi in two (1.1%) cultures. The isolated agents are presented in Table 3. The most common agent was *Staphylococcus aureus* (15.2%), followed by *Escherichia coli* (14.6%) (Table 3).

Overall, the mean CRP level was 127.02±101.49mg/L, the mean ESR was 72.07±32.55mm/h, the mean WBC count was 11.81±5.04 x 10⁹, the mean platelet count was 312.67±120.77 x10⁹, the mean lymphocyte count was 1704.26±884.93 x10⁹, the mean neutrophil percentage was 75.92±9.32%, the mean lymphocyte percentage was 16.08±7.96%, the mean NLR was 6.70±5.38, and the mean platelet/lymphocyte ratio was 214.02±116.68 (Table 4).

In comparison of patients with and without osteomyelitis, there was no difference in sex distribution, but the mean age was significantly lower in the osteomyelitis group (p=0.017). The CRP level and WBC count showed no significant difference between the groups (p>0.05). The ESR was significantly lower in the osteomyelitis group compared to the non-osteomyelitis group (p=0.010) (Figure 2a). The neutrophil percentage was also significantly lower in the osteomyelitis group compared to the non-osteomyelitis group (p=0.021) (Figure 2b). In contrast, the lymphocyte percentage was significantly higher in the osteomyelitis group (p=0.024) (Figure 2c). The NLR was also significantly lower in the osteomyelitis group compared to the non-

Table 3. Bacteria cultured from tissue biopsy specimens (bone and/or soft tissue) collected intraoperatively

Type of bacteria	n	Percentage of bacteria seen by gram staining (%)	Percentage of bacteria seen by total proliferation (%)
Gram-positive bacteria			
<i>Staphylococcus aureus</i>	26	34.21	15.2
<i>Corynebacterium striatum</i>	17	22.36	9.9
<i>Streptococcus agalactiae</i>	13	17.10	7.6
<i>Staphylococcus epidermidis</i>	8	10.5	4.7
<i>Staphylococcus haemolyticus</i>	7	9.2	4.1
<i>Staphylococcus hominis</i>	1	1.31	0.58
<i>Staphylococcus caprae</i>	1	1.31	0.58
<i>Streptococcus agalactiae</i>	1	1.31	0.58
<i>Streptococcus anginosus</i>	1	1.31	0.58
<i>Gemella morbillorum</i>	1	1.31	0.58
Gram-negative bacteria			
<i>Escherichia coli</i>	25	26.9	14.61
<i>Pseudomonas aeruginosa</i>	11	11.8	6.43
<i>Morganella morganii</i>	8	8.6	4.7
<i>Citrobacter freundii</i>	7	7.5	4.1
<i>Enterobacter faecalis</i>	6	6.5	3.5
<i>Enterococcus faecalis</i>	4	6.5	2.3
<i>Proteus mirabilis</i>	4	6.5	2.3
<i>Proteus vulgaris</i>	4	6.5	2.3
<i>Enterobacter cloacae</i>	4	6.5	2.3
<i>Klebsiella oxytoca</i>	4	6.5	2.3
<i>Acinetobacter baumannii</i>	3	3.2	1.8
<i>Alcaligenes faecalis</i>	3	3.2	1.8
<i>Klebsiella pneumoniae</i>	2	2.1	1.2
<i>Citrobacter koseri</i>	2	2.1	1.2
<i>Proteus penneri</i>	1	1.07	0.58
<i>Enterobacter hormaechei</i>	1	1.07	0.58
<i>Serratia marcescens</i>	1	1.07	0.58
<i>Aeromonas veronii</i>	1	1.07	0.58
<i>Providencia rettgeri</i>	1	1.07	0.58
<i>Providencia species</i>	1	1.07	0.58

Table 4. Patients' laboratory results

Pathology	Min-max (median)	Mean±SD
CRP (mg/L)	2.4-442 (109)	127.02±101.49
ESR (mm/h) (n=129)	1-145 (75)	72.07±32.55
WBC (109/L) (n=123)	4.2-37.3 (10.8)	11.81±5.04
Platelet count (x109/L)	22-831 (288)	312.67±120.77
Lymphocyte count (x109/L)	421-8583 (1533.5)	1704.26±884.93
Neutrophils (%)	46-94 (77.4)	75.92±9.32
Lymphocytes (%)	2.2-44.4 (14.7)	16.08±7.96
Neutrophil/lymphocyte ratio	1-42.7 (5.2)	6.70±5.38
Platelet/lymphocyte ratio	12.8-704 (180.9)	214.02±116.68

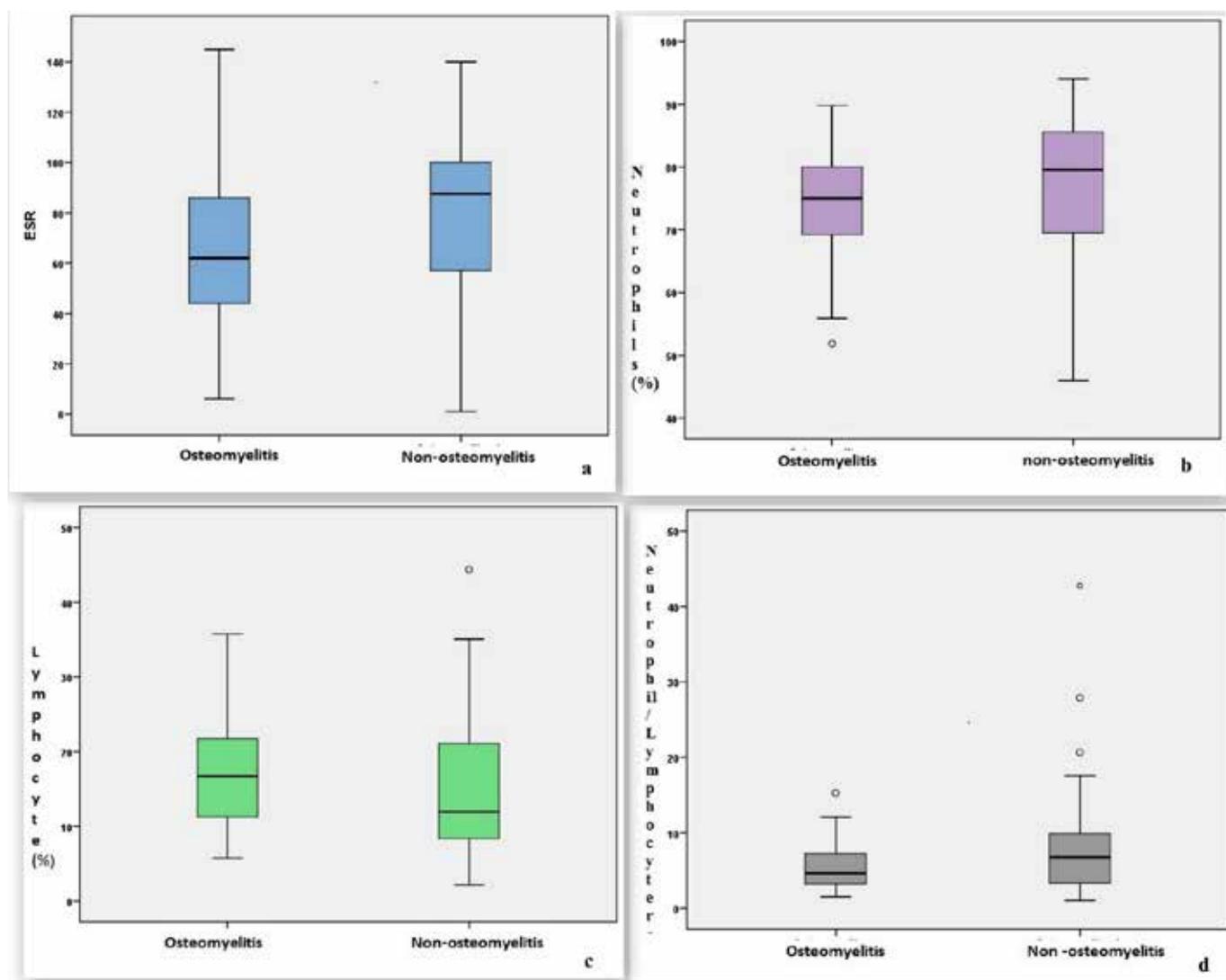


Figure 2. Distributions of a) ESR, b) neutrophil percentage, c) lymphocyte percentage and d) neutrophil/lymphocyte ratio in patients with and without osteomyelitis

osteomyelitis group ($p=0.025$) (Figure 2d). Comparisons of patient characteristics and biomarkers between the groups are shown in Table 5.

Determination of cut-off values

ROC curve analysis and diagnostic screening tests were used to determine cut-off values for the parameters that differed significantly between the groups (ESR, neutrophil percentage, lymphocyte percentage and NLR).

The cut-off point for ESR was identified as 79mm/h. An ESR of 79mm/h or lower had sensitivity of 72.88%, specificity of 53.13%, PPV of 58.90%, NPV of 68.00%, and accuracy of 62.60% in the diagnosis of osteomyelitis (positive LR=1.55, negative LR=0.5). Area under the ROC curve (AUC) was determined as 65.0% with standard error of 5.0% (Figure 3a). In addition, a statistically significant difference was detected between the groups based on the cut-off point obtained for ESR (≤ 79 mm/h) ($p=0.003$). Patients with ESR of 79mm/h or lower had 3.046-fold higher odds of osteomyelitis (odds ratio

[OR]: 3.046, 95% confidence interval [CI]: 1.431–6.482).

In ROC curve analysis of neutrophil percentage, the optimal cut-off point was 78%. At this cut-off value, neutrophil percentage had sensitivity of 69.12%, specificity of 57.35%, PPV of 61.84%, NPV of 65.00%, and accuracy of 63.24% (positive LR=1.62, negative LR=0.53). The ROC AUC was 61.5% with standard error of 4.9% (Figure 3b). There was a statistically significant difference between the groups based on the neutrophil percentage cut-off point (≤ 78) ($p=0.002$). Patients with neutrophil percentage of 78% or lower had 3.010-fold higher odds of osteomyelitis (OR: 3.010, 95% CI: 1.489–6.085).

For lymphocyte percentage, a cut-off point of 12.3% was identified. At this cut-off value, neutrophil percentage had sensitivity of 72.06%, specificity of 52.94%, PPV of 60.49%, NPV of 65.45%, and accuracy of 62.50% (positive LR=1.55, negative LR=0.5). The ROC AUC was 61.2% with standard error of 4.9% (Figure 3c). A statistically significant difference

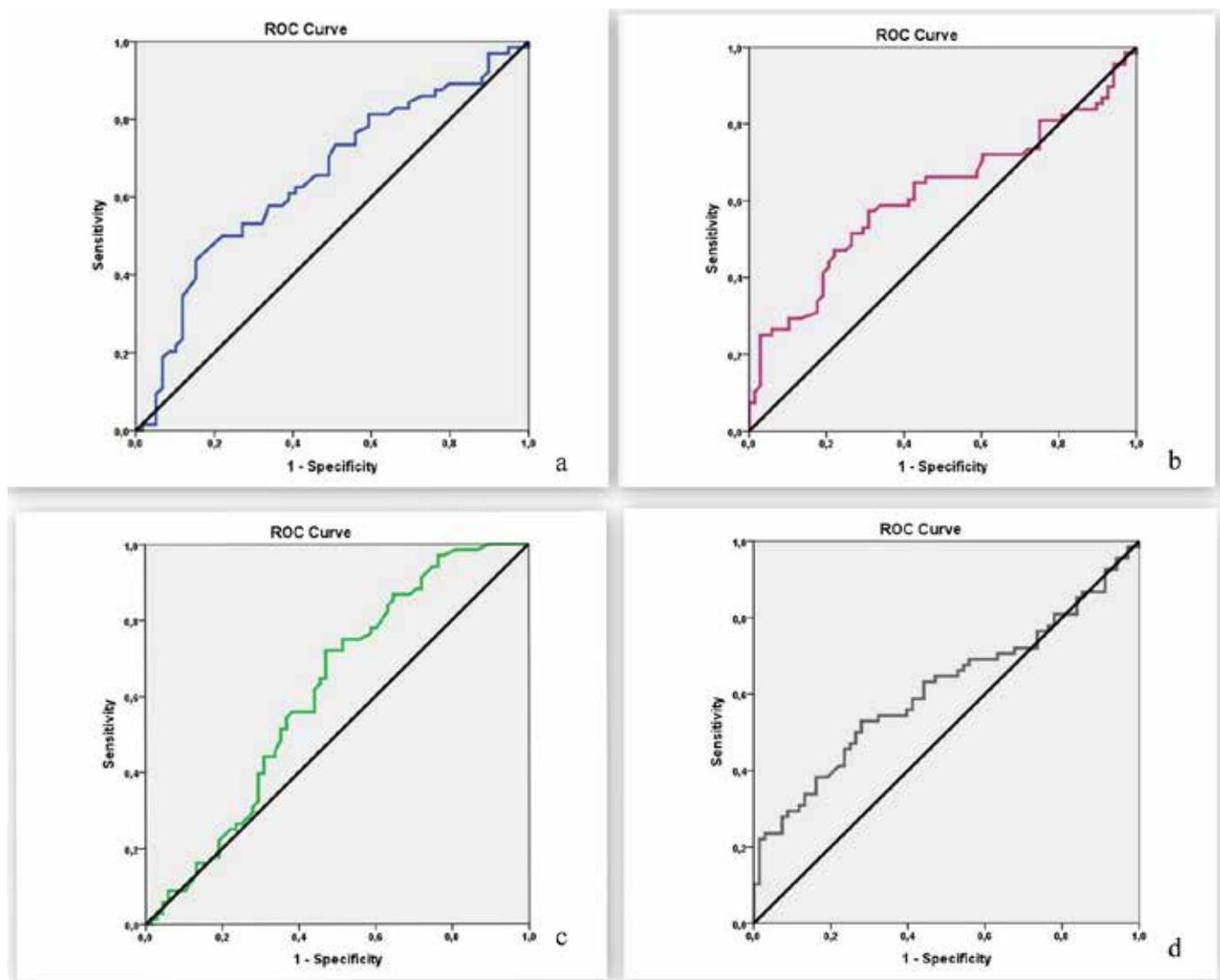


Figure 3. ROC curves for a) ESR, b) neutrophil percentage, c) lymphocyte percentage and d) neutrophil/lymphocyte ratio

was detected between the groups based on the lymphocyte percentage cut-off point ($\geq 12.3\%$) ($p=0.003$). The odds of having osteomyelitis were 2.901-fold higher at lymphocyte percentages of 12.3% or higher (OR: 2.901, 95% CI: 1.423–5.915).

The cut-off point for NLR was identified as 6.02, which had sensitivity of 72.06%, specificity of 52.94%, PPV of 60.49%, NPV of 65.45%, and accuracy of 62.50% (positive LR=1.53, negative LR=0.52). The ROC AUC was 61.1% with standard error of 4.9% (Figure 3d). There was a statistically significant difference between the groups based on the NLR cut-off point (≥ 6.02) ($p=0.003$). Patients with NLR of 6.02 or lower had a 2.901-fold risk of osteomyelitis (OR: 2.901, 95% CI: 1.423–5.915).

Analysis of the diagnostic accuracy of biomarker combinations showed that ESR ($\leq 79\text{mm/h}$) and NLR (≥ 6.02) considered together had sensitivity of 86.76%, specificity of 36.76%, PPV of 57.84%, NPV of 73.53%, and accuracy of 61.76% (positive LR=1.37, negative LR=0.36). The ROC AUC was

61.8% with a standard error of 5.3%.

The combination of ESR ($\leq 79\text{mm/h}$) and lymphocyte percentage ($\geq 12.3\%$) had sensitivity of 86.76%, specificity of 36.76%, PPV of 57.84%, NPV of 73.53%, and accuracy of 61.76% (positive LR=1.37, negative LR=0.36). The ROC AUC was 61.8% with standard error of 5.3%.

ESR ($\leq 79\text{mm/h}$) and neutrophil percentage ($\leq 78\%$) together had sensitivity of 86.76%, specificity of 36.76%, PPV of 59%, NPV of 50%, and accuracy of 57.63% (positive LR=1.37, negative LR=0.36). The ROC AUC was 61.8% with standard error of 5.3%. The indicators of diagnostic accuracy for the analysed biomarkers and their combinations are shown in Table 6.

Discussion

Osteomyelitis is associated with longer treatment duration and increased risk of amputation and mortality. A diagnosis of osteomyelitis is relevant to the duration of antibiotherapy and requires repeated debridement. The prevalence of foot

Table 5. Comparisons of patient characteristics and biomarkers based on presence of osteomyelitis

Patient characteristics		Osteomyelitis (n=68)	Non-osteomyelitis (n=68)	p
Age (years)	Min-max (median)	38-91 (65)	53-92 (66.5)	0.017^{†a}
	Mean±SD	63.69±11.37	67.79±8.06	
Sex, n (%)	Female	8 (11.8)	13 (19.1)	0.235 ^c
	Male	60 (88.2)	55 (80.9)	
CRP (mg/L)	Min-max (median)	3.9-442 (100)	2.4-376 (126)	0.159 ^b
	Mean±SD	113.04±94.21	139.96±106.87	
ESR (mm/h) (n=129)	Min-max (median)	6-145 (62)	1-140 (87.5)	0.010^{†a}
	Mean±SD	64.22±31.92	79.30±31.66	
WBC (n=123)	Min-max (median)	5.9-28.6 (10.8)	4.2-37.3 (10.7)	0.561 ^b
	Mean±SD	11.37±4.23	12.24±5.73	
Neutrophils (%)	Min-max (median)	51.9-89.8 (75.1)	46-94 (79.6)	0.021^{†b}
	Mean±SD	74.42±7.95	77.43±10.35	
Lymphocytes (%)	Min-max (median)	5.8-35.8 (16.8)	2.2-44.4 (12)	0.024^{†b}
	Mean±SD	17.29±6.99	14.86±8.70	
Neutrophil/lymphocyte ratio	Min-max (median)	1.5-15.3 (4.6)	1-42.7 (6.8)	0.025^{†b}
	Mean±SD	5.28±2.78	8.11±6.81	
Platelet/lymphocyte ratio	Min-max (median)	60.5-511.4 (180.1)	12.8-704 (181.6)	0.572 ^b
	Mean±SD	198.35±82.74	229.69±141.73	

[†]Student's t-test ^bMann-Whitney U test ^cPearson's Chi-Square test *p<0.05

Table 6. Diagnostic indicators and ROC curve results for biomarkers and their combinations

Pathology	Diagnostic indicator					ROC curve		p
	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	95% CI	
ESR (mm/h)	≤79	72.88	53.13	58.90	68.00	0.650	0.553-0.748	0.004^{**}
Neutrophils (%)	≤78	69.12	57.35	61.84	65.00	0.615	0.518-0.711	0.021[*]
Lymphocytes (%)	≥12.3	72.06	52.94	60.49	65.45	0.612	0.516-0.707	0.024[*]
NLR	≤6.02	72.06	52.94	60.49	65.45	0.611	0.515-0.707	0.025[*]
ESR & NLR		86.76	36.76	57.84	73.53	0.618	0.523-0.712	0.048[*]
ESR & lymphocytes (%)		86.76	36.76	57.84	73.53	0.618	0.523-0.712	0.048[*]
ESR & neutrophils (%)		86.76	36.76	59.00	50.00	0.632	0.538-0.726	0.048[*]

NLR: Neutrophil/lymphocyte ratio; *p<0.05, **p<0.01

ulcers among diabetic patients is approximately 4-10%, with up to 25% of people with diabetes developing a foot ulcer during their lifetime¹⁴. Infection is a problem in more than half of diabetic foot ulcers. Osteomyelitis develops in 20% of moderate infections and 50-60% of severe infections²⁰. Although osteomyelitis can be diagnosed using methods such as MRI and labelled WBC scintigraphy, the gold standard is pathologic examination.

Bone infections in diabetic osteomyelitis are histopathologically categorised as acute, chronic, chronic acute and fibrotic. The frequency of these types of osteomyelitis varies in different series. Cecilia-Matilla and Tardáguila-García et al reported chronic osteomyelitis as the most common type^{21,22}, while Aragón-Sánchez et al reported that acute osteomyelitis was

most common²³, as in our study. Fibrosis and sclerosis were detected on pathological examination of the bone marrow in three patients in our study. In one of the cases, these changes were reported together with acute osteomyelitis, while in the others they were reported as bone marrow changes in the absence of osteomyelitis.

The diagnostic sensitivity of histologic examination for the presence of osteomyelitis has been reported to be as high as 95%, with a diagnostic specificity of 99%⁸. The negative microbiological culture rate in histologically proven osteomyelitis is stated to be 40-60%^{24,25}. In another study, the rate of positive bone culture was 87.5% in patients with positive probe-to-bone test and was higher in patients with positive histopathology compared to those with negative results²⁶.

White et al reported that microbiological culture alone had 42% sensitivity in the diagnosis of osteomyelitis but that sensitivity increased to 84% when combined with histological examination²⁴. Tardáguila-García et al reported that bone culture had diagnostic specificity of 70% and sensitivity of 40%²². They emphasised that histopathological evaluation was more accurate in diagnosing diabetic osteomyelitis than microbiology, especially in patients with chronic diabetic osteomyelitis. These patients may be underdiagnosed because bone culture gives false negative results. Therefore, both histopathological evaluation and bone culture are recommended to confirm the presence of diabetic osteomyelitis²².

In our study, 20.5% of biopsies that were histopathologically diagnosed as osteomyelitis did not have microbiological growth in culture. However, we attribute this to the fact that cultures were performed using samples that included both bone and soft tissue. We also observed a 79.5% positive culture rate in patients without osteomyelitis, indicating that these percentages are more related to soft tissue infection than histopathological misdiagnosis. Because cultures were not done exclusively with bone, the differential diagnosis of osteomyelitis was based on histopathological findings in this study. Another reason for negative cultures may be a history of antibiotic use, which was unknown in our patients. However, it has been reported that the rate of positive bone culture did not differ between patients who received antibiotic therapy and those who did not²⁶.

Bone exposure may occur in diabetes-related foot wounds because of tissue loss, neurotrophic ulcer, or fistula formation. If a sterile device inserted into the wound contacts bone (i.e., the probe-to-bone test), it is considered exposed bone. The prevalence of osteomyelitis in these wounds was reported to be 66% and this method had 87% sensitivity and 83% specificity in the identification of patients with osteomyelitis²⁷.

It has been reported that 31.3% of the patients with osteomyelitis had exposed bone under the lesion and 94.6% had a positive probe-to-bone test²³. In another study, the prevalence of osteomyelitis was found to be 53%²⁸. Aragón-Sánchez et al reported this rate as 72.4%²⁶. In our series, the prevalence of osteomyelitis was 50%, while 67.6% of the patients without osteomyelitis exhibited bone marrow changes such as inflammation and oedema, which are used as criteria in the diagnosis of diabetic osteomyelitis²⁰. These changes in the bone marrow may be indicative of nascent but not yet fully developed osteomyelitis.

Grayson et al emphasised that foot ulcers with palpable bone also have a high prevalence of osteomyelitis. In fact, they stated that special x-ray and radionuclide tests are not necessary to diagnose osteomyelitis in these cases³. However, they noted that although a positive probe-to-bone test result supports the diagnosis of diabetic foot osteomyelitis, the reliability of this method is not high and that MRI or bone biopsy is required in suspicious cases²⁹.

It should be noted that the probe-to-bone test is especially valuable in neurotrophic ulcers³⁰.

Aragón-Sánchez et al reported that they could safely diagnose diabetic foot osteomyelitis in the presence of positive probe-to-bone test or plain x-ray, and especially when both are positive²⁶. In another study, Álvaro-Afonso et al emphasised the importance of evaluating the probe-to-bone test and simple radiography together for the diagnosis of diabetic foot osteomyelitis¹³. The reliability of the probe-to-bone test depends on the clinician's experience and location of the wound^{31,32}.

There have been numerous studies on serological markers such as CRP and ESR in the diagnosis and follow-up of osteomyelitis associated with diabetes-related foot wounds, and some of these studies have reported cut-off values³³⁻³⁶. The results of meta-analyses indicate that ESR is the parameter with the most evidence for the diagnosis of diabetic osteomyelitis^{11,37,38}.

Lymphocyte, ESR and CRP elevation may also be useful in identifying and diagnosing patients who are likely to develop complications. Serial blood tests can be an easy and useful tool for early detection and prevention of complications³⁸. In several studies, an ESR of 70mm/h or higher was accepted as the critical value for osteomyelitis^{17,39}. In a diabetic osteomyelitis series with a positive probe-to-bone test rate of 76.6%, an ESR cut-off value of 43mm/h or higher was reported to have 82.9% sensitivity and 70.5% specificity. The same study reported that when using the probe-to-bone test alone to diagnose diabetic foot osteomyelitis, the sensitivity was 76.6% and the specificity 93.2%, while both ESR >43mm/h and positive probe-to-bone test together had 98% specificity in the diagnosis of osteomyelitis¹¹. In our study, the cut-off value for ESR was 79mm/h, and the risk of osteomyelitis was about three times higher below this threshold.

Although additional morbidities of the patients could not be determined in this retrospective series, ESR may decrease in conditions such as haemoglobinopathy, extreme leukocytosis, and the use of steroids or non-steroidal anti-inflammatory drugs. Rabjohn et al investigated the effects of comorbidities that influence ESR in patients with diabetic foot osteomyelitis and reported that ESR values were not significantly affected by comorbidities in diabetic osteomyelitis⁴⁰. Therefore, we do not believe comorbidities contributed to low ESR values in our patients.

In general, the accepted cut-off value for ESR in diabetic osteomyelitis is 70mm/h^{17,39}. The much lower value reported in the abovementioned study may be related to the high rate of bone exposure and may be specific to their patient group. Damage to the bone tissue and periosteum as a result of exposure of bone to the external environment may cause changes in the inflammatory response. Therefore, the difference in findings between studies may be related to the inflammatory changes caused by bone exposure.

There are varying results in the literature on the role of CRP in the diagnosis of osteomyelitis. While some studies have reported that CRP is the best marker in the diagnosis of osteomyelitis, some other publications have reported that it is a poor marker^{41–45}.

Serum CRP levels have consistently been found to be significantly higher in infected diabetic foot ulcers than in non-infected diabetic foot ulcers, with levels increasing significantly in association with infection severity^{46–48}. In our patient group, we found that CRP and WBC count had no statistical significance in the diagnosis of osteomyelitis. This may indicate that infection was of similar severity in both groups.

Although ESR is a simple marker with high sensitivity and specificity in the diagnosis of diabetic foot osteomyelitis^{37,43–48}, CRP was found to be superior to ESR for evaluating early treatment response³⁰. It has also been emphasised that both neutrophil count and CRP values are higher in patients with soft tissue infection without osteomyelitis compared to osteomyelitis patients⁴⁴.

CRP, ESR and NLR were reported to have no diagnostic value in patients with diabetic foot infection, whereas the less costly and more accessible marker WBC count was found to be more useful during follow-up for the evaluation of diabetes-related foot wound severity¹⁸. However, in another study, it was emphasised that leukocytosis is a poor marker of diabetic foot osteomyelitis because WBC count is normal in 50% of patients with osteomyelitis and high WBC count may be associated with soft tissue or systemic infection⁴⁷. However, Eren et al stated that ESR, WBC and CRP values showed a positive correlation in the diagnosis of diabetic osteomyelitis⁴².

In our study, WBC count showed no statistically significant difference according to the diagnosis of osteomyelitis in diabetes-related foot wound patients with bone exposure and was not found to be a significant marker of osteomyelitis in our series.

There are many studies with conflicting results regarding the effect of diabetes mellitus on the immune system. Both in vivo and in vitro studies have demonstrated that chronic hyperglycaemia causes oxidative burst and anomalies in lymphocyte subpopulations and immunoglobulins^{49–56}. In a study with diabetic ulcer patients and diabetic controls, no significant difference was detected between the two groups in terms of total leukocyte count. No difference was detected between the two groups in markers of innate immunity other than low absolute natural killer cell counts. However, decreases in the absolute numbers of nearly all subpopulations of both B and T lymphocytes and changes in humoral immunity have been observed in patients with an infected diabetic foot ulcer. The main findings differentiating patients with a chronic infected diabetic foot ulcer from diabetic controls without a diabetic ulcer are the significant

decreases in percentages of lymphocyte and absolute lymphocyte count⁵⁶. Moreover, no significant difference was detected in patients with a diabetic foot infection and chronic bacterial infection in terms of active phagocytic polymorphonuclear leukocyte count and initial phagocytic activity or the humoral component of non-specific immunity, and it was concluded that the non-specific immune response is slightly altered in patients with a diabetic foot infection and a chronic bacterial infection⁵⁷.

In a study investigating the effect of osteomyelitis on immune response, there was no statistically significant difference between the osteomyelitis group and healthy control group in terms of lymphocyte count⁵⁸. In a study on patients with a diabetic foot infection and healthy controls, it was found that levels of CD3+ T cells and inhibitory/cytotoxic CD8+ T cells were significantly higher, while there was no difference in terms of serum monocyte and lymphocyte counts, CD4+ helper T cells, or CD4+/CD8+ T cell ratio⁵⁹.

In our study, diabetic osteomyelitis patients with positive probe-to-bone test results had higher lymphocyte percentage but lower neutrophil percentage compared to the group of patients with positive probe-to-bone test but without osteomyelitis, resulting in a lower NLR. Our patient population consisted entirely of patients with diabetes-related foot wounds, and although lymphocyte ratios and counts may be lower compared to patients without diabetes-related foot wounds, these findings indicate that diabetic patients with exposed bone respond to osteomyelitis with an increased lymphocyte percentage. In patients with a lymphocyte percentage of 12.3% or higher and those with a neutrophil percentage of 78% or lower, the risk of osteomyelitis was found to increase approximately threefold. However, our results contradict a previous report that increased NLR may be an indicator of osteomyelitis in patients with diabetes-related foot wounds¹⁹. Moreover, many studies have emphasised that high NLR is an independent risk factor for progression to amputation^{19,60,61}. The physiological response of circulating leukocytes to stress manifests as an increase in neutrophil count and decrease in lymphocyte count. High NLR results in neutrophilic hyperactivity, which leads to endothelial damage and dysfunction⁶². NLR may be an indicator of increased risk of microvascular complications of diabetes⁶³, and the relationship between high NLR and amputation risk may be associated with this vascular mechanism independent of osteomyelitis, which may be an additional factor that contributes to this relationship.

All of the patients in our series had exposed bones, suggesting that the immune response was caused by different factors such as bone desiccation and necrosis, which led to the differences in serological marker values. Moreover, while high platelet/lymphocyte ratio was previously found to be associated with osteomyelitis in diabetic foot infections⁶⁴, no statistically significant difference in this ratio was observed between the groups with and without osteomyelitis in our study. As no other studies on this specific patient group

could be found in the literature, we based our comparison on studies of general diabetes-related foot wounds.

Using a combination of diagnostic methods is recommended to increase the reliability of diagnoses¹. In our study, we evaluated parameters that were significant in terms of osteomyelitis in pairs (ESR ≥ 79 mm/h and NLR ≥ 6.02 ; ESR ≤ 79 mm/h and lymphocyte percentage $\geq 12.3\%$; and ESR ≤ 79 mm/h and neutrophil percentage $\leq 78\%$) and found that the sensitivity increased to 86.8% in patients where both were positive.

Looking at the microorganisms isolated in diabetic foot infections, generally aerobic gram-positive cocci are isolated^{65,66}. However, deep or chronic wounds often have both aerobic gram-negative and obligatory anaerobic bacteria together⁶⁷. In our series, gram-negative bacteria accounted for 54.3% and gram-positive bacteria for 44.4% of wound culture isolates. No microorganisms were observed in 19.9% of the cultures. Karthik et al reported in their study that 46% of bone culture samples were sterile and 58% yielded gram-negative organisms⁶⁸.

Machado et al divided patients with diabetes-related foot wounds into two time periods, 2010–2011 and 2016–2017⁶⁹. They reported that, overall, bacterial cultures yielded 59.6% gram-positive pathogens and 40.4% gram-negative pathogens, with 18.5% of cultures being negative. Monomicrobial infections were seen in 50.0% of cases in the earlier group, while this rate fell to 35.5% in the later group. They noted an increase in the median number of pathogens isolated per sample over the years⁶⁹.

In the present study, 36% of cultures yielded a single pathogen and 34.5% yielded two bacterial pathogens. Overall, polymicrobial infections were more common in our series. The higher rate of gram-negative bacteria in our series and the higher incidence of polymicrobial infections may be related to the deep infections associated with bone exposure to the external environment.

Aragón-Sánchez et al reported that *S. aureus* was the most common bacterium, detected in 47.5% of patients with osteomyelitis, and *Staphylococcus epidermidis* was the second most common, at 10%. A single organism was isolated in 64% of cultures²³. Machado et al also reported *S. aureus* as the most common gram-positive agent in their series, while the most common gram-negative agent was *Enterobacteriaceae*⁶⁹.

Karthik et al reported that the most common organisms detected in bone culture were *Staphylococcus* spp., followed by *Pseudomonas*, *Klebsiella* spp., *E. coli*, and *Enterococcus* spp., respectively⁶⁸. In our study, *S. aureus* was isolated most frequently, at a rate of 15.2%. However, *E. coli* was the second most frequent isolate (14.6%). The fact that the second most common bacterium was gram-negative and was similar in frequency to *S. aureus* may be due to the fact

that our case series included patients with deeper tissue infections.

In some studies examining the relationship between age and the appearance of osteomyelitis in diabetes-related foot wounds, a statistically significant relationship was not found^{11,43}. However, another study showed that lower mean age was significantly associated with the presence of osteomyelitis, with patients aged over 70 representing 33.3% of the osteomyelitis group and 54.4% of the group without osteomyelitis⁸. This is consistent with our finding of a lower mean age in our osteomyelitis patients.

Conclusions

The patient group examined in this study consisted of those with contact between bone tissue and the external environment as a result of a diabetes-related foot ulcer. We believe that this patient group is distinct from other diabetes-related foot ulcer groups. The results of this study demonstrated differences in the epidemiological and laboratory findings and calculated risk values in this patient group compared to the group with general diabetes-related foot wounds and osteomyelitis. We believe that the immune response caused by the exposure of bone to the environment is different than the immune response caused by soft tissue infection in classical diabetic foot. Experimental and clinical studies are needed to elucidate the underlying histopathogenesis.

The major limitation of this study is that it was retrospective. Another limitation is not knowing the patients' duration of bone exposure. Longer exposure may increase infection and necrosis and might alter inflammatory responses. In addition, the study included both patients with bone exposure and those with positive probe-to-bone test. The additional contact with the external environment and greater loss of soft tissue in wounds with bone exposure compared to those with positive probe-to-bone test will cause more necrosis due to fluid loss. Moreover, as mentioned earlier, bone biopsies were not cultured separately and the culture results are from bone and soft tissue together. However, considering the lack of a similar study, it may contribute to the literature.

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Conflict of interest

The authors declare no conflicts of interest.

Ethics statement

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Author contribution

OA took part in data collection and/or processing processes.

Other processes were carried out by HD.

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