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Evaluation of the Histopathological Outcomes Association of Proximal Bone Samples during Minor Amputations in Treatment of Diabetes-Related Foot Infections

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Abstract: The study was done to determine the histopathological results of proximal bone samples of DFIs during minor amputations and to determine the relationship of the results with postoperative treatment outcomes. A retrospective, single-center study was carried out on 113 patients who underwent minor amputation (toe, ray, or transmetatarsal) of moderate-to-severe DFIs and proved to have osteomyelitis. Histopathological assessment of the proximity bone margins was done to determine osteomyelitis, inflammatory infiltrates, bone necrosis, and viability. A proper comparison of outcomes was made between patients who had a positive (n=38) and negative histopathology at the resection margin through the relevant statistical tests. Osteomyelitis of the proximal bone margin was detected in 33.6% (38/113) of the patients by histopathological examination. Positive-margins patients had a much poorer prognosis than negative-margins with lower treatment success rates (50.0 vs. 82.7), higher levels of complete wound healing (55.3 vs. 77.3), treatment time (14.8 vs. 8.6 weeks), and unplanned reoperation (36.8 vs. 14.7), proximal re-amputated (28.9 vs. 10.7), and infection recurrence (31.6 vs. 12 Histopathologic signs of osteomyelitis at the apical bone margin in minor amputation in DFIs is a powerful, independent risk factor, such as the failure of treatment, retarded bone healing, and re-amputation.

Keywords:. Proximal bone, type of diabetes, microbiological outcomes, and mortality.

INTRODUCTION

Diabetes mellitus is a health menace that has affected the world, and its complications cause a lot of morbidity, mortality, and economic strain. Among them, the foot infections associated with diabetes (DFIs) are truly devastating, as they represent the most common hospitalization cause among diabetic patients and the most common antecedent to the occurrence of non-traumatic lower extremity amputations (Ramsey SD. 1999; Armstrong DG, 2017; Lázaro-Martínez JL, 2017). Pathophysiology of diabetic foot infection is multifactorial, which involves peripheral neuropathy, peripheral arterial disease, and immunological malfunction, which together form an environment conducive to where microorganisms can gain entry and infect the bones (Hicks CW, 2014; Kurowski JR, 2015; Lipsky BA, 2020). In mild or moderate diabetic foot infections, osteomyelitis, or bone infection, approximately 20 percent of the infections and about 60 percent of the severe cases are observed, and therefore, the treatment strategies are highly challenging, and the patient is predisposed to amputation. (Izumi Y, 2006). Limb-salvage operations such as toe, ray, and Tran's metatarsal amputations are examples of minor amputation in an attempt to avoid infected tissue but maintain as much functional capacity as possible (Khan SF, 2024). These procedures, however, depend on the extent to which the infected bone is excised; incomplete excision results in re-amputation risk, the need to take longer to achieve full treatment through antibiotic therapy, and transition to major amputation (Voon K, 2022). The existing preoperative diagnostic methods, such as plain radiography, magnetic resonance imaging, and bone biopsy cultures, have shown inconsistency in terms of their sensitivity and specificity and leave surgeons to consider intraoperative clinical judgment of bone resection adequacy (Kowalski TJ, 2011; Lázaro-Martínez JL, 2017). Bone samples taken when an amputation takes place can be examined histopathologically to give an unambiguous means to determine the presence or absence of osteomyelitis, and this gives objective information that can be used in future management. (Jin L, 2024)

METHODOLOGY

The aim of this retrospective, cohort, and single-center study was to assess the importance of the histopathological examination of proximal bone margins collected during minor amputations in diabetic patients with foot infections (DFIs). The purpose of the study was to establish the prevalence of residual osteomyelitis at the surgical border and its relationship with the postoperative outcomes, such as wound healing, reoperation, and length of antibiotics.

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Moreover, the sample population comprised of adult patients ([?] 30 years) who sustained a minor amputation due to a clinically diagnosed foot infection with suspected or confirmed osteomyelitis in the period of January 2024-25 at a vascular care and diabetic foot center of Al-Najaf - Iraq hospitals. The identification of patients was done by querying the hospital medical records. Inclusion criteria were major amputations above the ankle, incomplete medical records, not having the histopathological examination of the proximal bone margin, and amputation due to non-infective causes (e.g., trauma, tumor).

Also, data of all patients were extracted in a standardized manner through the use of a standardized case report form in all the health records. Demographic factors were the age, sex, body mass index (BMI), and smoking. The nature of infection was recorded in the Wagner and PEDIS classification systems, as well as the site of the ulcer, duration of time, size, and the presence of inflammatory factors (C - reactive protein [CRP], erythrocyte sedimentation rate, and white blood cell count). Perfusion was determined with the help of the ankle-brachial index (ABI).

Microbiological culture and histopathological sampling of intraoperative bone samples of the proximal resection margin were sent out as per the usual institutional practice. Microbiological processing entailed aerobic, anaerobic, and fungal cultures with conventional identification and antimicrobial susceptibility tests. The hematoxylin and eosin (H&E)-stained formalin-fixed, decalcified, paraffin-embedded bone sections were subject to histopathological analysis. The existence of neutrophilic infiltrates, bone necrosis, or formation of sequestrum led to osteomyelitis at the margin being diagnosed. Presence of acute and chronic inflammatory infiltrates in bone and bone viability were also measured.

The outcomes of the surgery and the postoperative period were taken into account, according to which all the amputations were conducted by experienced vascular or orthopedic surgeons. The extent of amputation and wound closure (primary, delayed primary, or secondary intention) was documented. The multidisciplinary protocol included the use of antibiotic therapy depending on culture results, wound care, and offloading, which were included in the postoperative management. Negative pressure wound therapy (NPWT) and revascularization procedures usage were reported.

Success of treatment at 12 months was the chief outcome, which was complete wound healing with no signs of further infection or re-amputation on a more level-proximate site. Time to complete healing, unplanned reoperation, proximal re-amputation, major amputation (below or above knee), recurrent infection, hospital readmission within 30 days, and mortality were the secondary outcomes. The stratification of patients was carried out into two groups according to the proximal margin histopathological results in the positive (presence of residual osteomyelitis) and negative groups. Continuous variables were given in the form of mean ± standard deviation and compared either by Student t-test or Mann-Whitney U test. The frequencies and percentages of categorical variables were presented and compared with the chi-square or Fisher test. Multivariate logistic regression analysis was conducted to determine the independent predictors of treatment failure and control the possible confounding variables, including ABI, dialysis dependence, Wagner grade, and glycemic control. The p-value below 0.05 was said to be statistically significant. All the analyses were done under SPSS version 24.0.

RESULTS

The sample was composed mainly of males (65.5%), with an average age of 62.4 years, extensive diabetes (average duration of 14.6 years), and inadequate glycemic control (mean HbA1c 8.9%). There were high levels of significant comorbidities, such as hypertension (78.8%), neuropathy (83.2%), peripheral artery disease (46.0%), and chronic kidney disease (41.6%). Most of the ulcers were of the toe or forefoot (79.6%), of Wagner grade 3 or 4 (59.3%), the bone affected was universal (PEDIS depth), and common clinical features of osteomyelitis (78.8% probe-to-bone positive).

Table 1. Demographic and baseline features of 113 patients.

Variable	Frequency, n = 113	Percentage, %
Age (years)	62.4 ± 11.8	
Sex		
- Male	74	(65.5%)
- Female	39	(34.5%)

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BMI (kg/m²)	28.7 ± 5.4	
Duration of Diabetes (years)	14.6 ± 8.2	
Type of Diabetes		
- Type 1	9	(8.0%)
- Type 2	104	(92.0%)
Smoking Status		
- Current smoker	31	(27.4%)
- Former smoker	42	(37.2%)
- Never smoked	40	(35.4%)
Alcohol Consumption		
- Yes	38	(33.6%)
- No	75	(66.4%)
Diabetes Duration (years, mean ± SD)	14.3 ± 6.7	

Table 2. Diagnostic and clinical data.

Variable	Outcomes
HbA1c (%)	8.9 ± 2.1
Fasting Blood Glucose (mg/dL)	186.4 ± 62.8
Serum Creatinine (mg/dL)	1.8 ± 1.4
eGFR (mL/min/1.73m²)	58.2 ± 28.6
Comorbidities	
- Hypertension	89 (78.8%)
- Coronary Artery Disease	41 (36.3%)
- Chronic Kidney Disease	47 (41.6%)
- End-Stage Renal Disease (Dialysis)	18 (15.9%)
- Peripheral Artery Disease	52 (46.0%)
- Retinopathy	58 (51.3%)
- Neuropathy	94 (83.2%)
Ankle-Brachial Index (ABI)	0.78 ± 0.24
- ABI < 0.6	29 (25.7%)
- ABI 0.6–0.9	48 (42.5%)
- ABI > 0.9	36 (31.9%)
Previous Foot Ulcer	61 (54.0%)
Previous Amputation	34 (30.1%)

Microbiological tests of the bone samples showed that the proportion of culture positivity (77.0%) and polymicrobial culture (36.3 percent) was high. Most of them were gram-positive, with Staphylococcus aureus (MSSA and MRSA) prevalent, and the gram-negative Pseudomonas aeruginosa. Of importance, almost twenty-five percent (24.8) of isolates were multidrug-resistant. Proximal bone margins, however, revealed a critical discordance that 77.0% of samples contained positive cultures; however, only 33.6% (n=38) contained histopathological evidence of osteomyelitis at the surgical margin. This implies that much culture positivity might be embodied by colonization or contamination and not gross invasion bone infection at the amputation level.

Table 3. Clinical features of infection and wounds related to the foot.

Variable	Findings
Ulcer Location	
- Toe(s)	58 (51.3%)
- Forefoot	32 (28.3%)
- Midfoot	15 (13.3%)
- Heel	8 (7.1%)
Ulcer Duration (weeks)	8.4 ± 6.2
Ulcer Size (cm²)	4.8 ± 3.6
Wagner Classification	

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- Grade 1	12 (10.6%)
- Grade 2	34 (30.1%)
- Grade 3	48 (42.5%)
- Grade 4	19 (16.8%)
PEDIS Classification	
- Perfusion (impaired)	52 (46.0%)
- Extent > 1 cm²	87 (77.0%)
- Depth (bone involvement)	113 (100.0%)
- Infection (moderate/severe)	98 (86.7%)
- Sensation (loss)	94 (83.2%)
Clinical Signs of Osteomyelitis	
- Probe-to-bone positive	89 (78.8%)
- Visible bone	42 (37.2%)
- Sausage toe	36 (31.9%)
Pre-operative CRP (mg/L)	78.6 ± 54.2
Pre-operative ESR (mm/hr)	68.4 ± 32.8
Pre-operative WBC (×10⁹/L)	12.4 ± 4.6

The results of the treatment in stratification by the status of the histopathology are sharp. The positive proximal margin histopathology patients suffered much less. They had a lower rate of 50.0 of success in their treatment at 12 months compared to 82.7 in the histology-negative group (p=0.001). They also had very prolonged wound healing (14.8 vs. 8.6 weeks, p<0.001), increased unplanned re-operation (36.8% vs. 14.7%, p=0.008), proximal re-amputation (28.9% vs. 10.7%, p=0.016), and 30-day readmission (26.3% vs. 10.7, p=0.034). In addition, they also needed much more time on total and intravenous antibiotic treatment.

Table 4. Microbiological outcomes.

Microorganism	Findings
Culture Results	
- Positive culture	87 (77.0%)
- Negative culture	26 (23.0%)
- Polymicrobial	41 (36.3%)
Gram-Positive Organisms	
- Staphylococcus aureus (MSSA)	32 (28.3%)
- Staphylococcus aureus (MRSA)	19 (16.8%)
- Coagulase-negative Staphylococci	14 (12.4%)
- Streptococcus spp.	11 (9.7%)
- Enterococcus spp.	16 (14.2%)
Gram-Negative Organisms	
- Pseudomonas aeruginosa	18 (15.9%)
- Escherichia coli	12 (10.6%)
- Klebsiella spp.	9 (8.0%)
- Proteus spp.	14 (12.4%)
- Enterobacter spp.	7 (6.2%)
Anaerobes	
- Bacteroides spp.	8 (7.1%)
- Peptostreptococcus spp.	6 (5.3%)
Fungi	
- Candida spp.	4 (3.5%)
Multidrug-Resistant Organisms	28 (24.8%)

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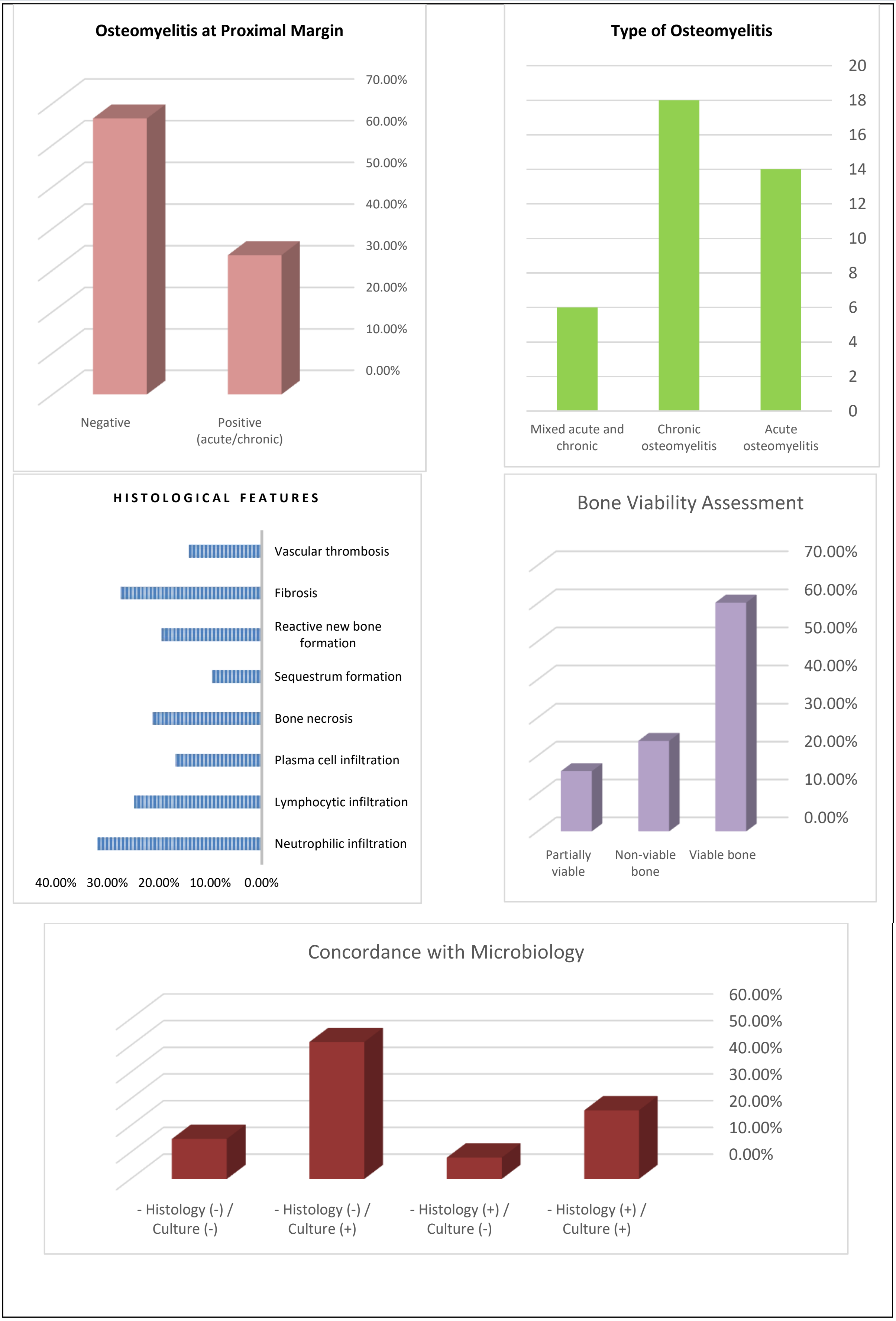

Figure 1. Classification of the histopathological features of proximal bone margins.

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Table 5. Surgical outcomes.

Variable	Characteristics
Type of Minor Amputation	
- Toe amputation (single)	42 (37.2%)
- Toe amputation (multiple)	18 (15.9%)
- Ray amputation	28 (24.8%)
- Transmetatarsal amputation	17 (15.0%)
- Lisfranc amputation	5 (4.4%)
- Chopart amputation	3 (2.7%)
Wound Closure	
- Primary closure	64 (56.6%)
- Secondary intention	38 (33.6%)
- Delayed primary closure	11 (9.7%)
Operative Time (minutes)	48.6 ± 22.4
Intraoperative Blood Loss (mL)	85.2 ± 62.8
Antibiotic Therapy	
- Pre-operative antibiotics	98 (86.7%)
- Post-operative IV antibiotics (days)	12.4 ± 8.6
- Post-operative oral antibiotics (days)	18.2 ± 14.8
- Total antibiotic duration (days)	28.6 ± 18.4
Revascularization Performed	24 (21.2%)
Negative Pressure Wound Therapy	46 (40.7%)
Hospital Length of Stay (days)	14.8 ± 9.6

Table 6. Post – treatment outcomes in association with histopathology status.

Variables	Positive Histopathology (n = 38)	Negative Histopathology (n = 75)	P-value
Primary Outcomes			
Treatment success at 6 months	19 (50.0%)	62 (82.7%)	0.001
Wound healing	21 (55.3%)	58 (77.3%)	0.018
Time to wound healing (weeks)	14.8 ± 8.4	8.6 ± 5.2	<0.001
Secondary Outcomes			
Unplanned reoperation	14 (36.8%)	11 (14.7%)	0.008
Proximal re-amputation	11 (28.9%)	8 (10.7%)	0.016
Major amputation (BKA/AKA)	6 (15.8%)	4 (5.3%)	0.074
Recurrent infection	12 (31.6%)	9 (12.0%)	0.012
Readmission within 30 days	10 (26.3%)	8 (10.7%)	0.034
Antibiotic Parameters			
Total antibiotic duration (days)	42.8 ± 16.4	21.4 ± 12.6	<0.001
IV antibiotic duration (days)	18.6 ± 8.2	9.2 ± 6.4	<0.001
Mortality			
30-day mortality	2 (5.3%)	1 (1.3%)	0.248
1-year mortality	5 (13.2%)	4 (5.3%)	0.144

Multivariate logistic regression analysis validates the fact that positive bone margin histopathology is the best independent predictor of treatment failure with an adjusted odds ratio of 3.86 (95% CI: 1.52-9.82, p=0.004). A significant independent risk factor was also an ankle-brachial index (ABI) <0.6, which indicated a severe peripheral arterial disease (adjusted OR 3.42, p=0.014). These results highlight that the occurrence of histologically determined osteomyelitis at the resection margin is a supreme prognostic factor, which is stronger than the impact of the microbiology findings, Wagner grade, or glycemic control in this model.

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Table 7. A multivariate logistic regression analysis of outcomes of risk factors that impact treatment.

Variable	Adjusted OR	95% CI	P-value
Positive histopathology at the margin	3.86	[1.52, 9.82]	0.004
ABI < 0.6	3.42	[1.28, 9.14]	0.014
Dialysis dependence	2.94	[0.98, 8.82]	0.054
Wagner grade ≥ 3	2.18	[0.78, 6.08]	0.138
Positive histopathology at the margin	3.48	[1.32, 9.18]	0.012
ABI < 0.6	2.96	[1.04, 8.42]	0.042
Dialysis dependence	2.68	[0.84, 8.54]	0.096
HbA1c > 8%	1.42	[0.54, 3.74]	0.478
CRP > 50 mg/L	1.86	[0.72, 4.82]	0.198
MRSA infection	2.12	[0.72, 6.24]	0.172
Secondary wound closure	1.94	[0.76, 4.96]	0.166

DISCUSSION

This proximal bone margin histopathology is crucial in clinical outcome prediction, as this single-center retrospective study of 113 patients undergoing minor amputation to treat diabetes-related foot infections (DFIs) demonstrates. The fact that we found that histopathological evidence of osteomyelitis at the resection margin occurred in 33.6% of cases, and that this was a strong, independent predictor of failure in treatment. Patients who had positive margins experienced much lower treatment success (50.0% vs. 82.7%), complete wound healing (55.3% vs. 77.3%), and a longer healing period, and re-amputation or re-hospitalization. This agrees with other studies (Atway S, 2012; Shiraev TP, 2019; Cecilia-Matilla A, 2013; Meyr AJ, 2011), which also found that residual osteomyelitis at the surgical margin was the strongest predictor of recurrent infection and re-amputation. Therefore, microbiological clearance is not adequate when viable infected bone is present.

The noted discrepancy between histopathology and intraoperative bone culture is an important observation. However, 77.0% of bone cultures were positive, but only 33.6% of proximal margins showed osteomyelitis by histology. Histology-negative/ culture-positive (51.3%) was the most frequent finding and may indicate that contamination occurred commonly, but no tissue invasion occurred in the proximal level. This inconsistency supports the concept that histology, rather than culture, is the gold standard of diagnosis of osteomyelitis (Weng B, 2023). Positive bone cultures with no histological findings are likely simply colonies that may not require a long period of specific antibiotic treatment, and may help minimize antimicrobial exposure. Our microbiological spectrum is typical of DFIs with Staphylococcus aureus (with 16.8% MRSA) and Gram-negative rods, such as Pseudomonas aeruginosa, predominating. The multidrug-resistant organisms (MDROs) rate (24.8 percent) is an indication of a difficult antimicrobial environment in chronic care.

Regardless of histopathology, the results of our multivariate analysis revealed that an Ankle-Brachial Index (ABI) below 0.6 is a strong independent risk factor of treatment failure (adjusted OR 3.42) that can help emphasize the ultimate significance of perfusion. Ischemia restricts the administration of antibiotics, hinders the healing process, and is a proven risk factor in amputations, as it is widely reported in a Canadian study [18]. This significant trend (adjusted OR 2.94, p=0.054) also indicates the increased risk of dialysis dependence on the end-stage renal disease patients who experience advanced microvascular disease, uremic immune dysfunction, and altered pharmacokinetics.

Our cohort experience of a positive correlation between positive histopathology and a significantly longer period of IV and total antibiotic treatment (p<0.001) is not unique to the clinical practice of adding antibiotic regimens to patients based on margin status (Johnson MJ, 2019; R Development Core Team. 2012; Mijuskovic B, 2018; Morawietz L, 2006; Spangehl MJ, 1999; Commons RJ, 2022). The fact that 8.0% of our patients had been found to have histology-positive/culture-negative margins is an indication that we have a subset with empirical broad-spectrum coverage that could overlook fastidious organisms. In addition, the prevalence of neuropathy (83.2) and peripheral artery disease (46.0) were high in our population, which once again confirms that infection alone rarely contributes to DFIs and amputations.

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CONCLUSION

This study confirms that histopathological examination of proximal bone margins in minor amputations in the case of diabetic foot infection is a critical prognostic data. Osteomyelitis at the resection margin, which was present in one-third of patients, was a strong and independent predictor of poor outcomes such as reduced successful treatment, worse wound healing, and increased reoperation and readmission. Such data support the fact that histology is the gold standard of osteomyelitis diagnosis and not microbiology, which was already tested. The constant discrepancy of positive bone cultures and negative histology at the proximal margin is the sign of the fact that colonization is extensive, and it refers to the danger of overtreatment based on the culture data alone.

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