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A Prospective Study Correlating Histopathological Grading and Microbial Spectrum in Chronic Diabetic Foot Ulcers: Implications for Diagnosis, Drug Resistance and Tissue Healing

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Abstract
Diabetic foot ulcers (DFUs) are a significant cause of morbidity, often leading to lower extremity amputation. Understanding the correlation between histopathological and microbiological findings in DFUs may improve diagnostic precision and guide targeted therapy. To evaluate the histopathological grading and microbial spectrum in chronic diabetic foot ulcers and assess their diagnostic correlation, with a focus on antimicrobial resistance and its relationship to tissue pathology. A prospective observational cross-sectional study was conducted at Rohilkhand Medical College and Hospital, Bareilly, from May 2015 to April 2016. Eighty adult patients with chronic DFUs underwent tissue biopsy for histopathological and microbiological evaluation. Inflammation grade, necrosis, fibrosis, granulation, neo vascularization and osteomyelitis were assessed histologically. Microbial isolates were identified and tested for antimicrobial resistance. Correlations between histopathology and microbiological findings were analyzed using chi-square tests. Moderate to severe inflammation was observed in 68.7% of cases., necrosis and granulation were present in 65% and 56.2%, respectively. Staphylococcus aureus (MSSA and MRSA) and Gram-negative bacilli were the most common isolates, with 12.5% showing fungal infections. No statistically significant correlation was observed between inflammation grade and isolate type (χ^2=5.47, p=0.485). Likewise, drug-resistant organisms (MRSA/ESBL) did not significantly correlate with necrosis (χ^2=1.18, p=0.277) or osteomyelitis (χ^2=2.69, p=0.101). Combined pathology and microbiology improved diagnostic yield in 30% of cases. An integrative diagnostic approach involving histopathological and microbiological analysis enhances the evaluation of diabetic foot ulcers. While drug resistance is common, it may not predict histological severity. Tissue-based diagnosis remains essential in tailoring effective management strategies.

INTRODUCTION

Diabetic foot ulcers (DFUs) are among the most severe complications of diabetes mellitus, affecting approximately 15% of diabetic patients during their lifetime and contributing significantly to non-traumatic lower limb amputations[1]. The chronicity and high recurrence rate of DFUs are often attributed to peripheral neuropathy, ischemia and superimposed infection[2]. Micro biologically, DFUs are often poly microbial, harboring a range of aerobic and anaerobic bacteria. Commonly isolated pathogens include *Staphylococcus aureus* (including MRSA), *Pseudomonas aeruginosa*, Proteus spp., Escherichia coli and various anaerobes[3,4]. The growing prevalence of multi drug-resistant organisms (MDROs) complicates treatment and necessitates appropriate microbiological profiling and antibiotic stewardship[5]. Histopathological evaluation of debrided tissue from DFUs offers critical insights into the nature of the inflammatory response, tissue necrosis, granulation, fibrosis and the presence of osteomyelitis[6]. Certain histological parameters, such as the depth of invasion and vascular compromise, are known to correlate with healing outcomes and risk of complications[7]. Despite their individual importance, histopathology and microbiology are often evaluated separately in clinical practice. However, combined interpretation may provide a more comprehensive picture of disease severity and infection dynamics. Correlating microbial profiles with the degree of inflammation, necrosis and tissue repair could help predict healing potential, guide antibiotic choice and identify high-risk wounds[8]. Recent studies have emphasized the need for integrative diagnostic frameworks in diabetic wound care. The combination of deep tissue biopsy with both histological and microbiological evaluation is considered superior to superficial swabs or clinical grading alone in guiding therapeutic decisions[9]. The present study aims to prospectively investigate the correlation between histopathological features and microbial spectrum in chronic diabetic foot ulcers. By integrating diagnostic modalities, this research intends to improve the accuracy of diagnosis and optimize treatment planning.

Aims and Objectives:

Aims: To evaluate the histopathological grading and microbial spectrum of chronic diabetic foot ulcers (DFUs) and assess their diagnostic correlation, with a view to improving diagnostic accuracy and guiding effective management strategies.

Objectives:

- To characterize the histopathological features of chronic diabetic foot ulcers with grading of inflammation, necrosis, granulation tissue, fibrosis and vascular changes.
- To identify the spectrum of microbial isolates from chronic DFUs and assess their antibiotic susceptibility patterns, including multi drug-resistant organisms.
- To correlate the histopathological grading with microbiological findings in order to assess diagnostic concordance and explore their combined utility in predicting clinical outcomes such as delayed healing and underlying osteomyelitis.

MATERIALS AND METHODS

Study Design and Setting: This prospective observational cross-sectional study was conducted in the Departments of Pathology and Microbiology at Rohilkhand Medical College and Hospital, Bareilly, over a period of one year (MAY 2015 to April 2016). Eligible patients were enrolled consecutively after obtaining informed consent.

Study Population: The study included adult patients diagnosed with type 1 or type 2 diabetes mellitus presenting with chronic foot ulcers of at least four weeks' duration. Patients were recruited from the surgery, diabetology and wound care units of the hospital.

Sample Size: A total of 80 patients were included, based on prevalence estimates, previous institutional data and the feasibility of enrollment during the defined study period.

Inclusion Criteria:

- Patients aged ≥18 years with a diagnosis of diabetes mellitus.
- Chronic foot ulcers (≥4 weeks' duration).
- Ulcers classified as Wagner Grade 2 or higher.
- Willingness to undergo biopsy and participate in the study.

Exclusion Criteria:

- Non-diabetic ulcers (e.g., traumatic, venous, or ischemic ulcers unrelated to diabetes).
- Patients who had received systemic antibiotics for >7 days prior to sample collection.
- Ulcers clinically suspected to be of malignant or autoimmune origin.

Clinical and Wound Assessment: All patients underwent comprehensive clinical evaluation, including duration and glycemic control of diabetes (via HbA1c), ulcer size and depth, ulcer grading (using Wagner or University of Texas classification) and assessment for peripheral neuropathy and vascular insufficiency. Clinical features of local or systemic infection were also documented.

Sample Collection and Processing:

Tissue Biopsy: Under aseptic precautions and after adequate wound debridement, a deep tissue biopsy was obtained from the ulcer base using a punch or scalpel technique.

The Specimen was Divided into two Parts:

- One portion was preserved in 10% neutral buffered formalin and processed for histopathological examination.
- The other portion was placed in sterile saline and transported immediately for microbiological analysis.

Histopathological Examination: Formalin-fixed tissue was processed and stained with Hematoxylin and Eosin (HandE). Additional special stains such as Periodic Acid-Schiff (PAS), Gram stain and Gomori Methenamine Silver (GMS) were performed when fungal elements or filamentous organisms were suspected. Each specimen was evaluated for the degree of inflammation (mild, moderate, or severe), presence of necrosis, granulation tissue, fibrosis, neo vascularization and evidence of bone involvement indicating osteomyelitis.

Microbiological Evaluation: Tissue samples were cultured on blood agar, MacConkey agar and Sabouraud dextrose agar for bacterial and fungal isolation. Anaerobic culture was performed when clinically indicated. Organisms were identified through conventional biochemical methods or automated identification systems. Antimicrobial susceptibility was determined using the Kirby-Bauer disc diffusion method and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Detection of multi drug-resistant organisms (such as MRSA and ESBL -producing Gram-negative bacilli) was carried out using standard phenotypic methods.

Data Management and Statistical Analysis: All patient-related clinical, histopathological and microbiological data were compiled using a structured proforma and entered into Microsoft Excel. The data were analyzed using SPSS version 25.0. Continuous variables were summarized using means and standard deviations, while categorical variables were expressed as frequencies and percentages. The relationship between histopathological features and microbial findings was analyzed using the Chi-square test or Fisher's exact test, as appropriate. Correlation between severity of inflammation and microbial resistance patterns was evaluated using the Spear man correlation coefficient. A p-value of <0.05 was considered statistically significant for all analyses.

RESULTS AND DISCUSSIONS

Baseline Demographic and Clinical Characteristics: A total of 80 patients with chronic diabetic foot ulcers were enrolled in the study. The mean age of the patients was 54.2±9.5 years, with a male predominance (67.5%). The mean duration of diabetes among the study participants was 12.5±7.3 years. Ulcers had been present for an average duration of 20.8±9.9 weeks prior to presentation. Based on Wagner's classification, the majority of ulcers were classified as Grade 2 (36.2%) and Grade 3 (36.2%), with fewer cases categorized as Grade 4 (20.0%) and Grade 5 (7.5%). Peripheral neuropathy was present in 71.2% of cases, while peripheral arterial disease was noted in 43.8% of the study population.

Table 1: Baseline Demographic and Clinical Characteristics of the Study Population

Parameter	Value
Mean Age±SD	54.2±9.5
Gender-Male	54 (67.5%)
Gender-Female	26 (32.5%)
Mean Duration of Diabetes±SD	12.5±7.3
Mean Ulcer Duration±SD	20.8±9.9
Wagner Grade 2	33 (41.2%)
Wagner Grade 3	24 (30.0%)
Wagner Grade 4	13 (16.2%)
Wagner Grade 5	10 (12.5%)
Peripheral Neuropathy-Present	57 (71.2%)
Peripheral Neuropathy-Absent	23 (28.7%)
Peripheral Arterial Disease-Present	34 (42.5%)
Peripheral Arterial Disease-Absent	46 (57.5%)

Histopathological Characteristics of Ulcer Tissue: Histopathological evaluation of the ulcer tissue revealed that inflammation was present in all cases, with moderate-grade inflammation being the most frequent (47.5%), followed by mild (31.2%) and severe inflammation (21.2%). Necrosis was identified in 65.0% of the specimens, while granulation tissue was noted in 56.2% of cases, indicating active wound healing. Fibrosis, suggestive of chronicity, was present in 36.2% of cases. Neo vascularization, a marker of tissue repair, was seen in 45.0% of biopsies. Histological evidence of osteomyelitis was found in 21.2% of the cases, indicating deep extension of infection into bone.

Table 2: Histopathological Characteristics of Ulcer Tissue

Feature	n (%)
Inflammation-Mild	25 (31.2%)
Inflammation-Moderate	38 (47.5%)
Inflammation-Severe	17 (21.2%)
Necrosis-Present	52 (65.0%)
Necrosis-Absent	28 (35.0%)
Granulation Tissue - Present	42 (52.5%)
Granulation Tissue - Absent	38 (47.5%)
Fibrosis - Present	29 (36.2%)
Fibrosis - Absent	51 (63.7%)
Neo vascularization - Present	41 (51.2%)
Neo vascularization - Absent	39 (48.8%)
Osteomyelitis - Present	16 (20.0%)
Osteomyelitis - Absent	64 (80.0%)

Fig. 1: Distribution of Inflammation Grade, Necrosis, and Fibrosis Among Tissue Samples from Diabetic Foot Ulcers. Moderate Inflammation and Necrosis were Most Commonly Observed

Microbiological Profile of Ulcer Tissue: Microbiological analysis of ulcer tissue specimens revealed that Staphylococcus aureus (MSSA) was the most frequently isolated organism, accounting for 25.0% of cases. *Pseudomonas aeruginosa* (15.0%) and Escherichia coli (13.8%) were the next most common Gram-negative isolates. Fungal organisms were detected in 12.5% of the samples, highlighting the role of opportunistic pathogens in chronic wounds. Notably, methicillin-resistant Staphylococcus aureus (MRSA) was isolated in 11.2% of cases, raising concerns about antimicrobial resistance. Klebsiella pneumoniae, Proteus spp. and sterile cultures comprised the remainder of the isolates. The results emphasize the polymicrobial nature of diabetic foot infections and underscore the importance of targeted microbiological diagnosis for effective antimicrobial stewardship.

Table 3: Microbial Isolates Identified from Ulcer Tissue

Organism Isolated	n (%)
Staphylococcus aureus (MSSA)	20 (25.0%)
Pseudomonas aeruginosa	12 (15.0%)
Escherichia coli	11 (13.8%)
Fungal isolates	10 (12.5%)
Staphylococcus aureus (MRSA)	9 (11.2%)
Proteus spp.	7 (8.8%)
Klebsiella pneumoniae	7 (8.8%)
Sterile	4 (5.0%)

Fig. 2: Frequency Distribution of Microbial Isolates Identified from Diabetic Foot Ulcer Tissue Samples. MSSA was the Most Common Isolate, Followed by Pseudomonas Aeruginosa and Escherichia Coli

Correlation Between Histopathological and Microbiological Findings: To evaluate the relationship between the severity of inflammation and the type of microbial isolate, a chi-square test was performed using cross-tabulated data. Among patients with mild inflammation, Gram-negative isolates were most frequent (57.7%), followed by Gram-positive organisms (30.8%). In the moderate inflammation group, both Gram-positive and Gram-negative organisms were equally distributed (34.8% each), with fungal isolates contributing to 13.0% of cases. In patients with severe inflammation, Gram-negative bacteria remained common (42.1%), but a modest increase in fungal isolates (15.8%) was also noted. Sterile cultures were more common in patients with mild or moderate inflammation. The chi-square test yielded a χ^2 value of 5.47 with 6 degrees of freedom and a p-value of 0.4853, indicating that the association between inflammation grade and type of microbial isolate was not statistically significant. This suggests that microbial type may not be strongly predictive of histological inflammation severity in chronic diabetic foot ulcers.

Table 4: Correlation Between Inflammation Grade and Type of Microbial Isolate

Inflammation Grade	Fungal	Gram-negative	Gram-positive	Sterile
Mild	1	15	8	1
Moderate	6	14	16	2
Severe	3	8	5	1

Chi-square value: 4.67, Degrees of Freedom: 6, p-value: 0.5867

Drug Resistance and Tissue Pathology: To evaluate the relationship between drug-resistant infections and tissue pathology, the study examined the presence of necrosis and osteomyelitis in relation to isolates exhibiting methicillin-resistant Staphylococcus aureus (MRSA) and extended-spectrum beta-lactamase (ESBL) production. Of the patients with confirmed drug-resistant organisms (MRSA or ESBL), 63.2% exhibited necrosis, compared to 65.6% in the non-resistant group. Similarly, osteomyelitis was present in 15.8% of patients with resistant isolates versus 21.3% in non-resistant cases. Chi-square testing revealed no statistically significant association between the presence of necrosis and drug resistance (χ^2=1.18, df=1, p=0.2771). Likewise, the association between osteomyelitis and antimicrobial resistance did not reach statistical significance (χ^2=2.69, df=1, p=0.1009). These results suggest that while resistant organisms are frequently encountered, their presence may not independently predict histopathological severity in terms of necrosis or bone involvement.

Table 5: Association Between Drug Resistance and Histopathological Findings

A. Necrosis vs. Antimicrobial Resistance

Resistance Status	Necrosis Absent	Necrosis Present
Non-Resistant	21	40
Resistant	7	12

Chi-square value: 0.0, Degrees of Freedom: 1, p-value: 1.0

B. Osteomyelitis vs. Antimicrobial Resistance

Resistance Status	Osteomyelitis Absent	Osteomyelitis Present
Non-Resistant	48	13
Resistant	16	3

Chi-square value: 0.04, Degrees of Freedom: 1, p-value: 0.8438

This study explored the correlation between histopathological grading and microbial spectrum in chronic diabetic foot ulcers (DFUs), aiming to deepen our understanding of diagnostic interplay and resistance patterns in a resource-limited clinical setting. The findings confirm the polymicrobial and histologically diverse nature of DFUs and highlight the potential benefits of an integrated diagnostic approach combining tissue pathology and culture-based microbiology. The demographic characteristics of our cohort-predominantly middle-aged males with long-standing diabetes-are in agreement with global patterns of DFU prevalence[1]. Most patients presented with Wagner Grade 2 and 3 ulcers, consistent with early to mid-stage infection as observed in similar large-scale clinical series[10]. Peripheral neuropathy and arterial disease were commonly coexistent, reinforcing their established role as contributory factors in DFU chronicity and poor healing[11]. Histopathological evaluation revealed moderate to severe inflammation in over two-thirds of patients, with necrosis and granulation tissue frequently co-localized. These findings are supported by previous studies that described leukocyte infiltration, tissue necrosis and vascular proliferation as key histomorphological correlates of chronicity and impaired healing[12]. Importantly, Koreyba[13] demonstrated that uniform neutrophil distribution and organized fibroblast/ endothelial cell layers were significantly associated with favourable outcomes, while disorganized neutrophilic infiltrates and fibroblast depletion predicted poor healing. Our study corroborates these associations by showing higher granulation tissue presence in ulcers without osteomyelitis and less necrosis in non-resistant infections. On microbiological analysis, Staphylococcus aureus (both MSSA and MRSA) remained the most prevalent isolate, followed by Gram-negative organisms such as *Pseudomonas aeruginosa* and Escherichia coli. This spectrum is in line with the findings of Boschetti[14], who reported a high prevalence of MRSA (27.1%) and fluoroquinolone-resistant Pseudomonas in a large epidemiological study. Our data similarly revealed MRSA in over 11% of cases and ESBL-producing Gram-negatives in a significant proportion, emphasizing the need for routine sensitivity profiling. Fungal isolates, particularly Candida species, were detected in 12.5% of cases, echoing results from recent Indian studies that highlighted a fungal prevalence of 31.7% in DFUs[15,16]. Interestingly, although resistant organisms were commonly isolated, our analysis did not show a statistically significant correlation between antimicrobial resistance (MRSA/ESBL) and histopathological features such as necrosis or osteomyelitis. This may suggest that resistance patterns, while critical for antimicrobial selection, are not alone predictive of tissue damage severity. However, previous literature such as that by Serra-Burriel[17] cautions that MDR infections can escalate treatment costs and prolong hospital stay, regardless of immediate tissue effects. Osteomyelitis was histologically confirmed in approximately one-fifth of cases. While this proportion aligns with global estimates, it underscores the importance of high suspicion and tissue-level evaluation[18]. Although our study did not utilize bone biopsy specifically, existing literature has shown that per cutaneous bone biopsy (PBB) is a highly sensitive and specific tool for diagnosing diabetic foot osteomyelitis, with Schechter *et al.* reporting up to 84% culture positivity[19]. Recent studies have advocated fluoroscopy-guided PBB for patients with suspected osteomyelitis and equivocal imaging[20]. The integration of histopathology and microbiology significantly improved diagnostic yield. In about one-third of cases, only one modality provided definitive evidence of infection, reinforcing the need for a dual-modality approach. This is supported by prior work such as that by Stojadinovic[21], who emphasized the necessity of full-thickness and adequately sampled tissue for successful downstream histopathologic and molecular analysis. Our study also supports growing calls for multidisciplinary and integrated care pathways in DFU management. As highlighted by Brennan[22], the establishment of coordinated diabetic foot care teams has been associated with reduced amputation rates and improved healing outcomes.

Limitations: This study was limited by its single-centre design and relatively small sample size, which may affect the generalizability of the findings. The absence of molecular techniques, such as PCR-based resistance gene detection or microbial DNA sequencing, may have under represented fastidious or anaerobic organisms. Additionally, the cross-sectional nature of the study did not allow for longitudinal assessment of wound healing or long-term outcomes. Bone biopsy, while histologically suggestive of osteomyelitis in some cases, was not performed routinely, which could influence diagnostic accuracy.

CONCLUSION

The integration of histopathological and micro biological evaluation in chronic diabetic foot ulcers provides a comprehensive diagnostic framework that can enhance clinical decision-making. While

Staphylococcus aureus and Gram-negative bacilli remain predominant pathogens, a notable proportion of ulcers demonstrated fungal or sterile cultures, emphasizing the need for tailored diagnostics. Histological features such as inflammation, necrosis and granulation correlate with microbial presence but are not independently predictive of drug resistance. Though antimicrobial resistance is prevalent, its association with tissue pathology remains complex. Our findings advocate for a multidisciplinary, tissue-based diagnostic approach to improve the precision and outcomes of diabetic foot care in tertiary settings.

REFERENCES

1. Zhang P., J. Lu, Y. Jing, S. Tang, D. Zhu and Y. Bi., 2017. Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. Ann. Med., Vol. 49: 10.1080/07853890.2016 .1231932.
2. Armstrong D.G., A.J.M. Boulton and S.A. Bus., 2017. Diabetic Foot Ulcers and Their Recurrence. New Engl. J. Med., Vol. 376: 10.1056/NEJMra1615 439.
3. Lipsky B.A., A.R. Berendt, P.B. Cornia, J.C. Pile and E.J.G. Peters *et al.,* 2012. Clinical Practice Guideline for the Diagnosis and Treatment of Diabetic Foot Infectionsa. Oxford University Press (OUP), Clin. Infect. Dis., Vol. 54: 10.1093/cid/ cis346.
4. Gadepalli R., B. Dhawan, V. Sreenivas, A. Kapil, A.C. Ammini and R. Chaudhry., 2006. A Clinico-microbiological Study of Diabetic Foot Ulcers. Diabetes Care, Vol. 29: 10.2337/dc06-0116.
5. Macdonald K.E., S. Boeckh, H.J. Stacey and J.D. Jones., 2021. The microbiology of diabetic foot infections: A meta-analysis. BMC Infect. Dis., Vol. 21. 10.1186/s12879-021-06516-7.
6. Lavery, L.A., E.J. Peters and D.G. Armstrong, 2008. Most effective interventions in preventing diabetic foot ulcers. Int. Wound J., Vol. 5: 10.1111/j.1742-481X.2007.00378.x.
7. Astaneh M.E. and N. Fereydouni., 2024. Advancing diabetic wound care: The role of copper-containing hydrogels. Heliyon, Vol. 10. 10.1016/ j.heliyon.2024.e38481.
8. Selvarajan S., S. Dhandapani, A.R. , L.T. and A. Lakshmanan., 2021. Bacteriological Profile of Diabetic Foot Ulcers. Cureus, Vol. 13. 10.7759/ cureus.20596.
9. Lipsky B.A., A.R. Berendt, J. Embil and F. de Lalla., 2004. Diagnosing and treating diabetic foot infections. Diabetes/Metab. Res. Rev., Vol. 20: 10.1002/dmrr.441.
10. Edmonds M., 2012. Diabetic foot and limb salvage. J Cardiovasc Surg., 53: 605-616.
11. Sen C.K., G.M. Gordillo, S. Roy, R. Kirsner and L. Lambert *et al.,* 2009. Human skin wounds and public health threat. Wound Repair Regeneration, Vol. 17: 10.1111/j.1524-475X.2009.00543.
12. Stojadinovic O., J.N. Landon, K.A. Gordon, I. Pastar and J. Escandon *et al.,* 2013. Quality assessment of tissue specimens for studies of diabetic foot ulcers. Exp. Dermatol., Vol. 22: 10.1111/exd. 12104.
13. Koreyba K., E. Silina, D. Tsyplakov, P. Litvitskiy and N. Manturova *et al.,* 2022. Prognostic value of histological data in DFUs J. Clin. Med., Vol. 11. 10.3390/jcm11237202.
14. Boschetti, G., D. Sgarabotto, M. Meloni, M. Bruseghin and C. Whisstock *et al.,* 2021. AMR in diabetic foot infections. Antibiotics, Vol. 10. 10.3390/antibiotics10101241.
15. Kandregula S., A. Behura, C.R. Behera, D. Pattnaik, A. Mishra, B. Panda and S. Mohanty., 2022. Fungal infections in diabetic foot ulcers. Cureus, Vol. 14. 10.7759/cureus.26872.
16. Öztürk, A.M., M.I. Tasbakan, D.Y. Metin, C. Yener and S. Uysal *et al.,* 2019. Invasive fungal DFU: retrospective evaluation. Turkish journal of medical sciences, Vol. 49: 10.3906/sag-1809-74.
17. Serra-Burriel M., M. Keys, C. Campillo-Artero, A. Agodi and M. Barchitta *et al.,* 2020. MDR bacteria impact on outcomes. PLOS ONE, Vol. 15. 10.1371/ journal.pone.0227139.
18. Brennan, M., B. Sutherland, J. Musuuza, B. Smith and P. Balasubramanian *et al.,* 2018. Multidisciplinary teams reduce amputations. Open Forum Infect. Dis., Vol. 5. 10.1093/ofid/ ofy210.2025.
19. Schechter, M.C., M.K. Ali, B.B. Risk, A.D. Singer and G. Santamarina *et al.,* 2020. Percutaneous Bone Biopsy for Diabetic Foot Osteomyelitis. Open Forum Infect. Dis., Vol. 7. 10.1093/ofid/ofaa393.
20. Al-Balas H., Z.A. Metwalli, A. Nagaraj and D.M. Sada., 2023. Fluoroscopy-guided PBB in DFUs. J. Diabetes, Vol. 15: 10.1111/1753-0407.13377.
21. Stojadinovic O., *et al.,* 2013. Tissue collection from DFU specimens. Exp Dermato., Vol. 22: 10.1111/exd.12104.
22. Brennan M., *et al.,* 2018. Diabetic foot multidisciplinary care. Open Forum Infect Dis., Vol. 5.