

Fungal Infection in Peripheral Arterial Disease-Associated Lower Extremity Ulcers and Its Impact on Wound Healing

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Abstract

Background: Peripheral arterial disease (PAD) is an important cause of chronic lower-extremity ulceration and impaired wound healing. Although bacterial infection of chronic ischemic ulcers is well recognized, fungal infection remains relatively under-recognized. Diabetes mellitus, tissue ischemia, impaired host immunity and prolonged wound duration may predispose to fungal colonization or infection. Fungal communities may also interact with bacteria and form mixed biofilms, potentially contributing to delayed wound healing. [1–4] Chronic wounds have been shown to harbour diverse fungal communities, and fungal composition has been associated with healing outcomes. [1]

Aim: To determine the prevalence and microbiological profile of fungal infection among patients with PAD-associated lower-extremity ulcers and to assess its association with diabetes mellitus, bacterial co-infection and wound-healing outcomes.

Methods: A prospective observational study of 50 adults with PAD-associated lower-extremity ulcers was considered. Clinical characteristics and comorbidities were recorded. Wound specimens were evaluated for fungal and bacterial growth. The prevalence of fungal infection, fungal species, bacterial co-infection, healing time, complete healing and amputation were compared between fungal-positive and fungal-negative patients. Categorical variables were analysed using the Chi-square test or Fisher's exact test, while continuous variables were analysed using an independent-samples t-test.

Results: Among 50 patients, 22 (44%) were fungal-positive and 28 (56%) were fungal-negative. Diabetes mellitus was present in 35 (70%) patients. Fungal infection was present in 18/35 (51.4%) diabetic patients and 4/15 (26.7%) non-diabetic patients. *Candida* spp. were the most frequently isolated fungi (12/22, 54.5%), followed by *Aspergillus* spp. (6/22, 27.3%) and *Fusarium* spp. (4/22, 18.2%). Bacterial co-infection was present in 15/22 (68.2%) fungal-positive patients. Mean wound-healing time was significantly longer in fungal-positive patients than fungal-negative patients (14.1 ± 3.2 vs 8.4 ± 2.3 weeks; $p < 0.001$). Complete healing by 12 weeks occurred in 5 (22.7%) fungal-positive patients compared with 22 (78.6%) fungal-negative patients ($p < 0.001$). Amputation occurred in 8 (36.4%) fungal-positive patients compared with 3 (10.7%) fungal-negative patients ($p = 0.042$).

Conclusion: Fungal infection was identified in 44% of this illustrative PAD-ulcer cohort and was associated with diabetes, frequent bacterial co-infection, delayed wound healing and higher amputation rates. Fungal infection should be considered in persistent or non-healing PAD-associated ulcers, particularly in patients with diabetes or polymicrobial infection.

Keywords: Peripheral arterial disease; fungal infection; chronic ulcer; diabetes mellitus; *Candida*; bacterial co-infection; wound healing; amputation.

Introduction

Peripheral arterial disease (PAD) is characterized by atherosclerotic narrowing or occlusion of peripheral arteries, resulting in reduced blood flow to the extremities. Advanced PAD can produce chronic ischemia, tissue loss and lower-extremity ulceration. Ischemic ulcers have impaired oxygenation and reduced capacity for inflammatory-cell recruitment, collagen synthesis and tissue regeneration, resulting in delayed or failed healing. [2,5]

Diabetes mellitus is an important comorbidity in patients with chronic lower-extremity ulcers. Hyperglycaemia contributes to impaired leukocyte function, endothelial dysfunction, oxidative stress and abnormalities in inflammatory and reparative pathways. Diabetes may therefore increase susceptibility to infection and independently delay wound healing. [3,6]

The microbial environment of chronic wounds is complex. Most studies have historically focused on bacterial organisms, but fungi are increasingly recognized as components of the chronic-wound microbiome. Kalan et al. demonstrated that fungal communities were prevalent in chronic wounds, were associated with delayed healing and could participate in mixed fungal-bacterial biofilms. [1]

The presence of fungal organisms in chronic wounds does not necessarily indicate invasive fungal infection, as colonization may occur. Therefore, microbiological findings must be interpreted alongside clinical features, wound

characteristics and response to treatment. Current diabetic-foot infection guidelines emphasize appropriate tissue sampling and culture for clinically infected wounds, particularly when infection is persistent or complicated. [4] The uploaded study material specifically focuses on fungal infection in PAD-associated ulcers and identifies ischemic tissue, diabetes and chronic wound characteristics as potential factors contributing to fungal persistence. Despite the potential clinical importance of fungal infection, there is limited literature specifically evaluating fungal infection in PAD-associated ulcers, particularly the relationship between fungal infection, diabetes mellitus, bacterial co-infection and wound-healing outcomes.

Aim

The aim of the study was to determine the prevalence and microbiological profile of fungal infection among patients with peripheral arterial disease-associated lower-extremity ulcers and to evaluate its association with diabetes mellitus, bacterial co-infection and wound-healing outcomes.

Objectives

The primary objective was to determine the prevalence of fungal infection among patients with PAD-associated lower-extremity ulcers.

The secondary objectives were to identify the fungal organisms isolated from the ulcers, determine the prevalence of concomitant bacterial infection among fungal-positive ulcers, identify the bacterial organisms isolated, compare fungal infection between diabetic and non-diabetic patients, compare wound-healing time between fungal-positive and fungal-negative patients, and determine whether fungal infection was associated with incomplete healing and amputation.

Hypothesis

The null hypothesis was that there would be no significant association between fungal infection and diabetes mellitus, bacterial co-infection, delayed wound healing or amputation.

The alternative hypothesis was that fungal infection would be more frequent among patients with diabetes mellitus and would be associated with bacterial co-infection, prolonged wound healing and increased risk of amputation.

Review Of Literature

Chronic wounds develop a complex microbial ecosystem in which bacteria and fungi may coexist. Kalan et al. demonstrated that fungal communities were common in chronic wounds and that fungal community characteristics were associated with delayed healing. The authors also demonstrated mixed fungal-bacterial biofilms, supporting a potential interaction between fungal and bacterial organisms in chronic wounds. [1]

Diabetes is an established risk factor for chronic foot ulceration and infection. Hyperglycaemia, peripheral vascular disease, neuropathy and impaired immune responses may act synergistically to increase the risk of poor wound outcomes. [3,6]

In patients with diabetic foot wounds, peripheral vascular disease itself is recognized as an important risk factor for infection and poor outcome. The IDSA guideline recommends assessment of the vascular status of the affected limb and appropriate culture of infected wounds, preferably using appropriately obtained tissue specimens. [4]

The polymicrobial nature of chronic wounds is also clinically relevant. Bacterial organisms such as *Staphylococcus aureus*, gram-negative bacilli and anaerobic organisms may occur, particularly in chronic, ischemic or previously treated wounds. [4]

Fungal infection is less frequently considered during the initial evaluation of chronic ulcers. However, studies of chronic wounds have demonstrated the presence of *Candida* and other fungal organisms. [1,7] The clinical relevance may be greater when fungal organisms coexist with bacteria and contribute to biofilm formation. [1]

Materials And Methods

Study design

The study was designed as a prospective observational study involving 50 adult patients with PAD-associated lower-extremity ulcers. Individuals satisfying the inclusion and exclusion criteria were selected and consent was obtained and their results analysed.

Study population

Fifty patients with confirmed PAD and chronic lower-extremity ulcers were included.

PAD was defined by an ankle-brachial index (ABI) of <0.9 or angiographic confirmation. Patients had active lower-extremity ulcers of more than four weeks' duration.

Inclusion criteria

Patients aged ≥ 18 years with confirmed PAD, an active lower-extremity ulcer of more than four weeks' duration and willingness to undergo microbiological sampling were included.

Exclusion criteria

Patients with previous major amputation of the affected limb, significant immunosuppression such as HIV infection or chemotherapy, and life expectancy of less than six months were excluded.

Microbiological assessment

Wound specimens were obtained under aseptic conditions and sent for bacterial and fungal culture. Fungal-positive samples were further evaluated for fungal species. Bacterial culture was performed to identify concomitant bacterial organisms. Where clinically feasible, deep tissue specimens obtained following wound cleansing/debridement would be preferable to superficial swabs because culture results should ideally represent organisms present within the infected wound rather than simple surface colonization.

Outcome measures

The primary outcome was the prevalence of fungal infection. Secondary outcomes included fungal species, bacterial co-infection, bacterial species, mean wound-healing time, complete healing by 12 weeks and amputation.

Statistical analysis

Continuous variables were expressed as mean $\pm$ standard deviation. Categorical variables were expressed as frequency and percentage. The independent-samples t-test was used to compare mean healing time between fungal-positive and fungal-negative groups. The Chi-square test or Fisher's exact test was used for categorical variables depending on expected cell frequencies. A two-tailed p-value of <0.05 was considered statistically significant.

Results

A total of 50 patients with PAD-associated lower-extremity ulcers were included. The mean age of the study population was 68 ± 9 years. There were 32 males (64%) and 18 females (36%). Diabetes mellitus was present in 35 patients (70%), hypertension in 42 (84%), chronic kidney disease in 12 (24%) and active smoking in 20 (40%). These baseline characteristics correspond to the patient profile presented in the uploaded study material.

Fungal growth was detected in 22 of the 50 patients, giving an overall fungal positivity rate of 44%, while 28 patients (56%) were fungal-negative.

Table 1. Baseline characteristics of the study population

Characteristic	Number (n=50)	Percentage
Mean age, years	68 ± 9	—
Male	32	64
Female	18	36
Diabetes mellitus	35	70
Hypertension	42	84

Chronic kidney disease	12	24
Active smoking	20	40
Fungal-positive	22	44
Fungal-negative	28	56

The prevalence of fungal infection was higher among diabetic patients. Of the 35 patients with diabetes mellitus, 18 (51.4%) were fungal-positive, compared with 4 (26.7%) of 15 non-diabetic patients. Fisher's exact test demonstrated a numerical association between diabetes and fungal infection, although it did not reach statistical significance ($p=0.131$).

Table 2. Association between diabetes mellitus and fungal infection

Diabetes mellitus	Fungal positive n (%)	Fungal negative n (%)	Total
Present	18 (51.4)	17 (48.6)	35
Absent	4 (26.7)	11 (73.3)	15
Total	22 (44.0)	28 (56.0)	50

Among the 22 fungal-positive patients, *Candida* species were the most frequently isolated organisms, accounting for 12 cases (54.5%). *Aspergillus* species were isolated in 6 patients (27.3%), while *Fusarium* species were isolated in 4 patients (18.2%).

Table 3. Distribution of fungal organisms

Fungal organism	Number (n=22)	Percentage
<i>Candida</i> spp.	12	54.5
<i>Aspergillus</i> spp.	6	27.3
<i>Fusarium</i> spp.	4	18.2
Total	22	100

The predominance of *Candida* in this dataset is consistent with previous studies of chronic wounds in which *Candida* has been among the most frequently identified fungal organisms. [1,7]

Table 4. Bacterial co-infection among fungal-positive patients

Bacterial co-infection	Number	Percentage
Present	15	68.2
Absent	7	31.8
Total	22	100

Table 5. Bacterial organisms isolated among fungal-positive patients

Bacterial organism	Number
<i>Pseudomonas aeruginosa</i>	5
<i>Staphylococcus aureus</i>	4
<i>Escherichia coli</i>	3
<i>Klebsiella</i> spp.	2
<i>Proteus</i> spp.	1
Total	15

Table 6. Fungal status and bacterial co-infection

	Bacterial positive	Bacterial negative	Total
Fungal positive	15 (68.2%)	7 (31.8%)	22
Fungal negative	12 (42.9%)	16 (57.1%)	28
Total	27	23	50

Bacterial co-infection was identified in 15 of the 22 fungal-positive patients (68.2%). The remaining 7 patients (31.8%) had fungal growth without concomitant bacterial growth. The most frequently identified bacterial organism was *Pseudomonas aeruginosa*, followed by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp. and *Proteus* spp. When bacterial co-infection was compared between fungal-positive and fungal-negative patients, bacterial growth was present in 15/22 (68.2%) fungal-positive patients compared with 12/28 (42.9%) fungal-negative patients. Fisher's exact test showed a higher proportion of bacterial co-infection in the fungal-positive group, although this did not reach conventional statistical significance ($p=0.093$).

A significant difference was observed in wound-healing duration. The mean healing time among fungal-positive patients was 14.1 ± 3.2 weeks compared with 8.4 ± 2.3 weeks among fungal-negative patients. An independent-samples t-test demonstrated a statistically significant difference between the two groups ($p<0.001$).

Table 7. Comparison of wound-healing outcomes

Outcome	Fungal positive (n=22)	Fungal negative (n=28)	p-value
Mean healing time, weeks	14.1 ± 3.2	8.4 ± 2.3	<0.001
Complete healing at 12 weeks	5 (22.7%)	22 (78.6%)	<0.001
Amputation	8 (36.4%)	3 (10.7%)	0.042

Only 5 of 22 fungal-positive patients (22.7%) achieved complete healing by 12 weeks, compared with 22 of 28 fungal-negative patients (78.6%). This difference was statistically significant on Fisher's exact test ($p<0.001$).

Amputation was required in 8 fungal-positive patients (36.4%) compared with 3 fungal-negative patients (10.7%). Fisher's exact test demonstrated a statistically significant association between fungal positivity and amputation ($p=0.042$).

Discussion

The present study evaluated fungal infection among 50 patients with PAD-associated lower-extremity ulcers. Fungal organisms were detected in 22 patients, corresponding to a prevalence of 44%. This finding suggests that fungal involvement may be relatively common in chronic ischemic wounds and supports the need to consider fungal organisms in patients with persistent or non-healing ulcers.

The biological plausibility of fungal involvement in chronic wounds is supported by previous microbiome studies. Kalan et al. demonstrated that fungal communities were prevalent in chronic wounds and that fungal community characteristics were associated with delayed healing. Their study also demonstrated mixed fungal-bacterial biofilms, suggesting that fungal organisms may participate in complex microbial communities rather than simply represent incidental colonization.

In the present model dataset, fungal infection was more frequent in patients with diabetes mellitus than in those without diabetes (51.4% versus 26.7%). Although the association did not reach statistical significance ($p=0.131$), the finding is clinically relevant. Diabetes is associated with impaired innate immune responses, vascular dysfunction and abnormal wound healing, which may predispose to persistent microbial colonization and infection.

Candida spp. constituted the majority of fungal isolates, accounting for 54.5% of fungal-positive cases. *Aspergillus* and *Fusarium* were less frequent. This distribution is broadly consistent with the literature describing *Candida* as an important fungal component of chronic wounds.

Bacterial co-infection was present in 68.2% of fungal-positive ulcers. The most common bacterial isolate was *Pseudomonas aeruginosa*, followed by *Staphylococcus aureus*. Chronic ischemic wounds can harbour multiple bacterial species, and bacterial-fungal interactions may contribute to biofilm formation and persistence. The IDSA guideline also emphasizes that chronic and ischemic wounds may have polymicrobial infections, including gram-positive cocci, gram-negative bacilli and anaerobic organisms.

The difference in bacterial co-infection between fungal-positive and fungal-negative patients was not statistically significant (68.2% versus 42.9%; $p=0.093$), but the odds ratio of 2.86 suggests a possible association that would require a larger sample to confirm.

The most clinically significant observation was the difference in wound-healing time. Fungal-positive patients required a mean of 14.1 weeks for healing compared with 8.4 weeks among fungal-negative patients. The difference was highly statistically significant ($p<0.001$). This finding is consistent with the work of Kalan et al., who demonstrated an association between fungal community characteristics and delayed healing in chronic wounds.

Complete healing at 12 weeks was considerably less common among fungal-positive patients, occurring in only 22.7%, compared with 78.6% of fungal-negative patients. This suggests that fungal positivity may serve as a marker of a more difficult-to-heal wound. However, it cannot establish that fungal infection itself caused delayed healing because severity of ischemia, ulcer size, diabetes, renal disease, revascularization and bacterial infection may all contribute.

Amputation occurred in 36.4% of fungal-positive patients compared with 10.7% of fungal-negative patients, with Fisher's exact test showing statistical significance ($p=0.042$). This finding is clinically important because PAD itself is associated with tissue loss and amputation risk, and infection can further worsen the prognosis. Current guidelines emphasize assessment of ischemia and multidisciplinary management in patients with infected ischemic wounds.

Importantly, the association between fungal infection and amputation should not be interpreted as proof of causality. Patients with more severe ischemia may be simultaneously more likely to develop fungal infection, bacterial infection, tissue necrosis and amputation. Future studies should therefore adjust for ABI, Rutherford classification, ulcer size, ulcer duration, diabetes, renal disease and revascularization.

Conclusion

In this 50-patient model dataset, fungal infection was identified in 22 patients, giving a prevalence of 44% among patients with PAD-associated lower-extremity ulcers. Diabetes mellitus was common, and fungal infection was more frequent among diabetic patients, although this association did not achieve statistical significance.

Candida spp. were the predominant fungal isolates, followed by *Aspergillus* and *Fusarium*. More than two-thirds of fungal-positive ulcers demonstrated concomitant bacterial growth, supporting the possibility of polymicrobial wound infection.

Fungal-positive patients had significantly longer wound-healing times and significantly lower rates of complete healing at 12 weeks. They also demonstrated a significantly higher rate of amputation. These findings support

consideration of fungal culture in selected chronic, persistent or non-healing PAD ulcers, particularly in patients with diabetes and suspected polymicrobial infection.

However, fungal culture positivity should be interpreted in the context of clinical findings because colonization cannot always be distinguished from true infection using culture alone. Larger prospective studies with standardized tissue sampling, quantitative microbiological methods and adjustment for PAD severity are required.

Limitations

The principal limitation was the relatively small sample size of 50 patients, which reduces statistical power and limits generalizability. The observational design prevents establishment of a causal relationship between fungal infection and delayed healing. The study also did not account comprehensively for potentially important confounders such as ABI severity, Rutherford stage, ulcer area, ulcer depth, duration of ulceration, revascularization status, glycaemic control and previous antibiotic exposure.

Another limitation is that fungal culture may detect colonization rather than invasive infection. Therefore, fungal positivity should not automatically be interpreted as evidence of pathogenic fungal infection. Molecular characterization and histopathological demonstration of tissue invasion could provide stronger evidence in future studies. The bacterial cultures similarly cannot always distinguish colonization from clinically meaningful infection. Finally, the organism-wise fungal and bacterial numbers presented in this version are illustrative/model data and require replacement with the actual microbiological results from the study.

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