Prevalence Analysis of Fungi in Chronic Lower Extremity Ulcers

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Abstract: Background. Lower extremity ulcers are a major cause of morbidity in elderly patients and can be colonized by many different microorganisms, including fungi. The purpose of this prospective study was to determine the prevalence of fungal colonization and/or infection at the ulcer site and the surrounding skin. Methods. Swabs were taken from 152 lower extremity ulcers and the surrounding skin. Direct microscopic examination and cultures for fungal and bacteriological investigations were obtained. The characteristics of the patients, ulcers, and surrounding skin were studied. Results. Fungi were isolated from 6% of ulcers and 27.6% of the skin samples. Three species were found: Candida albicans (2% of ulcers, 4.6% of surrounding skin), Candida parapsilosis (2% of ulcers, 11% of surrounding skin), and Candida ciferrii (0.6% of surrounding skin). Fungal infections were found in 2% of ulcers and 8.5% of skin samples from the surrounding skin. Conclusion. The prevalence of fungal colonization was less robust than that observed in previous studies. No relationship was found between fungal infection and patient or ulcer characteristics. However, there was significant Corynebacteria colonization in the fungal infection group (P = 0.02). It would be interesting to conduct similar studies in order to evaluate the effect of antifungal treatment on infected wounds.

Chronic lower extremity ulcers are one of the main reasons for dermatology consultation and are a major handicap for older patients. The prevalence of chronic lower extremity ulcers in France is estimated to range from 1%-3.6%, resulting in a significant socioeconomic impact and annual cost of nearly 1 million Euros. The healing process is usually long, and colonization by infectious agents (mostly polymicrobial) is frequent.

The influence of microorganisms on the healing process is a controversial issue. While some have described the negative effects of bacteria on the healing process, others consider these factors to be of little importance. The prevalence of fungal flora in chronic leg ulcers has been reported to range from 4.5%-50%. Cases of chron-
ic leg ulcers healing due to effective antifungal treatments have also been reported. Changes in the incidence of a pathogen can change the approach to treatment. To the authors' knowledge, there is no recent epidemiological study on the prevalence of fungal elements in chronic lower extremity ulcers; therefore, the authors performed the following descriptive study.

The primary objectives of this study were to determine the prevalence of fungal colonization and/or infection in chronic lower extremity ulcers and to identify the most frequently involved species.

The study's secondary objectives were to determine the prevalence of fungal elements and the colonizing species on skin surrounding the ulcer. The authors also sought to describe the prevalence of this colonization and/or infection according to the characteristics of the patients, ulcers, surrounding skin, and associated bacterial flora.

**Key Points**

- While some have described the negative effects of bacteria and the quantity of bacteria on the healing process, others consider these factors to be of little importance.
- The primary objectives of this study were to determine the prevalence of fungal colonization and/or infection in chronic lower extremity ulcers and to identify the most frequently involved species.

**Methods**

Patients who had been referred to the Dermatology department at a teaching hospital (Centre Hospitalier Universitaire [CHU], Nancy, France) for lower extremity ulcers from the beginning of January 2008 to the end of April 2008 were prospectively included in the study. Sample size was calculated by using nQuery Advisor Sample Size software, version 6 (Statistical Solutions, Saugus, MA) and the following parameters: power (80%), alpha level (0.05), CI (95%), and an estimated prevalence of 20% (based on previously reported prevalence rates of 4.5%–50%). The estimated sample size was 138 to which a 10% attrition rate was added.

The exclusion criteria included prior treatment with a local antifungal medication in the last 8 days, systemic antifungal therapy during the previous month, or use of a local antiseptic in the previous 24 hours.

The ethical committee of the East of France (Comité de Protection des Personnes Est-III; Brabois Hospital, Vandoeuvre-les-Nancy, France) approved the study parameters before the study began. All of the patients were informed about the study objectives and gave informed consent to participate.

The ulcers and the surrounding skin were sampled using swabs and scrapings for culture and direct microscopic examination. Sterile gloves were worn during sampling. The ulcers and the surrounding skin areas were not cleansed before sampling. Fungal samples were taken using cotton-tipped swabs applied to the entire ulcer area or surrounding skin in different directions using a simultaneous rotating and zig-zagging motion. Alginate swabs were used to collect bacterial samples. Swabs were placed either in an empty transport container (fungal samples) or transport medium (bacterial samples) and submitted to related laboratories on the same day. Once in the laboratory, the samples were either inoculated onto culture media or smeared onto a slide for subsequent direct microscopic examination (only fungal samples). Smears made from swabs and scrapings were stained with chlorozol black solution (Sigma-Aldrich, St. Louis, MO) for direct microscopic examination, which is more sensitive compared to the more routinely used potassium hydroxide preparation. Stained smears were examined under an optical microscope (Leica Microsystems, Wetzlar, Germany). The entire slide area was thoroughly scanned for fungal elements. A positive direct examination was considered if yeast cells (spores) and/or *Pseudobopyrhae* were present. The primary isolation medium was *Sabouraud dextrose agar* (SDA, Becton Dickinson, le pont de Claix, France) supplemented with chloramphenicol and cyclohexamide. All cultures were incubated at 30°C and maintained for up to 4 weeks. After 48 hours had passed, SDA plates were evaluated for positive cultures in terms of the number of colonies. Recovered yeast was subjected to further analysis for identification. The degree of fungal growth was quantitated according to the number of colonies per isolate: negative; a few (1–9); and numerous (≥ 10).

A consulting physician collected complementary data (ie, clinical and anthropometrical) during sampling from the clinical examinations and from the patients’ files. Clinical assessments included ulcer dimensions; cause (with the help of Doppler echography or ankle/brachial systolic pressure); description of the base, edges, and surrounding skin; importance and nature of exudate; and the treatment used.

**Colonization versus infection.** It was considered important to distinguish as clearly as possible between true ulcer infection and occasional colonization. The
mouth, female genitalia, gastrointestinal tracts, and the skin adjacent to body orifices are usually colonized by *C. albicans*. Therefore, all *C. albicans* at the ulcer site or in the surrounding skin were considered pathogenic. When a skin saprophyte (e.g., *C. parapsilosis*) was isolated, a rough estimation was made by analyzing the culture results (number of colonies per isolate) and microscopic examinations. A positive direct microscopy exam and culture with numerous colonies (≥10) was considered to show infection. Otherwise, colonization was considered.

### Statistical Analysis

Statistical analysis was conducted using Epidata V3.1 (The EpiData Association, Odense, Denmark) and SAS V9.1 (SAS Institute Inc., Cary, NC) software programs. Data were assessed using the two-tailed chi-square test or Fisher’s exact test (qualitative variables), *t* tests, and Wilcoxon or Kruskal-Wallis (quantitative variables) tests. *P* < 0.05 was considered the limit of significance. Confidence intervals (95%) were calculated using Minitab® V15 (Minitab Inc., State College, PA) statistical software.

### Results

Lower extremity ulcers (n = 152) of 69 men and 83 women (ratio male/female = 0.83) were investigated. Mean patient age was 75 years (range 35–95). The mean body mass index (BMI) was 28.2 (27.8 for men and 28.7 for women). Thirty-three (22%) of the patients had diabetes and three patients (2%) were being treated with antibiotics (Table 1).

Ulcers were a result of venous insufficiency in 78 (51%) cases; peripheral arterial disease in 15 (10%); mixed arterial/venous ulcer in 41 (27%); and other causes (e.g., diabetic ulcers, vasculitis, hypertensive [Martorell] ulcers) in 18 (12%) cases. The mean ulcer area was 36 cm² (± 69 cm²). The location of ulcers was at the external malleolus in 52 (34%) cases, internal malleolus in 30 (20%), and other regions (calf, anterior aspect of the leg, foot) in 70 (46%) cases. The wound bed was mostly covered with granulation tissue in 47 (31%) cases; fibrin (sloughy) was found in 103 (68%); and 2 (1%) were necrotic. The amount of exudate was significant in 28 (18%) cases, moderate in 77 (51%), and insignificant in 47 (31%). The mean ulcer duration was 32 months.

The clinical aspect of the surrounding skin was erythematous (n = 58, 38%), macerated (n = 38, 25%), desquamated (n = 4, 3%), and normal (n = 29, 19%). An association between erythema and maceration was found in 23 (15%) cases.

Hydrofiber, mainly Aquacel® (ConvaTec, La Garenne-Colombes, France) was used in 25 (16%) cases, silver-releasing dressings, mainly Release Ag® (Systegenix, Montigny-le-Bretonneux, France) in 15 (10%), hydrocellular foams, mainly Biatain® (Colopast, Rosny-sous-Bois, France) in 32 (21%), paraffin-impregnated gauze, mainly Jelonet® (Smith & Nephew, Le Mans, France) in 47 (31%), alginate, mainly Algosteril® (Brothier, Nanterre, France) in 21 (14%), and other dressings in 12 (8%). The frequency of dressing change was three times a week in 41 (27%) cases, every other day in 70 (46%) cases, every day in 26 (17%) cases, and less than three times a week in 15 (10%) cases.

Compression therapy was used in 99 (65%) cases. Monolayer compression bandaging, mainly Biflex® (Thuasne SA, Levallois-Perret, France) was used in 71 (72%) cases, and multilayer Profore® (Smith & Nephew, Le Mans, France) in 28 (28%) cases. In 65 (43%) cases, a topical corticosteroid was applied to the surrounding skin.

### Mycological results.

One hundred fifty-two (152) ulcers and their surrounding skin were sampled. In all, 45/152 (29.6%; 95% CI: 22%–37%) samples were positive upon direct examination and/or in culture. Three samples were positive only at the level of the ulcer, 35 only at the level of the surrounding skin, and 7 samples were positive at both sites (Figure 1). According to the defined study criteria, of the 45 positive samples, a fungal infection in 3 ulcers (3 observations of *C. albicans*) and 13 surrounding skin samples (7 *C. albicans* and 6 *C. parapsilosis*) were identified. Colonization was determined in 32 cases.

### Mycological characteristics of ulcers.

Ten samples (6.5%; 95% CI: 3.1%–11.7%) at the ulcer site (3 at the level of the ulcer only and 7 at the level of the ulcer and the surrounding skin) were found to be positive upon direct examination and/or in culture. Among these, 5 cultures (2%; 95% CI: 0.4%–5.6%) were positive for *C. albicans* and the other 3 (2%; 95% CI: 0.4%–5.6%) were positive for *C. parapsilosis* (Figure 1). No dermatophytes were observed.

### Mycological characteristics of surrounding skin.

Forty-two (42) samples (27.6%; 95% CI: 20.6%–35.4%) taken from the surrounding skin were positive (35 at the surrounding skin level only and 7 at the level of the ulcer and the surrounding skin) upon direct examination and/or in culture. Among these, 7 cultures (4.6%; 95% CI: 1.8%–9.2%) were positive for *C. albicans*, 17 (11.1%; 95% CI: 6.6%–17.3%) were positive for *C. parapsilosis*, and 1
(0.6%; 95% CI: 0%–3.6%) was positive for *C. ciferrii* (Figure 1). Both ulcer samples and surrounding skin samples presented the same fungal profiles.

**Bacteriological results.** One hundred eighteen (118) ulcers were swabbed for bacteriological study, of which 113 (95.7%; 95% CI: 90.3%–98.6%) were positive. The presence of anaerobic species was not investigated. The mean number of bacterial strains (species) found was 2.3 (± 1; range, 1–5). The main bacterial strains isolated were methicillin-sensitive *Staphylococcus aureus* in 64 cases (56.6%); *Corynebacteria* in 35 (31%); *P aeruginosa* in 35 (31%); *Streptococci* (group G, B, C) in 34 (30%); *Pseudomonas*

**Table 1.** Characteristics of patients, ulcers, and surrounding skin in the fungal infection, colonized, and negative groups.

<table>
<thead>
<tr>
<th></th>
<th>Fungal infection</th>
<th>Colonized</th>
<th>Negative</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. patients</strong></td>
<td>13</td>
<td>32</td>
<td>107</td>
<td>152</td>
</tr>
<tr>
<td><strong>Mean age (years)</strong></td>
<td>76</td>
<td>74</td>
<td>76</td>
<td>75</td>
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<tr>
<td><strong>Sex ratio (M/F)</strong></td>
<td>1.3</td>
<td>1.7</td>
<td>0.6</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>27.66</td>
<td>26.68</td>
<td>28.8</td>
<td>28.2</td>
</tr>
<tr>
<td><strong>Diabetes</strong></td>
<td>40%</td>
<td>14%</td>
<td>26%</td>
<td>22%</td>
</tr>
<tr>
<td><strong>Antibiotic therapy</strong></td>
<td>10%</td>
<td>3%</td>
<td>1%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Mean area (cm²)</strong></td>
<td>39</td>
<td>43</td>
<td>34</td>
<td>36</td>
</tr>
<tr>
<td><strong>Mean duration (months)</strong></td>
<td>32</td>
<td>52</td>
<td>27</td>
<td>32</td>
</tr>
<tr>
<td><strong>Venous</strong></td>
<td>45%</td>
<td>62%</td>
<td>48%</td>
<td>51%</td>
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<tr>
<td><strong>Arterial</strong></td>
<td>18%</td>
<td>10%</td>
<td>9%</td>
<td>10%</td>
</tr>
<tr>
<td><strong>Mixed (arterial/venous)</strong></td>
<td>36%</td>
<td>14%</td>
<td>30%</td>
<td>27%</td>
</tr>
<tr>
<td><strong>Sloughy (fibrinous)</strong></td>
<td>92%</td>
<td>55%</td>
<td>68%</td>
<td>68%</td>
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<tr>
<td><strong>Important</strong></td>
<td>38%</td>
<td>21%</td>
<td>14%</td>
<td>18%</td>
</tr>
<tr>
<td><strong>Erythematous and/or macerated</strong></td>
<td>92%</td>
<td>73%</td>
<td>78%</td>
<td>78%</td>
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<tr>
<td><strong>Paraffin gauze</strong></td>
<td>45%</td>
<td>34%</td>
<td>29%</td>
<td>31%</td>
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<tr>
<td><strong>Foam</strong></td>
<td>27%</td>
<td>7%</td>
<td>25%</td>
<td>21%</td>
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<tr>
<td><strong>Silver-releasing</strong></td>
<td>18%</td>
<td>7%</td>
<td>10%</td>
<td>10%</td>
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<tr>
<td><strong>3 times/week</strong></td>
<td>38%</td>
<td>41%</td>
<td>21%</td>
<td>27%</td>
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<tr>
<td><strong>46%</strong></td>
<td>35%</td>
<td>49%</td>
<td>46%</td>
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<td><strong>15%</strong></td>
<td>13%</td>
<td>26%</td>
<td>17%</td>
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<tr>
<td><strong>61%</strong></td>
<td>73%</td>
<td>63%</td>
<td>65%</td>
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<tr>
<td><strong>87%</strong></td>
<td>64%</td>
<td>70%</td>
<td>72%</td>
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<td><strong>13%</strong></td>
<td>36%</td>
<td>30%</td>
<td>28%</td>
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<tr>
<td><strong>63%</strong></td>
<td>44%</td>
<td>43%</td>
<td>43%</td>
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<tr>
<td><strong>2.9</strong></td>
<td>2.24</td>
<td>2.2</td>
<td>2.33</td>
<td></td>
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<tr>
<td><strong>38%</strong></td>
<td>45%</td>
<td>41%</td>
<td>56%</td>
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<tr>
<td><strong>8%</strong></td>
<td>9%</td>
<td>18%</td>
<td>20%</td>
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<tr>
<td><strong>38%</strong></td>
<td>21%</td>
<td>17%</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td><strong>Corynebacteria</strong></td>
<td>46%</td>
<td>21%</td>
<td>17%</td>
<td>31%</td>
</tr>
</tbody>
</table>

MSSA: Methicillin-sensitive *Staphylococcus aureus*
MRSA: Methicillin-resistant *Staphylococcus aureus*
aeruginosa in 30 (26.5%); and methicillin-resistant S aureus in 23 (20.3%). Twenty-one percent (24/113) of the patients who had a positive bacteriological culture were diabetic.

**Comparison of patients considered to have a fungal infection, fungal colonization, or negative results.** Table 1 summarizes the characteristics of the 13 patients in the group considered to exhibit a mycosic infection (fungal infection group), 32 patients in the group considered to exhibit colonization (colonized group), and 107 patients with negative results for both culture and direct examination (negative group).

The characteristics of the fungal infection group and the negative group were compared and statistically significant differences were not found concerning sex ratio, BMI, ulcer area, ulcer duration, frequency of diabetes, ulcer etiology, clinical aspects of the wound bed, exudation \((P = 0.09)\), clinical aspect of the surrounding skin, type of dressing, frequency of dressing changes, compression therapy, or application of topical corticosteroid on surrounding skin.

With regard to differences in bacterial co-infection between the fungal infection and negative groups, no differences were observed in the mean number of bacterial strains found \((P = 0.09)\) or in the type of bacteria isolated. Only the presence of Corynebacteria was significantly more frequent in the fungal infection group as compared to the negative group \((46\% \text{ versus } 17\%; P = 0.02)\).

With regard to differences in bacterial co-infection between the fungal infection and negative groups, no differences were observed in the mean number of bacterial strains found \((P = 0.09)\) or in the type of bacteria isolated. Only the presence of Corynebacteria was significantly more frequent in the fungal infection group as compared to the negative group \((46\% \text{ versus } 17\%; P = 0.02)\).

The comparison between the characteristics of the colonized group and the negative group showed statistically significant differences concerning the male:female ratio \((1.7:0.6; P = 0.009)\), duration of ulcer \((52 \text{ versus } 27 \text{ months}; P = 0.04)\), and frequency of dressing changes \((more frequent in the negative group; P = 0.02)\).

**Discussion**

In this series, 4\% (95\% CI: 1.4\%-8.3\%) of ulcer sample cultures were positive for the presence of C albicans (2\%) or C parapsilosis (2\%). These results are compatible with the data reported by Mallol et al\(^{14}\) who observed the presence of Candida in 4.5\% of 290 ulcers. The species of Candida was not determined.\(^{14}\)

However, the prevalence of fungal elements in the authors’ series is much lower than that described in other previous studies.\(^{15-17}\) In previous studies, C albicans was found in 20\% of 120 ulcers,\(^{15}\) 11\% of 58 ulcers,\(^{16}\)

![Figure 1. Flowchart of sampling and distribution of mycological findings.](Image)

- DME: Direct microscopic examination
- C alb = Candida albicans; C para = Candida parapsilosis; C cif = Candida ciferri
Fungi were isolated from both the ulcers and the surrounding skin samples. Three species were found: *Candida albicans*, *Candida parapsilosis*, and *Candida ciferrii*.

and 14.9% of 509 samples.\(^{17}\)

The higher prevalence of *C. albicans* in these series could be biased since a higher number of patients with diabetes were included (83% according to Simonart\(^ {15}\) and 100% in the Missoni et al\(^ {17}\) study); Hansson et al\(^ {16}\)'s study only included venous ulcers.

The present study found positive culture for fungal elements in the surrounding skin in 16.4% (95% CI: 10.9%–23.3%) of cases, of which 4.6% were positive for *C. albicans* and 11.1% were positive for *C. parapsilosis*. Our findings are lower than those found by English et al\(^ {18}\) who reported the presence of *Candida* in 58% of 29 samples of surrounding skin. The species of *Candida* revealed in that study were *C. parapsilosis* in 27%, association of *C. parapsilosis* and *Fusarium* in 27%, and *C. albicans* in 3%. Notably, the ulcers were not swabbed at the same time in that study.\(^ {18}\)

To the authors' knowledge, only one study has performed the sampling of ulcers and the surrounding skin concomitantly. Hansen et al\(^ {19}\) performed sampling on 49 ulcers and surrounding skin in 23 patients. *C. albicans* was found in 14% of ulcers and 10% of samples from surrounding skin. *C. parapsilosis* was found in only 4% of ulcers (2/49). The prevalence of *C. albicans* in this study was greater than that observed in the present study. These results can be explained by a lower frequency of the dressing changes (once or twice a week),\(^ {19}\) which provided a humid and warm environment and may have facilitated fungi growth. The high prevalence of *Candida* may have been biased since multiple ulcers from the same patients were included (49 ulcers in 23 patients).

The present study describes the presence of three species: *C. albicans*, *C. parapsilosis*, and *C. ciferrii*. *C. albicans* is an opportunistic, commensal organism of the natural cavities, particularly the digestive tract and the female genital tract.\(^ {21, 22}\) The presence of *C. albicans* on the skin (in non-periorificial regions) can be considered pathogenic.\(^ {23}\)

*C. parapsilosis* is a commensal species found on skin surfaces that is emerging as a major invasive pathogen in humans, particularly in the presence of a compromised immune system or intravenous catheters.\(^ {24–27}\)

Nevertheless, some experimental studies have shown its virulence with regard to human skin *in vitro*, leading to flattening and cleft formation at the basal layer, and increased intracellular edema and even apoptosis in a human skin model.\(^ {28}\) Like *C. parapsilosis*, *C. ciferrii* is found in a commensal state on the skin.\(^ {29}\)

Fungal infections were identified in 2% of ulcers and 8.5% of the surrounding skin, according to the present study criteria. Statistically significant differences between the fungal infection and negative groups were not found, with the exception of more frequent presence of *Corynebacteria* in the fungal infection group. One explanation for this increased incidence of *Corynebacteria* could be the ability of *Corynebacteria* to hydrolyze triglycerides, which liberates free fatty acids, decreases pH, and therefore, the reproduction of *Candida*. The absence of statistically significant differences for the other parameters could be explained by the disparity in the number of groups compared (106 in the negative group versus 13 in the fungal infection group).

The present study had some limitations. Potential contamination sources (eg, interdigital spaces and nails) were not examined for the presence of fungal elements. However, there may be a correlation between infection in these locations and secondary ulcer contamination. The distinction between fungal infection and colonization, as described in this study, remains debatable. The authors' judgment was based only on laboratory criteria. Perhaps only a tissue biopsy or an effective antifungal treatment can solve this dilemma. Swab sampling has been considered unsatisfactory by some authors on the basis that the superficial microbiology does not reflect that of deeper tissues. Some authors believe that a biopsy culture is the best sampling method.\(^ {30–33}\) Others believe that superficial wound fluid and tissue debris display a full spectrum of wound microflora.\(^ {29}\) Swab sampling is simple, inexpensive, noninvasive, and convenient for routine assessment of the majority of wounds and facilitates semiquantitative and qualitative analysis of wound microflora. The fungal flora of the hands of those recruited to take and treat samples may have biased the results of this study. To limit this effect, anyone who was involved in sampling wore gloves.

**Conclusion**

The presence of fungal elements was observed in 6% of ulcers and 27.6% of surrounding skin samples. In 2% of ulcers and 8.5% of surrounding skin samples, a fungal...
infection was considered. Given this frequency, the systematic characterization of fungal agents in chronic lower extremity ulcers remains controversial.

Fungal infection may be a factor that contributes to delayed healing. More trials are needed to confirm the interaction between fungal colonization and wound healing. One study showed that the quantity of granulation tissue in cases of contamination with *C. albicans* yeast decreased and that this decrease correlated with the quantity of contaminating yeast. The present study provides limited information about the effects of fungal colonization on the healing process. It would be interesting to pursue this study further with a larger number of patients in order to evaluate the evolution of fungally infected ulcers after antifungal treatment.

**Acknowledgments**

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**Key Points**

- Statistically significant differences between the fungal infection and negative groups were not found, with the exception of more frequent presence of *Corynebacteria* in the fungal infection group. This might be explained by the ability of *Corynebacteria* to hydrolyze triglycerides, which liberates free fatty acids, decreases pH, and therefore, the reproduction of *Candida*.
- Given the low frequency of fungal infection found, the systematic characterization of fungal agents in chronic lower extremity ulcers remains controversial.
- It would be interesting to pursue this study further with a larger number of patients by evaluating the evolution of fungally infected ulcers after antifungal treatment.

**References**


