Cutaneous Alternariosis in a Renal Transplant Recipient

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Practice Points

- Cutaneous alternariosis may present as painless purplish nodules.
- Histologic analysis of cutaneous alternariosis generally reveals yeastlike structures within an inflammatory process in the dermis; mycologic culture also is useful in making the diagnosis and identifying the clinical strain.
- In transplant recipients, cutaneous alternariosis should be treated with antifungal therapy (intravenous amphotericin B) along with decreased doses of immunosuppressive agents and cryotherapy.

Alternariosis is a fungal infection that is usually described in immunocompromised patients. We report a case of cutaneous alternariosis in a renal transplant recipient caused by Alternaria tenuissima*. The diagnosis was supported by histopathologic (ie, yeastlike cells, filamentous structures) and mycologic findings from a cutaneous biopsy. Cutaneous lesions regressed 1 month following a decrease in the dosage of immunosuppressive therapy. The patient also was treated with intravenous amphotericin B followed by oral fluconazole without improvement. Cryotherapy remarkably accelerated healing of the lesions.*

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The authors report no conflict of interest.

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Case Report

A 33-year-old woman who was born in Mahdia, a rural area on the coast of Tunisia, presented with numerous painless, purplish, 1- to 3-cm nodules on the left foot, legs, and right thigh of 3 months' duration. She had returned to the nephrology department for routine follow-up after undergoing a kidney transplant 1 year prior. Following surgery, the patient underwent immunosuppressive therapy with mycophenolate mofetil (1000 mg daily), tacrolimus (6 mg daily), and prednisone (2 mg daily). Insulin also was administered because of steroid-induced diabetes mellitus.

Physical examination revealed slightly scaly lesions with 1 ulcerated lesion on the foot (Figure 1). The patient was a farmer but did not recall any trauma to the affected sites. On admission, a complete blood cell count, blood electrolytes, serum glucose, creatinine, and blood urea nitrogen levels were normal. Human immunodeficiency virus serology was negative, and a chest radiograph showed no abnormalities.

Given the patient's occupation and birthplace, leishmaniasis was suspected, but direct examination and polymerase chain reaction were negative. A skin biopsy was performed. Histologic analysis on sections stained with hematoxylin and eosin showed an intense inflammatory process in the dermis that was vaguely granulomatous (Figure 2). The infiltrate was composed of macrophages, giant cells, and a few islets of polymorphonuclear neutrophils. Some

of these cells included thick-walled, yeastlike, oval structures and short septate hyphae, which were positive on periodic acid–Schiff staining. Consequently, the patient was empirically treated with amphotericin B perfusion (12 mg daily) over 3 hours; the dose was then reduced to 10 mg because of an increase of creatinine in the blood (151 μmol/L [reference range, 60–110 μmol/L]). Immunosuppressive therapy was reduced to 750 mg daily of mycophenolate mofetil; tacrolimus, which was supratherapeutic at more than 30 U/mL, was reduced to 4 mg daily to reach a level of 8.8 U/mL. Prednisone was maintained at 2 mg daily.

Mycologic examination of superficial lesion scrapings revealed the presence of rare hyaline septate hyphae with a regular section, evoking yeast pseudohyphae. Furthermore, a fragment of pigmented brown mycelium was observed. Microscopic examination of a second biopsy specimen after May-Grünwald-Giemsa staining showed thick-walled septate hyphae of varying widths (3–6 μm) budding from oval spores of 7 to 10 μm in diameter (Figure 3). The culture was carried out under laminar air flow onto Sabouraud dextrose agar with chloramphenicol, with and without cycloheximide, and blood agar to isolate a possible dimorphic fungus. Colonies appeared on day 2 on Sabouraud dextrose agar with chloramphenicol tubes incubated at 27°C and on day 7 on Sabouraud dextrose agar with chloramphenicol and cycloheximide. At first, the colonies were restrictive, cottony, filamentous, flat, and creamy white on the front with no reverse pigmentation; on day 7, the colonies became extended with grayish, dark brown to black reverse pigmentation. The culture was slower at 37°C, and it had a different macroscopic appearance with light bulging colonies. Microscopy showed dark septate hyphae and phragmospores of *Alternaria*.

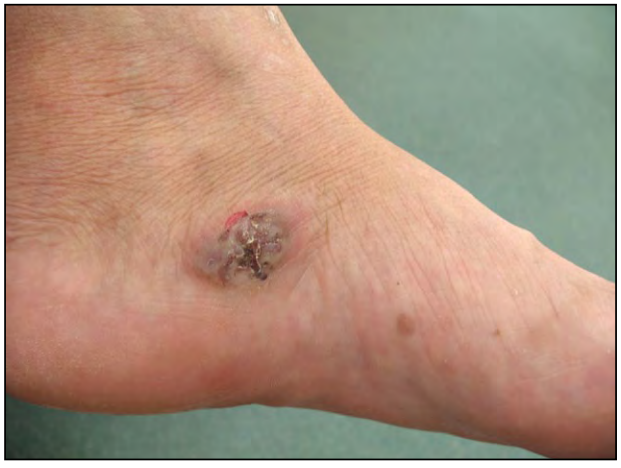

Figure 1. Crusted ulcerated lesion on the foot.

Slide culture on malt agar was performed. Within 4 days, branched, septate, dark conidiophores and several pigmented muriform obclavate conidia produced in short chains (2–4 items) were observed. The conidia were pyriform, 40- to 60-μm long, and 6- to 8-μm wide with a long beak that was half the length of the spore. The conidia cell walls were smooth to finely verrucose and septate with 4 to 7 transverse divisions and few longitudinal bulkheads (Figure 4). These macroscopic and microscopic phenotypic characteristics were consistent with *Alternaria tenuissima*.[1,2] These findings were confirmed on further biopsy specimen (day 60).

After a 10-day course of intravenous amphotericin B, the skin lesions remarkably regressed and the ulceration was almost completely healed. A second

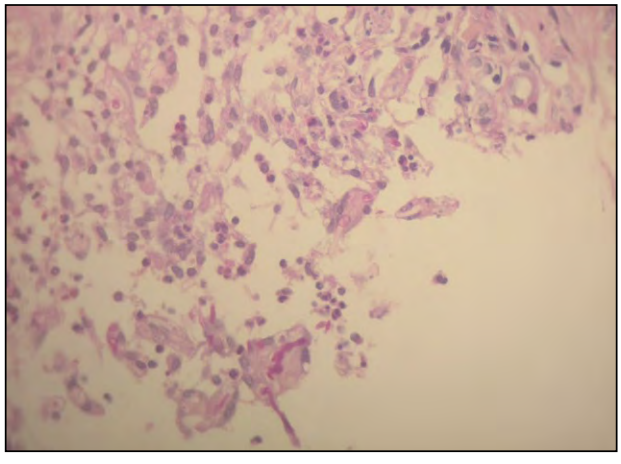

Figure 2. Histopathologic examination revealed filamentous and yeastlike structures with dermal infiltration of inflammatory cells (H&E, original magnification ×40).

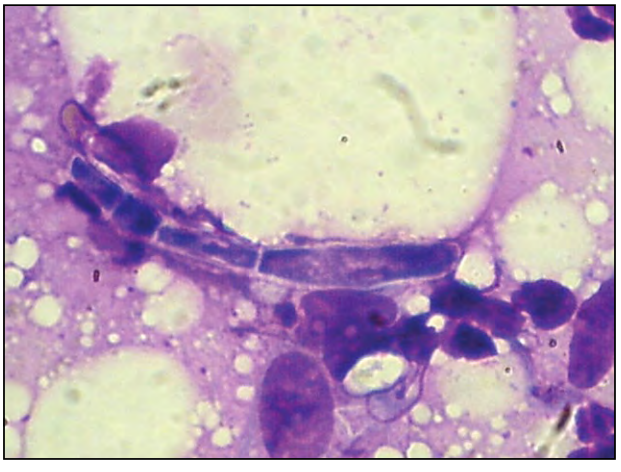

Figure 3. May-Grünwald-Giemsa staining of a smear from a cutaneous biopsy showed thick-walled irregular septate hyphae (original magnification ×100).

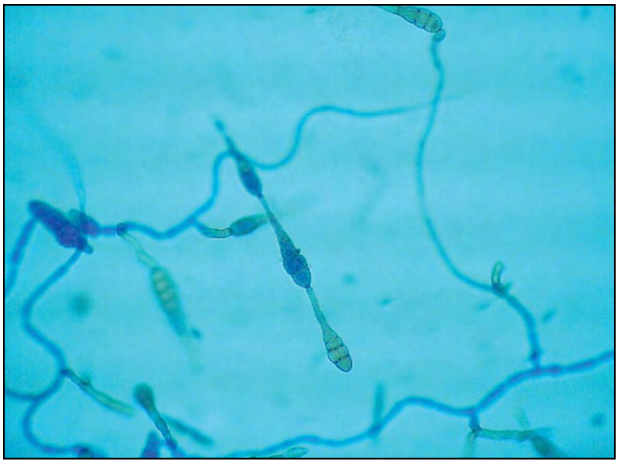

Figure 4. Macroconidia of *Alternaria tenuissima* obtained on malt agar medium (original magnification ×40).

course of amphotericin B (10 mg daily) was administered after 15 days but was discontinued due to intolerance. Tacrolimus treatment was maintained at 4 mg daily. Insulin (10 U daily) also was administered for treatment of diabetes. No notable improvement was noted after 3 months' follow-up. At this point, tacrolimus was reduced to 2 mg daily, and a 4-week course of oral fluconazole therapy (100 mg daily) was initiated but was not effective. At the end of the fluconazole course, once weekly liquid nitrogen application seemed to be useful because the lesions nearly healed after 6 months of cryotherapy.

Comment

Alternaria species are dematiaceous, ubiquitous, saprophytic fungi that cause a wide spectrum of opportunistic infections and can develop in both immunocompetent and immunosuppressed patients. The effects most often are superficial, rarely with visceral involvement.[3,4] Cutaneous alternariosis is common but has been poorly documented; 210 cases were reported in the literature from 1933 to 2008 with a small proportion of invasive forms.[5] It has been associated with several predisposing factors, mainly immunosuppressive therapy, steroids, solid organ transplants (ie, kidney, liver), and Cushing syndrome.[5] Moreover, alternariosis has been associated with diabetes,[6] hematologic disease,[7] and chronic granulomatous disease.[8] One case has been reported in an IgA-deficient patient.[9] Because of the natural habitat of the fungus, *Alternaria* infection most often affects farmers and gardeners. Rural life and local trauma can facilitate the implantation of the fungus in situ.[2] Our patient was a farmer, but she denied working in the fields following her kidney transplant. The patient did not recall any trauma to the affected areas, but she noted that she was at high risk for injury due to the nature of her work. Additionally, her immune system was compromised by steroids and tacrolimus overdose. Corticosteroid treatment induces skin fragility, which promotes dermal penetration of the fungus.[10] Although the majority of cases of cutaneous alternariosis in transplant recipients have been reported within 1 year of the procedure, 1 case was reported 8 years later.[11]

Cutaneous lesions associated with *Alternaria* infection usually are painless and develop either as single papules, plaques, or pustules; scaly erythema; purplish nodules; or ulcerative crusted kaposilike lesions occurring as single or multiple lesions on exposed sites.[4,12-14] They most commonly occur on the extremities, mainly on the lower limbs[6] as in our patient. Colonization of the skin by *Alternaria* is normal; therefore, epidermal infections remain controversial, and the dermis is most frequently involved.[6,13] Inoculated deep into the dermis, the fungi causes an inflammatory reaction.[13,14] Histologic preparations show mixed granulomatous dermal infiltrates in the upper dermis but may reach the deep dermis.[15] Our histologic findings were similar to this description.

The main diagnosis is based on the isolation and identification of the fungus. Because *Alternaria* species are prevalent in the environment (eg, trees, soil) and their conidia often are present in the air, laboratory contamination should be ruled out before a diagnosis is made; therefore, *Alternaria* infection should be demonstrated by the presence of the fungus in the sample tissue as well as a pure culture isolation (ie, no other fungi on the medium).[7,14] The fungus can take on various forms, including round, vesicular, or short septate hyphae, and stains positive using Grocott-Gomori methenamine-silver and periodic acid–Schiff stains.[13] The large oval budding spores observed on a smear of a section of the biopsy spread with saline water led to our initial suspicion of a dimorphic soil fungus (*Blastomyces dermatitidis*); however, the rapid appearance of colonies was not consistent with this hypothesis. Thus the mycologic culture was useful in making the diagnosis and identifying the clinical strains.

The genus *Alternaria* contains approximately 80 species, mainly known as plant parasites. Eight species currently are involved in human pathology.[5,6] The species most often isolated are *Alternaria alternata*, *A tenuissima*, *Alternaria infectoria*, and less frequently *Alternaria chartarum*.[5,6] These phaeohyphomycetes seem to evade environmental stress and immune defenses thanks to melanin production.[13] The *Alternaria* species usually are sensitive to cycloheximide,[2] but the strain that we isolated in our patient was not. The identification keys are the

culture shape (macroscopic), description of conidia (microscopic), and description of the way conidia rise from hyphae (conidiogenesis). The isolate we obtained was identified as *A tenuissima*.[1,16]

Therapeutic management is not standardized, though several treatments have been used, including local (intralesional) or systemic antifungal agents. Itraconazole,[17] ketoconazole,[12] amphotericin B,[18] fluconazole,[8] terbinafine, miconazole, and natamycin[15] have been successful. Some of these agents are not suitable with immunosuppressive therapy (eg, itraconazole). Resolution of the lesions typically is achieved in 2 to 18 months.[15,17] *Alternaria* species seem to be resistant to griseofulvin and flucytosine.[15] In some patients, simply withdrawing or reducing immunosuppressive therapy may be sufficient for regression of lesions.[19] Surgical excision can be suitable in cases of small lesions and can accelerate the healing process.[18] Povidone iodine also can produce positive results.[13,16] Some investigators have tried local thermotherapy with successful results.[5] Cryotherapy frequently is used for cutaneous leishmaniasis but to our knowledge has not been used for *Alternaria* lesions.[20] Cryotherapy was effective in our patient, and the skin lesions were healed within 10 months of presentation following a combination of treatment methods.

Conclusion

An increased awareness of the clinical and histopathologic features of cutaneous diseases in transplant recipients is important to avoid misdiagnosis and provide early treatment against opportunistic fungi such as *Alternaria*.

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