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SHORT COMMUNICATION

Subcutaneous Phaeohyphomycosis due to *Alternaria dennisii* in an Immunocompromised Patient

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Phaeohyphomycosis refers to infections caused by the dematiaceous (darkly pigmented) fungi. These fungi encompass a large, heterogeneous group with a high number of genera, e.g. *Alternaria, Bipolaris, Phialophora, Curvularia*, and *Exophiala*. The fungi have pigmented hyphae due to melanin deposition in the cell wall of hyphae, conidia or both. They are found in decomposing plant debris, wood and soil, and inoculation of these opportunistic pathogens is possible through a disrupted skin barrier. Diseases caused by dematiaceous fungi are most common in the tropics/subtropics and are typically divided into chromoblastomycoses, eumycetoma and phaeohyphomycoses. Phaeohyphomycoses have different clinical manifestations, e.g. subcutaneous phaeohyphomycosis. The initial subcutaneous cyst from this infection can ulcerate locally and/or become systemic and spread rapidly to renal, pulmonary and cerebral systems in an immunocompromised host.

We report here a case of subcutaneous phaeohyphomycosis caused by the dematiaceous fungus *Alternaria dennisii* (syn. *Embellisia dennisii*) in an immunocompromised patient. To our knowledge, this is the first report of human disease associated with this particular species.

CASE REPORT

An 85-year-old Caucasian woman of Scandinavian descent was referred to our department of dermatology due to a rapidly spreading, ulcerated and inflamed lesion on her left leg. A lesion had appeared on her left lower leg 6 months prior to referral. Initially, the lesion appeared indolent, but during the last month it had grown more rapidly, was sore and had started to ulcerate. Prior treatment with topical corticosteroid had not abrogated growth. No further complaints were elicited during a systematic enquiry, specifically no fever, chills, night sweats, weight loss, eye complaints, coughing, wheezing, chest pain, or claudication.

Prior medical history included long-standing hypertension, atrial fibrillation, diabetes mellitus, and muscle sarcoidosis without further organ involvement. There were no known prior skin diseases or allergies. Regular medications included olmesartan, metoprolol, warfarin, insulin, azathioprine, 100 mg daily, and prednisolone, 10 mg daily.

Clinical examination revealed involvement of almost the entire medial aspect of the lower left leg with extensive exudative inflammation, punctuated black and haemorrhagic changes, and punched-out ulcers (Fig. 1). The skin was sore and oedematous. The remainder of the examination was unremarkable. Histopathological examination of a skin biopsy prior to referral suggested fungal infection due to the presence of microabscesses and fungal elements. The patient was diagnosed with subcutaneous mycosis and was initially treated with oral terbinafine, 250 mg daily, because of a positive PCR for *Trichophyton rubrum* from the skin surface 3 months earlier.

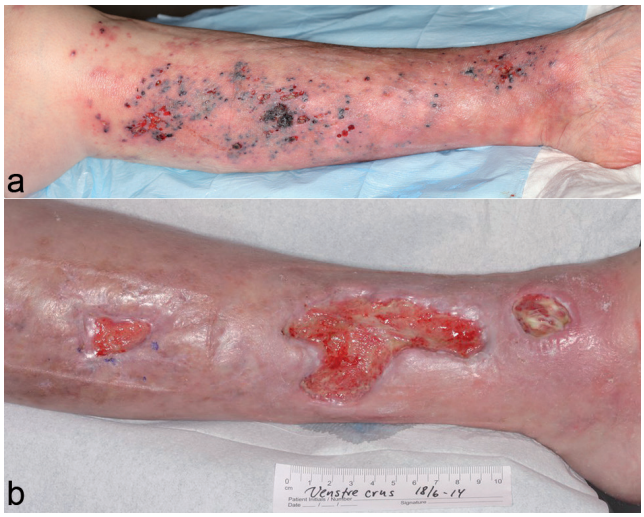

Fig. 1. (a) Initial presentation of phaeohyphomycosis of the lower left leg. (b) The same patient 7 months after initiation of itraconazole therapy.

Histological examination of new skin biopsies showed Periodic Acid Schiff-positive fungal elements, darkly pigmented spores, and granulomas consistent with fungal infection. There was no sign of vasculitis, pyoderma or necrobiosis. Direct immunofluorescence studies were negative. Microbiological examinations included a skin biopsy and scraping for microscopy and culture, and PCR analysis for dermatophytes. Routine blood work and blood cultures were unremarkable. PCR for dermatophytes from the skin samples were negative. Microscopy of blankophor-stained skin samples showed abundant hyphae and conidia. Samples were inoculated on Sabouraud agar, and the culture was positive after incubation for one week, showing grey-brown colonies with a dark-brown reverse. Microscopic examination showed brownish hyphae and after inoculation on cornmeal agar and rice and tween agar chains of club-shaped conidia. The conidia were multiseptate with transverse, longitudinal and oblique septa and some of the conidia had a terminal beak. The macroscopic and microscopic findings were consistent with *Alternaria* sp.

A new skin biopsy and scraping were performed one week later, with the same macroscopic and microscopic findings. The isolate was sent to the Mycology reference laboratory in Bristol where the *Alternaria* sp. diagnosis was confirmed and subsequent nuclear ribosomal repeat region sequencing suggested *Alternaria dennisii*. Antifungal susceptibility testing showed *in vitro* susceptibility (based on normal ranges as there are no established breakpoints for *Alternaria* spp.) for amphotericin B, itraconazole, posaconazole and voriconazole.

Long-term treatment (over 6 months) with systemic itraconazole, wound debridement and wound therapy was instituted and resulted in clearance of the offending pathogen. Follow-up 5 months later with repeated wound swab and skin biopsy for microscopy, culture, and histopathological examination revea-

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led no fungal infection. However, the course of wound healing was protracted by a rapidly deteriorating arterial perfusion pressure of both legs.

DISCUSSION

To our knowledge this is the first report of human pathology due to *Alternaria dennisii* manifesting as subcutaneous phaeohyphomycosis. A review of the literature in PubMed, EMBASE and Google Scholar with MeSH, EmTree headings and free-text searches for *Alternaria* resulted in many publications of subcutaneous phaeohyphomycosis caused by various fungal genera, but none in which a species diagnosis of *Alternaria dennisii* was made (as of 9 June 2015).

Alternaria is a ubiquitous mould with worldwide distribution in air, soil, and plants, and is well-known for its detrimental effects on crops. Certain species may cause airborne allergy and phaeohyphomycosis in humans (1, 2). The literature contains many references to opportunistic infections in immunocompromised patients, mostly organ-transplant recipients (3–5). *Exophiala* spp., *Bipolaris* spp. and *Phialophora* spp. are the organisms most commonly isolated in subcutaneous phaeohyphomycosis (6). The microbiological diagnosis of *Alternaria* rests on the macroscopic morphology and the characteristic microscopic findings. An exact species diagnosis is difficult based solely on morphology and molecular methods, e.g. nucleic sequence analysis, are increasingly used.

Our patient was immunocompromised due to diabetes and treatment with azathioprine and prednisolone for muscular sarcoidosis. Prednisolone has also been highlighted previously as a risk factor in phaeohyphomycosis due to its undesirable effects on the skin barrier, especially increased skin fragility (7). Considering our patient's medical history it is possible that the primary ulcer was trauma-related due to a fragile skin barrier and exacerbated by fungal infection. However, the patient denied any trauma. Differential diagnosis in these cases includes vasculitis, pyoderma gangrenosum, warfarin necrosis, cutaneous leishmaniasis, chromoblastomycosis and eumycetoma. In our case sarcoidosis was also considered and excluded.

There is no standard protocol for treating subcutaneous alternariosis, or indeed any of the phaeohyphomycoses (4). Surgical removal is generally the treatment of choice for small, single or non-numerous lesions (8).

A combination of surgical excision with oral antifungals is advised for larger or numerous lesions, as oral antifungals alone are usually insufficient. Itraconazole is usually the treatment of choice and long-term treatment is generally recommended. The recommended treatment durations varies widely (from 3 to longer than 12 months) and should be guided by clinical evaluation. Dose should be guided by regular serum concentration measurements of the chosen antifungal agent.

Additional treatment options include the newer triazoles, voriconazole and, posaconazole (3, 4, 9). Results of antifungal susceptibility testing may be helpful to determine the optimal antifungal agent. However, the evidence for *in vitro/in vivo* correlation is still sparse.

Close follow-up is required and should include evaluation by culture and histopathology to ensure mycological cure.

Physicians should be aware of the possibility of deep mycotic skin infections in patients presenting with atypical ulcerating skin lesions, especially in immunocompromised individuals. This case reminds us that deep skin mycosis is most effectively diagnosed by correlating clinical and histopathological findings with macroscopic and microscopic examination of cultures. Furthermore, molecular methods are a valuable tool in the identification process.

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The authors declare no conflicts of interest.

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