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Case Report. Cutaneous phaeohyphomycosis due to *Alternaria alternata*

Fallbericht. Kutane Phaeohyphomykose durch *Alternaria alternata*

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Schlüsselwörter. *Alterna*ria alternata, Alternariose, Phaeohyphomykose, ITS rDNA, Chirurgische Therapie.

Summary. A case of cutaneous alternariosis with a well-delimited lesion of traumatic origin is described in a renal transplant recipient. On the basis of histopathology the case was first thought to be cryptococcosis, but *Alternaria alternata* was identified after culturing by means of morphological and molecular examination. Surgical treatment, accompanied by prophylactic application of itraconazole (200 mg day^{-1} for 4 weeks), resulted in complete cure.

Zusammenfassung. Wir beschreiben eine isolierte, umschriebene Läsion einer kutanen Alternariose, die bei einer nierentransplantierten Patientin aufgetreten war. Die Histopathologie ähnelte zunächst der einer kutanen Cryptococcose, aber *Alternaria alternata* konnte mittels morphologischer und molekularbiologischer Methoden identifziert werden. Die chirurgische Entfernung, begleitet von einer prophylaktischen Itraconazolgabe (200 mg day^{-1} für 4 Wochen), führte zu einer vollständigen Heilung.

Introduction

Among the melanized hyphomycetes, the rapidly expanding genera *Alternaria*, *Curvularia*, *Bipolaris*, *Exserohilum* and *Ulocladium* are frequently referred to as 'Dematiaceae' [1]. They are anamorphs of ascomycetes of the order Pleosporales [1–3]. Most species are pathogens on grasses and produce large, airborne conidia. When inhaled by humans or animals, their large conidia are deposited in the sinus, which occasionally leads to colonization with chronic allergic reactions [4]. Alternatively, they may cause skin infections by two possible routes: either (a) exogenously by traumatic inoculation or colonisation of altered skin, or (b) via systemic spread with secondary cutaneous involvement. As they have a low degree of pathogenicity [5, 6], they primarily affect patients with impaired host resistance or other underlying disease, and both the clinicial course and the outcome may vary considerably [6–10].

Most infections by *Alternaria* species are attributed to the ubiquitous saprobe *A. alternata*, but three further species have been reported as potential agents of the disease [1]. As some of these may produce muriform, chlamydospore-like cells *in vitro*, particularly *A. infectoria* and *A. chlamydospora*, it is likely to expect that some of the species may be more therapy-resistant than others [11] and therefore adequate identification of the etiologic agent is mandatory. In the present paper, a localized infection by *A. alternata* in a renal transplant recipient is described, which could be controlled by surgery. The identification on the basis of morphology was confirmed by sequencing of the rDNA ITS domain [12].

Case report

A 68-year-old woman had developed a painless, papulonodular lesion of approximately 3 cm^2 on

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her right knee over a period of 6–8 weeks [Fig. 1]. On pressure it showed a yellowish infiltrate of the granuloma type. The first clinical diagnosis was *granuloma anulare*. Ten months ago, kidney transplantation had been performed, and 8 months previously, the patient had undergone mastectomy and dissection of the axillae due to breast cancer. Since then, the patient was under continuous treatment with prednisolone (10 mg day^{-1}) and tacrolimus (2.5 mg day^{-1}). The patient was otherwise in good condition, free of fever and had no other signs of infection. The lesion was excised completely. On histological examination, granulomas were detected in the dermis, with a mixed infiltrate consisting of macrophages, plasma cells and neutrophils. No foreign body was noted. PAS (Periodic acid Schiff) staining of a 4 mm punch-biopsy specimen revealed colourless fungal elements resembling large yeast cells; therefore cryptococcosis was initially suspected. With Grocott's stain, short septate hyphae and spherical bodies were found [Fig. 2].

Tissue specimens incubated at 30 °C on Kimmig's Agar after 3 days yielded a brownish-white, woolly, rapidly-expanding thallus. No growth could be observed on actidione containing media. The brownish-olivaceous colouration was more pronounced in subcultures on Kimmig's Agar. Conidiation was obtained within 48 h. The fungus had erect, brown, multicellular conidiophores producing unbranched conidial chains, the conidia having a round base, a short, cylindrical beak and muriform septation. Morphological identification lead to *A. alternata*. This was confirmed by sequencing of the ITS (Internal Transcribed Spacer) domain of the rDNA gene and comparison with sequences held at the Centraalbureau voor Schimmelcultures (CBS). The strain was enlisted in the CBS culture collection under accession number CBS 109803.

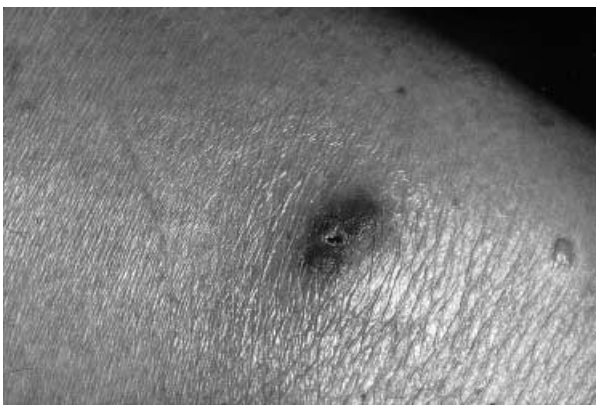

Figure 1. Alternariosis Erythematous nodule on right knee, which had been biopsied shortly before.

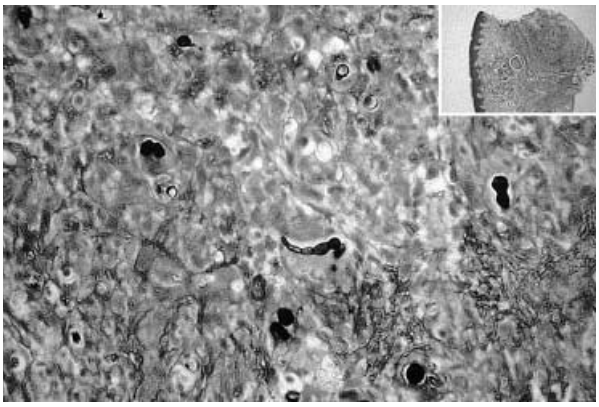

Figure 2. Grocott's stain shows short septate hyphae and spheroidal bodies × 400. At the margin, scanning magnification of the biopsy, which shows the location of the lesion.

After surgery, the lesion healed completely. An additional therapy with itraconazole 200 mg day^{-1} was performed for 4 weeks for safety reasons. As itraconazole can rise the tacrolimus serum level, monitoring was performed regularly. To date, after 10 months, no relapse has occurred.

Discussion

A. alternata species is a common saprobe found on various kinds of plant remains, in soil or on other substrata [1, 5]. Despite its ubiquitous presence in the environment and its world-wide distribution, human infections are rare: approximately 70 cases have been reported. Most of these concerned localized cutaneous infections resulting from direct, traumatic inoculation. Hence, lesions are mostly observed on the extremities. In our case an injury by a rose thorn in the patient's garden is the most probable source of contamination.

The majority of the reported cases occurred either in patients with severe underlying disease or in those receiving immunosuppressive therapy [6, 7, 9–11]; only rarely healthy patients are affected [8]. Clinically, lesions in debilitated patients are quite variable: they may appear as weeping, non-healing ulcers; as crusted, vegetative lesions; as erythematous macules; as reddish, subcutaneous nodules or as verruciform skin lesions resembling eczema. Histopathologically, microabscess formation or granulomatous inflammation may be observed in the dermis and subcutis. Hyphal elements in the tissue may be branched, thick-walled filaments (sub)spherical cells 10–15 μm in diameter, or short chains of oblong cells. As the rounded elements are mostly unpigmented, alternariosis is frequently misdiagnosed initially as cryptococcosis, as it was in our case and in nearly identical cases published by Wätzig and Schmidt [8] and Viera *et al.* [9], or as blastomycosis

[11]. The cellular walls may be brownish in colour, particularly when the fungal elements are deposited less deeply into the skin. The hyphae may be brown, but often are also unpigmented, as in our case.

For proper diagnosis of alternariosis, isolation of the etiologic agent is required. In addition, differentiation down to the species level is recommended, because species may have different virulence or resistance to antimycotic therapy due to the occurrence of chlamydospores [1]. Main groups to be distinguished are the *A. alternata* complex and *A. infectoria* (teleomorph: *Pleospora infectoria*). These species have marked differences in their rDNA ITS spacer domain [12]. Morphologically the two species differ by the formation of conidia with a distinctly verrucose wall and longer beak in *A. infectoria* [1], and culturally by growth responses to DRYES medium [1].

When the lesion is local and well-delimited, it may allow therapy by surgery. Additional antimycotic treatment is probably not required in such cases. With immunosuppression, local lesions tend to disseminate locally, taking a sporotrichoid course [11] and therefore adequate therapy is recommended. Systemic dissemination is rare and is dependent on the patient's immune status. A reduction of (iatrogenic) immunosuppression is then recommended, in addition to antimycotic therapy, preferably with systemic or intralesional [9] amphotericin B or itraconazole [12]. However, treatment of cutaneous alternariosis is not standardized.

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