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Case Report and Literature Review

Cutaneous infection caused by *Alternaria* in patients receiving tacrolimus

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Tacrolimus, an immunosuppressant used in organ transplant surgery, is inhibitory to some medically important fungi but also may obstruct azole monotherapy in the immunocompromised patient. We observed a case of cutaneous phaeohyphomycosis caused by *Alternaria alternata* in a liver transplant recipient who had been under tacrolimus immunosuppression for 6 months post-transplantation. At the onset of the infection, the patient presented with an increased whole-blood tacrolimus level. After a simple surgical excision the patient was discharged from the hospital without antifungal treatment but with an adjusted tacrolimus dosage. Literature review on fungal infections in patients receiving tacrolimus suggested these patients experience cutaneous and deep mould infections that are more frequent, severe and therapy-refractory than those seen in patients with other types of immunosuppression.

Keywords *Alternaria alternata*, drug-interactions, immunosuppression, molecular identification, tacrolimus

Introduction

Tacrolimus (FK-506) is an 822-kD macrolide antibiotic obtained from an actinomycete strain (often referred to as a fungus in related literature) given the nomenclaturally unrecognized proprietary label '*Streptomyces tsukubaensis*' [1,2]. The compound acts by blocking interleukin-2 messenger production and therefore T-cell activation [2,3]. Recent studies have shown that tacrolimus has antifungal activity against cutaneous *Malassezia* isolates (*Malassezia furfur* in the broad, pre-molecular sense of the name) [4]. This property may play a role in the demonstrated clinical efficacy of tacrolimus ointment in patients with atopic dermatitis. Tacrolimus alone, however, showed no antifungal activity against other yeasts such as *Candida albicans* [5]. In addition, 4.5–6% of organ transplant recipients receiving oral or systemic tacrolimus contracted cryptococcosis, and of these patients, 66–67% (based on sample sizes of between nine and 11 patients) were found to develop cutaneous, soft-tissue or osteoarticular manifestations [6,7] whereas only around 11–17% had central nervous system involvement. Tacrolimus-immunosuppressed patients were more likely to have cryptococcosis than patients receiving non-tacrolimus-based immunosuppression [6]. The risk of acquiring deep fungal infections in patients receiving tacrolimus was estimated to be similar to that in patients receiving cyclosporin A [8,9].

Alternaria species are uncommon opportunists responsible for a wide spectrum of infections ranging from non-invasive colonization to systemic involvement. The disorders have been referred to by the umbrella term alternariosis [10]. *Alternaria* species are melanized, and therefore are associated with the histopathological presentation called phaeohyphomycosis [11], characterized by dark-coloured elements in tissue. However, these elements may also be hyaline and

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yeast-like [12]. Clinical strains of *Alternaria* mostly show poor sporulation, and thus identification with conventional methods may be difficult. In such cases definitive identifications need to be carried out by means of molecular techniques [13].

Cutaneous and subcutaneous phaeohyphomycoses can develop after local injury, both in immunosuppressed and healthy subjects. The clinical picture depends on the causative agent and on host responses [10]. The appearance of cutaneous *Alternaria* infection can be quite variable. Manifestations include scaly, infiltrated erythema, plaques with papules, pustules and crusts, suppurative or crusted ulcers, nodular cystic masses, subcutaneous abscesses, and mycetoma, as well as tuberous masses with smooth or rouged surfaces [10,14–16].

Cutaneous *Alternaria* infection represents an emerging medical problem, in some *Alternaria* species are among the facultative pathogens that are favoured by iatrogenic immunosuppression. There are many reports of *Alternaria* infection in drug-induced immunosuppression, with most cases occurring as a result of high-dose corticosteroid therapy [17,18]. It has been suggested that cutaneous fragility in cases of skin disease secondary to corticosteroid use, such as autoimmune blistering diseases, contributes to the risk of percutaneous inoculation from the environment [17,18]. However, there are many cases of cutaneous *Alternaria* infection in non-corticosteroid-based immunosuppression [19]. Reported immunosuppressive drugs in cases of cutaneous *Alternaria* infection include prostacycline, azathioprine and cyclosporin [20–22].

To our knowledge, four cases of human cutaneous infection caused by *Alternaria* species in organ transplant recipients receiving tacrolimus have been reported [12,23–25]. Here we report another case of cutaneous phaeohyphomycosis due to *Alternaria* in a liver transplant recipient with tacrolimus-based immunosuppression. Clinical events and management of fungal cutaneous infections in transplant recipients receiving tacrolimus as primary immunosuppressive agent are discussed.

Case report

A 66-year-old man with a history of alcoholic cirrhosis and hepatic carcinoma underwent liver transplantation in November 2000. Immediately after surgery, he suffered from bacterial pneumonia, heart failure and tonic-clonic-convulsions. He was discharged from the hospital 112 days after transplantation, receiving tacrolimus (26 mg/day) and prednisone (5 mg/day). In May 2001, he presented with a general syndrome consisting of diarrhoea, asthenia, anorexia and weight loss without fever. Concomitantly, he developed an ulcerated lesion in the dorsum of the left foot. At his hospital re-entry, analytical findings were 3310 leucocytes, 8.3 haemoglobin, 24 haematocrit, 3.5 albumen, 131 urea, 2.1 creatinine, 8.4 ng/ml whole-blood tacrolimus level (oral dosage 23 mg/day). Chest roentgenography, electrocardiography and echocardiography showed findings compatible with left-side heart failure. Colonoscopy showed signs of colitis, probably related to pharmacological toxicity.

When our department was consulted to elucidate the nature of the skin lesion, the patient could not recall a prior injury. Physical exploration revealed a hypertrophic, ulcerated, infiltrated tuberous mass, easy to bleed (Fig. 1). With the suspicion of a squamous cell carcinoma or cutaneous fungal infection, a biopsy specimen was taken. Haematoxylin-eosin staining revealed the presence of a pseudo-epitheliomatous hyperplasia with epithelioid cells, lympho-histiocytic infiltrates with neutrophils, giant cells and micro-abscesses throughout all the epidermal layers. At high magnification, many fungal elements were observed both in haematoxylin-eosin and periodic acid-Schiff (PAS) stains. Fungal hyphae were also demonstrated in PAS stains (Fig. 2, 3). The patient was submitted to our in-patient department for management of the skin lesion.

The lesion was excised under local anaesthesia. Fungal cultures was performed on Sabouraud–gentamicin–chloramphenicol, potato dextrose, Sabouraud–cycloheximide and brain–heart infusion agars at 25 and 37°C. After 10 days, flat, velvety colonies developed which were olivaceous-black at 25°C and off-white at 37°C, both with blackish reverse. Colonies grew well on all media, though faster growth was observed at 25°C. Sporulation was poor. Microscopic examination in slide cultures revealed short

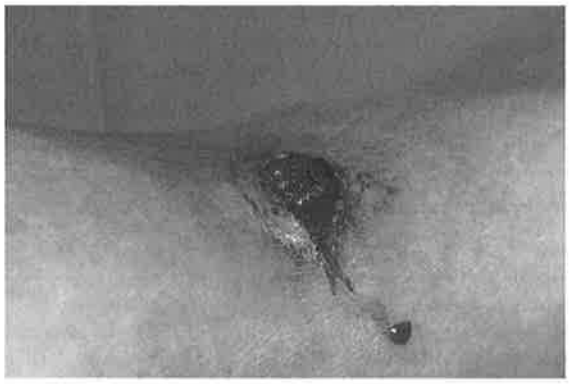

Fig. 1 Appearance of the lesion 3 days after admission.

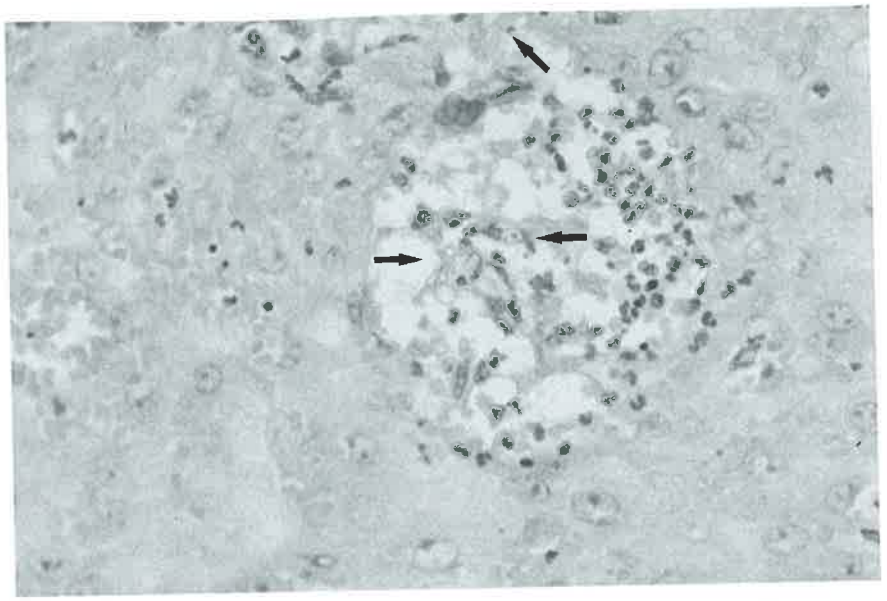

Fig. 2 Fungal hyphae interspersed in lympho-histiocytic infiltrate. A hyaline capsule surrounds yeast-like structures and hyphae (arrows) (H&E × 400).

chains composed of two- to five-celled dark smooth-walled conidia, with transverse or oblique septa, arising terminally on long septate, rugose, simple or geniculate conidiophores with scarce lateral branches (Figs. 4 and 5). Based on the morphology of conidia on Sabouraud media, we initially identified the isolate as *Alternaria infectoria*. The strain was analysed at the Centraalbureau voor Schimmelcultures (CBS) for possible molecular identification. After sequencing of the nuclear ribosomal internal transcribed spacer (ITS) domain, and comparison with 250 comparable sequences of other *Alternaria* and *Ulocladium* species held at CBS, including numerous *A. infectoria* isolates, 100% identity to *A. alternata* was found. Therefore, we consider the definitive identification of our strain to be *A. alternata* (Fr.) Keissl. [12]. The isolate was deposited in the CBS culture collection under accession number CBS 112018.

After the excision, the patient was submitted to his original department without antifungal therapy. In

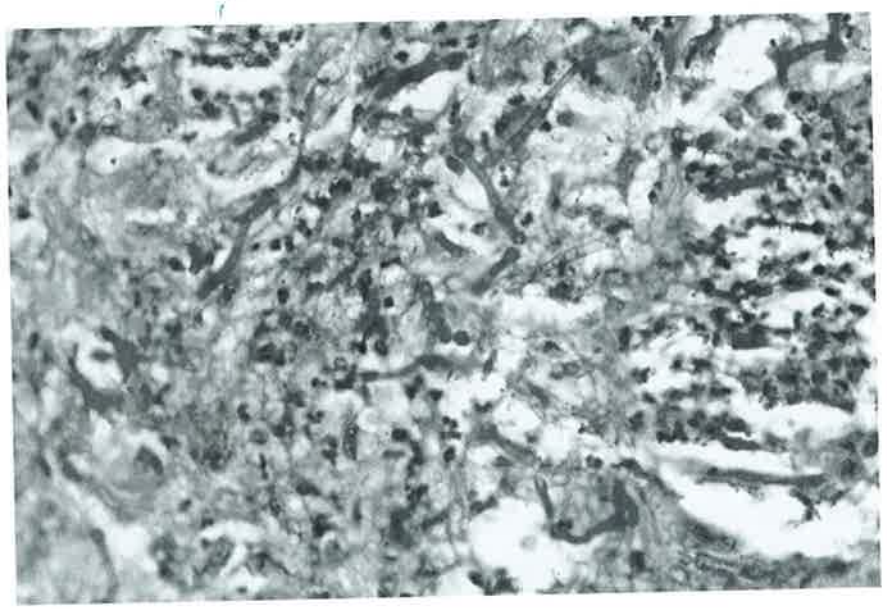

Fig. 3 PAS-positive, septate hyphae (× 400).

Fig. 4 Microscopic aspects of *Alternaria alternata* culture showing conidiophores and conidia.

June 2001, he was discharged from the hospital, receiving treatment with tacrolimus adjusted to whole-blood levels of 4.6 ng/ml (oral dosage 11 mg/day) and prednisone 5 mg/day. The patient remained under control in our department and 24 months after the resection no evidence of relapse was observed.

Discussion

Tacrolimus is currently considered to be an excellent immunosuppressant for organ transplant recipients [26]. However, its application is not without risks. A higher number of fungal infections was detected in patients receiving tacrolimus as sole immunosuppressant than in patients receiving a reduced dosage of tacrolimus supplementary to mycophenolate mofetil [27]. Despite the prophylactic use of amphotericin B, systemic infection and fungal pneumonia due to *Aspergillus* can reach 37% in organ transplant recipients receiving tacrolimus [28,29]. The incidence of fungal infections significantly increased the mortality rate of these patients [29,30]. Specific risk factors for

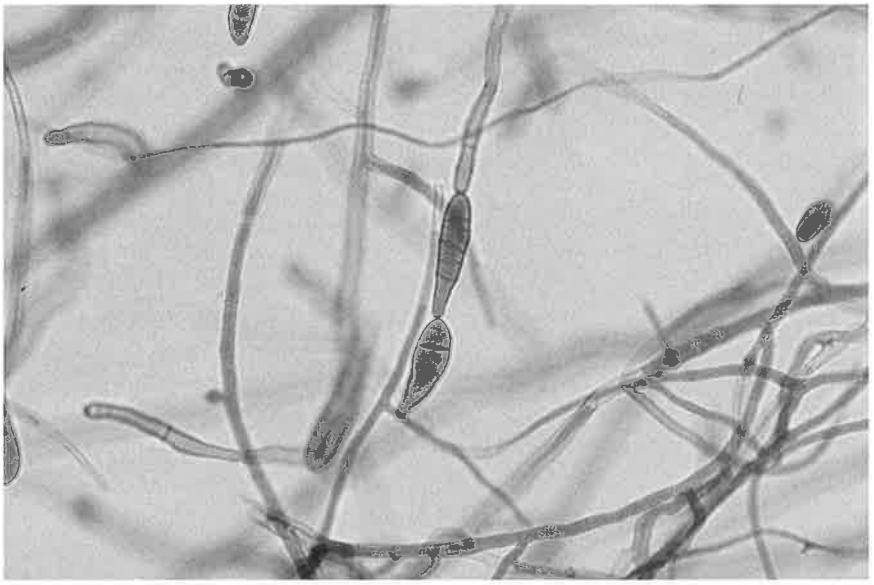

Fig. 5 Catenulate conidia.

fungal infections under tacrolimus were dialysis, elevated creatinine level, and recurrence of hepatitis C [30]. Central nervous system involvement due to cryptococcosis was uncommon in this patient group [7].

Cutaneous and subcutaneous infections reported to be caused by filamentous hyphomycetes in patients under tacrolimus immunosuppression include dermatophyte [31] and non-dermatophyte mould infections. In many of these cases, a poor response to azole therapy was seen. Non-dermatophyte moulds involved were *Phoma* sp. [31] *Scopulariopsis brevicaulis* [32], *Trichoderma longibrachiatum* [33], *Aspergillus ustus* [34] and *A. fumigatus* [35].

Considering the four previously reported cases known to us of cutaneous *Alternaria* infection in patients receiving tacrolimus [12,23–25], plus the present case, three cases thus far have concerned liver transplants, including one renal and one heart transplant recipient. A case in which a reduced tacrolimus dosage was applied in combination with mycophenolate mofetil showed a good response to itraconazole therapy [12]. The remaining patients on tacrolimus monotherapy, when infected by *Alternaria*, showed poor response to surgical excisions alone [22], itraconazole alone [23] and itraconazole plus 5-flucytosine [25].

The association of tacrolimus with ketoconazole or itraconazole was synergistic against azole-resistant *C. albicans* strains [5]. This is because tacrolimus inhibits *CDR1* and *CaMDR1* genes in *C. albicans*, which are thought to play a role in the antifungal resistance to azole derivates [5]. Therefore, it might be expected that patients under tacrolimus immunosuppression would exhibit a good response to azole therapy against a fungal infection. In contrast, it is well documented that in amphotericin B prophylaxis in organ transplant recipients under tacrolimus, a higher dosage of antifungal is needed for prevention of systemic aspergillosis than is required for prevention of yeast infections [7–9,27–30]. Many authors have reported failures with itraconazole therapy in uncommon mycoses under tacrolimus immunosuppression. Cases include disseminated nodular lesions due to *S. brevicaulis* [32], acute invasive sinusitis due to *T. longibrachiatum* [33], cutaneous *Alternaria* infection [21,24,25] and cutaneous infection due to *A. ustus* [34]. All these cases were cured with long term amphotericin-B therapy.

In the case reported here, successful cure was obtained by simple surgical excision and reduction of tacrolimus dosage. In patients under tacrolimus immunosuppression, antifungal treatment must always be accomplished by a carefully evaluation of whole-blood tacrolimus level. Itraconazole can be used in yeast infections when the patients have no renal or hepatic problems hindering normal metabolism of the drugs taken. In *Alternaria* infections, however, surgical excision followed by amphotericin B therapy until a total dosage of 1.5 g has been attained, combined with prolonged follow-up, must be considered the therapy of choice, though excision alone may be successful if the inoculum can be entirely removed.

Alternaria species are likely to be underdiagnosed as agents of opportunistic infections in humans. In part this is due to their unexpected histopathology, which may involve hyaline yeast cells rather than brown hyphal elements [12]. In addition, as mentioned above, the strains isolated often show highly reduced morphology and conidiation. This poorly developed morphology mostly does not allow the application of current criteria for identification of *Alternaria* species, such as the three-dimensional aspects of conidial structures [36]. For this reason de Hoog and Horré [37] have developed an identification system of clinical *Alternaria* species based on ITS rDNA data. They found that the great majority of cases could be ascribed to two species complexes of plurivorous saprobes, either *A. infectoria* or *A. alternata*. The ITS domains of these species are easily distinguishable, particularly due to the occurrence of a 30 bp insertion in ITS1 of *A. infectoria*. Primary colonies of members of this species mostly are cream-coloured and nearly sterile, with occasional, long-beaked conidia [13], whereas *A. alternata* produces olivaceous colonies with patches of conidial chains. The present isolate had the morphology of *A. infectoria*, but on the basis of its ITS sequence it was unambiguously identified as *A. alternata*. This underlines the need for non-morphological methods in the reliable identification of clinical *Alternaria* strains.

Acknowledgements

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