

Cutaneous infection by different *Alternaria* species in a liver transplant recipient



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ABSTRACT

Fungal invasive infections are rare in general population but are an emergent cause of infection in the immunocompromised population, especially in the solid organ transplant recipients. Herein the authors report a clinical case of a liver transplanted patient suffering a cutaneous co-existent infection with *A. alternata* as well as *A. infectoria*.

To our knowledge this is the first case of cutaneous concomitant infection due to those two species reported not only in Portugal but also worldwide. The patient was treated with surgical excision of the lesions and oral itraconazol without relapse.

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1. Introduction

Fungal infections are well-recognized life threatening problems in solid organ transplant (SOT), namely in liver transplant recipients [1]. Host and environmental factors are critical and important determinants regarding the epidemiology of fungal infections in transplantation [2].

The incidence and aetiology of fungal infections differ dramatically between hematopoietic stem cell transplantation and SOT. In the last group, those receiving lung grafts have the highest incidence (7.9%) followed by heart (3.4%), liver (3.1%), kidney (1.1%), and pancreas (0.7%) [2,3,4]. In liver transplanted patients, the incidence of invasive fungal infections ranges from 4 to 50% and *Candida* spp. and *Aspergillus* spp. are the most common aetiological fungal agents of those infections [2,3].

Fungal cutaneous and subcutaneous infections are frequently associated with people with professional activities related to agriculture or forestry work and the entry of the fungus is facilitated by penetrating injuries caused by thorns, splinters, nails, etc. The limbs are the most affected, but other body parts can also be involved. Infection can be localized or can spread through the blood or lymph [5].

Alternaria general characteristics include the production of dark-coloured phaeodictyospores in chains, and a beak of tapering apical cells [6, 7]. This genus comprises a large number of saprobic or plant pathogenic species. Some species are recognized to cause cutaneous phaeohyphomycoses and are described as emerging pathogens in immunocompromised patients, being transplantation the most common risk factor and also associated to the use of prednisone [7,8]. There are several cases of cutaneous *Alternaria* infections described in Portugal [19,10–13].

2. Case report

A 65-year-old male Caucasian was observed in a Dermatology consultation of a central Hospital in Lisbon on 16th April 2014. The patient presented with two painless, fast-growing nodules which had developed within 4-months. One of the nodules was located on the right leg and the other one on the dorsum of the left hand (Figs. 1 and 2). He had a clinical history of 6-months liver transplantation and at the time of observation was under immunosuppressive therapy with tacrolimus 8 mg once a day, mycophenolate mofetil 500 mg twice a day and prednisone 20 mg once a day. In addition, he was under therapy for diabetes. The patient referred a previous history (previous to the transplantation) of agricultural activities with frequent skin injuries.

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Fig. 1. Cutaneous lesion from the left hand.



Fig. 2. Cutaneous lesion from the right leg.

Physical examination revealed a well-defined erythematous nodule with 1.4 cm of maximum diameter, with central haematic crusts and a peripheral scaly collarette on the dorsum of the left hand and a violaceous nodule with 1.5 cm with central haematic

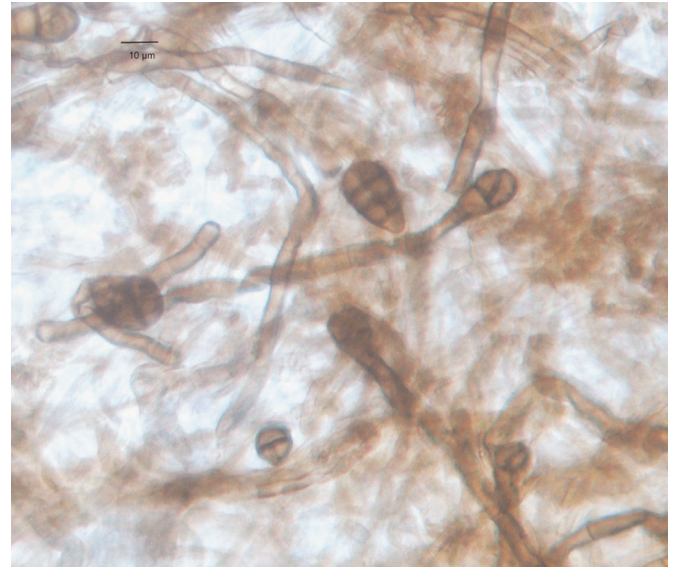


Fig. 3. Microscopy of the culture from clinical isolate of the hand showed prominent growth of *Alternaria infectoria*.

crusts and serous exudate on the right leg. Regional lymph node inspection was unremarkable.

Skin biopsies were collected on that day from both nodules using a 4 mm biopsy punch and sent for histological and mycological examination.

Histological sections of the biopsy were stained with periodic acid-Schiff and results retrieved on day +13 revealed an inflammatory infiltration of the dermis and the presence of multiple fungal spores and hyphae.

The mycological analysis of the biopsy material was performed using with calcofluor-white staining, followed by observation under fluorescence microscopy. Microscopic observations revealed the presence of septate hyphae. Clinical specimens were cultured on Sabouraud's dextrose agar containing chloramphenicol at 30 °C and 35 °C. Cultures yielded a grey velvety fungus, with a dark-brown reverse on day +14. When sub-cultured both on Malt extract agar for identification, the microscopic examination of the



Fig. 4. Microscopy of the culture from clinical isolate of the leg showed prominent growth of *Alternaria alternata*.

moulds performed by teased mount and lacto-phenol cotton blue preparation, showed different macroscopic features: green (leg biopsy) to grey (hand biopsy) colonies both with dark reverse. Microscopic examination showed chains of brown conidia with rounded base and beaked apex, with transverse and oblique septation. There were a slight difference between microscopy from leg and hand biopsy, in the first one, conidial chains were more branched than in the second one, and conidia were less tubular (Figs. 3 and 4). From the above microscopic and macroscopic features, the isolates were identified as *Alternaria* spp. on day +20 and sequenced for speciation.

The lesions were excised on day +34 and the patient started antifungal therapy on the same day with oral itraconazole (100 mg daily) for 3 months with adjustment of immunosuppressive therapy. The antifungal therapy ended on day +118. There was no relapse at 5-months follow up after surgery.

Total genomic DNA was extracted from purified colonies and the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) of these isolates was amplified using primer set ITS1 (5' TCCGTAGGTGAACCTGCGG3') and ITS4 (5'TCCTCCGCTTATTGATATGC3') [14].

Sequencing of both strands was performed and nucleotide sequences were edited using the programme Chromas2 and aligned using the programme CLUSTAL X2 [15]. The sequences obtained were compared with sequences deposited in the GenBank and CBS-KNAW Fungal Biodiversity Centre databases.

Molecular sequencing resulted in the identification of *Alternaria infectoria* from the culture obtained from the patient's hand and *Alternaria alternata* from the culture obtained from the patient's leg. The sequencing coverage was high (99–100%), as well as the homologies with reference strains ($\geq 98\%$), which is the value considered as an acceptable identification to species level. In fact, we obtained a sequence with a homology of 98% and 99.5% with *Alternaria infectoria* and *Alternaria alternata*, respectively.

Nucleotide sequences of the isolates were deposited in the GenBank database under the accession numbers KP171632 (*Alternaria infectoria*) and KP171633 (*Alternaria alternata*).

3. Discussion

Phaeohyphomycosis can be caused by different species, being *A. alternata* and *A. infectoria* the most commonly isolated [7]. Dematiaceous fungi have melanin in their cell walls, which represent a virulence factor for several pathogenic fungi [16]. The raise of patients under immunosuppressive drug therapy has increased and so the risk of fungal infections is likely to increase [10].

The patient of this case report lives in a rural area, but did not refer trauma after transplantation. However, after a detailed questionnaire, the patient referred that before the transplantation he carried out different agricultural activities and had skin injuries. Cutaneous fragility induced by hypercorticism could be an important co-factor permitting direct inoculation from the environment. Nevertheless, several fungi can also remain latent for long periods of time and the clinical symptoms of fungal infection may appear several years after the inoculation of the etiological agent [7,9].

There are currently no standardised treatments for cutaneous infections caused by *Alternaria* spp. In those cases and when it is possible, the reduction of the immunosuppression level can be enough to resolve the lesions, although the long-term use of systemic itraconazole is often recommended [7].

Co-existent fungal infections associated with *Alternaria* spp are sometimes referred [17–23], despite infrequently. Furthermore,

most of the published cutaneous fungal infection cases refer infection due to only one species as etiological agent. To our knowledge, this is the first case described of a cutaneous co-infection with two different species of *Alternaria*. Infection with different species can be under diagnosed, since when multiple lesions are observed, biopsy material is normally taken from only one of them. Early detection and appropriate antifungal therapy are essential in the treatment of this emergent infection, molecular identification is a useful tool to distinguish mixed fungal infection.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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