Successful Terbinafine Treatment for Cutaneous Phaeohyphomycosis Caused by Trematosphaeria grisea in a Heart Transplanted Man: Case Report and Literature Review

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Successful terbinafine treatment of cutaneous phaeohyphomycosis caused by *Trematosphaeria grisea* in a heart transplanted man: case report and literature review

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Abstract

Phaeohyphomycosis is chronic infectious disease caused by dematiaceous fungi. It is characterized by the presence of pigmented septate mycelia within tissues. In case of superficial infection, the lesion(s) chronically evolve towards painless pseudo-tumors of the soft parts. We report herein the original case of a heart transplanted man who exhibited phaeohyphomycosis of the left hand, with no mention of travels in endemic areas. *Trematosphaeria grisea* was identified as the causative agent, which is quite innovative since this species has been rather described in mycetoma. The antifungal treatment initially based on isavuconazole alone was not sufficient to cure the patient. In contrast, its association with local terbinafine ointment allowed totalclinical improvement. This finding is unusual as diagnosis of phaeohyphomycosis caused by *T. grisea* is uncommon in non-tropical countries, and as the outcome appeared successful by the means of add-on therapeutic strategy with terbinafine.

Keywords: phaeohyphomycosis – dematiaceous fungus – *Trematosphaeria grisea* – isavuconazole – terbinafine

Introduction

Dematiaceous fungi are ubiquitous molds colored in pale brown-to-black due to the presence of melanin within their cell wall. They can cause chromoblastomycosis, mycetoma or phaeohyphomycosis. Phaeohyphomycosis refers to as a group of infections characterized by the presence of pigmented septate mycelia - but neither grains (that are pathognomonic to mycetoma) nor Medlar sclerotic cells (pathognomonic to chromoblastomycosis) - within tissues [1]. It is potentially caused by a great variety of fungal genera [2]. Phaeohyphomycosis has been mostly reported in tropical areas, both in immunocompromised and non-immunocompromised hosts [3,4]. Clinical signs vary largely. Therefore, phaeohyphomycosis is nowadays usually classified in three distinct subtypes: local superficial infection, local deep infection or disseminated infection. In case of local superficial infections, phaeohyphomycosis is generally due to the inoculation of the causative agent directly into the skin following trauma; then, the fungus and the surrounding inflammation grow over several years to result in a painless pseudo-tumorous mass. Laboratory tests allow the species identification by the means of *in vitro* culture and molecular sequencing, but there is no diagnostic assay available for blood. The therapeutic management is challenging. We present herein an original case of phaeohyphomycosis in a heart transplanted man caused by *Trematosphaeria grisea* (syn. *Madurella grisea*), that is a fungal species usually rather involved in mycetoma [6]. Curative treatment with terbinafine was successful.

Observation

A 49-year-old man was referred for a painless tumorous lesion located at the left hand. It was significantly expanding over a one-month period. The patient had never left France, except for a short stay in Tunisia, ten years before. He also reported the inoculation of a foreign body into his left hand, following a road accident in metropolitan France few years ago. Three months before the present consultation, he underwent cardiac transplantation because of heart failure. He was currently given an immunosuppressive treatment based on mycophenolate mofetil, prednisone and tacrolimus monohydrate. At the physical examination, a bluish four centimeter-long abscessed and suppurated lesion of the soft parts was noticed on the dorsal side of the hand. The lesion was facing the third and fourth **Erreur ! Source du renvoi introuvable.**extensor muscle's tendon (Figure 1a, Figure 2). There was neither paresthesia nor sensory-motor deficiency. No satellite adenopathy was reported.

The abscess was drained (but the pus was not investigated), and a biopsy of the lesion was concomitantly performed. Histological examination showed pigmented mycelial structures without grains (Figure 3). The macroscopic features of colonies that were isolated *in vitro* first suggested *Madurella* genus (Figure 4). Eventually, the species *Trematosphaeria grisea* (syn. *Madurella grisea*) was confirmed by DNA sequencing through genomic amplification of the rDNA 28S D1-D2 region (Score GenBank = 1133; cover= 99%; identit.=100%; GenBank accession number MN581332). The absence of fructification and sporulation did not allow measurement of the antifungal MICs (minimum inhibitory concentrations). For the initial curative treatment, isavuconazole, at the conventional dosage of 200 mg *qd* (after an initial loading dose over 48h), was empirically chosen due to its theoretical efficiency against *Madurella* species and its drug-to-drug interactions that are assumed quite limited with immunosuppressive drugs [7]. Furthermore, isavuconazole does not require dosage adaptation in case of kidney insufficiency, the patient having herein a glomerular filtration rate at 47 mL/min. No clear clinical improvement was noticed over the next nine months; so, daily application of topic terbinafine ointment was added to oral isavuconazole. Two months later, substantial reduction of the lesion size was noticed (Figure 1b), so that the antifungal combination strategy was pursued for six additional months. Afterwards, side effects occurred with diarrhea, and the renal function decreased due to possible interaction of the oral antifungal with tacrolimus monohydrate. The therapeutic drug monitoring showed high residual concentrations of isavuconazole (5.20 mg /L in blood). Isavuconazole was then withdrawn, and instead oral therapy with terbinafine alone was initiated with a dosage at 250mg *per* day. The total excision was denied by the patient. After six months of terbinafine alone, the lesion almost disappeared (Figure 1c), while the tolerance was satisfactory, so that the dosage was reduced at 250 mg every other day. After one year of treatment with oral terbinafine, the lesion is no more visible.

Discussion

Burgeoning and invasive masses of the soft parts can evoke a skin tumour, like cutaneous carcinoma [8]. Phaeohyphomycosis - and more largely fungal infection of the skin and subcutaneous tissues - is often overlooked, because not well known by most of the practitioners. As there are no rapid and reliable serologic or immunologic tests that can be easily implemented for diagnosis of phaeohyphomycosis, biopsies with mycological and histological investigations must be systematically performed in case of any doubt.

Nowadays, the number of pathogenic fungi documented as causative agents of phaeohyphomycosis has reached 70 genera and 150 species [9]. The recent application of molecular and phylogenetic tools has largely revised the taxonomic placement of melanised mycetes. Using a multigene phylogenetic approach, Ahmed *et al.* concluded that *Trematosphaeria grisea* species (syn. *Madurella grisea*) is phylogenetically-affiliated to the Trematosphaeriaceae family, suborder Massarineae in the Pleosporales order [6]. Although not so common, *T. grisea* has long been acknowledged as an exclusive agent of mycetoma in South America, more precisely in Brazil [10,11] and in Chile (but also in a submandibular abscess in India [6]. However, demonstration of its involvement in phaeohyphomycosis is more recent, maybe because there was previously a lack of correct identification [12]. Morphologically, *T. grisea* colonies are usually described as rugose-cottony-brown of slow growth rate at 30° C. A brownish pigment diffuses into the agar. Microscopic examination reveals long septate hyphal elements with chains of arthroconidia. However, further analysis with yeast nitrogen base shows that *T. grisea* assimilates glucose, maltose and saccharose, but not lactose [11], whereas *M. mycetomatis* is able to assimilate glucose, maltose and lactose, but not saccharose [13]. Nowadays, modern tools available such as mass spectrometry or DNA sequencing can identify whole unusual kinds of microorganisms from the subculture, as long as the references for fragmentation mass patterns or DNA sequences exist in the data bank. So herein, we are able to distinguish between *T. grisea* and *Madurella mycetomatis, i.e.* the major species of the *Madurella* genus which is usually more frequently involved in mycetoma.

The present case report is original since the patient did not come from endemic tropical area and had been always living in France, except for a unique travel in Tunisia ten years before the heart transplantation. However, recent molecular studies showed that *T. grisea* can be found worldwide in the environmental, especially in water supplies and humid conditions in northern Europe [6,14]. Thus, one could suggest that the foreign body which was inoculated into the patient's left hand during the road accident, several years ago, likely represents the route of infection. Thereafter, the immunosuppressive treatment became an adequate underlying condition to enhance the development of the fungus, then subsequently generating local inflammation responsible for the macroscopic lesion without developing grains.

In practice, curative therapy for superficial phaeohyphomycosis is closed to the management of fungal mycetoma, based on oral antifungal drugs in first-line strategy, then on surgical excision (and, in some cases, on cryotherapy). Guidelines from Chowdary *et al.* recommend oral antifungals, mainly azoles, as co-adjunctive therapies particularly in immunocompromised patients and to prevent dissemination (recommendation BIII-

grade) [15]. In our case, an oral antifungal azole was not enough to allow complete or partial cure, so our second-line strategy finally included extended-spectrum azole drugs added with terbinafine, because MICs of the latter were demonstrated low for most dematiaceous fungi [12]. Terbinafine is actually a fungistatic agent that inhibits the ergosterol synthesis of the plasma membrane of fungi. While its therapeutic role has been longtime confined to treatment of dermatophyte infections, recent medical interest has been raised for subcutaneous or refractory tropical fungal infection [16, 17]. A review of the literature [Table 1] showed 22 cases of phaeohyphomycosis treated with terbinafine: 12 were successfully cured; in eight cases, terbinafine was prescribed in first-line, including seven times in monotherapy (two patients underwent concomitant surgery). Moreover, there were six cases of phaeohyphomycosis in solid organ recipients, including five who were cured with terbinafine. Durations of treatment are poorly codified, but they often extend beyond several months or even years [Table 1]. The main clinical results that one can expect consist in clearly delineating the lesion through the prolonged antifungal therapy. This is supposed to make easier the surgical excision, and also to reduce the subsequent risk of recurrence. A regular follow-up is encouraged throughout three years to ensure complete clinical cure.

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All authors have seen and approved the manuscript. They all contributed significantly to the work. They have no conflict of interest to declare.

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Figure legends

Figure 1 a) Aspect of the lesions on the dorsal side of the left hand, facing the third and fourth **Erreur ! Source du renvoi introuvable.**extensor muscle's tendon; b) Aspect of the lesion after two months of treatment by isavuconazole and terbinafine ointment on local apply; c) Aspect of the lesion after six month of treatment by isavuconazole and terbinafine ointment on local apply, followed by six months of oral terbinafine.

Figure 2 a) T1-weighted magnetic resonance imaging (MRI) of the left upper limb according to axial view exhibited a cutaneous injury measuring approximately 32 x 11 mm with bone contact (shown by the yellow arrow) but with neither bone edema nor osteitis; b) T1-weighted MRI of the left upper limb according to coronal view showed a lesion facing the third and fourth **Erreur ! Source du renvoi introuvable.**extensor muscle's tendon (shown by the yellow arrow)

Figure 3 a) Histological observation according to HPS staining (Hematoxylin Phloxine Saffron) showed inflammatory granulomatous giganto-epithelioid infiltrates, with polymorphonuclear cells and lymphocytes developed near mycelial structures. No obvious criterion of malignancy was observed. b) Gomori-Grocott methenamine silver staining, and c) PAS staining (Periodic Acid Schiff) preparations highlighted melanized hyphae and beadlike swellings. No grains were seen, so the diagnosis of mycetoma was clearly rejected.

Figure 4 Different colony morphologies (obverse and reverse) of *Trematosphaeria grisea* after ten days of *in vitro* growth at 30°C. Colonies were velvety, a) light grey and radially folded on Sabouraud-dextrose agar; b) light brown on oatmeal agar (OA); c) heaped with dark grey reverse on 2% malt extract agar (MEA) and d) light tan on potato-dextrose agar (PDA).