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Subcutaneous phaeohyphomycosis caused by *Exophiala xenobiotica* in a non-Hodgkin lymphoma patient

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Phaeohyphomycosis is a rare fungal infection that is more commonly associated with immunocompromised patients. We present a case in which a 77-year-old woman with non-Hodgkin lymphoma developed subcutaneous phaeohyphomycosis caused by *Exophiala xenobiotica*. *E. xenobiotica* is a dematiaceous hyphomycete that was recently identified as a segregant of the *E. jeanselmei* complex. The patient was successfully treated with local excision of the lesions and post-surgical oral itraconazole. The latter was administered with the aim of preventing systemic dissemination in this immunocompromised patient.

Keywords phaeohyphomycosis, *Exophiala xenobiotica*, *Exophiala jeanselmei*

Introduction

Phaeohyphomycosis, which was first defined by Ajello *et al*. in 1974 [1], encompasses a distinct, heterogeneous group of mycotic infections in which the etiologic agents occur in tissue as typically dematiaceous, yeastlike cells, pseudohyphae-like elements, hyphae that may be short or elongate, regular, distorted to swollen in shape, or any combination of these forms [2]. It comprises cutaneous, superficial, cutaneous and corneal, subcutaneous and systemic infections. Phaeohyphomycosis can be distinguished from chromoblastmycosis and mycetoma on the basis of its histological features rather than its clinical characteristics. Importantly, the term 'phaeohyphomycosis' is intended for opportunistic infections caused by dematiaceous fungi regardless of their taxonomic classification [2].

At least 104 species, belonging to 57 genera, are documented as agents of phaeohyphomycosis. The genus *Exophiala* is widely distributed in the environment and frequently isolated from phaeohyphomycotic cysts. However, the *E. jeanselmei* taxon is reportedly rather heterogeneous with various opportunistic *Exophiala* species that have been isolated from humans, and that have been previously identified as *E. jeanselmei*, differing in terms of predilection, clinical behavior and ecology. In recent years, diagnostic approaches have been supplemented by molecular tools, particularly sequence data for the rRNA internal transcribed spacer (ITS) regions. By using this methods, many strains identified as *E. jeanselmei* morphologically, have been re-identified as another *Exophiala* species [3]. In 2006 a new black yeast species, *E. xenobiotica*, was identified through molecular approaches as a segregant of the *E. jeanselmei* complex [4]. Furthermore, *E. xenobiotica* appeared to be the most frequently detected dematiaceous hyphomycete to cause subcutaneous or cutaneous infections [3]. In contrast to *E. xenobiotica*, *E. jeanselmei* sensu stricto appeared to be less common of these forms of the disease [3]. Further accumulation of the clinical features of cases in which the isolates are correctly identified is needed. Here, we report a case of phaeohyphomycosis caused by *E. xenobiotica* in an immunocompromised patient.

Case report

A 77-year-old Japanese woman presented with multiple nodules on her left hand of 2 months' duration. She had first noticed the lesions in July in 2007, but could not recall any recent trauma to the site. The lesions which were not painful continued to enlarge and increase in number without discharge. The patient had been diagnosed with non-Hodgkin lymphoma 8

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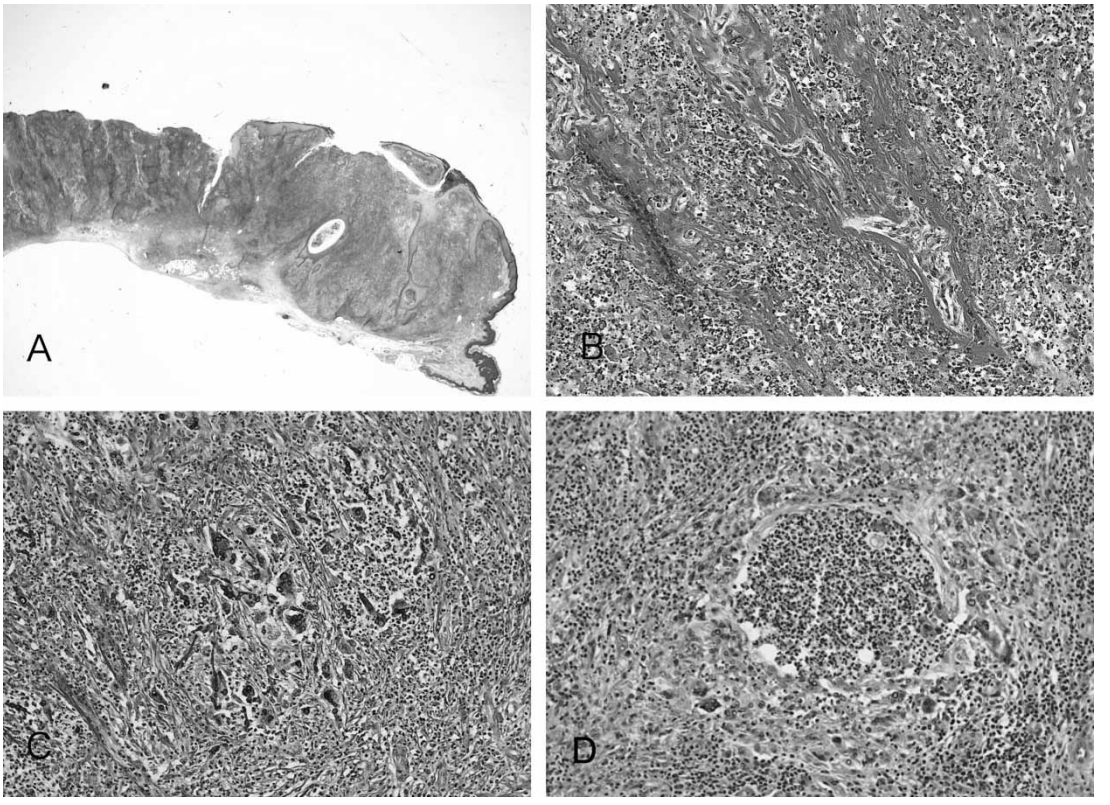

Fig. 2 Histological features of a biopsy specimen of the cutaneous lesion. (A) Abscesses surrounded by dense collagenous connective tissue in the dermis and subcutaneous tissue, with epidermal hyperkeratosis (hematoxylin-eosin ×40). (B) Chronic granulomatous inflammatory reaction and numerous pigmented hyphae in the papillary dermis (hematoxylin-eosin, ×400). (C & D) Various fungal organisms can be seen free in the dermis and within giant cells. PAS staining highlights the fungal organisms within the lesion (C, PAS stain, ×400). In the center of the granuloma, there is an abscess composed of aggregated neutrophils and pigmented hyphae, surrounded by epithelioid cells and multinucleated giant cells (D, hematoxylin-eosin stain, ×400).

years earlier and suffered from chronic renal and heart failure. Starting in January 2007, she had received several courses of chemotherapy, then follow-up treatment with various medications, including oral predonisolone (5 mg/day).

On presentation in August 2007, the patient had two verrucous-crusted nodules on the left hand, which were 3 cm and 2 cm in diameter, respectively. Both nodules had an elevated, dome-shaped appearance with a dry thick bloody crust and secondary ulceration on the surface (Fig. 1). Two red subcutaneous nodules, which were 1 cm in diameter, were also found beside these larger nodules. No superficial lymph nodes were palpable in the patient's left axilla. One of the largest nodules was surgically excised and the patient treated with itraconazole (200 mg per day) for 6 weeks. Although the remaining lesions reduced in size, they did not disappear completely, so they were also totally excised. The patient was treated with itraconazole (200 mg per day) for a total of 8 weeks after the second operation. The surgical sites healed well, and no recurrence had occurred until she died from her lymphoma in December 2007.

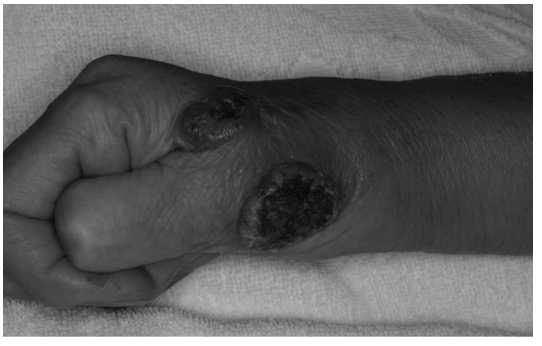

Fig. 1 Verrucous-crusted nodules with central ulceration on the back of the patient's left hand.

Laboratory investigation revealed anemia and hyperimmunoglobulinemia (IgG 2252 mg/dl) due to lymphoma. The leukocyte count was within normal limits.

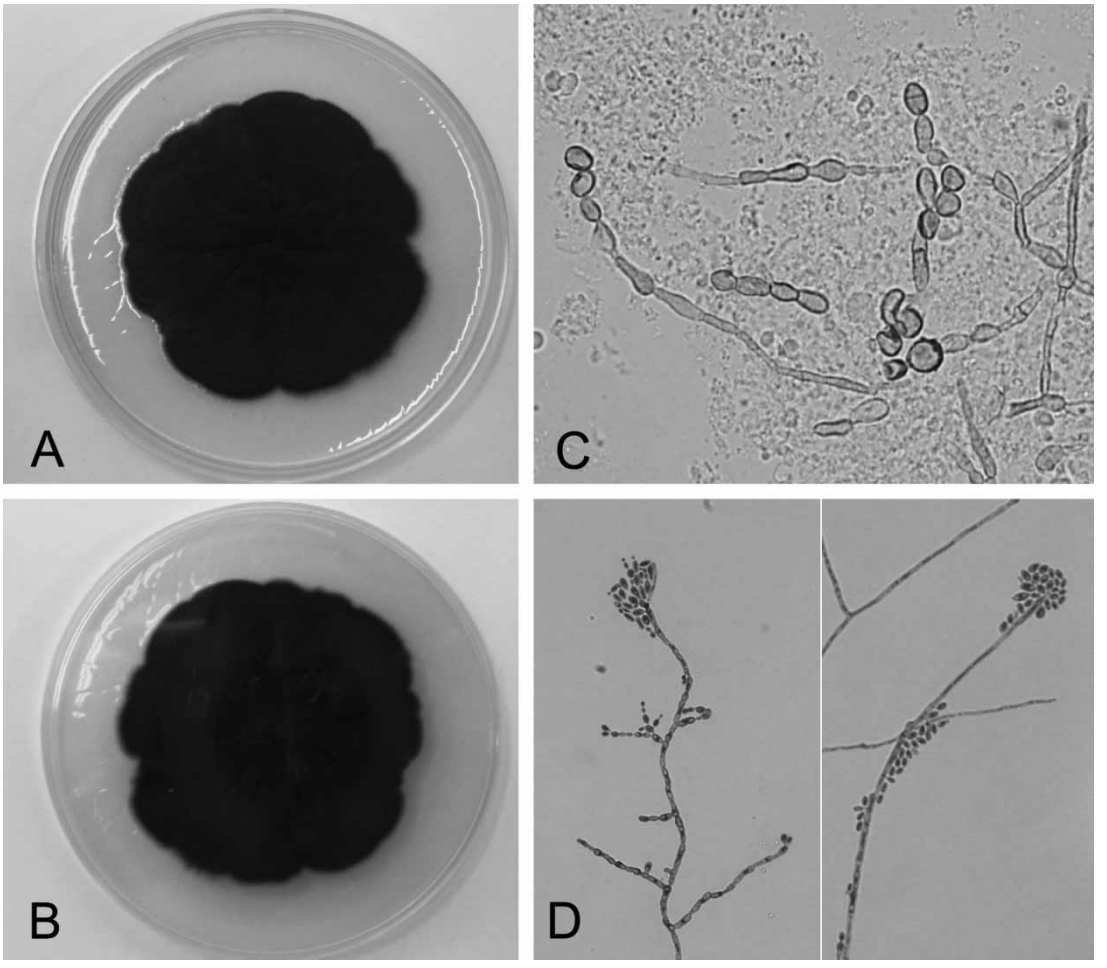

Fig. 3 Primary isolates cultured on SDA plate and light micrographs of a smeared sample of tissue and a slide culture. (A) Surface of an olivaceous black colony on an SDA plate grown at 24°C for 8 weeks. (B) The reverse side of the colony shown in A, which is also olivaceous black. (C) Brown hyphae in a smeared sample of tissue (KOH preparation, ×400). (D) Slide culture showing hyphae and conidiophores with numerous conidia (Lactophenol-cotton blue, ×400).

Biopsy of the larger nodules revealed multiple foci of granulomatous inflammation within the dermis, composed of histiocytes, epithelioid cells, multinucleated giant cells and lymphocytes (Fig. 2). In the centers of these granulomas there were abscesses composed of aggregated neutrophils and numerous fungal organisms, which appeared as dark brown, thick-walled, variable sized ovoid spores, present singly or in chains or clusters. Giant cells containing pigmented hyphae were also seen. The branching fungal organisms within the lesions stained with a blue-purple color upon periodic acid-Schiff (PAS) staining. The epidermis overlying the dermal granulomas was hyperplastic. On these results, a diagnosis was made of subcutaneous phaeohyphomycosis.

Table 1 Results of *in vitro* antifungal susceptibility testing

	MIC (g/ml)	
	Isolate 1	Isolate 2
Amphotericin B	0.5	2.0
5- Fluorocytosine	8.0	32.0
Fluconazole	16.0	64.0
Itraconazole	≦0.015	0.1
Miconazole	≦0.06	2.0
Micafungin	16.0	>16.0

Mycological findings

Physiological analysis and sequencing of the DNA ITS region of the isolates was done by First Laboratories (Chiba, Japan). Sequencing results of rDNA ITS region was confirmed following the method as described previously [3]. Antifungal susceptibility testing of antifungal agents was performed by BML (Saitama,

Japan) according to Clinical and Laboratory Standards Institute guidelines (M38-A).

Microscopic examination of the smeared sample of tissue revealed many fungal elements, consisting of thin, brown, ovoid spores and a brown budding yeast (Fig. 3 C). When tissue was inoculated onto Sabouraud dextrose agar (SDA) without antibiotics, many dark-colored colonies were recovered. Isolated colonies developing on SDA plates showed circular growth, and had an olivaceous black, yeast-like appearance. The colony was velvety on the surface at first, but became umbonate and felt-like later (Fig. 3 A). The reverse was also olivaceous black (Fig. 3 B). This colony was 30 mm in diameter after 8 weeks of growth at 24°C. Microscopic examination on SDA agar showed that the colony contained septate hyphae with numerous slender tubular, sometimes branched, conidiophores, which characteristically tapered to a narrow, elongate tip. The conidia were ellipsoidal, and gathered in clusters at the ends and sides of the conidiophores and at points along the hyphae (Fig. 3 D). The mold decomposed tyrosine, but not casein or xanthine, and assimilated potassium nitrate. It grew at 30°C and more slowly at 33°C, but did not grow at 36°C. The fungus was identified as belonging to the *E. jeanselmei* complex. Sequencing of the rDNA ITS region revealed a sequence identical to that of *E. xenobiotica* (CBS 118157), therefore this mold was determined to be *E. xenobiotica*.

The results of antifungal susceptibility testing for two fungal isolates, which were isolated from excised nodules from the first or second operation respectively, are shown in Table 1. Both isolates are sensitive to itraconazole, but resistant to fluconazol and micafungin.

Discussion

E. jeanselmei may cause phaeohyphomycosis, chromoblastmycosis, mycetoma and fungenia. According to a review of 54 dematiaceous infections cases caused by *E. jeanselmei*, which were reported in Japan in 2002, 50 of the patients had phaeohyphomycosis, and four chromoblastmycosis [5]. This suggests that *E. jeanselmei* may more frequently cause phaeohyphomycosis than chromoblastmycosis in Japan.

Black yeasts of the genus *Exophiala* are notoriously difficult to classify and identify. Relatively soon after *E. jeanselmei* was identified, it was found to be a rather heterogeneous taxon. Genotype analysis has shown that various genetically distinct strains exist among strains which are morphologically and physiologically indistinguishable. Forty-five *E. jeanselmei* isolates from Japanese patients have been classified into 15 strains based on mitochondrial DNA [6]. The isolate from our patient was also morphologically and physiologically indistinguishable from *E. jeanselmei*. DNA sequencing of the ITS region revealed that this mold was *E. xenobiotica.*

E. xenobiotica is a dematiaceous hyphomycete that was identified in 2006 as a segregant of *E. jeanselmei* [4]. Environmental strains of *E. xenobiotica* are frequently found in habitats rich in monoaromatic hydrocarbons and alkanes, such as the sites of industrial biofilters polluted by toxic xenobiotics [4]. In a retrospective analysis of 188 clinical isolates that had been previously identified as *Exophiala* species and that were preserved at the University of Texas Health Science Center (San Antonio, USA), DNA sequencing showed that *E. xenobiotica* was relatively common (19.7%), whereas *E. jeanselmei* (3.7%) was rare [3]. If a similar screening study were to be performed in Japan, it is feasible that similar results would be obtained, although our present case is the first confirmed case of *E. xenobiotica*.

Based on the results of the above-mentioned University of Texas study [3], *E. xenobiotica* seems to cause mild cutaneous and subcutaneous infections in humans, given that only one out of 37 *E. xenobiotica* strains was isolated from the blood sample of a patient as the causative agent of systemic infection. Of the other strains, two were isolated from dialysis fluid, one from an intraocular lesion, three from subcutaneous lesions, and 21 from cutaneous lesions (the origins of the remaining six strains were unknown). In contrast, 36 out of 55 strains of *E. dermatitidis* were isolated from samples of deep infection. Our present observations support the hypothesis that *E. xenobiotica* tends to cause superficial infections, as no systemic infection was generated in our patient, even though she was immunocompromised. *E. xenobiotica* seems to have relatively low virulence. In our case, an immunocompromised patient presented with multiple subcutaneous lesions, but did not present with any clinical features of systemic infection. This strain grew best at 30°C, more slowly at 33°C, and not at all at 36°C, which may explain why it did not cause a systemic infection. The present patient's immunocompromised status probably contributed to formation of the subcutaneous lesions following cutaneous infection. In the University of Texas study, the 37 strains that were later genetically identified as *E. xenobiotica* were initially identified morphologically as *E. dermatitidis* (3 strains), *E. jeanselmei* (28 strains) and *Exophiala* spp. (6 strains) [3]. Clearly, genetic analysis is needed for distinguishing between *E. xenobiotica, E. dermatitidis* and *E. jeanselmei.* Correct identification of *Exophiala* spp. is

clinically important, because *E. dermatitidis* frequently causes systemic infection, but *E. xenobiotica* and *E. jeanselmei* do not.

Although the optimal treatment for subcutaneous phaeohyphomycosis is still under debate, surgical excision reportedly produces good results. In our case, itraconazole (200 mg/day) was helpful in reducing the size of the lesions, but did not complete eliminate the nodules. It is known that itraconazole may not be absorbed from the stomach as well in patients who have low levels of or no stomach acid. It is possible that itraconazole levels in the blood and tissue of our case might not have reached a high enough level to cause the complete disappearance of the lesions. Eventually, all lesions were excised and the patient was treated with post-surgical itraconazole (200 mg/day) for two months. No local recurrence was observed prior to her death as a result of her underlying condition. The good clinical course of our patient after surgical excision of the lesions appears to support local excision followed by itraconazole being a suitable treatment for subcutaneous phaeohyphomycosis caused by *E. xenobiotica*.

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