Multiple subcutaneous cysts due to *Exophiala spinifera* in an immunocompetent patient

HAMID BADALI*,†, JAGDISH CHANDER‡, MANSOUR BAYAT†, SEYEDMOJTABA SEYEDMOUSAVI§, SHAILPREET SIDHU‡, HENA RANI‡, ASHOK ATTRI‡, UMA HANDA‡, JACQUES F. MEIS# & G. SYBREN DE HOOG^*  

*Department of Medical Mycology and Parasitology, School of Medicine/Molecular and Cell Biology Research Center, Mazandaran University of Medical Sciences, Sari, Iran, †Department of Medical and Veterinary Mycology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran, ‡Departments of Microbiology, Surgery and Pathology, Government Medical College Hospital, Chandigarh, India, §Department of Medical Mycology, Faculty of Medicine and Medical Sciences, Islamic Azad University, Ardalib Branch, Iran, #Department of Medical Microbiology and Infectious Diseases, Canisius Wilhelmina Hospital, Nijmegen, The Netherlands, and ^CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands

Here we report a case of a 55-year-old Indian male presenting with multiple subcutaneous cysts, which developed from painful nodules at the dorsal right wrist joint. Subsequently a painful nodule appeared on the left knee joint. Cytological examination of the knee swelling revealed a suppurative inflammatory lesion consisting of neutrophils, lymphocytes, multinucleated giant cells and few fungal elements, without involvement of the overlying skin. *Exophiala spinifera* was cultured (CBS 125607) and its identity was confirmed by sequencing of the internal transcribed spacer (ITS rDNA). The cysts were excised surgically, without need of additional antifungal therapy. There was no relapse during one-year follow-up and the patient was cured successfully. 

**Introduction**

In recent years the incidence of infections by opportunistic fungi has increased dramatically in immunocompromised individuals. However, black yeast-like fungi seem to infect human hosts relatively independent from underlying immune or metabolic disorders. Some systemic species even occur preferentially in immunocompetent individuals [1]. Routes of infection by black fungi have not been clarified in most cases and have been hypothesized to occur by inhalation [2], by ingestion [3] or by traumatic inoculation [4–7], the latter type leading to superficial, cutaneous or implantation mycoses. The most frequently encountered genus in routine clinical practice is *Exophiala* [8]. Fungi classified in this genus are characterized by slow growth with black or dark brown colonies, the presence of budding cells, and have conidia repetitively produced from annelides. The black yeast *Exophiala spinifera* is one of the most virulent species of this group and a potential agent of disseminated, osteotropis disease [8,9]. A difference has been noted in cases affecting adolescents without notable underlying disorders, cases being systemic and frequently fatal, and elderly individuals with various debilitating diseases, where infections remain as (sub)cutaneous lesions and taking a mild course [10,11]. The present report of multiple
subcutaneous phaeohyphomycotic cysts due to *E. spinifera* falls in the latter category, concerning a 55-year-old, apparently immunocompetent, male from India.

**Case report**

We present a case of a 55-year-old Indian male with a history of alcohol abuse and chronic smoking but without underlying immunosuppressive condition or disease who presented in 2009 to the Out Patient Department of General Surgery (OPD), Government Medical College Hospital, Chandigarh, India, with a localized, painful nodule dorsally on the right wrist joint that had appeared abruptly 3–4 months earlier. He did not recall any history of trauma or puncture to the wrist and denied any disease or relevant health problem in the past. He had an intensive contact with soil, as he was a laborer by profession. Dermatological examination was unremarkable except for a single 2×1.5 cm, soft to firm, well-defined, tender, slightly fluctuant nodule which did not adhere to the underlying tissues (Fig. 1A). No discharge or granules (the hallmark of mycetoma) were observed and no signs of skin inflammation above the lesion were apparent. Radiography of the site of infection was normal with no visible bone destruction or osteomyelitis and no significant regional lymphadenopathy was noted. Radiography revealed only a small swelling of soft tissue over the dorsum of the wrist joint with no evidence of recent or past fracture. Laboratory investigations including blood cell counts, blood chemistry, routine urine examination, liver and renal functions were within normal ranges. Chest X-ray and echocardiogram (ECG) were found to be normal and HIV antibodies were not detected. Fine needle aspiration of the mass was performed and showed an inflammatory cell infiltrate. Histopathological examination of the excised swelling has showed non-specific inflammation. All initial bacteriological and mycological investigations were negative.

Over the next few weeks, the patient noticed a small area of swelling on the anterior side of the left knee joint which slowly progressed into a painful nodule. The subcutaneous nodule was approximately 1.5×1.5 cm in size, soft to firm, tender, mobile in all directions and fluctuant on examination (Fig. 1B). Overlying skin appeared normal and there was no visible discharge and no sign of inflammation.

Fine needle aspiration of the swelling on the anterior side of the left knee joint was performed which yielded thick purulent fluid. Smears were prepared from the aspirated material and stained by May-Grünwald Giemsa, Haematoxylin and Eosin and Periodic Acid Schiff (PAS) stains. The remaining material was kept for mycological investigation. Direct examination (KOH 10%) revealed dark, septate hyphae, with no specific arrangement (Fig. 2A). Cytological examination revealed suppurative inflammation consisting of dense neutrophilic infiltrate, few

---

**Fig. 1** (A) Soft to firm, single, well-defined, tender, slightly fluctuant nodule on the dorsal aspect of right wrist joint measuring 2×1.5 cm. (B) Subcutaneous nodule (1.5×1.5 cm), soft to firm, tender, mobile in all directions on the anterior face of the left knee.
Multiple subcutaneous cysts due to *Exophiala spinifera*

After one week and these were morphologically classified as an *Exophiala* species. Stock cultures were maintained on slants of 2% malt extract agar (MEA, Difco) at 24°C [12], and the strain was deposited in the CBS-KNAW culture collection, Utrecht, The Netherlands, with accession number CBS 125607. Microscopic studies using slide culture techniques with PDA were conducted to induce sporulation and to suppress growth of aerial hyphae [13]. Slides of up to one-week-old cultures were made in lactic acid or lacto-phenol cotton blue and light micrographs were taken with a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Fi1 camera. Colonies growing restrictedly, slimy or at least mucoid at the centre, olivaceous grey to brownish black, with olivaceous black reverse (Fig. 3A, 3B). Microscopic features were the presence of erect, long, multi-celled conidiophores producing obovoidal conidia (1.8–3 × 2–4 μm) at the apices of the conidiogenous cells as well as from intercalary loci (Fig. 3C, 3D). Cardinal growth temperatures of strain CBS 125607 were: growth between 9 and 37°C, optimum at 27°C, scant growth at 37°C, no growth at 40°C. The isolate was subsequently

Lymphocytes, macrophages and multinucleated giant cells in the background of necrotic debris (Fig. 2B). A few branched and septate fungal structures were also seen which were highlighted by PAS stain (Fig. 2C–D). Muriform cells (the hallmark of chromoblastomycosis) were not detected. After complete local excision of the nodules, the patient completely recovered without additional antifungal therapy. There was no relapse during one-year follow-up and the patient was cured successfully.

**Mycology**

Clinical specimens of on the anterior side of the left knee joint were cultured from both cysts of the hand and the knee using standard techniques for the isolation of aerobic and anaerobic bacteria and on Sabouraud's glucose agar (SGA, Difco) and SGA supplemented with chloramphenicol (0.5 μg/ml) for fungi. Cultures were incubated at 25 and 37°C for up to one week. Results of bacteriological cultures were negative, while those for fungi were positive after 4–5 days of incubation. Growth of melanized fungi was recognizable after one week and these were morphologically classified as an *Exophiala* species. Stock cultures were maintained on slants of 2% malt extract agar (MEA, Difco) at 24°C [12], and the strain was deposited in the CBS-KNAW culture collection, Utrecht, The Netherlands, with accession number CBS 125607. Microscopic studies using slide culture techniques with PDA were conducted to induce sporulation and to suppress growth of aerial hyphae [13]. Slides of up to one-week-old cultures were made in lactic acid or lacto-phenol cotton blue and light micrographs were taken with a Nikon Eclipse 80i microscope equipped with a Nikon digital sight DS-Fi1 camera. Colonies growing restrictedly, slimy or at least mucoid at the centre, olivaceous grey to brownish black, with olivaceous black reverse (Fig. 3A, 3B). Microscopic features were the presence of erect, long, brown, multi-celled conidiophores producing obovoidal conidia (1.8–3 × 2–4 μm) at the apices of the conidiogenous cells as well as from intercalary loci (Fig. 3C, 3D). Cardinal growth temperatures of strain CBS 125607 were: growth between 9 and 37°C, optimum at 27°C, scant growth at 37°C, no growth at 40°C. The isolate was subsequently

Fig. 2 Direct microscopic examination (KOH 10%) of excised tissue from cyst revealed septate, irregular and branched hyphae (arrows) (40×). (B) Periodic acid shift staining (PAS) from biopsied material revealed a granulomatous and suppurative inflammatory lesion consisting of multinucleated giant cells with irregular, long, septate fungal hyphae (arrows). (C–D) May-Grünewald Giemsa staining show irregular septate hyphal elements.
sequenced. For molecular verification mycelia were grown on 2% (MEA) plates for 2 weeks at 27°C. DNA was extracted using an Ultra Clean Microbial DNA Isolation Kit (Mobio, Carlsbad, CA, USA) according to the manufacturer’s instructions. ITS rDNA was amplified using primers V9G (5′-TTACGTCCCTGCCCCTTTGTA-3′) and LS266 (5′-GCATTCCCAAACAAGTCGACTC-3′) and sequenced with the internal primers ITS5 (5′-GGAAGTAGGACGGCGCTGCGG-3′), ITS1 (5′-CCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATGATGC-3′). PCR amplification and sequencing were according to Badali et al. [6]. Sequences were compared with GenBank and through local blast with a molecular database maintained for research purposes at the CBS-KNAW Fungal Biodiversity Centre, Utrecht, The Netherlands. The obtained isolate (CBS 125607) was identified as *Exophiala spinifera* by showing 99.9% similarity with the ex-type strain of that species (CBS 899.68/H11005/AY156976), which had originally been isolated from a case of nasal granuloma in the USA. The sequence from isolate CBS 125607 determined in this study has been deposited in GenBank under accession number GU980971. The molecular investigation confirmed the mycological diagnosis, and histopathological observation led to the final diagnosis of a phaeohyphomycotic cyst due to *E. spinifera*.

**In vitro antifungal susceptibility testing**

The *in vitro* antifungal susceptibility of *E. spinifera* was determined by microbroth dilution according to the Clinical and Laboratory Standard Institute (CLSI) document M38-A2 [14]. Inoculum suspensions were prepared from 14 days potato dextrose agar (PDA; Difco) cultures by adding sterile saline solution with Tween 40 (0.05%) and slightly scraping the surface of mature colonies with a sterile cotton swab. The homogeneous conidial suspensions were then transferred to sterile tubes and the supernatants were adjusted spectrophotometrically at 530 nm wavelength to an optical density (OD) that ranged from 0.17–0.15 (68–71 T%). Therefore, the final size of the stock inoculum suspensions of the isolates tested ranged from $0.4 \times 10^3$ to $3.1 \times 10^4$ CFU/ml as performed by quantitative
Multiple subcutaneous cysts due to Exophiala spinifera

Discussion

Most infections with melanized hyphal elements in tissue are caused by black yeast-like fungi, which belong to the ascomycete order Chaetothyriales. Members of this order and particularly those in the family Herpotrichiellaceae are frequently encountered in human infections, causing disorders ranging from mild cutaneous lesions to fatal brain abscesses [4]. In tissue, the hyphae of these fungi sometimes remain hyaline, but melanin may then be visualized with Fontana-Masson staining [15], excluding for example, Aspergillus, dermatophytes and yeasts. In subcutaneous cases of infection, minor trauma, even when unrecognized by the patient, is supposed to be the inciting factor [16]. Lesions eventually occur at the site of inoculation and may become verrucous in the case of chromoblastomycosis, develop into a mycetoma, or remain a mild cutaneous infection. Cystic infections, which are encapsulated and thus cause limited destruction, are occasionally encountered [17].

In the environment Exophiala species are found in unusual (micro) habitats determined by the species’ oligotrophy and tolerance to toxins [18]. Confirmed strains of E. spinifera can be isolated from soil near animal burrows, from rotten sugary plant material and from fruits and juices [10]; repeated occurrence on pineapple in the tropics has been reported [3]. Most strains known as E. spinifera are, however, of clinical origin. Up until now there are reports of 22 cases of human disorder due to E. spinifera in the English literature (see Table 1). In a review, Harris et al. [11] reported 15 cases from the world of literature which are included in Table 1. Some infections in adolescents ended as fatal disseminated disease. De Hoog et al. [10] listed two more such cases from China and noted that the disseminated infections were mostly in children and adolescents without known underlying disease or immunosuppression. Infections in adults were local, irrespective of their immune status. This also holds true for the report by Harris et al. [11] and for the present case. Although, since the original isolates no longer are available from most cases published in the older literature, their identification down to the species level cannot be performed by molecular methods. It is possible that the etiological agent may have been misdiagnosed. In this article, a case of cysts caused by E. spinifera matching the original clinical concept of the species is presented.

At present no standard antifungal therapy can be recommended and little is known about the relation between MIC and clinical outcome for infections by melanized fungi.

Table 1 All cases of human disorder due to Exophiala spinifera reported in the English literature.

<table>
<thead>
<tr>
<th>Case number</th>
<th>Human disorder</th>
<th>Immunological status</th>
<th>Origin</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chromoblastomycosis</td>
<td>unknown</td>
<td>India</td>
<td>1958</td>
<td>[24]</td>
</tr>
<tr>
<td>2</td>
<td>Chromoblastomycosis</td>
<td>immunocompromised</td>
<td>USA</td>
<td>1992</td>
<td>[25]</td>
</tr>
<tr>
<td>3</td>
<td>Chromoblastomycosis</td>
<td>immunocompetent</td>
<td>USA</td>
<td>1996</td>
<td>[19]</td>
</tr>
<tr>
<td>4</td>
<td>Chromoblastomycosis</td>
<td>immunocompetent</td>
<td>Senegal</td>
<td>1998</td>
<td>[26]</td>
</tr>
<tr>
<td>5</td>
<td>Chromoblastomycosis</td>
<td>immunocompromised</td>
<td>–</td>
<td>1998</td>
<td>[27]</td>
</tr>
<tr>
<td>6</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompromised</td>
<td>USA</td>
<td>1967–68</td>
<td>[20]</td>
</tr>
<tr>
<td>7</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>El Salvador</td>
<td>1983</td>
<td>[28]</td>
</tr>
<tr>
<td>8</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>India</td>
<td>2003</td>
<td>[29]</td>
</tr>
<tr>
<td>9</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>Mexico</td>
<td>2005</td>
<td>[30]</td>
</tr>
<tr>
<td>10</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>India</td>
<td>2006</td>
<td>[31]</td>
</tr>
<tr>
<td>11</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>USA</td>
<td>2008</td>
<td>[32]</td>
</tr>
<tr>
<td>12</td>
<td>Cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>USA</td>
<td>2009</td>
<td>[11]</td>
</tr>
<tr>
<td>13</td>
<td>Sub-cutaneous phaeohyphomycosis</td>
<td>immunocompromised</td>
<td>USA</td>
<td>1984</td>
<td>[33]</td>
</tr>
<tr>
<td>14</td>
<td>Sub-cutaneous phaeohyphomycosis</td>
<td>immunocompromised</td>
<td>Argentina</td>
<td>1989</td>
<td>[34]</td>
</tr>
<tr>
<td>15</td>
<td>Sub-cutaneous phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>India</td>
<td>1993</td>
<td>[35]</td>
</tr>
<tr>
<td>16</td>
<td>Sub-cutaneous phaeohyphomycosis</td>
<td>immunocompromised</td>
<td>Pakistan</td>
<td>1994</td>
<td>[36]</td>
</tr>
<tr>
<td>17</td>
<td>Disseminated phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>–</td>
<td>1984</td>
<td>[37]</td>
</tr>
<tr>
<td>18</td>
<td>Disseminated phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>China</td>
<td>1987</td>
<td>[38]</td>
</tr>
<tr>
<td>19</td>
<td>Disseminated phaeohyphomycosis</td>
<td>immunocompetent</td>
<td>China</td>
<td>1987</td>
<td>[39]</td>
</tr>
<tr>
<td>20</td>
<td>Disseminated phaeohyphomycosis</td>
<td>immunocompromised</td>
<td>Japan</td>
<td>2004</td>
<td>[40]</td>
</tr>
<tr>
<td>21</td>
<td>Sub-cutaneous phaeohyphomycosis cyst</td>
<td>immunocompromised</td>
<td>France</td>
<td>2005</td>
<td>[41]</td>
</tr>
<tr>
<td>22</td>
<td>Sub-cutaneous phaeohyphomycosis cyst</td>
<td>immunocompetent</td>
<td>India</td>
<td>2010</td>
<td>Current case</td>
</tr>
</tbody>
</table>
and therefore surgical excision and cryosurgery have successfully been applied in a number of cases. A combination of surgical and antifungal treatment might be useful. The newer triazole agents, itraconazole and posaconazole expand the therapeutic options against this mycosis.

Correct identification of the causative agent of phaeohyphomycosis is clinically important, because different genera have different susceptibility patterns to antifungal agents [21]. Most patients infected with E. spinifera have received oral itraconazole at different dosages. Previous in vitro antifungal susceptibility testing of E. spinifera has shown that the widest range and the highest MICs were recorded for amphotericin B [21,22]. In contrast, quite uniform patterns were obtained for itraconazole and posaconazole [21,22]. Our results were in line with these studies, demonstrating that posaconazole and itraconazole have the highest in vitro antifungal activity (0.063 μg/ml and 0.125 μg/ml, respectively) against E. spinifera CBS 125607, whereas caspofungin (4 μg/ml) and anidulafungin (2 μg/ml) were less effective. Similar results have been recently reported for E. dermatitidis [23]. Posaconazole and itraconazole seem to be the best drugs for treating E. spinifera infections although, according to literature, E. spinifera can become resistant to itraconazole during treatment [15,25]. We have to take into account that systemic infections due to E. spinifera may present in otherwise healthy adolescents and thus principally are able to resist the ripening immune system. Despite the chronic course of the infection, adequate monitoring and therapy is recommended.

Acknowledgements

This study was supported by a grant (No. 13081) to H. Badali from the Ministry of Health and Medical Education of Islamic Republic of Iran and the School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

Declaration of interest: J. F. Meis received grants from Astellas, Merck, Basilea and Schering-Plough. He has been a consultant to Basilea, Merck and Schering-Plough and received speaker’s fees from Merck, Pfizer, Schering-Plough and Janssen Pharmaceutica. All the other authors report no potential conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Multiple subcutaneous cysts due to Exophiala spinifera


This paper was first published online on Early Online on 7 September 2011.