Invasive *Scytalidium dimidiatum* Infection in an Immune Competent Adult: Case Report and Review

Hila Elinav, 1 Uzi Izhar, 2 Shmuel Benenson, 1 Dan Admon, 3 Carlos Hidalgo-Grass, 1 Itzhack Polacheck, 1 and Maya Korem 1

1 Department of Clinical Microbiology and Infectious Diseases, 2 Department of Cardiothoracic Surgery and 3 Department of Cardiology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Running head: Invasive *Scytalidium dimidiatum* infection.

Keywords: *Scytalidium dimidiatum*; Immune-competent; Empyema; Invasive

Corresponding author:

Shmuel Benenson, MD

Department of Clinical Microbiology and Infectious Diseases

Hadassah-Hebrew University Medical Center, P.O. Box 12000

Jerusalem 91120

Israel.

Tel: 972-2-6776543

Fax: 972-2-6419545

E-mail: Benenson@Hadassah.org.il
Abstract

Scytalidium dimidiatum, a dematiaceous fungus, has been well established as an agent of dermatomycosis. There are few reports of invasive infection caused by S. dimidiatum, most in immunocompromised hosts. We present an immune-competent patient with pleural S. dimidiatum infection and review nine other published cases of invasive S. dimidiatum infections.
Case report

A 56-year-old male was admitted for evaluation after a few months of progressive dyspnea, fever, night sweats and loss of weight. His past medical history was significant for rheumatic heart disease which had necessitated mitral valve commissurotomy 20 years prior to his admission. As a consequence, he suffered from moderate mitral and tricuspid regurgitation and moderate right heart failure manifesting as chronic pleural effusion. He was treated with 40 mg/day furosemide, and warfarin due to chronic atrial fibrillation. The last thoracentesis, performed three years earlier, had yielded a bloody exudate but no pathogen was isolated.

On admission the patient appeared cachectic, had mild dyspnea and was afebrile. The jugular veins were maximally distended. Chest examination revealed dullness to percussion and reduced breath sounds over the right lung base, and a systolic heart murmur was heard at the apex, compatible with mitral regurgitation. Hepatosplenomegaly and moderate peripheral edema were noted as well.

Significant laboratory findings included: leukocyte count 5600 cells/μL with 61% neutrophils; hemoglobin level 11.7 g/dL; platelet count 164,000 platelets/μL; serum creatinine level 111 μmol/L; INR of 2.41; lactate dehydrogenase (LDH) 751 IU/L; γ-glutamyl transpeptidase 133 IU/L; alkaline phosphatase 257 IU/L. Chest computed tomography (CT) revealed a large loculated pleural effusion on the right with pleural thickening and total passive collapse of the right middle and lower lobes. Numerous enlarged mediastinal lymph nodes, up to 1.5 cm, were noted. Echocardiography showed enlargement of the right atrium and ventricle, in addition to mitral valve stenosis and regurgitation and moderate pulmonary hypertension.
A diagnostic thoracentesis revealed bloody exudative fluid with hemoglobin of 4.3 g/dL and WBC count of 2600 cells/μL with 66% neutrophils. The LDH level was 22,600 IU/L. Septate hyphae were detected by Calcofluor staining (Figure 1A). Culture on blood agar plates yielded a white hairy mold (Figure 1B), which progressed into a grayish-black fungus (Figure 1C). Minimum inhibitory concentrations (MICs) were determined by E-test® (AB Biodisk, Solna, Sweden). The mold was found to be susceptible to amphotericin B and voriconazole (MIC 0.032 mg/L) and to posaconazole (MIC 0.75 mg/L), but resistant to fluconazole (MIC >256 mg/L), itraconazole and caspofungin (MICs >32 mg/L).

The patient was treated for two weeks with intravenous voriconazole at 4 mg/kg q12h with no clinical or radiological improvement and an exploratory right thoracotomy was performed. Necrotic tissue covering both pleural surfaces and widespread fibrosis over the atelectatic right middle and lower lobes and over the entire mediastinum were seen. Extensive debridement and irrigation of the pleural space was performed, resulting in partial expansion of the right upper and lower lobes. Cultures taken during the procedure again yielded S. dimidiatum. The patient's clinical condition gradually improved and he was discharged 24 days after the surgery with a recommendation for continuance of the voriconazole treatment.

A month later, the patient was readmitted for dyspnea and recurrence of pleural effusion. Although septate hyphae were evident upon Calcofluor staining of the pleural fluid, no mold growth was detected. The serum and pleural fluid voriconazole levels were within therapeutic range, 1.45 mg/L and 1.51 mg/L, respectively (therapeutic range 1-5 mg/L (17)). Daily irrigation with amphotericin B (30 mg/day) injected through a pleural...
catheter was added to the treatment for three weeks and resulted in containment of the pleural effusion and clinical improvement. Six months later, still on voriconazole, the pleural effusion recurred.

*Morphological and molecular identification:* Culture on Sabouraud dextrose agar revealed effuse hairy colonies, dark gray to blackish-brown, with a deep ochraceous-yellow colony reverse. Microscopic examination demonstrated septate hyphae that were constricted at their prominent thick septations, giving the appearance of pseudohyphae (Figure 1D). Chains of arthroconidia with brown walls were produced in abundance on the aerial mycelium; many had two cells separated by a thick septum. Smooth- and thick-walled pycnidia formed after 2 weeks, showing typical pycnidioconidia with two-septate conidia and a darkened central cell upon dissection. The morphological features were compatible with the diagnosis of *S. dimidiatum*, also known as *Fusicoccum dimidiatum* (8); the pycnidial state is referred to as *Nattrassia mangiferae*. For molecular identification, two analyses were carried out: 28S rDNA and intergenic transcribed spacer (ITS). The sequence obtained by amplification and bidirectional sequencing of the 28S rDNA (region D1/D2) using primers NL1/NL4 (4) was compared with those in the GenBank DNA database (BLAST). The results gave 100% query coverage and 100% identity with *Scytalidium hyalinum* strain ATCC 38906 and 98% query coverage and 99% identity with *F. dimidiatum* (syn. *S. dimidiatum*) strain CBS 312.90. Alignment of the ITS region, amplified using the primer pair LR1/SR6R (23), resulted in 100% identity with *S. hyalinum* strain ATCC 38906 as well as with *F. dimidiatum* isolate CBS 251.49. *S. hyalinum* have been reported as a melanin-deficient cultural mutant of *S. dimidiatum* (19). Consequently the molecular methods based on ribosomal sequence, cannot
distinguish between these species and the only difference is based upon the pigment production. The nucleotide sequence of the case isolate has been submitted to the GenBank (Accession FJ648577).

The patient underwent a comprehensive investigation for immunodeficiency. HIV and nitro blue tetrazolium (NBT) tests were negative and total body CT scan failed to find any underlying malignancy. As free light chains were detected in the urine, bone marrow biopsy and rectal and abdominal fat biopsies were performed, ruling out multiple myeloma or amyloidosis. Blood IgG Kappa to IgG Lambda free light chains ratio was 1.5, compatible with polyclonal gammopathy, indicating an infectious origin.
Discussion

The dematiaceous (black-colored) fungi are a large and heterogeneous group of molds that cause mostly cutaneous, subcutaneous, and corneal infections (2, 7, 9). These organisms are widespread in the environment, found in soil, wood, and decomposing plant debris, and human infection results mainly after traumatic implantation (8, 13). *S. dimidiatum*, one of the human pathogens in this group, was proven to be a major pathogen of onychomycosis and tinea pedis, 34.6% and 46%, respectively, in a large study (22). *S. dimidiatum* is distinguished from dermatophytes by its characteristic sinuous, irregular hyphal appearance on direct microscopy of cutaneous specimens, its fast-growing colonies and its sensitivity to cycloheximide (21).

A search of the English-language literature up until 2008 produced only nine additional cases of invasive *S. dimidiatum* infection (cases of cutaneous or subcutaneous onychomycosis and keratitis were excluded), mostly in immunosuppressed patients (1, 3, 6, 10, 11, 15, 20, 21, 24) (Table 1). The underlying conditions reported, similar to those of invasive mucormycosis, were diabetes mellitus, cirrhosis, trauma, immunosuppressive therapy and chemotherapy, and transplantation. No predisposing immune deficiency was reported in only two cases (1, 15), one of them occurring after traumatic implantation (1). Variable clinical forms have been described including central nervous system abscess, endophthalmitis, sinusitis, osteomyelitis and fungemia. Five out of 10 patients died (50%) and one patient that suffered from endophthalmitis was cured only after enucleation.

Invasive mold infection is often difficult to eradicate by antifungal agents alone and usually necessitates surgical debridement. In the present case, the *S. dimidiatum* was isolated during the operation after two weeks of intravenous voriconazole treatment.
Assessment of in-vitro activity of various antifungal agents has demonstrated that amphotericin B and voriconazole exhibit the lowest MIC values for *Scytalidium* spp. (14), though amphotericin B alone was found ineffective in treating cerebral infection in one case (10). It is thought that melanization in *S. dimidiatum* may play a role in drug resistance (16); however, it appears that the white and black isolates of *Scytalidium* spp. are equally resistant to antifungals (18).

Fungal thoracic empyema, which usually affects hospitalized patients, most often in intensive-care units, is associated with high morbidity and mortality rates and its incidence has increased in recent years (12). In this retrospective analysis from Taiwan, overall mortality was estimated at 73%. Repeated thoracentesis was found to be among the major causes of fungal empyema, suggesting direct inoculation during the procedure. Those patients treated with systemic antifungal agents and surgery had a higher survival rate. In the present case of invasive pleural *S. dimidiatum* infection, the port of entry of the mold into the pleural space is unknown. The previous thoracentesis was performed three years prior to admission, the patient had no onychomycosis and no history of trauma was reported. Although the root of invasion to the pleural space is unknown, the chronocity of the disease as evidenced by hepatosplenomegaly and polyclonal gammopathy, suggests that it was introduced to the pleural space during the thoracentesis of three years previously. It is possible that the chronic pleural effusion and the presence of blood in the pleural space encouraged fungal growth and invasiveness.

Despite extensive debridement and irrigation of the pleural space and prolonged systemic antifungal therapy, local symptomatic disease recurred a month later. Re-operating was not an option due to the patient’s poor general condition, and an alternative
treatment was required. Intrapleural amphotericin B injection via pleural catheter was added to the treatment based upon a recent publication regarding the use of indwelling pleural catheters for chronic pleural infection (5). Clinical laboratory and radiographic studies performed six months later suggested stabilization of the patient’s pleural disease, with marked diminution of the fluid and absence of *S. dimidiatum*.

This case of invasive *S. dimidiatum* infection is unique in the organ involved, the chronicity of the infection and the absence of immunodeficiency. Along with the reviewed cases, it emphasizes the rarity of this entity and its relatively poor prognosis. In addition to appropriate antifungal therapy, it would be prudent to consider surgical intervention early in the course of the disease in order to improve outcome.
Acknowledgements

Potential conflict of interest. All authors: no conflicts.

No financial support was obtained.
Figure Legends:

Figure 1A
Calcofluor staining of the pleural effusion, demonstrating septate hyphae.

Figure 1B
Culture on blood agar demonstrating young white-colored mold.

Figure 1C
Culture on Sabouraud dextrose agar demonstrating mature effuse hairy colonies, dark gray to blackish-brown.

Figure 1D
Microscopic examination demonstrating septate hyphae with constrictions at their prominent thick septations, giving the appearance of pseudohyphae.
References


13
Table 1. Case reports of patients with invasive *Scytalidium dimidiatum* infection.

<table>
<thead>
<tr>
<th>Author (reference)</th>
<th>Age/Year</th>
<th>Country</th>
<th>Clinical form</th>
<th>Immune status</th>
<th>Treatment</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan et al (21)</td>
<td>31/M 2008</td>
<td>Canada</td>
<td>CNS abscess</td>
<td>Renal transplant</td>
<td>Voriconazole</td>
<td>Died</td>
</tr>
<tr>
<td>Mani et al (15)</td>
<td>18/M 2008</td>
<td>India</td>
<td>CNS abscess</td>
<td>Immune competent</td>
<td>Amphotericin B/Posaconazole&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Died</td>
</tr>
<tr>
<td>Sadeghi Tari et al (20)</td>
<td>60/M 2005</td>
<td>Iran</td>
<td>Endophthalmitis</td>
<td>Post-trauma + diabetes + cirrhosis</td>
<td>Amphotericin B</td>
<td>Died</td>
</tr>
<tr>
<td>Willinger et al (24)</td>
<td>62/M 2004</td>
<td>Austria</td>
<td>Skin + vertebra + lungs</td>
<td>Renal transplantation</td>
<td>Amphotericin B/Voriconazole&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Died</td>
</tr>
<tr>
<td>Geramishoar et al (10)</td>
<td>17/M 2004</td>
<td>Iran</td>
<td>CNS abscess</td>
<td>Immunosuppressive therapy</td>
<td>Amphotericin B</td>
<td>Died</td>
</tr>
<tr>
<td>Dunn et al (6)</td>
<td>51/F 2003</td>
<td>USA</td>
<td>Sinusitis</td>
<td>Lung transplantation</td>
<td>Amphotericin B/Voriconazole&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Cured</td>
</tr>
<tr>
<td>Gumbo et al (11)</td>
<td>60/M 2002</td>
<td>Zimbabwe</td>
<td>Endophthalmitis</td>
<td>Post trauma + diabetes + cirrhosis</td>
<td>Ketoconazole</td>
<td>Cured</td>
</tr>
<tr>
<td>al-Rajhi et al (1)</td>
<td>46/M 1993</td>
<td>Saudi Arabia</td>
<td>Endophthalmitis</td>
<td>Post trauma</td>
<td>IO Miconazole + Amphotericin B</td>
<td>Enucleation</td>
</tr>
<tr>
<td>Benne et al (3)</td>
<td>13/M 1993</td>
<td>Netherlands</td>
<td>Bacteremia</td>
<td>Chemotherapy</td>
<td>Amphotericin B</td>
<td>Cured</td>
</tr>
<tr>
<td>Present case</td>
<td>56/M 2007</td>
<td>Israel</td>
<td>Empyema</td>
<td>Immune competent</td>
<td>Voriconazole</td>
<td>Alive</td>
</tr>
</tbody>
</table>

CNS, central nervous system; Amphotericin B, amphotericin B; IO, intraocular; Top., topical

<sup>a</sup>Amphotericin B was changed to posaconazole

<sup>b</sup>Amphotericin B was changed to voriconazole