Fatal Invasive Infection with Fungemia Due to Microascus cirrosus after Heart and Lung Transplantation in a Patient with Cystic Fibrosis

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Received 21 January 2011/Returned for modification 28 February 2011/Accepted 20 April 2011

Scopulariopsis species are rarely but increasingly recognized as opportunistic pathogens in immunocompromised patients. We report on a patient suffering from cystic fibrosis who developed disseminated fungal infection due to a rare Scopulariopsis species, Microascus cirrosus, after heart and lung transplantation. Despite antifungal combination therapy with voriconazole and caspofungin, the patient died 4 weeks after transplantation. Diagnostic difficulties and optimal management of disseminated Scopulariopsis/Microascus infections are discussed.

CASE REPORT

A 36-year-old man suffering from cystic fibrosis was admitted to our hospital in June 2009 for a heart and bilateral lung transplantation. He had a first lung transplantation in 1997 but had chronic rejection since 2001 in spite of several immunosuppressive regimens. His medical history also included renal failure (hemodialysis since 2007), diabetes mellitus, and airway colonization with a multiresistant Pseudomonas aeruginosa. Short antifungal therapy courses had been prescribed over the past years for various yeasts (Candida spp.) and molds (Aspergillus, Paecilomyces, and Fusarium species) colonizing his respiratory tract, but there was no evidence of previous isolation of a Scopulariopsis species. At the time of admission, sputum cultures grew only Candida albicans, and fluconazole was thus prescribed (200 mg intravenously [i.v.] after each dialysis).

Following graft surgery, the immunosuppressive regimen included induction therapy with basiliximab (anti-CD25 antibody [20 mg i.v. on the day of transplantation and on day 4]) and solumedrol (10 mg/kg of body weight on the day of transplantation, followed by three 120-mg doses per day), followed by cyclosporine adjusted according to dialysis parameters to reach therapeutic blood levels (300 to 350 ng/ml) and solumedrol. Antimicrobial therapy consisted of a broad-spectrum antibiotic therapy with tobramycin, vancomycin, piperacillin, and tazobactam. Although the patient seemed to do well in the early postoperative period, with apyrexia and extubation on day 3, his white blood cell count (WBC) increased slowly starting on the day after transplantation, reaching $20 \times 10^9$ cells/liter by day 8 postsurgery. On day 11 after the operation (WBC = $30 \times 10^9$ cells/liter), a thoracic computed tomodensitometry (TDM) revealed bilateral pulmonary effusions (Fig. 1A), which were drained on day 19 without clinical improvement. Bilateral hemorrhagic pleural fluid specimens revealed several regular, hyaline, and branched septate hyphae on direct examination (Fig. 2A), leading to the diagnosis of proven invasive fungal infection (IFI) according to the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) criteria (8). The patient was given an antifungal combination therapy of voriconazole i.v. (6 mg/kg every 12 h as a loading dose followed by two 4-mg/kg doses a day) and caspofungin (70 mg/day as a loading dose followed by 50 mg/day) while awaiting mycological identification. A bronchial fibroscopy, performed on day 23 because of subcutaneous emphysema and air bubbles coming from the pleural drain, highlighted pseudomembranes on the surgery scar. A thoracic TDM showed the persistence of the bilateral pleural effusions associated with pneumothorax and a slight pneumomediastinum (Fig. 1B).

Surgery was decided on day 25 to suture the left pulmonary membranes on the surgery scar. A thoracic TDM showed the persistence of the bilateral pleural effusions associated with pneumothorax and a slight pneumomediastinum (Fig. 1B). Surgery was decided on day 25 to suture the left pulmonary parenchyma and to remove pleural clots. A blood culture (BacCee Mycosis IC/F; Becton Dickinson, Sparks, MD) was positive on day 27. Direct smear examination revealed several filamentous and septate hyphae with intercalary chlamydospore-like cells (Fig. 2B). The patient died of multiorgan failure on day 28.

All serum Aspergillus galactomannan antigen assays (GM), performed over time on days 11, 14, 15, 24, and 25, were negative (Platelia Aspergillus; Bio-Rad, Marnes-la-Coquette, France). Fungal cultures of the bilateral pleural specimens...
withdrawn on day 19, and incubated at both 26°C and 35°C on Sabouraud’s dextrose agar, revealed restricted, white and velvety colonies after 6 days of incubation. Microscopic examination revealed branched, hyaline, regular, and septate but sterile hyphae. All other specimens recovered on day 22 (bronchial secretions) and during surgery (intrapericardial fluid and a blood clot resulting from hemothorax and bronchial secretions) also revealed several septate hyphae after microscopic examination and Gomori-Grocott methenamine silver (GMS) stain and similar colonies after a few days of culture as well as for the blood culture. Identification was finally obtained after death, by sequencing the D1/D2 region of the 28S rDNA. Comparison of the nucleotide sequences (567 bp) with the GenBank database revealed 99.6% similarity with Microascus cirrosus reference strain CBS 217.31 (accession number AF275539) and 96% with Scopulariopsis brevicaulis strain IFM 54315 (accession number AB36375).

Several weeks later, phenotypic characteristics consistent with the genus Scopulariopsis were obtained using agar block culture on slides: annellidic and ampulliform conidiogenous cells produced chains of truncated, bullet-shaped, and smooth hyaline conidia, which became melanized in old cultures (Fig. 3). Unfortunately, and despite prolonged incubation for several months on different media (Sabouraud’s dextrose agar, potato-dextrose agar [PDA], potato-carrot agar [PCA], and oatmeal agar [OA]) and different temperatures (25°C and 30°C), no sexual stage (production of ascocarps, teleomorph) was obtained. Antifungal susceptibility testing was performed using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standardized methodology and Etest (21). Both techniques uncovered high MICs for all antifungal agents tested (8 μg/ml for amphotericin B, 4 μg/ml for caspofungin and terbinafine, and more than 8 μg/ml for itraconazole, voriconazole, and posaconazole).

Although invasive aspergillosis (IA) remains, with candidiasis, the most frequent cause of invasive fungal infections (IFIs) worldwide, severe invasive infections due to other opportunistic hyphomycetes molds (including Scedosporium, Fusarium, and Scopulariopsis) are emerging in the ever-growing population of immunocompromised patients (32). In two recent multicenter prospective studies that investigated the incidence of IFIs in hematopoietic stem cell transplant (HSCT) recipients and solid-organ transplant (SOT) recipients, these molds accounted for up to 8% of all IFIs in both populations and up to 20% in lung transplant recipients (13, 19). Fungi belonging to the genus Scopulariopsis are widely found in our environment (soil, food, paper, and other substrates) (7). As a
result of its keratinolytic activity, superficial infections, such as onychomycosis in immunocompetent subjects (1 to 10% of such infections), are the most common clinical setting (4, 9). Scopulariopsis spp. have also been involved in a large spectrum of infections in immunocompromised subjects, including nasal infections, peritonitis, pulmonary infections, endocarditis as well as cerebral infections, and diagnosis is sometimes made at autopsy (2, 11, 12, 14, 18, 30, 31). Whereas Scopulariopsis brevicaulis accounts for most of the cases, other Scopulariopsis species, some existing as a sexual state or teleomorph (most in the genus Microascus), have also recently been described as human pathogens: Scopulariopsis acremonium, S. brumptii, S. candida, and S. cinerea (Microascus cinereus) (2, 3, 14, 20).

Herein, we report on a disseminated M. cirrosus infection after a heart and lung transplantation in a patient suffering from cystic fibrosis. In light of this case and previously reported cases, optimal management of these infections and diagnostic issues are discussed.

Disseminated infections (involving at least two noncontiguous sites and proved by histology and/or culture) due to Scopulariopsis/Microascus are uncommon (only eight cases have been reported since 1987). As shown in Table 1, most patients with disseminated infection were transplant recipients (five HSCT recipients or bone marrow transplant [BMT] recipients for hematological malignancy, and three SOT recipients, including two lung recipients). Dissemination to the skin seems to be a common event, being noted in more than half of the patients with disseminated Scopulariopsis infections (patients 2, 3, 4, 6, and 7 in Table 1). In the present case, infection occurred early (day 19) in the heart and bilateral-lung transplantation period in comparison to the case described by Wuyts et al., which involved a single-lung-transplant recipient (34). The hypothesis of possible transmission from the donor to our patient was excluded, as fungal cultures from the graft preservation fluid were negative. Such a difference in the median time to the onset of infection/diagnosis after solid-organ transplantation, according to the type of transplant, has been reported previously for IA (24). In this study, IA occurred earlier in the heart-lung transplant recipients (0.7 months) than in patients receiving bilateral (3.9 months) or single-lung transplants (5 months).

Disseminated Scopulariopsis infections are almost invariably fatal. Underlying conditions, delayed diagnosis, and a high level of resistance of Scopulariopsis to conventional antifungal agents are probably responsible for the poor outcome. MICs of amphotericin B, azoles, caspofungin, and terbinafine were high against our M. cirrosus isolate comparable to what is reported for clinical strains of S. brevicaulis (1, 5, 10). Potent synergistic activities between antifungal agents against S. brevicaulis have been reported with the highest synergistic effect for posaconazole and terbinafine (6). Other combinations, such as terbinafine with voriconazole or caspofungin with azoles (posaconazole and voriconazole), show synergy for some strains. Synergy of voriconazole with caspofungin was also obtained in vitro for a clinical isolate of S. brevicaulis recovered from a patient with disseminated infection (26). However, the patient died, as did our patient, when treated with the same antifungal combination, showing once again the lack of correlation between in vitro results and treatment efficacy.

The scarcity of Scopulariopsis infections explains the lack of consensus regarding the best antifungal regimen. Most patients (7/9) were prescribed various combination therapies with no obvious best regimen based on survival (Table 1). The contribution of surgery is also difficult to assess. Other compounds, such as miltefosine, widely used in the treatment of visceral leishmaniasis and exhibiting in vitro fungicidal activity against several molds, may need evaluation (29, 33). Adjunct therapies with cytokines, such as gamma interferon, and growth factors, such as granulocyte colony-stimulating factor (G-CSF), have been used to treat IFIs, including Scopulariopsis infections (14, 25, 27, 30).

Difficulties encountered in the management of these disseminated infections underline the need for early diagnosis of these infections. As shown in Table 1, blood cultures may be important to diagnose invasive Scopulariopsis infections, as they are during infections by Scedosporium and Fusarium spp. Here, direct smear of the positive blood culture revealed several septate hyphae, some with an unusual aspect, harboring intercalary and balloononed, chlamydospore-like structures. This interesting and unusual feature has previously been described in M. cirrosus cultures, but to the best of our knowledge, it has never been reported in clinical samples (17). Antigen detection is potentially interesting. Aspergillus galactomannan was detected during a case of disseminated S. brevicaulis when there was no evidence of Aspergillus infection (22). Here, as in other cases of disseminated infection, all the GMs performed since the day of transplantation were negative. Although histopathological examination showing septate hyphae is an important step in diagnosis, species identification is mandatory and can require molecular methods, as in our case. Over the past 15 years, only two cases involving M. cirrosus (not included in our review because of focal infection or dissemination not proven) have been reported (15, 30).

The present case is the third description of invasive infection caused by M. cirrosus in humans and the first in a SOT recipient patient. It underlines the need for increased awareness of infections caused by uncommon molds in immunocompro-
TABLE 1. Clinical and mycological characteristics of previously published cases of disseminated *Scopulariopsis/Microascus* infection

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Type of transplant (if any) and underlying disease</th>
<th>Time to diagnosis after transplantation</th>
<th>Mycological identification</th>
<th>Involved sites</th>
<th>Positive blood cultures</th>
<th>Antifungal agent(s)</th>
<th>Surgery</th>
<th>Outcome (survival time after diagnosis)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>M</td>
<td>BMT for CML</td>
<td>29 days</td>
<td><em>Scopulariopsis</em> sp.</td>
<td>Nasal septum, trachea, lungs, brain</td>
<td>Yes</td>
<td>AMB</td>
<td>Yes</td>
<td>Death (26 days)</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>M</td>
<td>SOT (liver) for primary sclerosing cholangitis</td>
<td>3–4 mo</td>
<td><em>S. brevicaulis</em></td>
<td>Skin, brain, lungs</td>
<td>No</td>
<td>AMB-MIC</td>
<td>Yes</td>
<td>Death (6–7 wk)</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>F</td>
<td>No underlying disease</td>
<td>3–4 mo</td>
<td><em>S. brevicaulis</em></td>
<td>Skin, lymph nodes, lungs</td>
<td>No</td>
<td>AMB-TRB, followed by TRB</td>
<td>Yes</td>
<td>Cure</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>M</td>
<td>HSCT (AML)</td>
<td>8–9 mo</td>
<td><em>S. brevicaulis</em></td>
<td>Skin, heart, lungs, kidneys, thorax</td>
<td>Yes</td>
<td>VRC-CAS</td>
<td>No</td>
<td>Death (11 days)</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>63</td>
<td>M</td>
<td>SOT (lungs) for chronic obstructive pulmonary disease</td>
<td>7–8 mo</td>
<td><em>S. acremonium</em></td>
<td>Heart, thyroid gland, stomach, kidneys, lungs</td>
<td>No</td>
<td>VRC-CAS</td>
<td>No</td>
<td>Death (1 day)</td>
<td>34</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>F</td>
<td>HSCT for AML</td>
<td>190 days</td>
<td><em>S. brevicaulis</em></td>
<td>Skin, lungs</td>
<td>No</td>
<td>AMBL, followed by AMBL-VRC</td>
<td>No</td>
<td>Death (22 days)</td>
<td>22</td>
</tr>
<tr>
<td>7</td>
<td>56</td>
<td>M</td>
<td>HSCT for CML</td>
<td>5–6 mo</td>
<td><em>Scopulariopsis</em> sp.</td>
<td>Skin, lungs</td>
<td>No</td>
<td>AMBL-CAS, followed by AMBL-VRC</td>
<td>No</td>
<td>Death (20 days)</td>
<td>28</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>F</td>
<td>HSCT for MM</td>
<td>499 days</td>
<td><em>S. acremonium</em></td>
<td>Sinus, vessel walls of carotid artery and middle cerebral artery</td>
<td>No</td>
<td>AMBL-POS, followed by CAS</td>
<td>No</td>
<td>Death (9 days)</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>M</td>
<td>SOT (heart and lung) for cystic fibrosis</td>
<td>19 days</td>
<td><em>M. cirrosus</em></td>
<td>Pleural fluid, intrapericardial fluid, blood clots, bronchial secretions</td>
<td>Yes</td>
<td>VRC-CAS</td>
<td>No</td>
<td>Present case</td>
<td>Present</td>
</tr>
</tbody>
</table>

*a Only cases involving at least two noncontiguous sites proved by histology and/or culture were considered.
*b M, male; F, female.
*c Abbreviations: BMT, bone marrow transplant; CML, chronic myelogenous leukemia; SOT, solid organ transplant; HSCT, hematopoietic stem cell transplant; AML, acute myeloid leukemia; MM, multiple myeloma.
*d Antifungal agent abbreviations: AMB, amphotericin B; AMBL, liposomal amphotericin B; CAS, caspofungin; ITC, itraconazole; MIC, miconazole; POS, posaconazole; VRC, voriconazole; TRB, terbinafine.
*e Death occurred early in the days following the diagnosis.
mised patients. Finally, in light of this and previously reported cases, several questions are raised. (i) Which antifungal(s) should be administered? (ii) Is there a place for adjuvant therapy, such as immunomodulatory regimens or new agents such as miltefosine, in the management of these highly life-threatening infections? Larger case series or prospective studies will probably help answer these questions and allow us to establish guidelines for the management of invasive Scopulariopsis/Microascus infections in immunocompromised patients.

Nucleotide sequence accession numbers. The nucleotide sequence of the isolate has been deposited in GenBank database under accession number HQ676488.

We are grateful to Francoise Dromer for fruitful discussions on the manuscript. We acknowledge Bernard Besse, Olivier Barre, and Damien Hoignard for technical assistance.

REFERENCES