Breakthrough Disseminated *Aspergillus ustus* Infection in Allogeneic Hematopoietic Stem Cell Transplant Recipients Receiving Voriconazole or Caspofungin Prophylaxis

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*Aspergillus ustus* is an uncommon clinical species which is poorly susceptible to antifungals. We report two cases of *A. ustus* infections that occurred in allogeneic stem cell transplant recipients while they were receiving either voriconazole or caspofungin. Prolonged use of these new antifungal agents may increase the risk of the emergence of resistant organisms.

CASE REPORT

Patient 1 was a 29-year-old male, weighing 80 kg, who was diagnosed with acute lymphoblastic leukemia in October 2002. During initial chemotherapy-induced neutropenia, he developed fever, cough, and nodular pulmonary infiltrates visible by computed tomography (CT) scan, suggestive of possible lung-invasive aspergillosis (2). Cultures of sputum were negative for *Aspergillus*, and *Aspergillus* antigenemia (Platelia *Aspergillus* assay; Bio-Rad, Marnes-la-Coquette, France) remained negative (index < 0.5). Treatment with oral voriconazole (200 mg twice a day after the loading dose) was initiated on 22 December 2002. Granulocyte recovery occurred 4 days later and was associated with the complete resolution of pulmonary lesions. A myeloablative genoidentical hematopoietic stem cell transplant (HSCT) was performed during the first complete remission, on 13 March 2003. A pre-HSCT lung CT scan revealed no abnormality. Voriconazole was continued as a secondary prophylaxis. Between April 2003 and January 2004, the patient experienced three episodes of severe graft-versus-host disease (GVHD), which were treated with cyclosporine, an increased dosages of steroids, and mycophenolate mofetil. In September 2003, the patient, who was afebrile, developed several necrotic cutaneous lesions for which he was admitted to the hospital. The chest CT scan revealed a nonspecific infiltrate in the right inferior pulmonary lobe. Mycological examination of a cutaneous biopsy specimen revealed septate and branched hyphae consistent with *Aspergillus* spp. A culture yielded *Aspergillus ustus*, which was identified by morphological methods, with confirmation by sequencing of the ITS1-5.8S-ITS2 region (Fig. 1). Cultures of sputum were also positive for *A. ustus*. Several *Aspergillus* antigenemia tests were positive, with galactomannan index values of up to 3. Voriconazole was discontinued, and amphotericin B deoxycholate (1 mg/kg of body weight/day) was started. Six days later, the patient developed right-leg paresis. Brain magnetic resonance imaging (MRI) revealed a single frontal lesion compatible with aspergillosis. Treatment was switched to liposomal amphotericin B (3 mg/kg/day) and caspofungin (70 mg/day). The patient clinically improved, with complete regression of both cutaneous lesions and neurologic abnormalities. Subsequent brain MRIs showed progressive regression of the lesion with scar formation. Subsequent lung CT scans showed a size decrease and excavitation of the pulmonary lesion. *Aspergillus* antigenemia decreased and remained negative after 18 December 2003. The patient later experienced several episodes of hemoptysis. Fungal hyphae compatible with a zygomycete were observed on direct mycological examination of sputum. Treatment with posaconazole was initiated, and a wedge lung resection was performed. Histological examination revealed characteristic broad, irregularly shaped, paucisephtated hyphae suggestive of a zygomycete. Cultures were negative. The patient was considered to be in complete remission of both *A. ustus* and zygomycete infections following this combined medical and surgical management. Unfortunately, he ultimately died from GVHD and *Escherichia coli* sepsis on 3 February 2004. An autopsy was not performed.

Patient 2 was a 17-year-old boy, weighing 64 kg, who was diagnosed with acute lymphoblastic leukemia in August 2001. He experienced a first complete remission but relapsed in March 2003. A second complete remission was obtained in September 2003. In August 2003, while he was neutropenic, he developed a proven disseminated aspergillosis due to *Aspergillus flavus* with cutaneous, pulmonary, hepatic, and esophageal involvement (2). He was treated with a combination of liposomal amphotericin B (5 mg/kg/day) and caspofungin (70 mg/day on day 1, 50 mg/day thereafter) for 25 days and then received caspofungin alone. A complete response of the aspergillosis to the therapy was obtained after 6 weeks. A matched unrelated peripheral stem cell transplant was performed on 9 January 2004. A pre-HSCT lung CT scan revealed micronodules in the right and left inferior lobes, which were considered to be scars.
Caspofungin was continued as a secondary prophylaxis. Granulocyte recovery occurred on day 26 posttransplant. He experienced severe acute GVHD beginning on day 7 posttransplant. First-line treatment of the GVHD included cyclosporine and steroids and thereafter mycophenolate mofetil, tacrolimus, and steroids. On 3 March 2004, the patient suddenly experienced bilateral conjunctivitis with retinitis and inflammation of the vitreous body. The patient’s Aspergillus antigenemia index increased to 2.85. The Aspergillus galactomannan index in the vitreous aspirate was 8.14, and cultures yielded A. ustus. Caspofungin was discontinued (total duration of caspofungin treatment, 197 days). Intravenous voriconazole (8 mg/kg/day after the loading dose) and liposomal amphotericin B (5 mg/kg/day) were initiated on 4 March 2004. Despite this treatment, the ocular lesions worsened and nodular, necrotic lesions of the skin appeared. Histological examination of a cutaneous biopsy showed hyphae compatible with Aspergillus spp. A brain MRI scan revealed one frontal lesion compatible with aspergillosis. The chest CT scan was normal. The patient died from disseminated aspergillosis with GVHD on 25 March 2004.

Invasive aspergillosis is an important cause of morbidity and mortality in HSCT recipients (12). Recent progress has been made in the treatment of aspergillosis, and voriconazole is now considered the first choice treatment for these patients (8). However, recent reports from different centers have documented the emergence of breakthrough fungal infections, mostly zygomycosis, in patients receiving voriconazole (9, 13, 15).

We report here on two cases of breakthrough invasive aspergillosis due to A. ustus in HSCT recipients while they were receiving secondary antifungal prophylaxis. Of note, no A. ustus strain had ever been previously isolated from clinical specimens or the environment in our hospital, although A. ustus is commonly found in both temperate and tropical soils (10). A. ustus is indeed an uncommon clinical species that may have a decreased susceptibility to voriconazole (16). As of January 2005, only 13 cases of invasive aspergillosis due to A. ustus have been reported in the medical literature, of which 6 cases were reported in allogeneic HSCT recipients (Table 1) (3–5, 7, 9, 10, 14, 16). Outcomes of infections were generally dismal. Ten patients died due to progressive aspergillosis. Only three patients were cured, none of whom were HSCT recipients. As previously described for A. ustus infections in HSCT patients, disseminated disease with skin involvement and increased Aspergillus antigenemia were observed in our two cases. It is noteworthy that the detection of Aspergillus antigen in the vitreous aspirate made an early diagnosis possible in the second case, 2 days before the results of cultures became known. The utility of Aspergillus antigen detection in samples other than serum specimens is currently being assessed (11).

The development of these unusual fungal infections in patients receiving antifungal treatment could be explained by a decreased susceptibility to the prescribed antifungals or by ineffective local or plasma concentrations. Using the EUCAST method adapted for filamentous fungi, we recorded MICs of amphotericin B of 1 µg/ml for both isolates (6). Itraconazole, voriconazole, and caspofungin MICs/minimal effective concentrations were ≥4 µg/ml, unusually high for Aspergillus species in our experience. However, these results are consistent with the data from the literature for A. ustus. Indeed, the 14 isolates of A. ustus tested in three different studies showed high MICs of itraconazole (a range of 1 to 8 µg/ml, with MICs of ≥2 µg/ml for 13 of 14 isolates) and voriconazole (a range of 0.25 to 8 µg/ml, with MICs of ≥4 µg/ml for 11 of 14 isolates) (3, 7, 16). Also, both isolates exhibited high minimal effective concentrations of caspofungin compared to our own data or to published data on Aspergillus species other than A. ustus (0.5 µg/ml) (1). In addition, the voriconazole plasma concentration determined in the first patient 3 h after oral dosing was 2.4 µg/ml, lower than the corresponding MIC, adding another factor for a breakthrough infection with a less “susceptible” isolate.

These two cases are another illustration of the risk of emergence of resistant organisms during the course of prolonged antifungal secondary prophylaxis in patients with severe and persisting immunodepression. Zygomycetes and A. ustus infec-

FIG. 1. Left panel: pigmented and smooth-walled conidiophore stipe of A. ustus with spherical vesicle and biseriate conidiogenous cells. Spherical conidia with very rough walls (magnification, ×1,000). Right panel: irregular ßülle cells of A. ustus (magnification, ×1,000).
TABLE 1. Summary of published cases of invasive infection in allogeneic HSCT recipients

<table>
<thead>
<tr>
<th>Duration (days)</th>
<th>Author</th>
<th>Type of transplant</th>
<th>Type or site of infection</th>
<th>Previous systemic antifungal diagnosis</th>
<th>Treatment</th>
<th>Outcome/cause of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>Imhof, A.</td>
<td>MM-UR</td>
<td>Disseminated</td>
<td>Voriconazole/primary prophylaxis</td>
<td>Liposomal amphotericin B</td>
<td>Death, date NA</td>
</tr>
<tr>
<td>40</td>
<td>Nakai, K.</td>
<td>RIST-MM-R</td>
<td>Disseminated</td>
<td>Fluconazole/primary prophylaxis</td>
<td>Amphotericin B</td>
<td>Death, day 68/aspergillosis and multiorgan failure</td>
</tr>
<tr>
<td>33</td>
<td>Verweij, J.</td>
<td>M-UR</td>
<td>Lung</td>
<td>None</td>
<td>Amphotericin B</td>
<td>Death, day 51/GI and aspergillosis</td>
</tr>
<tr>
<td>12</td>
<td>Baddley, J. W.</td>
<td>NA</td>
<td>Disseminated</td>
<td>Amphotericin B/primary prophylaxis</td>
<td>NA</td>
<td>Liposomal amphotericin B</td>
</tr>
<tr>
<td>285</td>
<td>Patient 1 (PR)</td>
<td>MM-UR</td>
<td>Disseminated</td>
<td>Voriconazole/secondary prophylaxis</td>
<td>Liposomal amphotericin B</td>
<td>Death, day 327/GVHD and sepsis E. coli</td>
</tr>
<tr>
<td>197</td>
<td>Patient 2 (PR)</td>
<td>MM-UR</td>
<td>Disseminated</td>
<td>Caspofungin/secondary prophylaxis</td>
<td>Voriconazole</td>
<td>Death, day 100/aspergillosis</td>
</tr>
</tbody>
</table>

*PR, present report. **MM-UR, matched/matched; UR, unrelated; RIST, reduced-intensity stem cell transplantation; T-, T-depleted.*