Scedosporium apiospermum Pneumonia after Autologous Bone Marrow Transplantation

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Although opportunistic infections after bone marrow transplantation (BMT) are very common, only five cases of *Pseudallescheria boydii* infection have been reported in the literature, two of which were autopsy findings. A case of *Scedosporium apiospermum* infection after BMT, treated initially with amphotericin B (total dose of 2.5 g) and then with itraconazole (for 25 days), is reported here. When the patient failed to improve, *Scedosporium apiospermum* pneumonia was diagnosed and therapy was changed. The patient was treated successfully with miconazole (600 mg/8h for 32 days) and ketoconazole (200 mg/8h for 7 days) plus surgery.

Scedosporium apiospermum is a ubiquitous saprophyte fungus distributed worldwide. It is a perfect fungus; i.e., it can reproduce by sexual or asexual reproduction. In its sexual form it is named *Pseudallescheria boydii* (but also has been named *Allescheria* or *Petriellidium boydii*). In human infections and cultures in the laboratory, it usually appears in its asexual form, named *Scedosporium apiospermum*. In special media, however, such as cornmeal agar and others, the fungus can develop its sexual phase, so identification in the lab can be performed with great accuracy.

This fungus is considered an opportunistic pathogen belonging to the *Ascomycete* family. Occasionally, *Pseudallescheria boydii* causes infections of cutaneous or subcutaneous tissues in the immunocompetent host (1). The most common extracutaneous site of this infection is the lung, manifesting as pulmonary fungus balls in a preformed cavity (1, 2). Invasive infection due to *Scedosporium apiospermum* is more commonly recognized in the immunocompromised host. Infections occur predominantly in the lower respiratory tract, though less frequently, cases have been reported in eyes, ears, sinuses, prostate gland, thyroid, endocardium, bone, and the central nervous system (1, 3–6).

Although opportunistic infections are very common after bone marrow transplantation (BMT), only few cases of *Pseudallescheria boydii* infection have been reported (6–10), two of which were autopsy findings (7, 8). We report a case of *Scedosporium apiospermum* infection treated successfully with miconazole and surgery. To our knowledge, only one similar case has been reported (9).

Case Report. A 20-year-old Caucasian woman received an autologous BMT for acute myeloid leukemia (AML) subtype M_1 in first remission. The diagnosis of AML had been established after examination of smears from bone marrow and blood in October 1991. Complete remission was achieved one month later with induction therapy (2 cycles of daunomycin plus C-Ara) followed by intrathecal chemotherapy (methotrexate plus C-Ara plus steroids). Remission was consolidated and maintained with the same drugs, but at a lower dosage. Two episodes of infection during the course of remission were recorded: sepsis and urinary tract infection with *Propionibacterium* spp. and *Escherichia coli*, respectively. Both were treated successfully with antimicrobial agents.

Bone marrow of the patient was harvested in March 1992 and purged ex vivo with Asta-Z. Day 0 designates the day of marrow infusion. The conditioning regimen included busulphan and cyclophosphamide. The patient was placed in a laminar air-flow room during her hospital stay and received no fungal chemoprophylaxis. Empiric antibiotic treatment with imipenem and teicoplanin was started on day 5 for fever (38.2°C) without microbiological positive cultures. She continued to be febrile, although all cultures remained negative; therefore, amikacin and amphotericin B (0.5 mg/kg/day) were added empirically.

On day 36 a chest radiograph showed a left upper pulmonary lobe infiltrate. The development of air crescents two days later suggested tissue invasion and necrosis by a fungal agent. Physical examination was within normal limits. The clinical findings were initially thought to be consistent with asper-

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gillosis, and thus treatment with amphotericin B was continued. Radiological improvement was observed on day 57, and when signs of marrow engraftment (> 500 granulocytes/μl and 20,000 platelets/μl) appeared on day 62, the patient was discharged.

The patient received a total dose of amphotericin B of 2.5 g. On day 81 treatment with oral itraconazole (400 mg/day) was started. A chest radiograph revealed a lower degree of infiltrate without cavitation, but on day 116 the patient developed cough without expectoration, left pleuritic pain, and low-grade fever. Itraconazole treatment was stopped on day 116. A chest radiograph showed progression of pulmonary lesions and left pleural effusion. This fluid was transudative in nature, and cultures for bacteria and fungi were negative. Protected specimen brush bronchoscopy and bronchoalveolar lavage were performed. Culture results were positive for *Pseudallescheria boydii*. Computed tomography showed an infiltrate in the lower section of the upper left and lower left lobes. Treatment with intravenous miconazole (600 mg/8h) was started on day 139 and maintained for 32 days. As miconazole was no longer available in the hospital, treatment had to be changed to ketoconazole on day 171 (200 mg/8 h for 7 days). Surgery was performed on day 146 with resection of the upper lobe and two nodes in the lower lobe. The patient improved clinically and radiologically and was discharged on day 181. After follow-up for 18 months, the hematologic disease of the patient remained in remission and no other pulmonary infection was present.

Bronchial samples were Gram stained and cultured on different types of media: blood and chocolate agars for bacteria and Sabouraud dextrose agar for fungi. The microscopical examination of the Gram-stained bronchial samples revealed dichotomous branching septate hyphae 3 to 4 μm wide, resembling those of *Aspergillus* spp. White colonies were noted after three days of incubation at 35°C in all three culture media.

The microscopical examination of colonies grown in these media stained with lactophenol cotton blue revealed branching septate hyphae of 1–3 μm in diameter with large, brownish, truncate base ovoid annelloconidia at the ends or on the short sides of conidiophores (Figure 1). The conidia, which usually occurred individually, measured 3–5 μm x 7–10 μm in size. All of these features led to an identification of *Scedosporium apiospermum*, the asexual form of *Pseudallescheria boydii*. A three-week subculture in cornmeal agar grew the sexual form of *Pseudallescheria boydii*: dark brown, thick-walled cleistothecia measuring 25 to 200 μm in diameter, which, upon rupture, released ascospores of a uniform size (Figure 2).

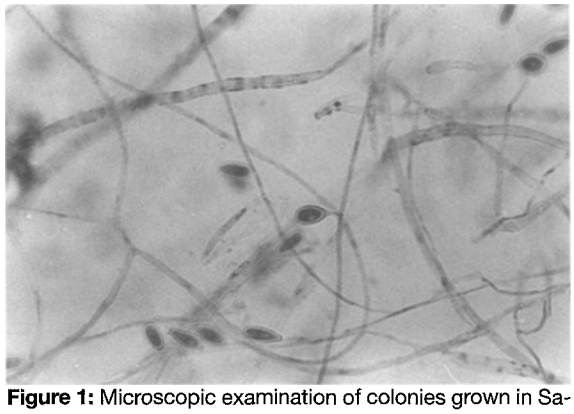

Figure 1: Microscopic examination of colonies grown in Sabouraud dextrose agar stained with lactophenol cotton blue. Branching septate hyphae of 1 to 3 μm in diameter with large, brownish, truncate base ovoid annelloconidia at the ends of conidiophores are seen.

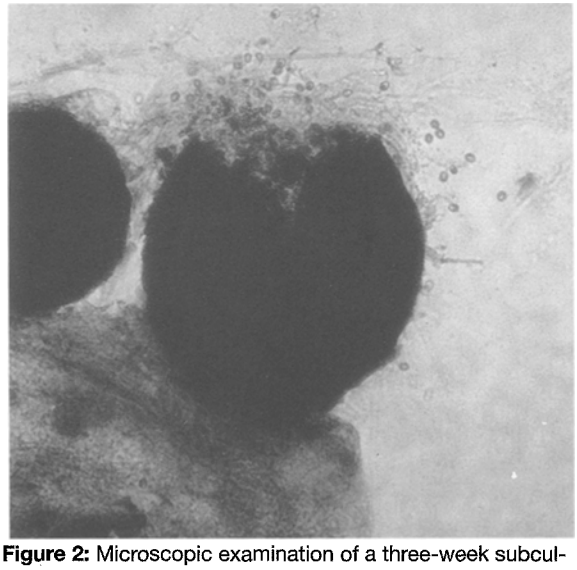

Figure 2: Microscopic examination of a three-week subculture in cornmeal agar, showing the sexual form of *Pseudallescheria boydii*: dark brown, thick-walled cleistothecia measuring 25 to 200 μm in diameter, which, upon rupture, released ascospores of a uniform size.

Minimal inhibitory concentrations were determined for amphotericin B (2 mg/l), flucytosine (16 mg/l), and the azoles ketoconazole (0.5 mg/l), itraconazole (1 mg/l), fluconazole (16 mg/l), and miconazole (2 mg/l).

Discussion. The most common clinical condition involving *Pseudallescheria boydii* is pedal mycetoma, although involvement in lung and lower

respiratory tract infections is also known. To our knowledge, only five cases of *Pseudallescheria boydii* infections after BMT have been reported (6–10). Two of the cases reported were autopsy findings (7, 8), and in one case the patient was treated successfully (9).

Amphotericin B, the treatment of choice for aspergillosis, is often not effective against *Pseudallescheria boydii*. As infections involving *Pseudallescheria boydii* require a treatment different from that used for infections involving *Aspergillus* spp., it is important to distinguish accurately between these two organisms. Histologic findings are insufficient for identification of these two fungi. The regularly and dichotomously septated mycelium of *Pseudallescheria boydii* (Figure 3) resembles that of *Aspergillus* (the most frequent fungal agent in these kind of infections) and other hyphomycete opportunists. Unless conidia are seen in the specimen, it is not possible to distinguish the mycelium from other fungi (1). Microbiological cultures of the samples are mandatory for confirmation of the causative agent.

Pseudallescheria boydii presents a serious threat to the immunocompromised patient because of its potentially pathogenic nature, but, more important, because little success has been achieved in these patients once infected (4, 10, 11). Miconazole appears to be the antifungal agent of choice. Regarding treatment with itraconazole, there are few studies conducted in immunocompromised patients. Some cases of successful treatment in the immunocompetent host have been reported (12). In the case reported here, the fungal disease showed evidence of progression after itraconazole treatment was started. This suggests that itraconazole did not have in vivo activity against this fungus, though a case similar to the one presented here, in which a patient was treated successfully with this drug at a similar dose, has been reported (9).

Itraconazole was administered at the maximum tolerated dosage (13), although some authors have suggested increasing the dosage to up to 600 mg/day in immunocompromised patients with deep-seated mycoses (14). Successful treatment with miconazole plus surgery resection has also been reported (15).

Pseudallescheria boydii grows on all laboratory media. Amphotericin B may not be effective against this fungus, but early diagnosis and rapid treatment appear to increase the chances of a favorable outcome.

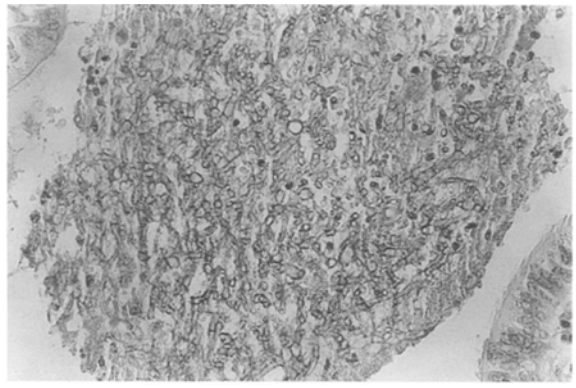

Figure 3: Histopathology section of bronchial lumen showing the regularly and dichotomously septated mycelium of *Pseudallescheria boydii*, resembling *Aspergillus* (PAS stained).

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Specific and Sensitive Two-Step Polymerase Chain Reaction Assay for the Detection of *Salmonella* Species

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A polymerase chain reaction (PCR) assay was applied for the selective amplification of a characteristic sequence within a *Salmonella*-specific chromosomal fragment. A two-temperature PCR cycle enhanced both the speed and overall sensitivity of the amplification procedure. Twenty-one well-characterized *Salmonella* strains and a number of non-*Salmonella* strains were tested. With the exception of the rarely isolated *Salmonella arizonae* strain, the PCR-based approach enabled the specific identification of *Salmonella* with a detection limit of 10^3 organisms. In combination with a nested PCR assay, as few as ten organisms were detectable. Specificity was demonstrated as no distinct amplification products were detectable with any of the tested non-*Salmonella* strains. With a pre-enrichment step using paramagnetic anti-*Salmonella* beads, an increase in sensitivity was observed in the case of clinical samples while the amplification process was not influenced.

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Salmonellae are among the most important agents of food-borne infections in Europe and the USA, causing infections such as typhoid fever, septicaemia, and, primarily, gastroenteritis. The incidence of enteric infections caused by *Salmonella* spp. has increased markedly in several Western countries over the past decades (1, 2). Epidemics, which often occur in institutional or other large-scale kitchens, may be fatal, especially for elderly people and hospital patients. Therefore, it has become increasingly important to detect *Salmonella* quickly in environmental, food, and clinical samples. The complex ecology of *Salmonella* spp, however, provides an obstacle to their isolation. These bacteria may be found in low numbers in a stressed state among large numbers of contaminating microorganisms. They are able to enter into a viable but nonculturable state after long exposure to ground water and soil at unfavourable temperatures and with a low concentration of nutrients (3).

The traditional method for detection of *Salmonella* in food involves a series of incubations in pre-enrichment and enrichment broths to multiply selectively the target organisms. These procedures are laborious, time-consuming, and of limited utility in respect to the resulting ratio of target to contaminating organisms (4). A promising alternative to traditional techniques is provided by nucleic acid-based assays. Of these, the polymerase chain reaction (PCR) provides the most specific and sensitive tool for the detection of small amounts of DNA. We evaluated the diagnostic capability of a nested, two-step PCR assay on a representative number of hygienic samples. The two-step PCR is a more rapid and sensitive variant of the conventional three-step PCR. By use of relatively long PCR primers, the annealing and extension steps can be combined, resulting in significantly enhanced sensitivity and overall processing speed. The possibility of specific pre-enrichment by immunomagnetic separation was also tested. This method has already been successfully applied to many other bacterial species, including *Escherichia coli* (5) and *Listeria monocytogenes* (6, 7),