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Disseminated Infection with *Prototheca zopfii* after Unrelated Stem Cell Transplantation for Leukemia

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Disseminated infection with *Prototheca zopfii* is a rare disease in immunosuppressed patients. We here report the first case of lethal infection with *P. zopfii* following unrelated stem cell transplantation for leukemia. Breakthrough protothecosis occurred during long-term administration of voriconazole in the case of pulmonary aspergillosis.

CASE REPORT

A 58-year-old man was diagnosed with osteomyelofibrosis in 1998. Four years later, the patient developed chemotherapy-refractory secondary acute leukemia (FAB-M1), and a one-antigen mismatched bone marrow transplantation from an unrelated donor was performed after reduced-intensity conditioning despite proven aspergillosis. Pulmonary aspergillosis was controlled by administering voriconazole (Pfizer, Vienna, Austria) in combination with caspofungin (MSD, Vienna, Austria) during the neutropenic period. Antifungal maintenance therapy consisted of monotherapy with voriconazole (400 mg/day). Three months later, leukemic relapse occurred while the patient was still under immunosuppression with cyclosporine. After immunosuppression was discontinued, reinduction with fludarabine, high-dose cytarabine, and granulocyte colony-stimulating factor was started but without success. The patient's refractory leukemia was treated with gemtuzumab ozogamicin (Mylotarg; Wyeth, Vienna, Austria) in order to induce remission for subsequent donor lymphocyte infusion since no graft-versus-host disease had yet been diagnosed. During severe pancytopenia after gemtuzumab ozogamicin the patient developed fever of up to 41°C, which was unresponsive to ceftazidime, vancomycin, and netilmicin. Concurrently, spotted pulmonary infiltrates were visible in a computerized tomography scan, raising suspicion of aspergillosis reactivation. Antifungal therapy was extended to caspofungin. At that time, erythematous skin papules appeared on the upper extremities and spread rapidly over the whole body. Multiple blood cultures with aerobic, anaerobic, and fungal broths were taken and incubated in a BACTEC 9050 blood culture system (Becton Dickinson, Cockeysville, Md.).

After 3 days of incubation at 37°C yeast-like fungi were grown on Sabouraud dextrose agar (Merck, Vienna, Austria) from several blood cultures. *Prototheca zopfii* was identified with Vitek 2 (bioMerieux, Marcy l'Etoile, France) and confirmed with the RapID Yeast Plus (code 730010) system (Remel, Santa Fe, N.Mex.) and microscopic examination. Cornmeal agar showed typical findings for *P. zopfii*: small spherules in aggregates with multiple internal cleavage. Susceptibility testing was performed by E-test as described by the manufacturer (AB Biodisk, Solna, Sweden). The medium consisted of RPMI 1640 agar with 2% glucose; the inoculum suspension was adjusted to the turbidity of a 0.5 McFarland standard and incubated at 37°C for 48 h. In vitro susceptibility gave the following MICs: amphotericin B, 0.125 μg/ml; voriconazole, >64 μg/ml. These results correlated with the MICs obtained with the National Committee for Clinical Laboratory Standards reference broth microdilution method, M27-A (12), as *P. zopfii* showed growth in medium containing 16 μg of voriconazole/ml. No data are available to define breakpoints for *P. zopfii*. Because the MIC of amphotericin B was low, the isolate was assumed to be susceptible to this drug. Immediately, antifungal therapy was changed to liposomal amphotericin B (5 mg/kg of body weight/day) and the central venous catheter was removed, because the blood cultures remained positive for *P. zopfii*. However, the clinical situation deteriorated, and the patient died. At autopsy, dissemination of *P. zopfii* was demonstrated in several organs such as lung, kidney, heart, and liver by means of postmortem cultures positive for this alga.

The genus *Prototheca* belongs to the family *Chlorellaceae*. It is a unicellular organism that reproduces asexually by internal septation and cleavage to form sporangia containing sporangiospores (14). *Prototheca wickerhamii* and *P. zopfii* have been associated with human disease. The first case of human protothecal infection due to *P. zopfii* was described by Davies et al. in 1964 (4). The patient was a rice farmer in Sierra Leone with a chronic localized skin lesion. The first case of multiorgan systemic infection was described by Cox et al. in 1974 (2). Since then, few cases of protothecosis have been described, mainly in patients with diabetes mellitus, continuous peritoneal dialysis, renal transplantation, steroid use, AIDS, or immunologic defects involving lymphocytes or neutrophils (6, 8, 9, 11, 15). Protothecosis is an uncommon infection in cancer patients, as only 13 cases have been reported in the literature (15). Most

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infections involve the skin or soft-tissue structures and were due to *P. wickerhamii* (1, 6, 8, 9, 16). We here report the first case of disseminated and ultimately fatal infection due to *P. zopfii* in a severely immunosuppressed leukemic patient following mismatched unrelated stem cell transplantation.

Prototheca spp. are ubiquitous in nature and have been found water, sewage, soil, potato skins, cow milk, cattle, dogs, and fruit bats (7, 13, 14, 17). In humans, *Prototheca* spp. have been found to colonize the fingernails, skin, respiratory tract, and digestive system (7, 10, 16). It is presumed that infection is exogenous and the result of traumatic inoculation; proven transmission from human to human or from animal to human is unknown. In this case, we were unable to trace the portal of entry of *P. zopfii*. Several environmental samples and the hospital water were free of *P. zopfii*. We speculate that skin lesions may have been the portal of entry; alternatively, infection could have resulted from endogenous colonization followed by dissemination during prolonged immunosuppression and neutropenia.

Several antifungal agents, including amphotericin B and azoles, have been used to treat protothecosis (5, 9). Administration of amphotericin B appears to be the most effective modality for protothecosis in immunocompromised patients. To date, there is no standard treatment regimen. In our case, this breakthrough infection occurred despite systemic long-term treatment with voriconazole (<120 days) and even after adding caspofungin during neutropenia. This clinical outcome agrees with the in vitro finding, as the infection was shown to be resistant to voriconazole; no detailed information is available on caspofungin, because there is a lack of applicable susceptibility tests. Identification of the pathogen is highly warranted for optimal therapy, as these new antifungal agents are ineffective against, for example, zygomycetes (3). However, treatment with amphotericin B also failed to produce a clinical response, despite the fact that it has been shown to be active against *P. zopfii*. The reason for this is unknown; it is conceivable that a 5-day regimen of amphotericin B therapy does not suffice to clear fungal elements. Also, no breakpoints have been established, and no clinical correlation studies have been performed.

This case illustrates the potential for disseminated infection and pathogenicity of this fungus when underlying conditions are given. Awareness for less-common fungal pathogens with resistance to new antifungal agents is of major importance. Antifungal treatment should be reassessed if fever persists or progression becomes apparent during therapy: the regimen should be changed or another antifungal drug should be added to the regimen. Because of its broad antifungal spectrum, amphotericin B or lipid-based amphotericin B formulations should be considered whenever the causative agent is unknown. Correct laboratory diagnosis is highly warranted, and susceptibility testing helps determine therapeutic options.

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