Rhizomucor variabilis var regularior and Hormographiella aspergillata Infections in a Leukemic Bone Marrow Transplant Recipient with Refractory Neutropenia

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Abstract

*Rhizomucor variabilis* and *Hormographiella aspergillata* rarely cause human infections.

This report details a fatal case in a 14 year old female with leukemia post hematopoietic cell transplant and relapse with refractory pancytopenia. The patient first developed *R. variabilis* var. *variabilis* palate infection and later developed a cutaneous *H. aspergillata* infection while on posaconazole and caspofungin therapy.
Case Report

The patient was a 14 year old female with history of acute myelogenous leukemia diagnosed in July 2007. The patient underwent an allogenic HLA matched bone marrow transplant in December 2007 and experienced relapse in April 2008 with failure of re-induction chemotherapy with a bone marrow aspirate showing a preponderance of blasts. In September 2008, a CT scan of the chest two weeks prior to admission revealed patchy ground glass opacities with tiny peripheral nodular densities in both lung fields sparing the left upper lobe and a 1.2 cm nodule in the right upper lobe. The patient's condition was deemed too fragile to tolerate a diagnostic lung biopsy. She was empirically treated with broad antibacterial and antifungal coverage with Voriconazole. Of interest, the patient also started drinking an herbal tea remedy of unknown variety. Shortly afterwards, the patient presented with a two week history of odynophagia and persistent febrile neutropenia. On exam the patient had white plaques involving the soft palate and pharynx. A smear stained with Calcofluor white from a throat culture showed hyphal elements and conidophores. A biopsy of the palate lesion showed submucosa and mucosa infiltrated with hyphal forms with sparse septation, rare branching and chamydoconidia. The patient was started empirically on posaconazole 800 mg PO BID. A CT scan of the head and sinuses was negative while a CT scan of the lungs confirmed pulmonary nodules that had been previously visualized. Serial galactomannan assays were negative. The fungal isolate obtained in culture from palate biopsy was tentatively identified as a *Rhizomucor spp* by the Mount Sinai clinical microbiology laboratory. The isolate was sent to the Mycology Laboratory at NYS Department of Health for further characterization. The option of surgical debridement was declined by the family due to potentially severe morbidity. On week two of therapy, caspofungin was added as adjunct therapy. By week two of therapy, the lesion
decreased in size with improved symptoms. On week three of therapy, she began receiving a new regimen of chemotherapy along with granulocyte infusions. By week four, the patient’s symptoms had resolved. By week five of therapy, the palate lesion was no longer visualized. Throughout, the patient had persistent severe refractory pancytopenia. One and one half months after initiation of therapy, she developed altered mental status and had a generalized seizure. A CT scan of the brain showed multiple hypodense lesions of the cerebral hemispheres and cerebellum. Serum and CSF *Toxoplasma* and Cryptococcal studies were negative. A repeat CT scan of the lungs showed cavitating lesions in the right upper lobe/right middle lobe. At that time the patient was switched to liposomal amphotericin (Ambisome) for better CNS penetration, caspofungin was maintained, and posaconazole discontinued. At this time, she developed high fevers, and a new small erythematous skin papule developed on the right knee. The skin was biopsied. Ten days later, a new skin lesion appeared on the left arm. The patient’s respiratory status progressively deteriorated and she expired from respiratory failure two weeks after the appearance of the initial skin lesion. Cultures obtained from the skin biopsy yielded a white mold, which was identified postmortem as *Hormographiella aspergillata* and sent to Mycology Laboratory, NYS Department of Health for confirmation and susceptibility testing. No autopsy was performed.

The throat biopsy specimen was cultured on Sabouraud Dextrose Agar (SDA), Mycosel and Inhibitory Mould Agar. All cultures were incubated at 30°C in ambient air. Growth of a mould from this culture was evident on the SDA agar plate after three days. The isolate was transferred to a Potato Dextrose Agar (PDA) plate and overlayed with sterile coverslips and incubated as above. The mould was examined by placing the coverslip onto a glass slide with
lactophenol cotton blue. The slide culture revealed an organism with branched round sporangia arising from hyphae which possessed rhizoids between the stolons. The identification of *Rhizomucor spp.* was made based on morphology and was confirmed and speciated as *R. variabilis* by genetic analysis. ITS2-PCR, nucleotide sequencing and BLAST search using two databases - Genbank (www.ncbi.nlm.nih.gov/genank/index.html), and Centraalbureau voor Schimmelcultures (www.cbs.knaw.nl/yeast/BioloMICSSequences.aspx) revealed *Rhizomucor* isolate to be 100% identical to *Rhizomucor variabilis* var. *regularior* (CBS 384.95). The ITS2 gene sequence of this isolate was deposited in GenBank (GQ338323). Growth from the skin biopsy specimen was evident first on the blood agar plate from the bacteriology culture. The mould was subcultured onto a PDA plate with sterile coverslips and examined as above. Macroscopically there was rapid growth of a white cottony mould with a brownish reverse. Microscopically the mould exhibited septate hyphae with cylindrical arthroconidia that formed whorls at the apex. The mould was identified as *Hormographiella spp.* based on its morphological characteristic and as *Corrinus cinereus* (of which *H. aspergillata* is the anamorph) by genetic analysis. This isolate was 100% identical to *Coprinus cinereus* (GenBank AB097562; Anamorph *Hormographiella aspergillata*). Its ITS2 gene sequence was also deposited in GenBank (GQ338322). Molecular identifications in these two instances were reconciled with characteristics morphological descriptions (2). Antifungal susceptibility tests (single drugs and two-drug combinations) were performed on both isolates according to CLSI M-38A2 protocol and checkerboard titrations (1). *Rhizomucor variabilis* var. *regularior* was susceptible to amphotericin B (1.0 µg/ml), posaconazole (1.0 µg/ml) and voriconazole (1.0 µg/ml), and non-susceptible to echinocandins (>4.0 µg/ml). The combination of echinocandins with amphotericin B was synergistic (ΣFICi = 0.265). *Hormographiella aspergillata* was
susceptible to posaconazole (0.5 µg/ml) and voriconazole (0.25 µg/ml), and resistant to amphotericin B (4.0 µg/ml) and echinocandins (>4.0 µg/ml). The combination of echinocandins with amphotericin B was synergistic ($\Sigma\text{FIC}_i = 0.075$).

*Rhizomucor* species are infrequent cause of human disease while *Rhizomucor variabilis* is rarely reported as an etiologic agent with few case reports published recently (8, 14). In one retrospective review of zygomycosis in children, among 77 culture confirmed cases, only one was found to be due to *Rhizomucor* species (13) while another review of 929 cases documented only 19 secondary infectious due to *Rhizomucor* species (9). Our patient’s localized palate infection responded to the combination of posaconazole and caspofungin with granulocyte infusions. Although it is not possible to establish the relative contribution of each of these therapies, it is important to note that the granulocyte infusions were initiated one week after the antifungal agents, and that a decrease in the size of the lesion along with alleviation of the patient’s symptoms were seen prior to their initiation. The patient’s isolate showed an MIC of 1 µg/mL for both posaconazole and amphotericin, and was resistant to caspofungin. Several papers demonstrate the efficacy of posaconazole as salvage therapy for Zygomycosis in patients refractory to standard therapy (4, 6). Our patient’s *R. variabilis* isolate had a summation fractional inhibitory concentration index ($\Sigma\text{FIC}_i$) of 0.265 for the combinations of amphotericin - caspofungin and amphotericin - posaconazole, and 0.18 for the combination of caspofungin-posaconazole, which underscores the fact that the combination used in this case was synergistic at least *in vitro*.

The patient’s second isolate *H. aspergillata* isolated from a cutaneous lesion is also a rare cause of human pathology. It is probably under diagnosed, as it is hard to identify by routine methods. Published reports on this pathogen include a case of prosthetic mitral valve
endocarditis that was successfully treated with amphotericin B (5). Cases of pulmonary disease include a post stem cell transplant AML patient who expired while on caspofungin therapy, and a Non-Hodgkin’s lymphoma who had recovered from a neutropenic episode and was successfully treated with amphotericin B (7, 10, 12). In vitro studies have shown that *H. aspergillata* shows variable susceptibility to amphotericin B, resistance to flucytosine, and uniform susceptibility to the azoles with the exception of fluconazole (3). Our patient’s isolate was in fact resistant to amphotericin with an MIC of 4 µg/ml, resistant to caspofungin with an MIC of >2, resistant to micafungin with an MIC of > 4, and susceptible to voriconazole with an MIC of 0.25, and posaconazole MIC 0.5 µg/ml.

Due to the lack of an autopsy, it is impossible to know definitively whether this patient’s disseminated disease with brain and lung involvement was secondary to the *R. variabilis* or *H. aspergillata*. But given the resolution of her initial palate lesion while on therapy, the timing of the development of the brain lesions which coincided with her cutaneous lesions, and her clinical deterioration after the appearance of the skin lesions, one can hypothesize that the disseminated disease was likely secondary to *H. aspergillata*. The possibility of two different simultaneous fungal infections in the immuno-compromised host is underscored in this report. This possibility along with data showing synergy among different agents illustrates the importance of considering combination therapy in cases of invasive fungal infections. Posaconazole as illustrated by our patient’s isolate and other in vitro susceptibility studies seems to have one of the lower MICs for Zygomyccosis, although the clinical significance of such MICs is yet to be established (13). Although better, larger *in vivo* studies and clinical trials are needed, posaconazole with its broad spectrum shows promise in combination with other agents as a potential treatment option for invasive fungal infections.
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References


Figure Legends

**Figure 1-1:** *Rhizomucor variabilis* var. *variabilis* (A-B) Macroscopic morphology potato dextrose agar: Colonies were filling the entire plate in 5 days at 30° C, and restricted growth at 37° C, respectively. Colonies – brown to tan color, hairy with reverse buff to brown color. (C) Microscopic morphology in lactophenol cotton blue after 3 days (magnification 200X): hyaline, unbranched ribbon like hyphae, sporangiophores are long, simple, arising from hyphae, ending in sporangium. Sporangia: spherical with globose columella, no apophysis. Sporangiospores: hyaline, ellipsoidal with smooth walled. Inset: (magnification 400X) showing details of spherical columella (arrow) with sporangiospores.

**Figure 1-2:** *Hormographiella aspergillata* (A-B) Macroscopic morphology on potato dextrose agar – 3 cm in 10 days at 30° C, and at 37° C. Colonies were white to cream colored, velvety with cottony tufts, with irregular margin with pale reverse color. (C) Microscopic morphology in lactophenol cotton blue after 10 days: Hyaline septate hyphae, conidiophores: simple, well differentiated. Arthroconidia: catenate, hyaline smooth walled, single cell, produced at the end of conidiophores either in clusters (single arrow) or in irregular groups (double arrow).
Figure 1-1

30°C

37°C

A

B

C
ERRATUM

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