Empyema thoracis due to *Rhizopus oryzae* in an allogenic bone marrow transplant recipient


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We describe a case of empyema thoracis caused by *Rhizopus oryzae* diagnosed in an allogenic bone marrow transplant patient with acute lymphocytic leukemia. The isolate of *R. oryzae* was recovered from three pleural effusion specimens, which were black in color. It was identified on the basis of characteristic colonial appearance and microscopic findings, as well as the partial sequencing of rRNA genes. The patient died of uncontrolled *R. oryzae* empyema thoracis and concomitant nosocomial infection.

**Keywords**  empyema thoracis, *Rhizopus oryzae*, bone marrow transplant

**Introduction**

Zygomycosis is the third most common invasive fungal infection after aspergillosis and candidiasis [1]. *Rhizopus* and *Mucor* species are the predominant etiologic agents which generally enter the human host through inhalation, percutaneous inoculation or ingestion [2]. Risk factors associated with zygomycosis include diabetic ketoacidosis, cancer, antibiotic or prednisolone use, deferoxamine and desferrioxamine therapy, transplantation, renal failure, extensive burns, trauma, and intravenous drug abuse [1,2]. There are six types of infection (rhino-orbito-cerebral, pulmonary, disseminated, cutaneous, gastrointestinal, and miscellaneous), with the lungs being the second most common site of involvement. *Rhizopus oryzae* is the principal cause of zygomycosis, but other *Rhizopus* species (*R. azygosporus, R. microsporus, R. schipperae* and *R. stolonifer*) have been reported associated with the disease [1,2]. Although a case of *Rhizopus* empyema (not identified to species) has been previously described in the literature in a series on fungal empyema thoracis [3], *R. oryzae* has never been described in empyema thoracis in the English literature.

**Case report**

A 46-year-old man was in good health until October 2003 when symptoms of exertional dyspnea and bloody stool developed. Acute lymphoblastic leukemia (L2, B cell lineage) was diagnosed in November 2003. He received induction therapy with Cancer and Leukemia Group B protocol 8811 (CALGB 8811) in December 2003 and early intensifications with CALGB 8811 and intrathecal chemotherapy. After complete remission was achieved, he received allogenic bone marrow transplantation from his sister on 13 April 2004. Bone marrow examination three weeks after transplantation showed engraftment of all blood cell lineages. He was discharged 49 days after transplantation without any complications.

Fifty-nine days after transplantation, he was readmitted due to symptoms of hematuria and dysuria caused by hemorrhagic cystitis. After admission, he underwent cystoscopy and hyperbaric oxygen therapy. At 90 days post-transplant, lymphoproliferative disease was diagnosed and rituximab (anti-CD20 monoclonal antibody) was administered. Severe watery diarrhea, vomiting and skin rash developed at 133 days after transplantation, leading to the diagnosis of graft-versus-host disease (GVHD). Tacrolimus (5 mg/day) and steroid were given and his symptoms gradually improved. During the post-transplant period, he did not receive prophylactic voriconazole therapy. He once received thoracocentesis on day 159 after transplanta-
tion. At the same time, prolonged prothrombin time (PT) of 15.1 s (normal range, 9.9–12.3 s), activated partial thromboplastin time (APTT) of 43.5 s (normal range, 24.2–35.1 s), and elevated values of fibrin degradation product (FDP) of 40–80 μg/mL (normal range, <5 μg/mL) and d-dimer of 2.12 μg/mL (normal range, <0.5 μg/mL) indicated the presence of coagulopathy. The pleural effusion was yellowish and was transudate with white blood cell count of 350/mm³ and a lymphocyte predominance (92%), lactate dehydrogenase value of 415 U/L, and total protein value of 0.9 g/dL. He began to experience dyspnea and fever at day 161 after transplantation, with a white blood cell (WBC) count of 3080/mm³ (neutrophils 93%), but gram and acid-fast stained sputum samples did not reveal any pathogen. Chest radiography and computed tomography (Fig. 1) revealed multiple areas of consolidations and massive bilateral pleural effusions. Thoracentesis performed on day 168 after transplantation obtained 630 ml of black-colored pleural fluid. Analysis of the pleural fluid revealed white blood cell count of 2500/mm³ with a lymphocyte predominance (93%), lactate dehydrogenase value of 11,230 U/L, total protein value of 3.4 g/dL, and glucose level of 86 mg/dL. A chest tube was not used due to lack of purulence and negative results of gram and acid-fast staining of pleural fluid. Urine was red in color at this time due to bleeding. Sputum specimen was purulent. He was treated with imipenem (500 mg every 8 h), amikacin (250 mg every 12 h), gancyclovir (150 mg every 12 h) and anti-cytomegalovirus (anti-CMV) immunoglobulin G. Progressive dyspnea with desaturation developed despite of broad-spectrum antimicrobial agents and he was intubated due to acute hypoxic respiratory failure. Sulfamethoxazole/trimethoprim (400 mg/80mg every 8 h) was added after Stenotrophomonas maltophilia bacteremia was diagnosed, as well as suspected Pneumocystis jirovecii pneumonia. Empirical anti-fungal therapy with caspofungin (50 mg loading and followed by 35 mg daily intravenously) was also administered. Profound shock developed and he died on day 172 after transplantation.

Microbiology

All three pleural effusion specimens collected on days 168 and 170 after transplantation were black in color and yielded a mold three to five days after incubation. Cultures of endotracheal aspirate specimens grew S. maltophilia but were negative for fungi. The same mold was recovered from all effusion specimens and was identified as Rhizopus species based on characteristic colonial appearance (whitish-to-yellowish cotton candy-like colonies grown on Sabouraud dextrose agar at 25°C for three days) and microscopic findings (broad hyphae without septa and the presence of rhizoids in the meeting points of stolons and long sporangiophores (Fig. 2). The isolates were further identified to the species level by partial sequencing of rRNA genes and found to have 100% identity for 5.8S-28S rRNA (accession number AB097383 by ITS3 [5'-GCATC GATGAAAGCCGACG-3']/ITS4 [5'-CTCTCCGCT TATGTATGC-3']), 18S-28S rRNA (accession number AB097383 by ITS1 [5'-TCCGTAGGTGAACCT GCGG-3']/ITS4, and 28S rRNA (accession number AB097382 by 5'-GGTAAAATTGTGGAAAAGGGA-3'/ 5'-GACTCCCTTGTCGGTGT-3'), respectively [4].

Fig. 1 Chest of computed tomography revealed areas of dense consolidation with necrosis and massive pleural effusions bilaterally.
Infections with non-*Aspergillus fumigatus* *Aspergillus* species, *Fusarium* species, and Zygomycetes increased during the late 1990s, especially in patients who received multiple transplants. Zygomycosis is now becoming the typical presentation in the area of allo-transplantation [5,6]. In GVHD patients, the use of immunosuppressive therapy is the predominant risk factor for zygomycosis (like our patient), particularly those receiving voriconazole prophylaxis [5,6]. In the present case, the patient died of uncontrolled *R. oryzae* empyema thoracis and a concomitant nosocomial *S. maltophilia* septicemia. Neither empyema thoracis due to *R. oryzae* nor black color of an *R. oryzae*-containing pleural effusion has been previously reported. Although *Rhizopus* is a very well known cause of laboratory contamination, there is little likelihood that it was such in the present case as it was recovered from three pleural effusion specimens. The black color of the pleural effusion might have resulted from liquefaction of old blood due to previous thoracentesis with the presence of underlying coagulopathy, or to necrotic debris caused by the *R. oryzae* infection. This case thus suggests a wide spectrum of clinical manifestations as a result of the *R. oryzae* and specifically, that *R. oryzae* is possibly involved in the etiology of empyema thoracis.

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A previous study revealed that the use of cultures in the recovery of the etiologic agent had a low sensitivity, with lower respiratory specimens proving to be negative in 75% of histopathologically proven pulmonary cases [7]. In addition, data suggested that more aggressive bronchoscopic or surgical approaches were needed to obtain adequate specimens for microbiological and histological diagnosis [8].

*In vitro* activity of amphotericin B, ketoconazole, and voriconazole against isolates of *R. oryzae* has been previously reported [9,10]. Amphotericin B deoxycholate or its lipid formulations remain the standard agents approved for the therapy of invasive zygomycosis [1,2]. Ibrahim et al. demonstrated that caspofungin, a novel antifungal echinocandin, has significant but limited activity against *R. oryzae in vivo* and had an inverse dose-response effect [11,12]. Combination therapy with amphotericin B lipid complex and caspofungin has also been demonstrated to be more effective than amphotericin B lipid complex monotherapy in *R. oryzae* infected mice with diabetic ketoacidosis. Amphotericin B was not used in this patient because he died prior to notification of positive culture result for *R. oryzae*. Inadequate coverage of caspofungin monotherapy against this isolate likely contributed to the poor outcome of the patient [11–14].

In this study, the isolates were confirmed as *R. oryzae* using sequence analysis of the rRNA genes. PCR amplification using the ITS1 and ITS4 primers produce an approximately 600-bp amplicon, which contains conserved regions among fungi, including the sequence for ITS3 [15]. The rRNA gene contains both unique and conserved regions and has been used for molecular identification of many fungi [4,15].

In conclusion, we have described a unique case of empyema thoracis with black-colored pleural effusion caused by *R. oryzae* in an allogenic bone marrow transplantation recipient. Although it is an infrequent pathogen, this organism should be considered a possible cause of empyema thoracis, especially in immunocompromised patients.
References