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Case Report

Pulmonary mucormycosis with embolism: two autopsied cases of acute myeloid leukemia

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Abstract: Mucormycosis is an increasingly important cause of morbidity and mortality for patients with hematological malignancies. The diagnosis of mucormycosis usually requires mycological evidence through tissue biopsy or autopsy because the signs and symptoms are nonspecific and there are currently no biomarkers to identify the disease. We herein present two autopsied cases of acute myeloid leukemia with prolonged neutropenia who developed invasive mucormycosis accompanied by pulmonary artery embolism. Our cases were featured by unexplained fever and rapidly progressive dyspnea. Computed tomography scan detected nodular lesions or nonspecific consolidations in the lungs. Cultures, cytological study, and serum fungal markers consistently gave negative results. Autopsy revealed embolism of the pulmonary artery which consisted of fibrin clots by filamentous fungi. Genomic DNA was extracted from the paraffin-embedded clots and was applied to polymerase chain reaction amplification, leading to the diagnosis of infection by *Rhizopus microsporus*. We should carefully search for life-threatening pulmonary embolism when patients with hematological malignancies develop pulmonary mucormycosis.

Keywords: *Rhizopus microsporus*, mucormycosis, pulmonary embolism, acute myeloid leukemia, neutropenia

Introduction

Invasive fungal disease (IFD) is an important cause of morbidity and mortality among patients with hematological malignancies [1, 2]. Mucormycosis is defined as IFD due to various members of the class Zygomycetes, order Mucorales, which is subdivided into the genera *Mucor, Rhizopus, Absidia, Apophysomyces, Cunninghamella,* and *Saksenaea* [3]. Following to candidiasis and aspergillosis, mucormycosis accounts for the third most frequent cause of IFD [1]. The incidence of mucormycosis has been increasing among patients with hematological diseases especially acute myeloid leukemia (AML) as a result of recent advances in treatment and prolonged survival [4]. Major risk factors for mucormycosis include hematological malignancies with neutropenia, high-risk hematological stem cell transplant (HSCT), severe graft versus host disease (GVHD) treated with corticosteroids, and iron overload with deferoxamine use. The diagnosis of mucormycosis is challenging because the signs and symptoms are nonspecific and there are currently no biomarkers to identify the disease [5, 6]. Histopathological evaluation is usually required to obtain mycological evidence, and approximately half of the cases are diagnosed by post-mortem examination [7]. Hence, autopsy still plays a crucial role in elucidating its pathogenesis. We herein describe two autopsied cases of AML with mucormycosis accompanied by pulmonary artery embolism.

Case report

Case 1

A 44-year-old man presented with anemia and was diagnosed as AML with myelodysplasia-related changes (AML-MRC). Cytogenetic study showed a complex karyotype harboring trisomy 8 and a deletion of chromosome 5. Complete

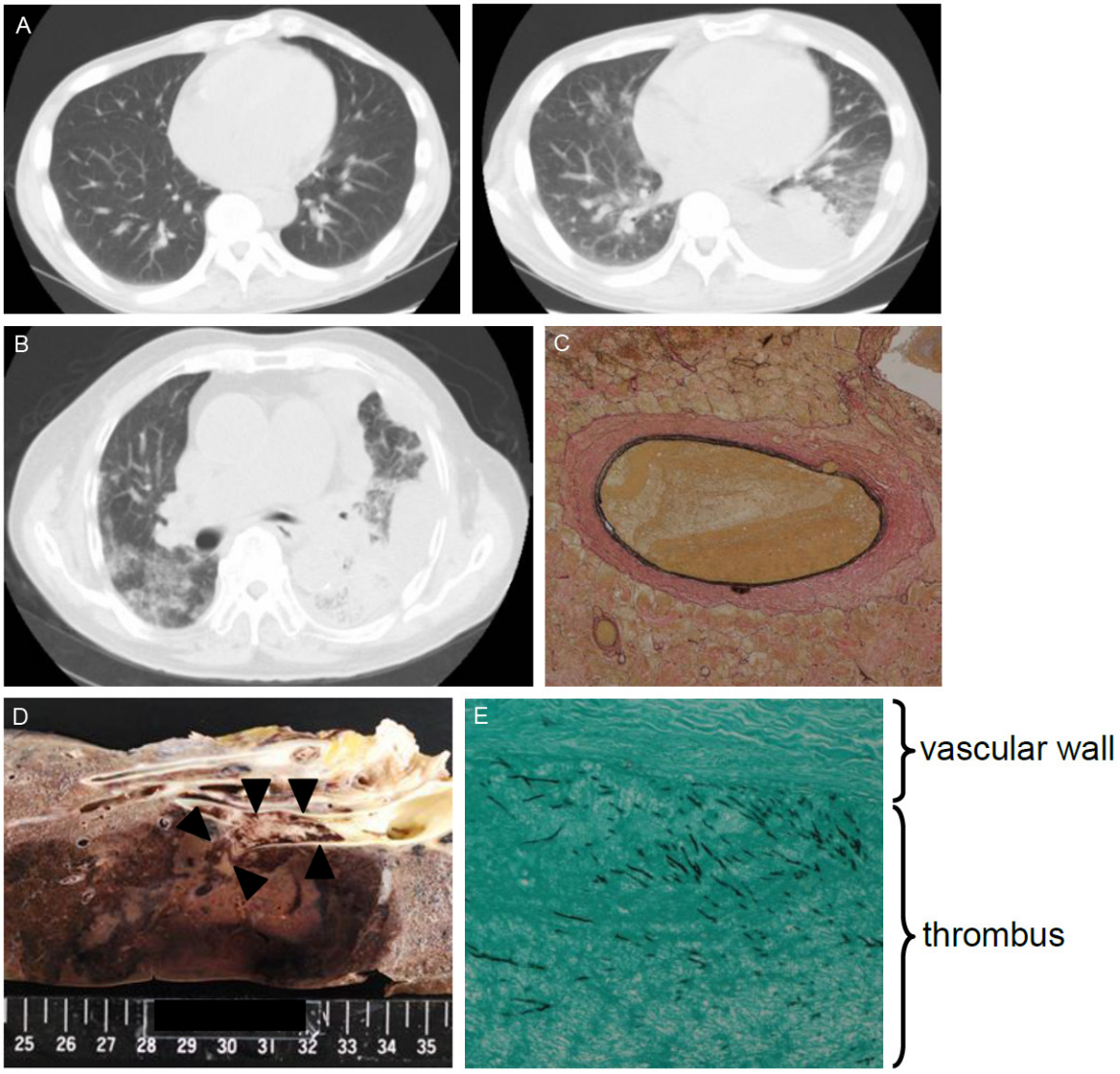

Figure 1. Pulmonary mucormycosis with emboli in the pulmonary artery. A. Computed tomography (CT) scan of Case 1 showed nodular lesions in the lungs (*left panel*). A follow-up CT scan after one month of antifungal treatment indicated deterioration of the lesions (*right panel*). B. CT scan of Case 2 showed nonspecific pneumonia and pleural effusion. C. Microscopic examination of Case 1 indicated the left pulmonary artery filled with fibrin clots (EVG stain, × 20). D. Macroscopic study of Case 2 was remarkable for fungal emboli in the left pulmonary artery (arrowheads) accompanied by hemorrhagic infarction of the surrounding parenchyma. E. Filamentous fungi were detected in the emboli (GMS stain, × 100).

remission was not achieved by several courses of chemotherapy and, five months after the onset, he underwent allogeneic bone marrow transplant from an HLA-matched unrelated donor after conditioning with cyclophosphamide and fractionated total body irradiation. GVHD prophylaxis consisted of cyclosporine A (CsA) and short-term methotrexate. Oral itraconazole was prophylactically administered against fungal infection. Neutrophil engraftment was achieved on day +30 after the transplant until when there had been no evidence of documented infection. Bone marrow examination on day +42 showed relapse. Leukemic blasts subsequently appeared in the peripheral blood, and absolute neutrophil count again fell below 0.5 × 10^9/L. After six months of the transplant, the patient suffered from unexplained fever. Blood counts showed hemoglobin level of 51 g/L, leukocyte count 5.2 × 10^9/L with 97% blasts and 0% neutrophils, and platelet count 3 × 10^9/L. Computed tomography (CT)

scan of the chest without contrast enhancement revealed nodular lesions of the lungs (**Figure 1A**, *left panel*). Blood cultures and serum fungal markers gave negative results. Smear and culture of bronchoalveolar lavage fluid could not detect a pathogen. Comprehensively, the patient was clinically diagnosed with possible IFD. Antifungal agents were switched from itraconazole to liposomal amphotericin B (L-AmB) at a daily dose of 150 mg, which was escalated to 300 mg daily one week later. The patient subsequently developed subacute progressive dyspnea. A follow-up CT at one month after initiation of L-AmB indicated a salient aggravation of parenchymal lesions and the presence of pleural effusion (**Figure 1A**, *right panel*). He succumbed to death two weeks later.

Case 2

An 88-year-old man presented with gradually progressive pancytopenia for the previous four years. Laboratory findings included hemoglobin level of 75 g/L, leukocyte count 1.4 × 10^9/L with 1% blasts and 40% neutrophils, and platelet count 24 × 10^9/L. The bone marrow was normocellular with trilineage dysplasia and 12% blasts, leading to the diagnosis of MDS (refractory anemia with excess blasts-2 according to the World Health Organization classification). He soon became dependent on red cell and platelet transfusions. Leukemic transformation was recorded at one year after the diagnosis of MDS. Although absolute neutrophil counts ranged from 0.2 to 0.4 × 10^9/L, the patient refused to take prophylactic antifungals.

Finally, at six months after the transformation, he presented with fever with mild dyspnea. Laboratory findings then showed leukocyte count of 0.4 × 10^9/L with 60% neutrophils and platelet count of 8 × 10^9/L. CT scan without contrast enhancement disclosed nonspecific multilobar consolidation and pleural effusion (**Figure 1B**). Blood cultures gave negative results. Sputum cultures recovered Enterococcus faecalis, methicillin-resistant Staphylococcus aureus, and coagulase-negative Staphylococci, but no fungi. Serum β-D-glucan level was below the cutoff value. He was diagnosed with bacterial pneumonia, and treatment was initiated with cefepime and teicoplanin. Because there was no evidence of fungal infections, oral fluconazole and subsequent micafungin were empirically administered. The patient exhibited rapidly progressive dyspnea with bloody phlegm, and succumbed to death four days after the onset of pneumonia.

Autopsy findings

Autopsy of our cases was performed with written informed consent from the patients' family. Macroscopic and microscopic findings of the lungs were similar across the cases. The left pulmonary artery was fulfilled with emboli, which was accompanied by hemorrhagic infarction of the surrounding parenchyma (**Figure 1C** & **1D**). Microscopic examination showed that the emboli were composed of fibrin clots. Grocott's methenamine silver (GMS) stain of revealed filamentous fungi in the emboli (**Figure 1E**). Because these microscopic findings were insufficient to distinguish Mucorales from Aspergillus, we decided to perform molecular analysis. Genomic DNA was extracted from paraffin-embedded specimen and was subjected to polymerase chain reaction (PCR) amplification of ribosomal DNA (rDNA) D1/D2 domain using a set of primers specific to Mucorales. In both cases, the microorganism was identified as *Rhizopus microsporus*. There was no evidence of leukemic infiltration to the pulmonary parenchyma. Taken together, we concluded that pulmonary embolism and hemorrhagic infarction were the cause of the deaths.

Discussion

AML is the most common hematological disease that underlies invasive mucormycosis. The lungs are the most frequent anatomical site of mucormycosis in association with hematological diseases, accounting for 64% of cases [7]. The signs and symptoms of pulmonary mucormycosis include fever, cough, dyspnea, chest pain, all of which are not specific to the disease. Radiological findings consist of various patterns including focal consolidation, widespread infiltration, and nodules. In our series, mycological evidence was not obtained before the patients' deaths because tissue biopsy was not available due to poor conditions. Although the diagnosis of *Rhizopus microsporus* infection was confirmed by PCR amplification of genomic rDNA, this method has not been applied to daily clinical practice. Establishment of biomarkers for early diagno-

sis of invasive mucormycosis is strongly encouraged.

One of the hallmarks of mucormycosis is vascular invasion by hyphae, leading to embolism and infarction. However, thrombotic events have rarely been documented in invasive mucormycosis with hematological diseases. Even a retrospective study of 59 cases of hematological malignancies with invasive mucormycosis did not comment on the thrombotic events [7]. Indeed, there have been only a few case reports that have focused on this complication [8-10]. In our series, AML was the underlying hematological disease, and neutropenia had lasted for several months before the onset of mucormycosis. Physical findings were featured by fever and rapidly progressive dyspnea. Autopsy revealed embolism of the pulmonary artery by *Rhizopus microsporus* and hemorrhagic infarction of the surrounding parenchyma, which were probably responsible for dyspnea. Our cases indicate that pulmonary embolism is recurrent in hematological malignancies with invasive mucormycosis. We should suspect this life-threatening complication when patients with hematological malignancies develop fever and deteriorating dyspnea under prolonged neutropenia. CT scan with contrast enhancement and lung perfusion scintigraphy may be useful for the detection of pulmonary embolism. Hemodynamic evaluation by cardiac echography may also be considered.

Invasive mucormycosis in patients with hematological diseases predicts dismal outcomes. The mortality rate within three months after diagnosis reaches 79% [7]. It is partly because Mucorales generally show resistance to echinocandins and most azoles. Furthermore, first-line antifungal agents such as deoxycholate AmB and its lipid derivative L-AmB are frequently insufficient to control invasive mucormycosis. In our series, treatment with L-AmB at 5 mg/kg daily was not successful in Case 1. In such occasions, the treatment of mucormycosis may also require surgical resection and management of neutropenia. Notably, the efficacy of posaconazole against mucormycosis has been described in recent years. L-AmB plus posaconazole for patients with mucormycosis resulted in a partial response rate of 46% [11]. The combination therapy is worth consideration when surgical resection is not feasible.

In conclusion, pulmonary embolism is a recurrent complication of pulmonary mucormycosis in patients with hematological malignancies. A large study is required to reveal the genuine incidence of the life-threatening sequelae.

Disclosure of conflict of interest

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