Int J Clin Exp Pathol 2017;10(2):2219-2223
www.ijcep.com /ISSN:1936-2625/IJCEP0040985

Case Report

A pneumonectomized case of *cunninghamella bertholletiae* infection detected by the polymerase chain reaction

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Received September 30, 2016; Accepted October 25, 2016; Epub February 1, 2017; Published February 15, 2017

Abstract: A 57-year-old male with acute myelogenous leukemia suffered from left-sided pneumonia and a pulmonary cavity. He underwent a transbronchial lung biopsy examination and was diagnosed with mucormycosis. He was treated with left pneumonectomy, and *Cunninghamella bertholletiae* was detected in formalin-fixed paraffin-embedded lung tissue samples using the polymerase chain reaction (PCR). To the best of our knowledge, this is the first case report about a patient with a PCR-detected pulmonary *C. bertholletiae* infection who underwent pneumonectomy.

Keywords: Pneumonectomy, *Cunninghamella bertholletiae*, PCR

Introduction

Mucormycosis can be a fatal invasive fungal infection in immunocompromised hosts [1, 2]. Mucormycosis is the common name given to several different diseases caused by filamentous fungi belonging to the order Mucorales [3]. Mucormycosis has a high mortality rate when left untreated. In general, surgical resection is the best treatment option for severe pulmonary mucormycosis.

We describe in detail the histological detection of mucormycosis in a patient with acute myelogenous leukemia (AML). In this case, *Cunninghamella bertholletiae* was detected in a DNA sample extracted from formalin-fixed paraffin-embedded (FFPE) lung tissue using the polymerase chain reaction (PCR).

Case report

Case

A 57-year-old male with a history of AML, M2, according to the French-American-British classification, was admitted to the hematological department of our hospital to receive treatment for his AML. He was administered intensive chemotherapy for his AML and was scheduled to undergo an additional chemotherapy regimen and an allogeneic bone marrow transplant from his brother. Although his AML was in remission, and his peripheral cell count was normal, he developed pneumonia, which was treated with antibiotics. However, his pneumonia got worse, and a pulmonary cavity formed.

The patient was otherwise healthy, except for diabetes mellitus, which was treated with insulin therapy. He had not suffered from any pulmonary infections before his AML.

The day after the chemotherapy, he developed neutropenic fever. He did not exhibit dyspnea, difficulty breathing, phlegm, or hemoptysis. During the period of his pneumonia, his body temperature was 38.7°C, his blood pressure was 128/72 mmHg, his pulse rate was 81 beats/min, his respiratory rate was 20 breaths/min, and his degree of oxygen saturation was 95%. Laboratory tests revealed a white blood

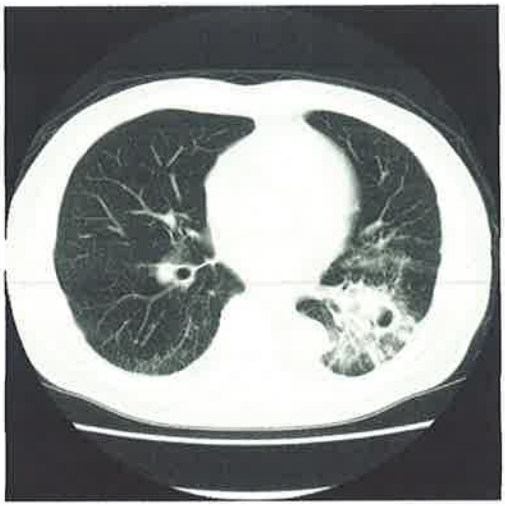

Figure 1. The CT findings of the patient's lungs. CT showed pneumonia and a cavity in the left lung.

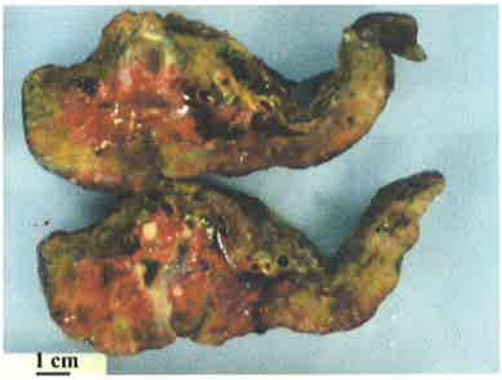

Figure 2. Gross findings of the pneumonectomized left lung. Purulent inflammation, necrosis, hemorrhaging, and a cavity were seen.

count of 100 cell/μl without peripheral blasts, a hemoglobin level of 8.6 g/dl, and a platelet count of 91,000 cell/μl. Serological analysis showed a C-reactive protein level of 25.24 mg/dl and a beta-D glucan concentration of <3.2 pg/ml. A chest x-ray and contrast-enhanced thoracic computed tomography (CT) scan detected in the pneumonia lower left lung together with a pulmonary cavity (**Figure 1**). Although the patient was given antibactericidal and antifungal therapy (liposomal amphotericin B) for his febrile neutropenia, his pulmonary infiltration did not improve. As we had to rule out the differential diagnoses, e.g., a non-bacterial infection, such as by fungi or mycobacterium, the hematologists asked the respiratory department of our hospital to obtain a transbronchial lung biopsy (TBLB) sample. Thus, the patient underwent a biopsy examination of his pneumonia on day 74 of his illness. A histopathological examination of the TBLB specimens detected inflammation and a filamentous fungal infection. Irregularly branched, thick, and aseptate hyphae were also detected. Periodic acid-Schiff staining and Grocott's staining depicted these hyphae more clearly, which was consistent with mucormycosis. No organisms, including Mucor spp., were detected in lung tissue cultures.

In order to allow the patient to complete his chemotherapy and undergo an allogeneic bone marrow transplant for his AML, he underwent left pneumonectomy to remove the pneumonia lesion and pulmonary cavity on day 102 of his illness. Grossly, the resected left lung specimen measured 20 × 14 cm along its longest axes and exhibited pneumonia together with necrosis and a pulmonary cavity (**Figure 2**). A histopathological examination showed mycotic emboli in the pulmonary arteries (**Figure 3**; × 40). Mucormycosis involving the characteristics of fungi; i.e., broad, pleomorphic, and aseptate hyphae that predominantly branched at right angles, was also detected. These findings were compatible with mucormycosis (**Figure 4**; × 400, Grocott's staining). However, no organisms were detected during a pathological examination or lung tissue cultures.

Finally, we detected *Cunninghamella bertholletiae* in the lung tissue using a PCR-based method [4]. Briefly, DNA was extracted from FFPE samples of the resected lung tissue using the QIAmp DNA FFPE tissue kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). PCR targeting the internal transcribed spacer and D1/D2 regions were performed as described previously [4]. The PCR products were electrophoresed in 2% ME agarose gel, followed by staining with ethidium bromide. Specific bands were cut and directly sequenced. Sequencing analysis revealed that the sequences of the bands were 98.9% identical to those of *C. bertholletiae* (GenBank AF113421).

After the pneumonectomy, the patient's postoperative course was stable, and his AML went into complete remission after the allogeneic bone marrow transplant; however, he subse-

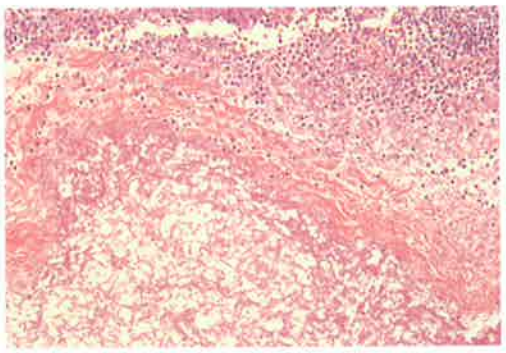

Figure 3. Microscopic findings of the hilar pulmonary artery of the left lung. Severe inflammation and mycotic emboli were seen (hematoxylin & eosin staining, original magnification: × 40).

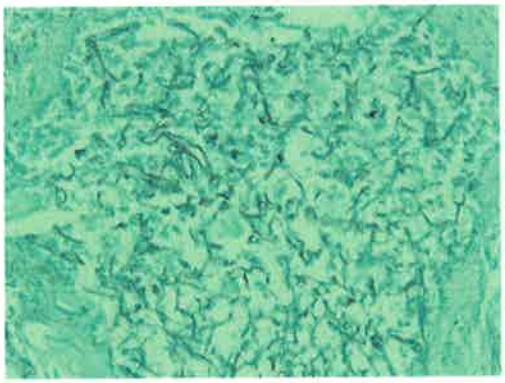

Figure 4. Microscopic findings of the *C. bertholletiae* hyphae. Irregularly shaped, broad, and aseptate hyphae with right-angle branching were seen (Grocott's staining, original magnification: × 400).

quently died of complications associated with graft versus host disease on postoperative day 265. The autopsy examination did not reveal any recurrence of the *C. bertholletiae* infection or relapse of the AML.

Discussion

To the best of our knowledge, this is the first reported case of a patient with a pulmonary *C. bertholletiae* infection who underwent pneumonectomy and in whom *C. bertholletiae* was detected by the PCR. Mucor spp., including Cunninghamella spp., are well-known environmental organisms and have been isolated from soil, water, air, flowers, and other vegetation worldwide, predominantly in more temperate climates [3]. The major route of transmission for *C. bertholletiae* is presumed to be the inhalation of spores through the respiratory tract [3, 5]. The first case of *C. bertholletiae* infection was described in 1958 and involved a patient with lymphosarcoma who had received strong immunosuppressive therapy [6]. Most patients with invasive *C. bertholletiae* infections are immunocompromised and exhibit an acute and progressive course [1, 3]. The lungs are a common site of Cunninghamella infections, and disseminated, cutaneous-articular, rhino-orbito-cerebral, and endo-cardiovascular infections have also been observed [3, 5]. The risk factors for Cunninghamella infections include hematological malignancies with neutropenia; immunosuppressive chemotherapy; or undergoing leukemia, diabetes mellitus, or a bone marrow or organ transplant. Mucormycosis exhibits a high mortality rate (40-76%) [2, 7, 8]. However, surgical treatment has a strong impact on the outcomes of the condition. The overall mortality rate of mucormycosis is 60% [9], but it is 11% in patients who undergo surgical treatment. Although surgical treatment seems to improve the clinical outcomes of patients with pulmonary mucormycosis, they frequently suffer complications, such as thrombocytopenia, multiple pulmonary infiltrations, or dissemination [9-11]. Cases involving multiple infectious foci in one organ or disseminated disease are not indicated for surgery. Among patients with hematological disease, about 40% have disseminated disease, and the mortality rate of disseminated mucormycosis is 96% [10]. Five cases of pulmonary *C. bertholletiae* infections (including those involving pulmonary emboli) in which surgical interventions were employed have been reported. In these cases, the causative species were identified via both morphological examinations and microbiological cultures [12-16] (**Table 1**). All five reported patients had underlying hematological malignancies, and only two patients survived [13, 15]. Of the five patients who underwent lung surgery for pulmonary *C. bertholletiae*, two underwent lobectomy, and two underwent partial resection of the lung (segmentectomy and the resection of a lung nodule); however, there were no cases in which pneumonectomy was performed. All five patients received high-dose amphotericin B or liposomal amphotericin B. The current patient was treated with liposomal amphotericin B and underwent pneumonectomy, which resulted in his *C. bertholletiae* infection being completely controlled.

Table 1. Reported cases of *C. bertholletiae* infection involving surgical interventions

Case number/ Reference	Age/ Sex	Underlying disease	Infected lung lesions	Surgery	Intravenous antifungal treatment	Histology/Culture for fungus	Genetic detection	Outcome
1 [12] (1993)	3/M	AML	LLL	Lobectomy	Amp B	Hemorrhaging, infarction, and mycotic thrombi/ Lung tissue culture+	None	Fatal, 11.5 postoperative mos
2 [13] (1998)	16/F	ALL	RLL/LLL	Wedge resection/Lobectomy	Amp B	Necrosis and mycotic thrombi/Lung tissue culture+	None	Alive
3 [14] (2001)	52/M	CLL/BMT	RUL/RML	Lobectomy	L-Amp B	Acute and chronic inflammation, and necrosis/ Lung tissue culture+	None	Fatal, POD 27
4 [15] (2006)	57/F	ALL	RUL	Apical segmentectomy	L-Amp B, Pos	Necrosis and cavitation/Lung tissue culture+	None	Alive
5 [16] (2010)	10/F	AML	RLL	Surgical resection of the lung nodule	L-Amp B, Pos	Nodules and thrombosis/Lung tissue culture+	None	Fatal, POD 154
Current case	57/M	AML/DM	LL	Pneumonectomy	L-Amp B	Cavitation and mycotic thrombi /Negative	PCR+	Fatal, POD 265

ALL: Acute lymphoblastic leukemia, AML: Acute myelogenous leukemia, Amp B: Amphotericin B, BMT: Bone marrow transplant, CLL: Chronic lymphocytic leukemia, DM: Diabetes mellitus, L-Amp B: Liposomal amphotericin B, LL: Left lung, LLL: Left lower lobe, mos: Months, RLL: Right lower lobe, RML: Right middle lobe, RUL: Right upper lobe, POD: Postoperative day, Pos: Posaconazole.

In the current case, pathological TBLB tissue specimens and the resected lung tissue demonstrated the typical morphological features of mucormycosis, e.g., irregularly shaped, broad, and aseptate hyphae with right-angle branching [3]. The lung tissue also displayed hemorrhagic necrosis, angioinvasion, mycotic thrombi, and infarction. The diagnosis of *C. bertholletiae* was confirmed by subjecting FFPE lung tissue samples to PCR.

The pathological detection of invasive fugal infections might be useful for diagnostic purposes and can help to ensure that appropriate treatments are administered earlier.

Acknowledgements

The authors thank Dr. Hitomi Fukumoto, Harutaka Katano, and Hideki Hasegawa of the Department of Pathology, National Institute of Infectious Diseases.

Disclosure of conflict of interest

None.

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