



Coinfection pulmonary mucormycosis and aspergillosis with disseminated mucormycosis involving gastrointestinal in an acute B-lymphoblastic leukemia patient

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Abstract

Pulmonary mucormycosis and aspergillosis with disseminated mucormycosis involving gastrointestinal is a very rare but lethal infection leading to extreme mortality. Herein, we present a unique case of pulmonary coinfection with *Cunninghamella bertholletiae* and *Aspergillus flavus*, with disseminated mucormycosis involving the jejunum caused by *C. bertholletiae* in an acute B-lymphocytic leukemia (B-ALL) patient with familial diabetes. Early administration of active antifungal agents at optimal doses and complete resection of all infected tissues led to improved therapeutic outcomes.

Keywords Mucormycosis · Aspergillosis · Disseminated mucormycosis · Acute lymphoblastic leukemia · *Cunninghamella bertholletiae* · *Aspergillus flavus*

Mucormycosis is a life-threatening and opportunistic infection leading to high mortality in immunocompromised individuals [1–3]. This lethal infection usually occurs in patients with uncontrolled diabetes, neutropenia, hematologic malignancies (HM), or corticosteroid treatment [4]. The incidence of mucormycosis has been increasing in recent decades,

mainly due to the growth of the number of patients presenting with these predisposing conditions and medical advances in diagnosis [1, 5, 6]. In patients with HM, the main clinical form is pulmonary mucormycosis (PM) [7–9]. The onset of PM is acute, the progression is rapid [10], and the reported mortality ranges from 20 to 100% in adults, depending on the underlying risk factors, site of infection, and treatment [11, 12]. Gastrointestinal mucormycosis (GIM) is the least frequent form, constituting only 4–7% of all cases [13]. Because of the nonspecific clinical hallmarks of GIM, the diagnosis is often delayed or missed, and mortality remains high at 57% [14]. However, in patients with prolonged neutropenia and in those with disseminated disease, mortality is 90–100% [4, 15].

A 51-year-old female presented to the hematology clinic complaining of an approximately 1-month history of fatigue and reported a fever lasting for 24 h. On admission, physical examination revealed a distended spleen. Other systemic examinations were unremarkable. At presentation, her body temperature was 37.4 °C, her blood pressure was 115/71 mmHg, and her pulse was 80 bpm. Her blood work showed an elevated leukocyte count of $33.17 \times 10^9/L$, hemoglobin 68 g/L, and platelets $44 \times 10^9/L$, and the percentage of primitive cells was 95% in peripheral blood.

The timeline of diagnosis and targeted therapy is shown in Table 1. Fever was relieved by anti-biotherapy introduction. The common type of acute B-lymphocytic leukemia

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Table 1 Timeline of events

Time	Clinical features	Biology results	Therapy strategies
D1	Fever	High level of CRP	Levofloxacin 0.6 g/day and cefoperazone/sulbactam 9 g/day IV
D3	Bone marrow pathology	B-ALL	Chemotherapy introduction (vindesine sulfate 4 mg per week and dexamethasone 10 mg/day IV); prophylactic therapy (fluconazole 0.1 g/day p.o.)
D33	Repeated bone marrow pathology	Not completely relieved	Chemotherapy switched to IVCP (idarubicin 10 mg/day 1–3, vindesine sulfate 4 mg per week, CTX 1.2 g/day 1, 15, and dexamethasone 10 mg/d IV)
D39	Neutropenia		Antifungal combined therapy (voriconazole 0.4 g/day p.o.)
D41	Persistence of fever	High level of CRP	Levofloxacin 0.6 g/day and cefoperazone/sulbactam 9 g/day IV
D49	Febrile neutropenia and cough	Rising of CRP rate; abnormal chest CT scan	Switched anti-biotherapy therapy (meropenem 3 g/day and linezolid 1.2 g/day IV)
D51		Filamentous fungi detected in BALF, serum GM test positive	Switched antifungal therapy (d-AmB 0.4 mg/kg/day IV)
D57	Fever resolution		
D62	Abdominal pain and fever	Blood pressure 85/33 mmHg; high level of CRP	Adjusted to tigecycline 0.1 g/day, combined L-AmB 0.5 mg/kg/day and voriconazole 0.4 g/day IV
D63		Abnormal abdominal CT scan, acute peritonitis	Emergency surgery
D64		Hypha detected in jejunum histopathology	Adding L-AmB dose to 1.0 mg/kg/day IV
D66	Fever resolution		
D83	Repeated bone marrow pathology	Completely relieved	Adding L-AmB dose to 1.2 mg/kg/day IV
D100	Regression of lesions on imagery		Switched antifungal therapy (posaconazole 0.8 g/day p.o.)
D130	Complete remission	Negative BALF	

D day, CRP C-reactive protein, IV intravenous, B-ALL acute B-lymphocytic leukemia, p.o. per os, CT computed tomographic, CTX cyclophosphamide, d-AmB amphotericin B deoxycholate, L-AmB liposomal amphotericin B, BALF bronchoalveolar lavage fluid, GM galactomannan

(B-ALL) with the IKZF1 mutation was diagnosed by bone marrow pathology. Considering her history of familial diabetes and percutaneous coronary intervention, the chemotherapy program was initiated with a low dose of vindesine sulfate and dexamethasone and oral prophylactic treatment with fluconazole simultaneously. Serologies for (1,3)-beta-D-glucan, galactomannan (GM) (Dynamiker Biotechnology Co., Ltd. Tianjin, China), syphilis, acquired immunodeficiency syndrome, and hepatitis A–E were negative. One month later, bone marrow pathology was repeated and showed 12% blast cells. A high-intensity IVCP program was performed. After 5 days, broad-spectrum antibiotics and voriconazole were started due to febrile neutropenia.

On day 49, significant pulmonary symptoms, such as productive cough, occurred, along with a persistent fever. Computed tomography (CT) showed a massive high-density shadow in the right superior lobe (Fig. 1) and rising levels of C-reactive protein (CRP). Blood culture was sterile, and polymerase chain reaction for cytomegalovirus and EB virus were negative. Anti-biotherapy was switched to meropenem and linezolid, but there was no obvious relief in



Fig. 1 A close-up chest CT scan of the right lung shows a massive high-density shadow (arrow) in the superior lobe

symptoms. On day 51, the serum GM antigen test was positive (2.38), and the microbiological tests were implemented with bronchoalveolar lavage fluid (BALF). Classically, microscopic evaluation with Gram (Fig. 2A) and calcofluor white (Fig. 2B) staining revealed filamentous hyphae; one type was uniformly thinner, septate, and branching at acute angles, and the other had a variable width, was nonseptate, and had branching filamentous hyphae and a ribbon-like appearance. Cultures of specimens on Sabouraud Dextrose

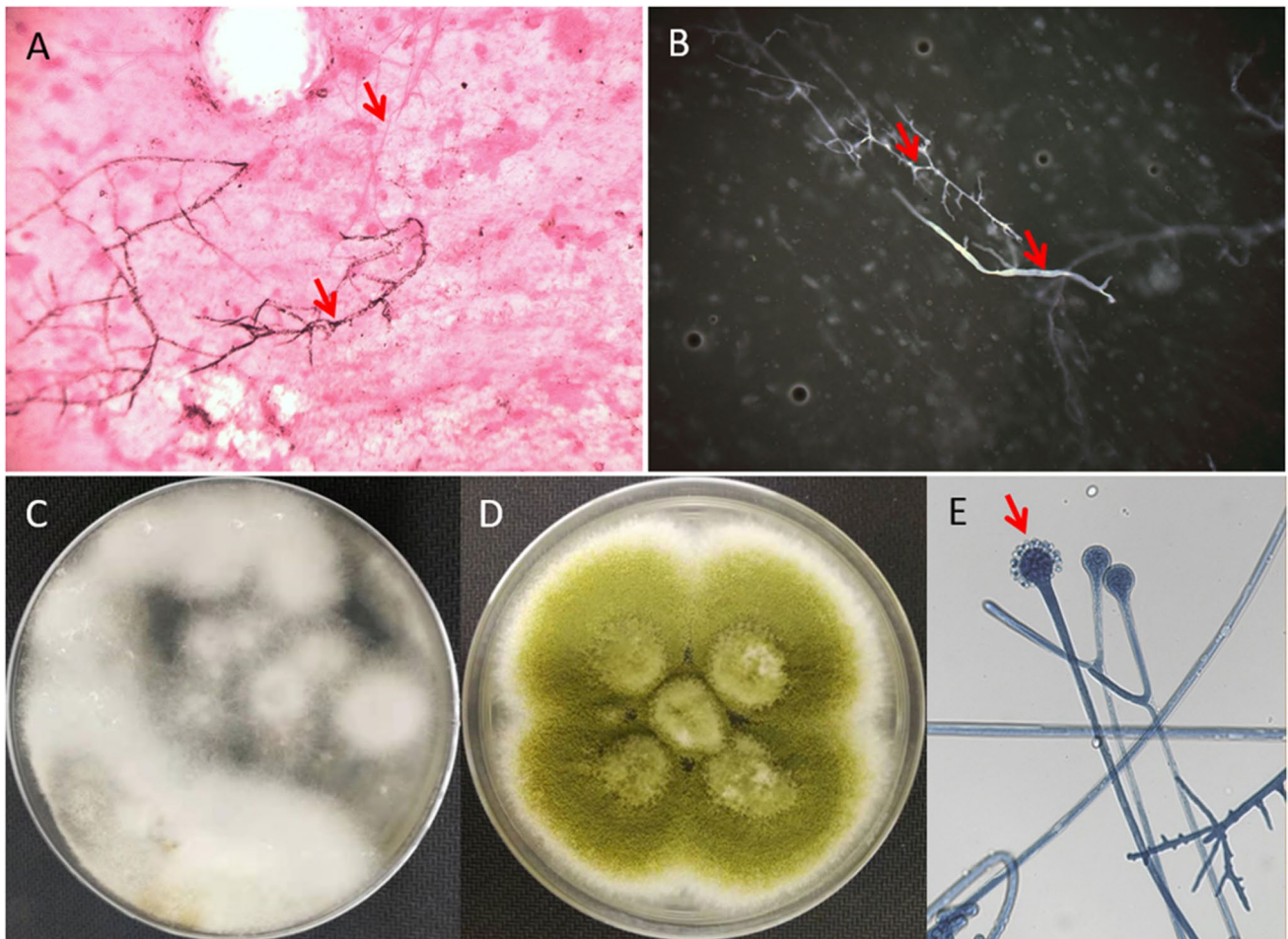


Fig. 2 (A) Gram staining and (B) calcofluor white staining showed two different hyphae: one is uniformly thinner, septate, and branching at acute angles. The other is a variable width with ribbon-like appearance (400× magnification). (C) *Cunninghamella bertholletiae* and

(D) *A. flavus* isolated from BALF cultured on a SDA medium plate for 48 h at 35 °C. (E) *Cunninghamella bertholletiae* sporangioophores in terminal swellings called vesicles, with sporangioles (lactophenol cotton blue staining, 400× magnification)

Agar (SDA) showed the features as *Mucorales*. Colonies appeared cotton and white–gray, both on the surface and reverse side (Fig. 2C). Lactophenol cotton blue staining revealed irregularly branching sporangioophores terminating prominently, and sporangioles borne off the vesicles (Fig. 2E). *Cunninghamella bertholletiae* was identified by mycological characteristics and internal transcribed spacer (ITS)–based sequencing (accession no. MT470208). DNA sequences were analyzed using NCBI BLAST (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Another pathogen isolated from BALF was *Aspergillus flavus* (accession no. MW911813).

Based on the characteristics of the filamentous hyphae, we switched the antifungal therapy to intravenous amphotericin B deoxycholate (d-AmB) with an initial dose of 0.5 mg/kg/day. Persistent fever was resolved, but unexpectedly, acute abdominal pain with high fever and a “sudden drop” in blood pressure appeared on day 62. Anti-biotherapy was adjusted to tigecycline combined with liposomal

amphotericin B (L-AmB). At midnight, the abdominal pain worsened, and acute diffuse peritonitis was considered. CT showed some free abdominal gas under the diaphragm, and peritoneal fluid was detected (Fig. 3). Emergency surgical management, including partial resection of the jejunum and ileum, was performed. There were 9 perforations in the jejunum 190–210 cm from the curved ligament, with an aperture of approximately 1–2 cm, and a perforated ileum was detected approximately 25 cm from the ileocecal part.

Histopathology of specimens from the jejunum and ileum showed broad septate fungal hyphae (Fig. 4). Cultures of specimens from the jejunum also showed features such as *Mucorales*, and *C. bertholletiae* (accession no. MW915438) was identified according to the same protocols mentioned above. Antifungal susceptibility tests according to the Clinical and Laboratory Standards Institute (CLSI) M38-A2 broth microdilution document [16] were implemented. The susceptibility profiles of *C. bertholletiae* isolated from BALF

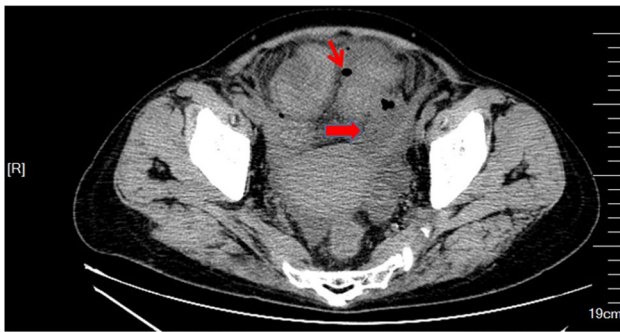


Fig. 3 The thinner arrow shows free abdominal gas under the diaphragm, and the wider arrow shows peritoneal fluid

and tissue showed fluconazole 256 $\mu\text{g/ml}$, itraconazole 0.5 $\mu\text{g/ml}$, posaconazole 0.5 $\mu\text{g/ml}$, voriconazole 8 $\mu\text{g/ml}$, AmB 2 $\mu\text{g/ml}$, and flucytosine 64 $\mu\text{g/ml}$. The susceptibility

profiles of *A. flavus* isolated from BALF showed itraconazole 1 $\mu\text{g/ml}$, posaconazole 0.5 $\mu\text{g/ml}$, voriconazole 0.25 $\mu\text{g/ml}$, and AmB 2 $\mu\text{g/ml}$.

L-AmB was added to 1.0 mg/kg/day for 1 week, followed by fever resolution. She was covered pre- and postsurgery with L-AmB for 8 weeks. Considering the relief of symptoms and regression of lesions on imagery, our strategy switched to oral posaconazole 0.8 g/day. The patient was discharged in good condition for continuous therapy with oral posaconazole 0.8 g/day for almost 6 months. Due to the COVID-19 pandemic and other reasons, the patient's family finally gave up and the patient passed away at home last year. Mucormycosis and aspergillosis are opportunistic fungal infections that can lead to life-threatening complications and occur most commonly in individuals with neutropenia and prolonged immunosuppressive therapy [17]. An epidemiological article of 929 cases of mucormycosis found a correlation between the patient survival and the species within

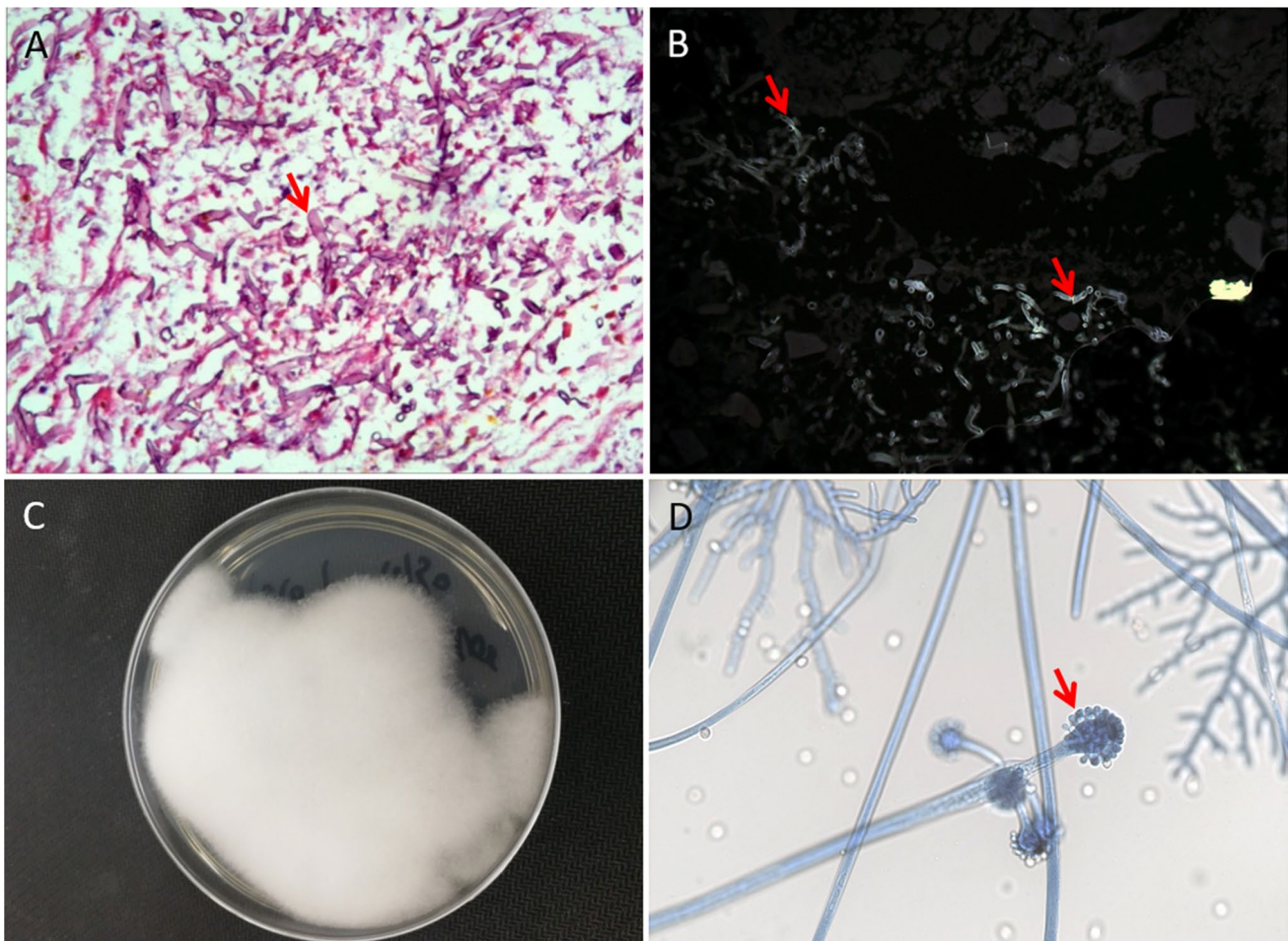


Fig. 4 Photomicrographs from the jejunum showed acute necrotizing angioinvasion with abundant broad, nonseptate fungal hyphae (arrow) consistent with mucormycosis ((A) hematoxylin and eosin staining; (B) calcofluor white staining; 400 \times magnification). (C) *Cunningham-*

mella bertholletiae colony isolated from tissue cultured on a SDA medium plate for 48 h at 35 $^{\circ}\text{C}$. (D) Lactophenol cotton blue staining revealed irregularly branching sporangiophores terminating prominently, and sporangioles borne off the vesicles (400 \times magnification)

the *Mucorales*, given the conclusion of *Cunninghamella* spp. causing the highest percentage of crude mortalities and being an independent risk factor for death in the multivariate analysis [18]. As the most representative etiologic agent, *C. bertholletiae* occurs less frequently but causes refractory and fatal infections. A review of 15 cases of mucormycosis caused by *Cunninghamella* spp. indicated a patient population mainly consisting of neutropenia and transplantation [19]. GIM is the rarest type of *Mucorales* infection, and the successful management of the aggressive illness requires early surgical debridement, control of underlying disease, and suitable antifungal therapy [20]. A typical characteristic of pathophysiology of *Mucorales* infection is angioinvasion with thrombosis and thus necrosis of an affected part of the intestine. This will produce acute abdominal pain, possible bleeding, or perforation [20, 21]. To the best of our knowledge, this is the first report of pulmonary coinfection with *C. bertholletiae* and *A. flavus* with disseminated mucormycosis involving the jejunum caused by *C. bertholletiae* in a B-ALL patient in China.

Cunninghamella bertholletiae demonstrated to be the most resistant species among zygomycetes. Literature on *C. bertholletiae* indicates higher minimum inhibitory concentrations (MICs); 37% of the isolates had MICs of 2 µg/ml for AmB, while approximately 75% of the isolates appeared to be susceptible to posaconazole [22]. The high MICs to AMB and the low MICs for itraconazole and posaconazole against *C. bertholletiae* have been reported [22–25]. In general, our results agree with other studies [22–25]. Although decreased susceptibility to amphotericin B in vitro, the lipid formulations of AMB may achieve higher concentrations in vivo [22]. According to the epidemiologic cutoff values (ECVs) of antifungals for *A. flavus* [26], the strain isolated from BALF could be considered a wild-type. Early diagnosis of mucormycosis is the key to treatment and prognosis. And the definitive diagnosis of mucormycosis depends on a combination of histopathological findings and standard mycological methods, as well as DNA sequencing of the ITS region, which has been suggested as a valuable target for identification at the genus and the species level by the CLSI guidelines [27]. Successful management of mucormycosis is on the basis of a multimodal manner, including reversal or revocation of underlying predisposing factors, early administration of suitable antifungal agents, and thorough resection of all infected tissues [28, 29]. According to the guidelines of European Conference on Infections in Leukaemia (ECIL-6) and European Confederation of Medical Mycology-The European Society for Clinical Microbiology and Infectious Diseases (ECMM-ESCMID), d-AmB and L-AmB are recommended as the first-line antifungal agent approved for the therapy of invasive mucormycosis [30]. High-dose L-AmB (10 mg/kg/day) immediately administered upon suspicion of mucormycosis greatly suppressed the infection in its early

stage [31]. However, in the absence of surgical debridement for infected tissue, antifungal therapy alone is rarely curative [4].

Our aim in this report is to highlight the need for a high clinical suspicion for *Mucorales* infection in neutropenic, immunocompromised, and diabetic patients. Direct microscopic testing with calcofluor white is the key to rapid diagnosis. In addition, effective multidisciplinary communication with consulting physicians, such as hematologists, pulmonologists, and microbiologists, as well as immediate initiation of treatment, including surgical resection, can lead to improved patient outcomes in managing this rare but devastating disease and lay a solid foundation for the subsequent treatment of original disease.

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Declarations

Conflict of interest The authors declare no competing interests.

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