Disseminated pulmonary mucormycosis involving jejunum in an acute 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 an extremely mortality. Herein, we present a unique case of pulmonary co-infection with Cunninghamella bertholletiae and Aspergillus flavus, with disseminated mycomycosis involving jejunum caused by Cunninghamella bertholletiae in a B-ALL patient with familial diabetes. Early administration of active antifungal agents at optimal doses, complete resection of all infected tissues led to improved therapeutic outcomes.

Keywords Mucormycosis • Pulmonary • jejunum • Acute lymphoblastic leukemia • Disseminated • Mucorales • Cunninghamella bertholletiae

Introduction
Mucormycosis is a life-threatening and opportunistic infection leading to a remarkably mortality in the immunocompromised individuals [1-3]. This lethal infection usually occurs in patients with uncontrolled diabetes, neutropenia, haematologic 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 patient groups presenting with these pre-disposing conditions and our medical advances in diagnosing the infection [1, 5, 6]. In patients with HM, the main clinical form is pulmonary mucormycosis (PM) [7-9] and the most representative risk factors associated with PM included neutropenia and corticosteroids [8]. The onset of pulmonary mucormycosis is acute and the progression is rapid [10], and the reported mortality ranges in adult from 20% to 100%, 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 non-specific 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].

Case report
On July 18, 2019, a 51-year-old female presented to the hematology clinic complained of approximately one month history of fatigue, and reported a fever lasting for 24 hours. On admission, physical examination revealed a distended spleen. Other systemic examinations were unremarkable. At presentation, her body temperature was 37.4°C, blood pressure was 115/71 mmHg, and pulse was 80 bpm. Her blood work showed an elevated white blood count of $33.17 \times 10^9/L$, hemoglobin 68 g/L, platelets $44 \times 10^9/L$, the percentage of primitive cells is 95% in peripheral blood.

Timeline of diagnosis and targeted therapy as showed in table 1. Fever were relieved by anti-biotherapy introduction. Common type of acute B-lymphocytic leukemia (B-ALL) with IKZF1 mutation was diagnosis by bone marrow pathology. Considered of her history of familial diabetes and percutaneous coronary intervention (PCI), chemotherapy program was initiated with low dose of vindesine sulphate and dexamethasone, oral prophylactic treatment with fluconazole simultaneously. One month later, bone marrow pathology was repeated, and showed 12% blast cells. A high-intensity of 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, and presented a persistence of fever. Computed tomographic (CT) showed a massive high density shadow in the right superior lobe (Fig. 1), and rising lever of C-reaction protein (CRP). Blood culture was sterile and serologies for (1,3)-beta-D-Glucan, galactomannan (GM), syphilis, acquired immunodeficiency syndrome and hepatitis A–E were negative. Polymerase chain reaction for cytomegalovirus and EB virus were negative. Anti-biotherapy was switch to meropenem and linezolid, but the symptoms had no obviously relief. Microbiological tests were implemented with low respiratory tract specimens. Classically, microscopic evaluation with Gram (Fig. 2a) and calcofluor white (Fig. 2b) stain reveals filamentous hyphae, one type have a uniform thinner, septate, and branch at acute angles and another have a variable width, non-septate, branching filamentous hyphae and show a ribbon-like appearance. Cultures of specimens on Sabouraud dextrose agar (SDA) showed the features as Mucorales. Colonies appeared cotton and white-gray, both on the surface and reverse (Fig. 2c).
Lactophenol cotton blue stain revealed irregularly branching sporangiophores terminating in prominent and sporangioles borne off the vesicles (Fig. 2d). *Cunninghamella bertholletiae* was identified by mycological characteristics and ITS-based sequencing (Accession No. MT470208). DNA sequences were analysed using NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Another pathogen isolated from specimen was *Aspergillus flavus*.

Based the characteristic of filamentous hypha, we switched the antifungal therapy to intravenous amphotericin B (AmB) with initial dose 0.5mg/kg/d. Persist fever was resolved, but unexpectedly an acute abdomen pain with high fever and “sudden drop” in blood pressure appeared on day 62. Anti-biotherapy adjusted to tigecycline combined with liposomal amphotericin B (L-AmB). At the 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 (Figure 3). Emergency surgical management including partial resection of the jejunum and ileum was performed. There were 9 perforations from the jejunum 190cm-210cm from the curved ligament, with an aperture of about 1-2cm, and about 25cm from the ileocecal part perforated ileum was detected.

Histopathology of specimens from jejunum and ileum showed broad septate fungal hyphae (Fig. 4). Cultures of specimens from jejunum also showed the features as *Mucorales*, and *Cunninghamella bertholletiae* was identified according to the same protocols mentioned above. Antifungal susceptibility tests according to Clinical and Laboratory Standards Institute (CLSI) M38-A2 [16] were implemented. The susceptibility profiles of *C.bertholletiae* showed fluconazole 256μg/ml, itraconazole 0.5μg/ml, posaconazole 0.5μg/ml, voriconazole 8μg/ml, AmB 2μg/ml, flucytosine 64μg/ml and echinocandins all 8μg/ml. The susceptibility profiles of *A.flavus* showed itraconazole 1μg/ml, posaconazole 0.5μg/ml, voriconazole 0.25μg/ml, and AmB 2μg/ml.

L-AmB was added to 1.0mg/kg/d for one week, then fever resolution. She was covered pre- and post-surgery with L-AmB for 8 weeks. Considering the relief of
symptoms and regression of lesions on imagery, our strategy switched to oral posaconazole 0.8g/d. The patient was discharged in good condition for continuous therapy of antifungal agents, and for follow up at outpatient clinic.

Discussion

Mucormycosis and aspergilllosis 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 review of 929 cases of mucormycosis found a correlation between survival and the species within the Mucorales that was the etiologic agent, with 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 more aggressive, refractory, and fatal infections despite antifungal therapy. A review of 15 well-described cases of mucormycosis caused by Cunninghamella revealed a patient population predominantly consisting with neutropenia, transplantation, and fatal outcome [19]. Gastrointestinal mucormycosis (GIM) is the rarest variant, constituting only 4–7% of all cases and causing a mortality rate of 85–90% [14]. Its successful management requires early surgical debridement, control of underlying illness and prompt antifungal therapy [20]. The jejunum is the least affected and only few cases of jejunal mucormycosis have been reported so far. Angioinvasion characteristic of Mucorales may cause thrombosis and thus necrosis of an affected segment of bowel. This will produce acute abdominal pain, possible bleeding or perforation [20, 21]. To the best of our knowledge, this is the first report of disseminated mycomycosis involving jejunum in B-ALL patient caused by C. bertholletiae in China.

The diagnosis and treatment of mucormycosis are challenging. Clinical approach to diagnosis lacks sensitivity and specificity. A definitive diagnosis of mucormycosis depends on a combination of histopathological findings and standard mycological methods, as well as DNA sequencing of the internal transcribed spacer (ITS) region, which has been proposed as a valuable target for resolution to genus and usually to the
species level by the CLSI guidelines for fungal identification [22]. Successful management of mucormycosis is based on a multimodal approach, including reversal or discontinuation of underlying predisposing factors, early administration of active antifungal agents at optimal doses, complete resection of all infected tissues, and use of various adjuncive therapies [23, 24]. Although AmB remains the only antifungal agent approved for the treatment of invasive mucormycosis, it is widely accepted that L-AmB, which recommended as the first line therapy for mycormycosis, according to the recently published guidelines by ECIL-6, as well as those published by ECMM/ESCMID [25]. High-dose L-AmB (10 mg/kg/day) immediately administered upon suspicion of mucormycosis greatly suppressed the infection in its early stage [26]. 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 neutropenia, immunocompromised and diabetic patients. Direct microscopic test with calcofluor white is the key to rapid diagnosis. Meanwhile, effective multi-departmental communication with consulting physicians such as hematology, pulmonology and microbiology, 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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Compliance with Ethical Standards  This work was carried out in accordance with relevant institutional and national guidelines. The patient gave written informed consent in accordance with the Declaration of Helsinki.

Conflict of interest  None of the authors has any potential financial or non-financial
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References


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<td>D1</td>
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<td>High level of CRP</td>
<td>Levofloxacin 0.6g/d and Cefoperazone/Subbactam 9g/d IV</td>
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<td>D3</td>
<td>Bone marrow pathology</td>
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<td>Chemotherapy introduction (Vindesine sulphate 4mg per week and Dexamethasone 10mg/d IV); Prophylactic therapy (fluconazole 0.1g/d p.o)</td>
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<td>Repeated bone marrow pathology</td>
<td>Not complete relieved</td>
<td>Chemotherapy switch IVCP (Idarubicin 10mg/d1-3, Vindesine sulphate 4mg per week, CTX 1.2g/d1,15, and Dexamethasone 10mg/d IV)</td>
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<td>D39</td>
<td>Neutropenia</td>
<td></td>
<td>Antifungal combined therapy (Voriconazole 0.4g/d p.o)</td>
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<tr>
<td>D41</td>
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<td>Levofloxacin 0.6g/d and Cefoperazone/Subbactam 9g/d IV</td>
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<tr>
<td>D49</td>
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<td>Rising of CRP rate; Abnormal chest CT scan</td>
<td>Switch antibiotherapy therapy (Meropenem 3g/d and Linezolid 1.2g/d IV)</td>
</tr>
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<td></td>
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<td>Switch antifungal therapy (AmB 0.4mg/kg/d IV)</td>
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<td>Fever resolution</td>
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<td>D62</td>
<td>Abdominal pain and fever</td>
<td>Blood pressure 85/33mmHg; High level of CRP</td>
<td>Adjust to tigecycline 0.1g/d, combined L-AmB 0.5mg/kg/d and Voriconazole 0.4g/d IV</td>
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<td>D63</td>
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<td>Abnormal abdominal CT scan, acute peritonitis</td>
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<td>D64</td>
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<tr>
<td>D66</td>
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<td></td>
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<td>D83</td>
<td>Repeated bone marrow pathology</td>
<td>Complete relieved</td>
<td>Adding L-AmB dose to 1.2mg/kg/d IV</td>
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<tr>
<td>D100</td>
<td>Regression of lesions on imagery</td>
<td></td>
<td>Switch antifungal therapy (posaconazole 0.8g/d p.o)</td>
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<tr>
<td>D130</td>
<td>Complete remission</td>
<td>Negative Sputrum</td>
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D day, CRP C-Reactive protein, IV intra-venous, B-ALL acute B-lymphocytic leukemia, p.o per os, CT computed tomographie, CTX Cyclophosphamide, AmB amphotericin B, L-AmB liposomal amphotericin B
Fig.1 Close-up chest CT scan of the right lung shows a massive high density shadow (arrow) in the superior lobe.

Fig.2 a Gram stain and b calcofluor white stain showed two different hyphae: one is uniform thinner, septate, branch at acute angles, another is a variable width with ribbon-like appearance (20× magnification). c Macroscopic and d microscopic appearances of C.bertholettiiae.
Fig. 3 The thinner arrow shows free abdominal gas under the diaphragm, and the wider arrow shows peritoneal fluid.

Fig. 4 Photomicrograph from jejunum showed an acute necrotizing angioinvasion with
abundant broad, nonseptate fungal hyphae (arrow) consistent with mucormycosis (a, Hematoxylin and eosin stain; b, calcofluor white stain; 20× magnification).