Cunninghamella bertholletiae pneumonia showing a reversed halo sign on chest computed tomography scan following cord blood transplantation

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This is the first reported case of a patient who developed fungal pneumonia caused by Cunninghamella bertholletiae (= C. elegans) following cord blood transplantation and who showed a reversed halo sign on a chest computed tomography scan (CT). In addition, the pathological findings related to the reversed halo sign are described in detail for the first time. The patient died due to respiratory failure and at autopsy, a consolidation corresponding to the reversed halo sign noted on CT was found histologically to be composed of a central infarct with some retained air spaces surrounded by a peripheral ring-like hemorrhagic band. Pulmonary vasculatures were occluded by thrombi containing numerous Zygomycetes hyphae within the central infarct and less frequently along the surrounding hemorrhagic band. A reversed halo sign may be an early marker to initiate preemptive therapy against Zygomycetes including C. bertholletiae.

Keywords Cunninghamella bertholletiae, zygomycosis, reversed halo sign, cord blood transplantation

Introduction

Immunocompromised patients with zygomycosis (= mucormycosis) have unfavorable prognoses [1]. Recently, the reversed halo sign has been reported as a chest computed tomography scan (CT) finding corresponding to an early phase of zygomycosis in immunocompromised patients [2]. This is a report of a patient who developed Cunninghamella bertholletiae pneumonia following cord blood transplantation and who showed the reversed halo sign on chest CT.

Case report

In October 2008, a 53-year-old woman underwent a second cord blood transplantation (CBT) due to a relapse of her acute myeloid leukemia following the first CBT. She had been treated with 10 mg prednisolone for chronic skin graft-versus-host disease (GVHD). She had no history of diabetes mellitus (hyper glycemia), chronic kidney disease, or chronic liver disease. Her serum ferritin level was 11980 ng/ml and no abnormal finding was noted on chest CT before transplantation. On day – 10 (10 days before CBT), prophylactic oral tosufloxacin, voriconazole, and acyclovir were started. Dry cough and fever developed on day – 2 and arterial blood oxygen was decreased. Chest CT revealed thickened interlobular septa and ground-glass attenuation in the right lower lobe of the lung. Respiratory syncytial virus (RSV) was detected using sputum polymerase chain reaction (PCR) analysis. Sputum Gram staining and culture were negative, and the serum Aspergillus galactomannan antigen and β-D glucan levels were within normal ranges. On the basis of these findings, RSV pneumonia was diagnosed. Because the clinical course of this infection was stable, cord blood with total nucleated cell dose of 2.43 × 10^7/kg was transplanted on day 0. GVHD prophylaxis consisted of tacrolimus and mycophenolate mofetil.

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On day 13 (13 days after CBT), fever, dry cough, and bloody sputum developed, during persistent neutropenia of less than 100/μl which had occurred since day −1. Chest CT revealed a reversed halo sign in the left upper lobe of the lung (Fig. 1), and prophylactic 8 mg/kg/day voriconazole was changed to 3 mg/kg/day liposomal amphotericin B on the same day. Breakthrough invasive pulmonary aspergillosis due to azole-resistant or low susceptible Aspergillus spp. or a low voriconazole trough blood level was taken into consideration. The neutrophil level was successfully restored to >500/μl on day 20 but the patient’s respiratory condition rapidly deteriorated and massive hemoptysis and confusion developed. Cytomegalovirus antigen assay was negative. Chest CT revealed that the reversed halo sign had increased in size on the same day (Fig. 2a, 2b). The results of brain magnetic resonance imaging, brain magnetic resonance angiography and cerebrospinal fluid examination, including attempts to recover fungi in culture did not reveal evidence of abscess, embolism, aneurysms or meningitis. The patient died of respiratory failure on day 25. An ill-demarcated hemorrhagic consolidation, 70 mm in diameters, observed in the left upper lobe (Fig. 3a, 3b) was noted at autopsy, which corresponded to the reversed halo sign observed on CT. Histologically, the lesion was composed of a central infarct surrounded by a peripheral ring-like hemorrhagic band. In the central infarct, multiple levels of pulmonary arteries and veins were extensively occluded by thrombi containing numerous Zygomycetes containing thrombi which were subsequently identified as C. bertholletiae on the basis of its growth at 42–45°C and examination of lactophenol cotton blue mounts (Fig. 3d) on 17 November 2008. Identification was further supported by the sequencing of the internal transcribed spacer (ITS) region and D1/D2 region of the ribosomal RNA gene of the isolated fungus. Briefly, cultures of the fungus were initially mechanically disrupted with glass beads and genomic DNA was extracted and purified using the DNeasy plant mini kit (Qiagen, Germany) according to the manufacture’s protocol. The ITS region of rRNA gene was amplified with the primer pair ITS1 (5′-TCCGTAGGTGAAACCTGCGG-3′) and ITS4 (5′-TCCGCTATTGATATGC-3′) [3], and D1/D2 region of large subunit rRNA gene was also amplified with the primer pair NL1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) [4] by PCR. PCR was performed using 5 μl of template DNA, with 1.25U of Takara EX tag (Takara, Otsu, Japan) in a total 50 μl of reaction mixture supplemented with buffer and dNTPs solutions supplied by Takara. PCR condition was as follows; initial denaturation at 95°C for 5 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min, final extension at 72°C for 3 min. PCR amplicons were purified using QIA quick PCR purification kit (Qiagen), and sequenced directly using BigDye Terminator v3.1 Cycle sequencing kit (Applied Biosystems, Austin, TX, USA) following the manufacture’s recommended reaction condition. The DNA fragments were sequenced using an Applied Biosystems 3130 genetic Analyzer (Life Technologies, Carlsbad, CA, USA). Subsequently, each ITS and D1/D2 region sequence was verified using database of the National Center for Biotechnology Information, Basic Local Alignment Search Tool. As a result, sequences of both the ITS and D1/D2 regions were found to have 99% similarity with C. bertholletiae, ATCC42115. Antifungal susceptibility testing was performed using broth microdilution method according to the Clinical and Laboratory Standard Institute (CLSI) M38-A2 standard [5]. Candida parapsilosis ATCC22019 was used as the quality control strain in this study. These in vitro tests revealed minimal inhibitory concentrations (MICs) of 4 μg/ml for amphotericin B, >64 μg/ml for flucytosine, >64 μg/ml for fluconazole, 8 μg/ml for itraconazole, >32 μg/ml for miconafungin, >16 μg/ml

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for caspofungin, > 8 μg/ml for voriconazole and 1 μg/ml for posaconazole.

Discussion

The reversed halo sign, a focal round area of ground-glass attenuation surrounded by a ring of consolidation, is found in CT studies. In immunocompromised patients, particularly in those with hematological malignancies, the reversed halo sign has been reported in the early phase of zygomycosis [2]. While the reversed halo sign is present in 19% of patients with zygomycosis, it is found in < 1% of patients with aspergillosis [2]. Therefore, this finding is probably an early highly specific diagnostic marker of pulmonary zygomycosis that exhibits low sensitivity in immunocompromised patients. The pathogenesis giving rise to the reversed halo sign can be hypothesized on the basis of histopathologic observation. First, hyphae grow in and around the blood vessels at the infection site during the neutropenic phase after CBT. Next, multiple blood vessels become occluded by thrombi containing numerous hyphae, resulting in extensive infarct and thus the development of air spaces and no demarcation (radiographically lucent) in the absence of an immune response. Then during and after neutrophil recovery and immune reconstitution, neutrophil infiltration occurs only from outside the lesion where blood flow is intact, but not from within the infarct where blood flow is absent. The infarct becomes surrounded by zones of inflammation and hemorrhage presumably caused by the host immune response against vascular infiltrating hyphae. Rapid thrombotic occlusion of the blood vessels, resulting in infarct, near the site of infection during the neutropenic phase may be the first and essential step in developing the reversed halo sign. It may be relevant that Zygomycetes are associated with a greater capacity of vascular invasion and grow faster than Aspergillus species in culture. The reversed halo sign has also been observed in patients with cryptogenic organizing pneumonia, Pneumocystis jirovecii pneumonia, tuberculosis, pneumococcal pneumonia, paracoccidioidomycosis, psittacosis, dermatomyositis, pulmonary sarcoidosis and lymphomatoid granulomatosis [6–15]. When clinicians observe a reversed halo sign, they should also include zygomycosis in the differential diagnosis because in severely immunocompromised patients, particularly in those with hematological malignancies, early optimal treatment against invasive fungal infection before the diagnosis is essential to improve the clinical outcome. Thus, initiating amphotericin B-based preemptive therapy against Zygomycetes and performing diagnostic procedures such as early bronchoscopy are probably justified when a clinician observes a severely immunocompromised patient whose chest CT show a reversed halo sign.

This is the first reported case of a patient with pneumonia caused by C. bertholletiae who showed the reversed halo sign on CT scan following CBT. The pathological findings of this effect are described in detail for the first time. Cunninghamella spp. have been reported to cause breakthrough fungal infections following hematopoietic stem cell transplantation during voriconazole therapy [16], which is typically the initial option for treating invasive aspergillosis. Moreover, Triflon et al. observed a high mortality rate in patients with zygomycosis during voriconazole administration [17]. C. bertholletiae infection of immunocompromised patients, including those who have...
undergone hematopoietic stem cell transplantation, have unfavorable prognoses [18]. The MIC level of amphotericin B against *C. bertholletiae* showed a higher trend than that of the other Zygomycetes [19]. This may be one of the reasons why the outcomes of *C. bertholletiae* infections are so poor. The *C. bertholletiae* isolate in our case had a high MIC level for amphotericin B. In previous reports, 5–10 mg/kg/day liposomal amphotericin B has been used successfully to treat Zygomycete infections other than those caused by *Cunninghamella* spp. [20]. Additionally, there is only one case report of a pediatric patient who acquired pneumonia due to *C. bertholletiae* after bone marrow transplantation who was successfully treated by 5 mg/kg/day liposomal amphotericin B followed by posaconazole therapy and surgical debridement [21]. Since our patient was treated with 3 mg/kg/day liposomal amphotericin B, this level of the antifungal was probably insufficient for a positive outcome. Instead of voriconazole treatment, a higher dose of liposomal amphotericin B with or without posaconazole could have been administered when the reversed halo sign was first revealed on chest CT on day 13.

The reversed halo sign in our case increased in thickness even after neutrophil recovery. At the same time, the consolidation and ground-glass attenuation of the right lung and left lower lobe worsened. Both effects may have been caused by immune reconstitution after neutrophil recovery. The pathology of the right lung and left lower lobe revealed diffuse alveolar damage with no evidence of zygomycosis or other infections. Additionally, the results of autopsy showed no evidence of hyphae infiltration into any other organs. Therefore, acute respiratory distress syndrome induced by *C. bertholletiae* pneumonia was thought to be the cause of the fatal outcome.

This is the first reported case of a patient with *C. bertholletiae* pneumonia who showed the reversed halo sign on CT scan following CBT. Additionally, the pathological findings of the reversed halo sign are described in

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Fig. 3 Gross cut-surface view (a) and low-power microscopic view: the lesion which corresponded to the reversed halo sign seen on CT; central (C) area and peripheral (P) ring-like area of the lesion (b, hematoxylin-eosin staining, ×5) of the left upper lobe of the lung, revealing a central (C) infarct with some retained air spaces surrounded by peripheral (P) ring-like hemorrhagic band, corresponding to the reversed halo sign observed on chest CT; (c) Numerous hyphae (arrows) infiltrating the pulmonary vasculatures (Grocott methenamine-silver staining, ×200); (d) Cultured *Cunninghamella bertholletiae* (Lactophenol cotton blue staining).
detail for the first time. In patients with hematological malignancy, the reversed halo sign may be an early marker to initiate preemptive therapy for infections caused by Zygomycetes including C. bertholletiae.

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