Detection of *Mucor velutinosus* in a Blood Culture After Autologous Peripheral Blood Stem Cell Transplantation: A Pediatric Case Report

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ABSTRACT

Filamentous fungi were detected in the blood culture of a one-year-old boy after autologous peripheral blood stem cell transplantation. The patient was suspected to have aspergillosis and received micafungin. Fungi were isolated on potato dextrose agar medium and incubated at 37°C for 2-5 days. Grayish, cottony colonies formed. A slide culture showed a spherical sporangium at the tips of the sporangiophores. The fungus could have been a zygomycete. The zygomycete was isolated from three blood cultures. The antifungal drug was changed from micafungin to liposomal amphotericin B, which resulted in an improvement in the patient's symptoms. Growth was observed at 37°C, but not 42°C in a growth temperature test. Gene sequence analysis identified the fungus as *Mucor velutinosus*. To the best of our knowledge, this is the first time *M. velutinosus* has been detected in Japan, and this case is very rare. Zygomycetes are known to be pathogens that cause fungal infections in immunodeficient patients such as those with leukemia. They are difficult to identify by culture and are identified at autopsy in many cases. Therefore, culture examinations should be performed for immunodeficient patients with the consideration of zygomycetes.

Key words: *Mucor velutinosus*, liposomal amphotericin B, autologous peripheral blood stem cell transplantation

Introduction

Zygomycetes are fungi that are widely distributed in nature. In Japan, zygomycosis is commonly mucormycosis, and zygomycosis is often used synonymously with mucormycosis. Risk factors for zygomycosis include prolonged neutropenia, use of corticosteroids, lymphopenia, bone marrow transplantation, and diabetes mellitus. Many severe cases of deep mycosis have been associated with bone marrow transplantation and malignant hematological diseases, and these cases commonly end in death. We here described a pediatric case in which *Mucor velutinosus* was detected in a blood culture after autologous peripheral blood stem cell transplantation.

Case report

The patient was a one-year-old boy with a non-contributory previous medical history. The history of the present illness included a tumor in the left...
The patient was diagnosed with cerebellar medulloblastoma and subsequently received 6 courses of chemotherapy and underwent autologous peripheral blood stem cell transplantation. There were no complications of infection after transplantation and a rapid recovery of the bone marrow was observed (Fig. 1). Forty days after transplantation, the patient had a slight fever upon returning to the hospital after an overnight stay outside the hospital. Forty-two days after transplantation, the patient had a fever of 38.7℃ and an elevated C-reactive protein (CRP) level. Blood was cultured, and the antibiotic used was cefozopran (150 mg/kg/day). Laboratory tests revealed a white blood count of 3,860/μl (66% neutrophils and 21% lymphocytes), CRP of 4.62 mg/dl, negative procalcitonin result, negative (1 → 3) -β-D glucan result, and negative Aspergillus antigen result. There were no abnormal findings in other biochemical tests. An otolaryngological examination did not identify any abnormal findings. The blood culture became positive after 24 hours of incubation. Since it was difficult to observe microorganisms using Gram staining, the culture was continued. Filamentous fungus-like colonies were observed in the culture medium. The patient was suspected of having aspergillosis and received 3 mg/kg/day of micafungin (MCFG) from 45 days after transplantation. Chest CT imaging was performed 49 days after transplantation and revealed nodules mainly in the subpleural area (Fig. 2). When the culture was continued further, the results showed that the fungus was a zygomycete. The zygomycete was isolated from the blood culture and was determined to be a zygomycete.
three blood cultures. The antifungal drug was changed to 3 mg/kg/day of liposomal amphotericin B (L-AMB) 51 days after transplantation. However, the patient continued to have a slight fever. L-AMB was administered up to 83 days after transplantation, which alleviated the fever. The antifungal drug was changed to itraconazole (ITCZ) and the patient was discharged from the hospital 121 days after transplantation.

Although Gram staining was performed in culture-positive blood cultures 43 days after transplantation, microorganisms were not observed. The culture solution was inoculated on a Vitalmedia Twin plate 6 (TSA No. 2/chocolate HP agar) and incubated in a 5% carbon dioxide atmosphere. Filamentous fungus-like colonies formed after 48 hours of incubation, and inoculated on potato dextrose agar (PDA) medium at 37°C for 5 days. After 48 hours of incubation, gray and black cottony colonies formed in the center of the PDA medium (Fig. 3a). After 5 days of incubation, black cottony colonies covered the entire PDA medium (Fig. 3b). In addition, a growth temperature test was performed to macroscopically observe fungal growth. This test used PDA and incubation for 3 days at 25°C, 30°C, 37°C, and 42°C. The growth of the colonies was the fastest on PDA when incubated at 30°C, followed by 25°C and 37°C (Fig. 3c). No growth was observed at 42°C. The maximum growth temperature was 37°C.

Slide cultures were prepared on PDA, incubated at 37°C for 5 days, and stained with lactophenol cotton blue for a morphological examination using a microscope. Wide hyphae of approximately 12 μm were observed on the slide cultures with PDA. The hyphae had no septa. At
the hyphal tips, spherical sporangia were observed with diameters ranging from approximately 20 to 30 μm. Sporangiophores extended from the sporangia and were branched. There was no rhizoid at the bases of the sporangiophores. Although no apophysis was detected at the lower area of sporangia, dome-shaped columellae were observed (Fig. 4a). Sporangiospores were spherical with diameters ranging from approximately 4 to 6 μm (Fig. 4b). A series of chlamydospores formed on the hyphae. The chlamydospores were cylindrical and the width of each of these was approximately 10 μm (Fig. 4c).

Fungal identification was performed by gene sequence analysis and antifungal susceptibility tests were conducted. The results of the gene analysis were compared with data on Genbank, and the fungus was identified as *M. velutinosus* by gene sequence analysis.

Antifungal susceptibility tests were performed using the broth microdilution method. The minimum inhibitory concentration (MIC) and minimum effective concentration (MEC) were measured for MCFG, amphotericin B (AMPH), flucytosine,

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**Table 1. Results of the antifungal susceptibility test**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Endpoint</th>
<th>48hr MIC (μg/ml)</th>
<th>72hr MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>micafungin</td>
<td>MEC</td>
<td>&gt; 16</td>
<td>&gt; 16</td>
</tr>
<tr>
<td>amphotericin B</td>
<td>IC100</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>flucytosine</td>
<td>IC50</td>
<td>&gt; 64</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>fluconazole</td>
<td>IC50</td>
<td>&gt; 64</td>
<td>&gt; 64</td>
</tr>
<tr>
<td>itraconazole</td>
<td>IC100</td>
<td>2</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>voriconazole</td>
<td>IC100</td>
<td>&gt; 8</td>
<td>&gt; 8</td>
</tr>
<tr>
<td>miconazole</td>
<td>IC50</td>
<td>4</td>
<td>4</td>
</tr>
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</table>
Discussion

The clinical forms of zygomycosis include pulmonary, rhinocerebral, gastrointestinal, cutaneous, and disseminated forms. Pulmonary zygomycosis and disseminated zygomycosis account for the majority of zygomycosis patients with hematological diseases. When zygomycosis occurs in patients with hematopoietic malignancies, hematopoietic stem cell transplantation, organ transplantation, or cell-mediated immune deficiency, it is most frequently the pulmonary type. The symptoms reported are similar to those of invasive pulmonary aspergillosis. Fever and coughing occur in the initial stage. As the disease progresses, chest pain, bloody sputum, and respiratory distress develop. In some cases, the main blood vessels are affected and pulmonary hemorrhage occurs, resulting in sudden death. Pulmonary mucormycosis develops when a host with reduced immune function inhales spores. The mortality rate of the pulmonary type is 67% and the prognosis of patients is poor. The prognosis worsens when the initiation of treatment is delayed, the condition of the underlying disease is poor at diagnosis, or the number of monocytes is low, and improves when the recovery of neutrophils occurs.

The route of infection in our patient was speculated to have been through the airway. In the early stages, the administration of L-AMB improved his condition.

Kume et al. analyzed the rate of mycosis in autopsy cases with leukemia using data from the Annual of the Pathological Autopsy Cases in Japan. Data obtained in 2001 were examined for recipients of hematopoietic stem cell transplantation. Among these autopsy cases, the predominant causative agents of mycosis were Aspergillus spp. in 55.3% of the recipients, Candida spp. in 21.1%, and Zygomycetes in 7.9%. Thus, zygomycetes were the third most common causative agent. The causative agent was zygomycetes in 79.2% of the severe visceral mycosis cases among autopsy cases with leukemia. Although zygomycetes were rarely the causative agent of mycosis in recipients of hematopoietic stem cell transplantation, mycosis was highly likely to be severe when zygomycetes caused mycosis. Therefore, it is necessary to consider the possibility of zygomycosis in fungal infection cases after hematopoietic stem cell transplantation.

Based on the rRNA sequence, M. velutinosus was originally classified as Mucor circinelloides. It was then newly classified as M. velutinosus. In 2011, Sugui et al. reported, for the first time, a case of mucormycosis due to M. velutinosus, which has since not been reported case in Japan. Its ecological niche has been speculated to be soil, fruits, and decaying vegetables, which are the same as other Mucor species. The microbiological characteristics of M. velutinosus include grayish cottony colonies, spherical sporangiospores, and columnar chlamydospores. The optimum temperature for growth is 30°C, followed by 37°C and 23°C, but it does not grow at 42°C. In the present study, the growth temperature test showed that the fungus grew best at 30°C but showed no growth at 42°C. These characteristics were consistent with those of M. velutinosus. Different zygomycete species have different growth temperatures, and the maximum growth temperature is important. Among Mucor species, the maximum growth temperature is 42°C for Mucor indicus; 37°C for Mucor circinelloides, a major pathogenic species; 36°C for Mucor ramosissimus; and 30-32°C for other Mucor species. A combination of the morphological characteristics of colonies in slide cultures and a growth temperature test is considered to be necessary for the identification of zygomycetes species.

Only a small number of zygomycosis cases have been definitively diagnosed from the causative fungus being detected before death. The diagnosis is made in many cases by autopsy. These fungi have been cultured and isolated in Japan, and the genus and species have only been identified in a very small percentage of zygomycosis cases (6.8%). This has been attributed to the differentiation of fungal species being difficult, the lack of culturing before death and at autopsy, and the low positive rate in a culture test of specimens such as sputum. In our patient, a zygomycete was isolated from three of five blood cultures, which enabled us to make a definitive diagnosis and provide early treatment. Zygomycosis and invasive aspergillosis have similar manifestations and are difficult to differentiate from one another. As in our patient, fungal infection should be suspected if a patient is...
immunodeficient after hematopoietic stem cell transplantation, has fever refractory to antibacterial agents, and has nodules in chest CT. In such a case, a culture test should be performed to differentiate between aspergillosis and zygomycosis. Early diagnosis and the initiation of appropriate antifungal therapy are thought to improve the prognosis of these patients.

References

1) Guidelines for management of deep-seated mycoses 2007, Shinzaiseishinkinsho no gaidorain sakuseiinikai