

## Suspected Pulmonary Infection with *Trichoderma longibrachiatum* after Allogeneic Stem Cell Transplantation

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### Abstract

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*Aspergillus* and *Candida* species are the main causative agents of invasive fungal infections in immunocompromised human hosts. However, saprophytic fungi are now increasingly being recognized as serious pathogens. *Trichoderma longibrachiatum* has recently been described as an emerging pathogen in immunocompromised patients. We herein report a case of isolated suspected invasive pulmonary infection with *T. longibrachiatum* in a 29-year-old man with severe aplastic anemia who underwent allogeneic stem cell transplantation. A direct microscopic examination of sputum, bronchoaspiration, and bronchoalveolar lavage fluid samples revealed the presence of fungal septate hyphae. The infection was successfully treated with 1 mg/kg/day liposomal amphotericin B.

**Key words:** *Trichoderma longibrachiatum*, allogeneic stem cell transplantation, liposomal amphotericin B, immunocompromised human hosts

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### Introduction

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Over the past decade, infections caused by opportunistic filamentous fungi have become increasingly common among patients after allogeneic stem cell transplantation (allo-HSCT). *Trichoderma* species are common fungi usually found in humid soil, decaying wood, and water-related sites. They are considered plant saprophytes but have recently been linked to severe cases of invasive infection in immunocompromised human hosts (1-4). We herein report a suspected case of invasive pulmonary infection with *T. longibrachiatum* in a patient with severe aplastic anemia who received allo-HSCT and was successfully treated with liposomal amphotericin B (L-AmB).

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### Case Report

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A 29-year-old man was diagnosed with severe aplastic anemia in May 2008. He was unresponsive to intravenous anti-thymocyte globulin (ATG), recombinant granulocyte

colony-stimulating factor, and cyclosporine. He was admitted for allogeneic matched unrelated donor bone marrow transplantation in March 2009. The conditioning regimen consisted of cyclophosphamide, ATG, and fludarabine. Tacrolimus and short-term methotrexate were used as prophylaxis against graft-versus-host disease (GVHD). Preemptive antifungal therapy with micafungin was started until a neutrophil count of >500/ $\mu$ L was achieved. On Day 37 after transplantation, tacrolimus was discontinued because of a deteriorating renal function, and predonine (0.5 mg/kg/day) was introduced. On Day 69, the patient developed a diffuse skin rash, consistent with an acute GVHD grade II. He received a combination of predonine and mycophenolate mofetil. The immunosuppressive therapy was eventually tapered to 15 mg predonine and 1,500 mg mycophenolate mofetil.

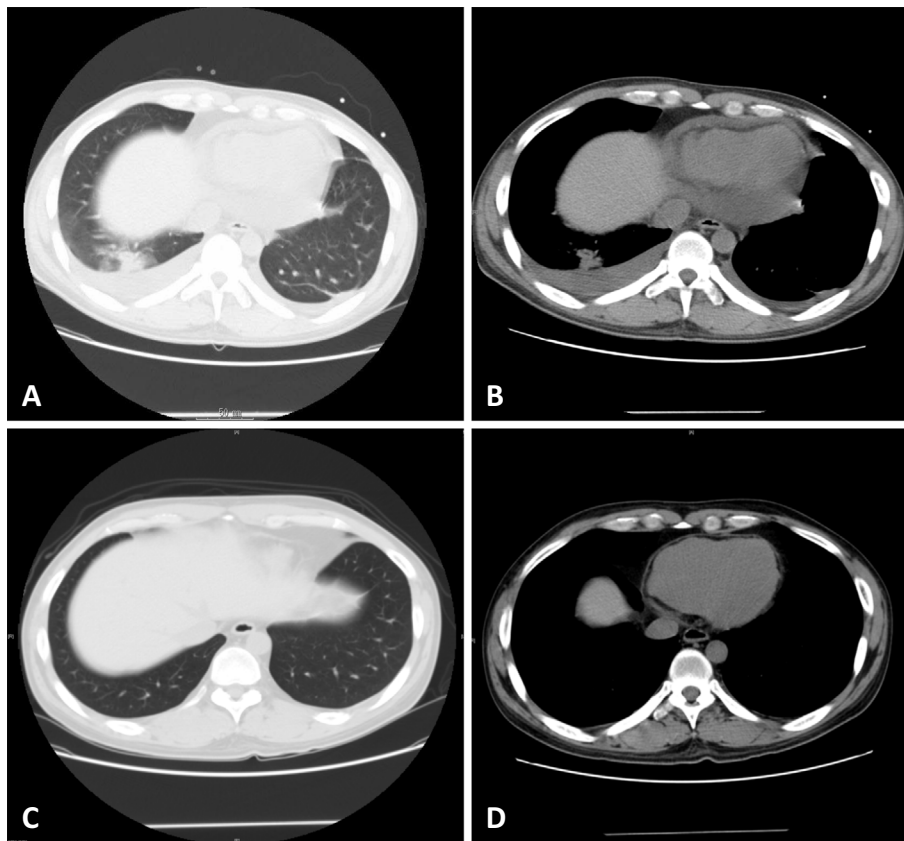
Over the subsequent months, the clinical course was complicated by recurrent cytomegalovirus reactivations which required antiviral treatment with gancyclovir. On Day 151 the patient developed painful grouped vesicles on erythematous skin of the right arm and a fever of 38°C, consistent with

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**Figure 1.** CT scan of the chest. (A, B) on day 180 from transplantation. (C, D) on day 204 from transplantation.

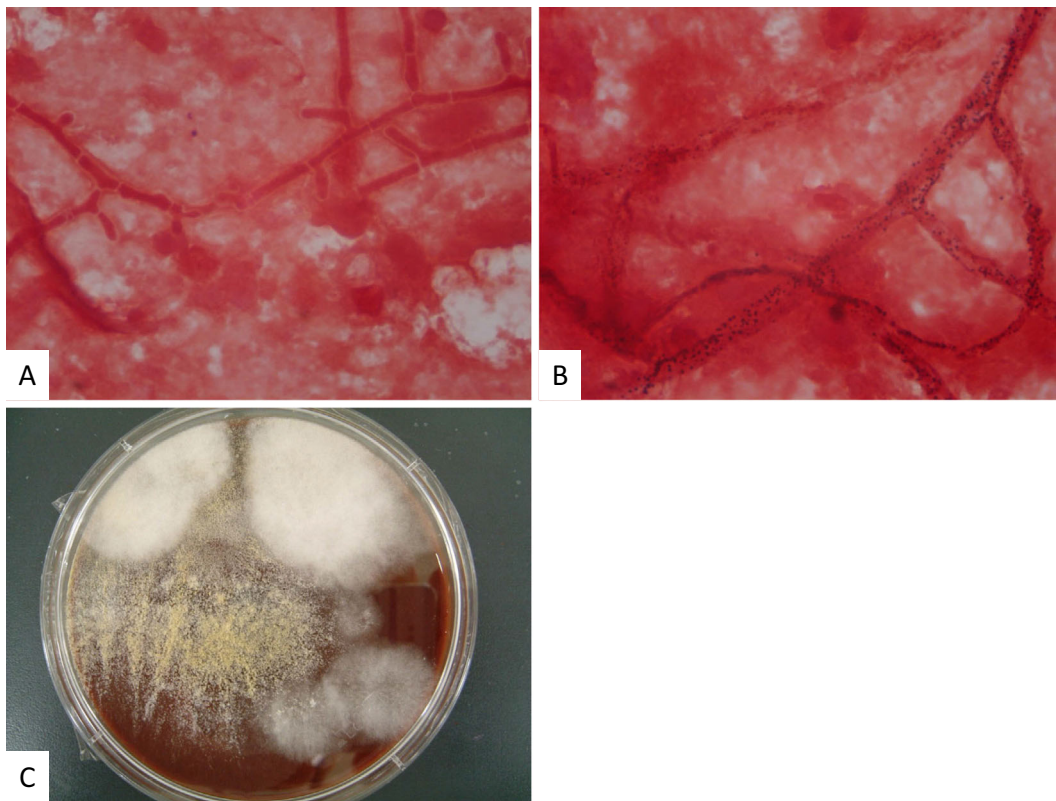
herpes zoster. Despite the administration of intravenous acyclovir, a spiking fever above 39°C persisted. Computed tomographic (CT) scans of the lungs showed a peripheral nodule in the right upper lobe. Tests for *Aspergillus* antigen were negative, and the findings for serum 1,3- $\beta$ -D-glucan, a marker of fungal infection, were also negative. On Day 168, treatment with 1 g/day imipenem-cilastatin and 400 mg/day fluconazole was initiated, but the spiking fever was unresponsive to the antibacterial and antifungal therapy. On Day 180, the patient developed dyspnea and a cough. Thoracic CT scans showed nodule growth in the right upper lobe, consolidation of the right inferior lobe, pleural effusion, and pericardial effusion (Fig. 1A and B). The serum 1,3- $\beta$ -D-glucan level was not elevated. A direct microscopic examination of sputum, bronchoaspiration, and bronchoalveolar lavage fluid samples revealed the presence of fungal septate and aseptate hyphae (Fig. 2A and B). The antifungal therapy was switched from fluconazole to L-AmB at a dose of 1 mg/kg/day the same day. The dosage was not increased because of his deteriorating renal function. Fungal growth occurred within two days on Sabouraud dextrose agar and chocolate agar at 30°C and 37°C, respectively (Fig. 2C). The isolate was forwarded to Gifu University Graduate School of Medicine for further examination. A polymerase chain reaction and sequencing of the internal transcribed spacer regions 1 and 2 (ITS1/2) from the fungal rRNA genes revealed *T. longibrachiatum*, and *Cunninghamella*

*bertholletiae*, respectively.

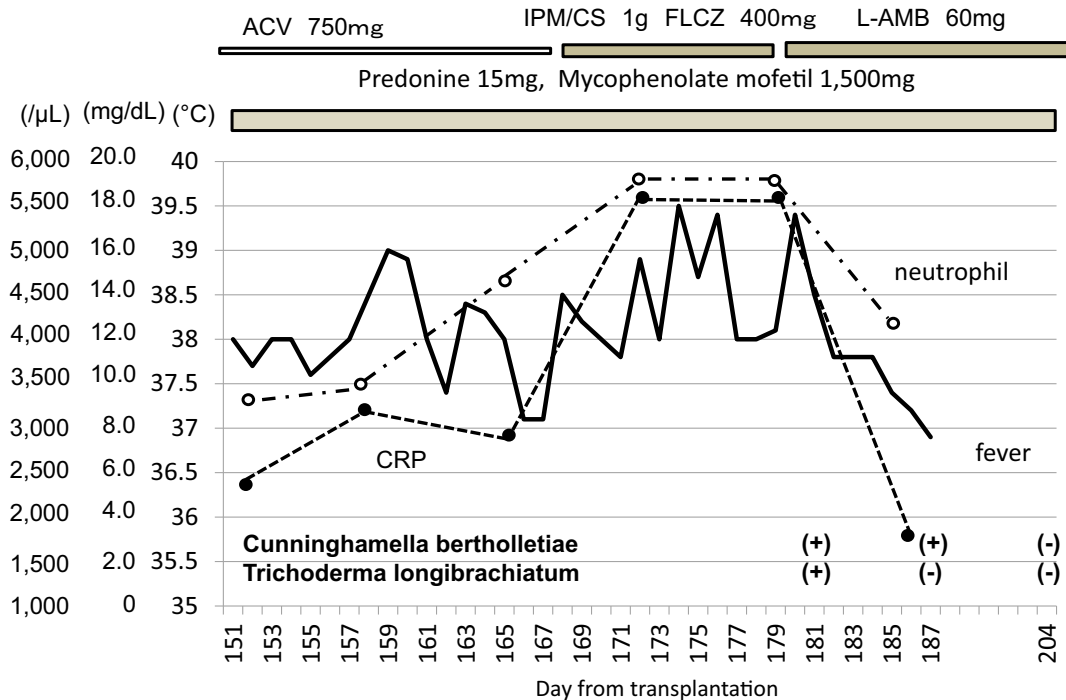
We were unable to examine the susceptibility of the filamentous fungi to antifungal drugs due to our inability to maintain an appropriate concentration of filamentous fungi. After 3 days of L-AmB administration, his fever declined, and 5 days thereafter, the sputum culture was negative for *T. longibrachiatum*, but positive for *C. bertholletiae* (Fig. 3). After 24 days of the antifungal therapy, a follow-up thoracic CT scan showed resolution of the pulmonary nodules, consolidation, pleural effusion, and pericardial effusion (Fig. 1C and D), and the sputum culture was negative for *C. bertholletiae*. The total dose of L-AmB administered was 1,600 mg, and the patient was discharged 235 days after the transplantation.

## Discussion

Disseminated fungal infections continue to cause substantial morbidity and mortality in recipients of bone marrow transplants, despite advances in patient management (5). *Aspergillus* and *Candida* species are the most common invasive fungal pathogens identified in such recipients. However, to our knowledge, invasive pulmonary infection due to *T. longibrachiatum* after allo-HSCT has not been previously reported. Kulhs et al. (6) showed that most *Trichoderma* infections in humans are caused by *T. longibrachiatum* and, rarely by *T. citrinoviride*. In recipients of bone marrow



**Figure 2.** (A) *Trichoderma longibrachiatum* shows septate hypha. (B) *Cunninghamella bertholletiae* shows aseptate hypha with gram stain. Magnification×1,000. (C) Culture of the bronchoaspiration samples on chocolate agar.



**Figure 3.** Clinical course of infection: relationship of fever (solid line), CRP (dot), neutrophil count (white dot) and L-AmB administration.

transplants, *T. longibrachiatum* causes a fatal disseminated infection, suggesting that the gastrointestinal tract is the portal of entry (1). Our patient's features were different. The in-

vasive fungal infection was confined to the lungs, and then only to the respiratory samples. The only similar reported case of *T. longibrachiatum* pulmonary infection concerned a

case of acute lymphoblastic leukemia (4). A definitive diagnosis of invasive pulmonary filamentous fungal infection may be difficult, since it requires the demonstration of hyphae in tissue sections, as is the case for the diagnosis of other hyalohyphomycosis infections. Transbronchial biopsy may be difficult to perform in many patients who are thrombocytopenic. Given that culture of blood samples does not appear to be a valuable diagnostic tool, the diagnosis is frequently based on the demonstration of hyphae associated with positive culture results in non-biopsy specimens obtained from accessible sites. Fortunately, in our case, the sputum, bronchoaspiration, and bronchoalveolar lavage fluid samples showed fungal septate and aseptate hyphae.

On a direct examination, *T. longibrachiatum* infection can be misdiagnosed as aspergillosis, because the hyphae are morphologically quite similar. If a test for *Aspergillus* antigens is negative, we need to consider the possibility of a false-negative antigen test as well as the presence of other filamentous fungal species, including *Trichoderma* species. In the present case, the sputum, bronchoaspiration, and bronchoalveolar lavage fluid samples also showed *C. bertholletiae*. After L-AmB administration, the patient's fever declined, and the sputum culture was negative for *T. longibrachiatum*, but positive for *C. bertholletiae*. We therefore considered that *T. longibrachiatum* might be the main causative agent in this invasive pulmonary infection.

Amphotericin B (AmB) is the most widely used antifungal agent. A starting dosage of 1 mg/kg/day for AmB and 5-7.5 mg/kg/day L-AmB is commonly used for adults and children. Patients receiving allo-HSCT require the administration of nephrotoxic drugs such as immunosuppressive, antiviral, and antibiotic regimens. The major problem with using L-AmB in allo-HSCT recipients is nephrotoxicity. Ringdén et al. (7) reported that the serum creatinine level was increased in 31% of patients who received cyclosporine A and L-AmB. Krüger et al. (8) described a single-center experience with L-AmB in 74 patients undergoing autologous or allogeneic stem cell transplantation. The median (range) dose of L-AmB was 2.8 (0.64-5.09) mg/kg. A total of 11 patients died from mycosis, and 52 were discharged without evidence of mycosis.

In our case, the suspected invasive pulmonary infection was successfully treated with 1 mg/kg/day L-AmB. Several factors might have contributed to the efficacy. First, most *T. longibrachiatum* isolates have shown resistance to fluconazole and flucytosine but have been found to be susceptible to AmB *in vitro* (9-11). Second, AmB may achieve satisfactory levels in an infected lung lesion. Several investigations have indicated that L-AmB accumulates in the lesions of a fungal infection (12, 13). Watanabe et al. (14) determined the concentrations of AmB in plasma and infected tissues of resected lung and found that the AmB concentration in the infected lung lesion was approximately 5.2 times higher than that in the plasma. Further studies are needed to clarify the distribution of L-AmB.

In conclusion, careful clinical and radiologic examinations

are the key to early diagnosis of an invasive pulmonary infection, especially in the disseminated form, in immunocompromised patients. We believe that early confirmation will lead to early initiation of the proper aggressive treatment and thereby decrease the mortality rate from such infections.

**The authors state that they have no Conflict of Interest (COI).**

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