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Case Report

Successful treatment of invasive pulmonary aspergillosis caused by *Aspergillus felis*, *a* cryptic species within the *Aspergillus* section *Fumigati*: A case report☆

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ABSTRACT

Aspergillus species are a major cause of life-threatening infections in immunocompromised hosts, and the most common pathogen of invasive aspergillosis is *Aspergillus fumigatus*. Recently, the development of molecular identification has revealed cryptic *Aspergillus* species, and *A. felis* is one such species within the *Aspergillus* section *Fumigati* reported in 2013.

We describe a case of invasive pulmonary aspergillosis caused by *A. felis* in a 41-year-old Japanese woman diagnosed with myelodysplastic syndrome. She presented with fever 19 days after undergoing autologous peripheral blood stem cell transplantation and was clinically diagnosed with invasive pulmonary aspergillosis. Bronchoscopy and bronchoalveolar lavage were performed for definitive diagnosis. The β-tubulin genes of the mold isolated from the bronchoalveolar lavage fluid, and sequenced directly from the PCR products using a primer pair were found to have 100% homology with *A. felis*. We successfully treated the patient with echinocandin following careful susceptibility testing.

To the best of our knowledge, this is the first published case reporting the clinical course for diagnosis and successful treatment of invasive aspergillosis by *A. felis*.

1. Introduction

Invasive aspergillosis (IA) is a major problem in hematologic malignancies and hematopoietic cell transplantation [1]. The most common pathogen of IA is *Aspergillus fumigatus*, followed by *A. niger*, *A. flavus*, and *A. terreus*.

Recently, the development of molecular identification has revealed cryptic *Aspergillus* species that are very closely related and almost indistinguishable by morphologic methods [2,3]. Some cryptic *Aspergillus* species are resistant and require higher minimum inhibitory concentrations (MIC) of azoles or amphotericin B deoxycholate, which are considered as first-line therapy for IA [4]. *A. felis* is one of the cryptic species within the *Aspergillus* section *Fumigati* described in 2013 [5]. It is known to cause IA in cats, dogs, and humans. There are few case reports of IA caused by cryptic *Aspergillus* species, especially *A. felis*.

Here, we report a clinical case of invasive pulmonary aspergillosis caused by *A. felis* that was successfully treated.

☆ All authors meet the ICMJE authorship criteria.

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2. Case report

The patient was a 41-year-old Japanese woman diagnosed with myelodysplastic syndrome. She underwent autologous peripheral blood stem cell transplantation and had started taking fluconazole (FLCZ) to prevent fungal infection 4 months before as a part of transplantation conditioning. Neutrophil engraftment occurred on day 20 after transplantation. On day 19 after transplantation and 162 days of neutropenia (less than 500 neutrophils per microliter), she developed a fever. She tested positive for *Aspergillus* antigen, and thoracic computed tomography (CT) image showed an air crescent sign, suggesting invasive pulmonary aspergillosis in the lung on day 27 after transplantation (Fig. 1). We clinically diagnosed her with invasive pulmonary aspergillosis, and initially started intravenous treatment with voriconazole (VRCZ) (6 mg/kg twice daily for 2 days, followed by 4 mg/kg every 12 h). Bronchoscopy and bronchoalveolar lavage (BAL) was performed for definitive diagnosis on day 37 after transplantation.

There was no change in the lung lesion after one month, and the fever persisted (Fig. 1). Considering the low voriconazole trough blood level, we switched to treatment with liposomal amphotericin-B (L-AMB) (2.5 mg/kg per day). However, she developed nausea as a side effect, and therefore, L-AMB therapy was stopped after one week. Subsequently, we reintroduced combination therapy with VRCZ (4 mg/kg every 12h) and caspofungin (CPFG) (70 mg loading dose, followed by 50 mg daily). The lesion gradually decreased in size.

Filamentous fungi were isolated from the bronchoalveolar lavage fluid (BAL) on day 10 of the culture. Compared to *A. fumigatus*, white colonies of the isolates grew slowly, and there was no morphologically special point (Fig. 2). The β-tubulin genes of the mold isolated from BAL were sequenced directly from the PCR products using a primer pair published previously for species identification [6]. The sequence showed 100% similarity with *A. felis* genes, using the BLAST database (www.ncbi.nlm.nih.gov/BLAST).

Susceptibility testing was performed according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI) document M38-A2 [7]. The MICs for amphotericin B, VRCZ, itraconazole, and FLCZ were found to be 2.0, >8.0, >8.0, and 64 μg/ml, respectively (Table 1). The minimal effective concentration (MEC) for micafungin (MCFG) was <0.015 μg/ml (Table 1). CPFG was not tested.

We stopped treatment with VRCZ and CPFG and switched to a single dose of MCFG (150 mg per day) based on its low MEC value. We continued to treat her as an outpatient successfully for a total of 5 months.

3. Discussion

Aspergillus species are a major cause of life-threatening infections in immunocompromised hosts, including patients with hematologic malignancies and those undergoing hematopoietic cell transplantation [1]. Studies have revealed the presence of cryptic *Aspergillus* species in clinical samples using advanced genetic identification technology. In a recent study, over 10% of all aspergilli clinically isolated were cryptic species [8,9].

A. felis, an emerging causative agent of invasive aspergillosis in humans, is one of the cryptic *Aspergillus* species in the section *Fumigati* and was genotypically identified as a new species in 2013 [5]. Only two human cases of IA caused by *A. felis* have been published so far. These cases, which were initially reported to be caused by *A. viridinutans* were subsequently attributed to *A. felis* following a reinvestigation [10–12]. However, both patients died during the course of the treatment. To the best of our best knowledge, this is the first reported case of invasive pulmonary aspergillosis caused by *A. felis* which has been treated successfully.

Like some other drug-resistant cryptic *Aspergillus* species, *A. felis* also requires higher MICs of azoles including VRCZ, which are considered the first-line therapy for IA [13]. Additionally, multi-azole resistant cases of *A. felis* such as this one have been reported [5]. Previous studies have reported amphotericin B to have MICs of ≦1 μg/ml in *A. felis* [5,10,11]. However, we found that the MIC of amphotericin B for *A. felis*, in this case, was 2 μg/ml, which is relatively high. Additionally, we could not determine

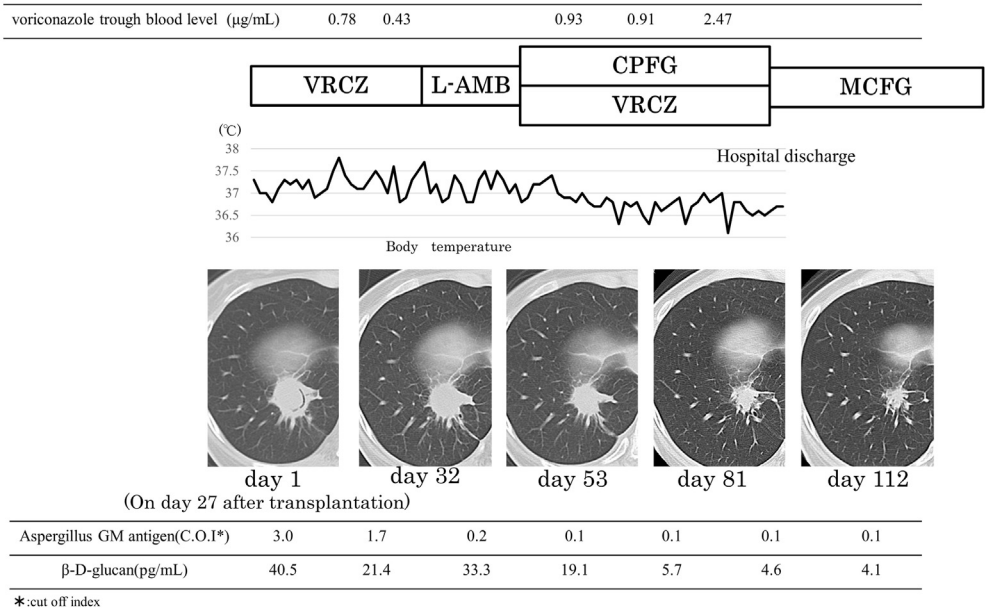

Fig. 1. The clinical course used to treat invasive pulmonary aspergillosis caused by *Aspergillus felis*. VRCZ: voriconazole, L-AMB: liposomal amphotericin B, CPFG: caspofungin, MCFG: micafungin.

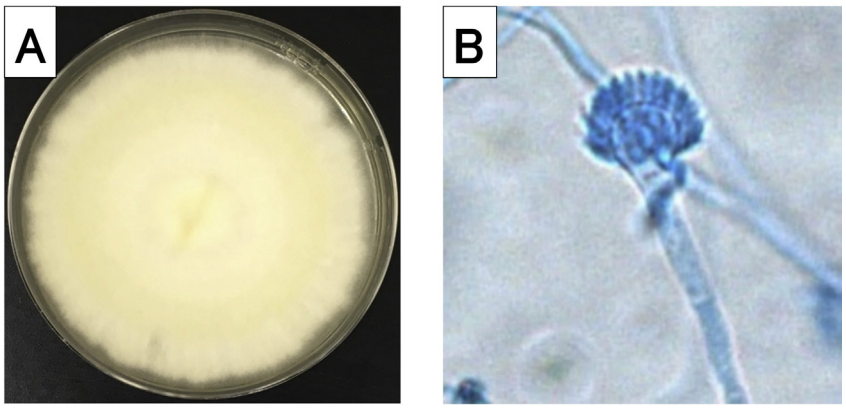

Fig. 2. *Aspergillus* culture. (A) Image of *Aspergillus felis* culture in Sabouraud Dextrose agar at 30 °C on day 9. (B) Microscopical examination of lactophenol cotton blue stain of *Aspergillus felis*.

Table 1
Susceptibility testing of a clinical isolate of *A. felis* based on the recommendations of the Clinical and Laboratory Standard Institute (CLSI) document M38-A2.

	MCFG**	AMPH*	5-FC*	FLCZ*	ITCZ*	VRCZ*
A. felis	0.015	2	>64	>64	>8	>8

*MIC, minimum inhibitory concentration; **MEC, minimum effective concentration; MCFG, micafungin; AMPH, Amphotericin B; 5-FC, 5-fluorocytosine; FLCZ, fluconazole; ITCZ, itraconazole; VRCZ, voriconazole.

the clinical efficacy of liposomal amphotericin B (L-AMB) since we used it only for a short time due to side effects. High MICs of amphotericin B are required for some resistant cryptic *Aspergillus* species, which have been associated with poor clinical outcomes [2,5,14–16]. Previous studies have reported relatively low MECs of echinocandin for *A. felis*. Especially, the MEC of MCFG was lower than that of the CPFG [5]. The regression of the lung lesion in response to echinocandin, in this case, suggests that echinocandin is effective against *A. felis*.

However, clinical data on the treatment of cryptic *Aspergillus* species including *A. felis* is limited. Hence, an effective treatment for invasive aspergillosis caused by cryptic *Aspergillus* species is not well established [17]. In this case, the combination of antifungal treatments using VRCZ plus CPFG was effective. Though combination therapies seem attractive, there is no data available to confirm their effectiveness [17]. Furthermore, in the two cases of IA reported previously, *A. felis* was refractory to combination antifungal treatments using VRCZ plus CPFG and L-AMB plus CPFG respectively, and the outcomes were fatal in both cases [9,10]. Therefore, for a treatment to be successful, it is important to determine the exact nature of the underlying disease in order to administer the right dose of appropriate antifungal agents [17].

Since susceptibility to antifungal agents varies largely among different fungal isolates and species [18,19], it is important to consider the choice of these agents based on the correct identification of the fungal species and their susceptibilities.

In Japan, there are only a few institutions that have the capability to identify fungal species and antifungal susceptibility accurately. It is, therefore, necessary to maintain core facilities that can help with this identification process.

Conflicts of interest

Hiroshi Kakeya has received grant support from Pfizer Japan Inc., MSD K.K., Astellas Pharma, Dainippon Sumitomo Pharma Co., Ltd.

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