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【 CASE REPORT 】

Exophiala dermatitidis Fungemia Diagnosed Using Time-of-flight Mass Spectrometry during Chemotherapy for Malignant Lymphoma and Successful Treatment with Voriconazole

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Abstract:

Infectious diseases, including those caused by fungi, remain important issues in patients receiving malignant lymphoma chemotherapy. We herein report a rare case of *Exophiala dermatitidis* fungemia during chemotherapy in a 67-year-old woman admitted to our hospital. She had a fever that could not be resolved despite antifungal therapy. Yeast-like fungi were detected in blood culture samples, but biochemical identification was difficult. *E. dermatitidis*, a black mold, was identified using time-of-flight mass spectrometry. The patient finally improved after her treatment was switched to voriconazole. Fungal infection is difficult to diagnose and treat, but this novel approach can improve patients' outcomes.

Key words: (2 to 6): malignant lymphoma, *Exophiala dermatitidis*, mass spectrometry, voriconazole

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Introduction

Malignant lymphoma is a heterogeneous hematological malignancy. Follicular lymphoma (FL) is the most common indolent B-cell lymphoma. In a previous Japanese cohort study, FL accounted for 15.7% of malignant lymphomas (1). The prognosis of FL is improving because of advances in chemotherapy, such as anti-CD20 monoclonal antibody therapy, rituximab, and supportive therapy (2). Examples of advances in supportive therapy include the emergence of new antibiotics and granulocyte colony-stimulating factor. Choices of antifungal therapy are also increasing, including the advent of agents such as voriconazole and caspofungin (3). However, diagnosing mycoses is challenging due to their slow growth and the similarity among species.

We recently experienced the usefulness of a new identification method, matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) (4). With this approach, a sample, such as from a cultured colony, is mixed with the matrix, ionized with laser absorption, detected using TOF MS, and matched with the database for identification. This sophisticated analysis can be conducted within a few hours and is faster than biochemical identification (4). The technique has shown favorable results for identification in comparison with biochemical methods (5), and it is cost effective (6). The benefits of this method may help improve the clinical patient outcome.

Case Report

A 69-year old woman was admitted to our hospital because of lymphoma recurrence. She had first visited our hospital when she noticed a chest mass six years earlier. Computed tomography (CT) showed a breast mass, splenomegaly, and enlarged para-aortic lymph nodes. Her

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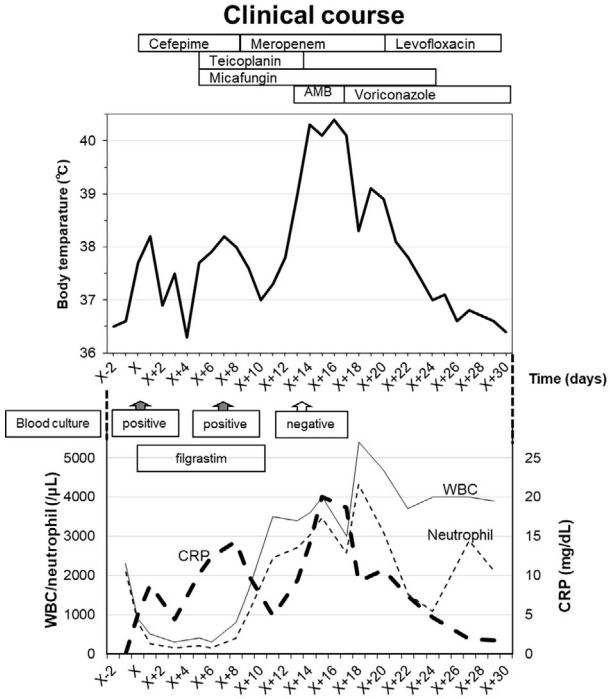

Figure 1. Clinical course of the patient. Cefepime 2 g i.v. q12h; meropenem 1 g i.v. q8h; levofloxacin 500 mg/d orally; teicoplanin i.v. loading dose of 600 mg q12h of the first 3 doses followed by the maintenance dose of 600 mg q24h; micafungin 150 mg i.v. q24h for 11 days, and then 300 mg q24h; AMB, liposomal-amphotericin B 5 mg/kg i.v. q24h; voriconazole 300 mg q12h for the first 24 h followed by 150 mg q12h orally.

breast mass was biopsied, and the pathological diagnosis of the section was low-grade FL. Because her breast mass was growing, we started systemic chemotherapy with eight cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP) therapy, and she achieved a complete response.

For five years after completing chemotherapy, she regularly visited the hospital for medical examination. Her lymph nodes regrew slowly, and a new site of lymphoma invasion was noted on follow-up CT. She complained of ocular pain and proptosis. Gadolinium-enhanced magnetic resonance imaging was performed, which showed bilateral orbital masses, and thickening of the dura was detected; there was no obvious invasion of the brain. In addition, whole-body CT and positron emission tomography/CT revealed systemic lymphadenopathy. She was diagnosed with recurrence of FL, but a biopsy of the orbital masses was not done because of the high risk for vision loss. Lumbar puncture was also performed, but the study showed no involvement of malignancy. Bone marrow aspiration and a biopsy also revealed no apparent abnormality. Before we started salvage chemotherapy, we placed a peripherally inserted central catheter (PICC). We planned salvage chemotherapy as a sequential combination of high-dose methotrexate and rituximab, dexamethasone, etoposide, ifosfamide, and carboplatin (R-DeVIC) therapies because the dural thickening and orbital lesion were thought to indicate a high risk of the cancer invading the central nervous system. Intrathecal chemotherapy was also performed.

After two cycles of R-DeVIC therapy and high-dose methotrexate therapy, we started the third cycle of R-DeVIC therapy (day X-8) in a planned manner after her leukocyte count had recovered (WBC 3800/μL; neutrophil 64%, lymphocyte 26%), with prophylactic oral fluconazole and trimethoprim/sulfamethoxazole. On day X-1, occlusion of the PICC occurred; the catheter was removed immediately, and no unusual findings were seen. The next day (day X), the patient had a fever of 38℃ and neutropenia (neutrophil 455/μL). We determined that the patient had developed febrile neutropenia and started cefepime (2 g q12h) with filgrastim (75 μg/body) after collecting two sets of blood culture bottles (Fig. 1). Her fever did not resolve, and yeast-like fungi were detected in aerobic blood culture bottles (Fig. 2A). Beta-D-glucan and aspergillus antigen tests were repeated and showed negative results. There were no obvious skin findings, and CT showed trabecular shadows in the upper lobe of the right lung at day X+5 (Fig. 3). Although the fungus could not be identified biochemically using YST® cards in the VITEK2® system (bioMérieux, Lyon, France), we started micafungin (150 mg q24h) for suspected candidemia and teicoplanin (loading dose of 600 mg q12h of the first 3 doses followed by the maintenance dose of 600 mg q24h) to cover methicillin-resistant *Staphylococcus aureus* at day X+5, adjusted by trough concentration.

Transthoracic echocardiography did not detect valve vegetation. We consulted an ophthalmologist to exclude fungal endophthalmitis, but there were no suspicious findings. The patient's high fever persisted, and a re-examination of blood cultures showed consistent fungemia. We decided to begin a combination of increased micafungin (300 mg q24h) and liposomal-amphotericin B (5 mg/kg q24h) in day X+12, but the addition of anti-fungal therapy did not improve her condition. Because a biochemical analysis could not identify the fungal pathogen, we next performed MALDI-TOF MS with VITEK-MS® (bioMérieux); the spectrum pattern indicated a high probability of *Exophiala dermatitidis* (Fig. 4). The colony color change from white to black supported this result (Fig. 2B and C). A drug susceptibility test of *E. dermatitidis* after 48-hour incubation was done (Table), although the kit used (ASTY®, Kyokuto Seiyaku, Tokyo, Japan) was designed to assay *Candida* species. We switched from liposomal-amphotericin B to voriconazole (300 mg q12h for the first 24 hours followed by 150 mg q12h orally). The next day, the patient's body temperature decreased, and her condition gradually improved, including the laboratory findings. Voriconazole was continued, with adjustment of the drug dose according to the trough concentration. The patient finally recovered and was discharged from the hospital. She received voriconazole orally in ambulatory care and her lymphoma remained in remission.

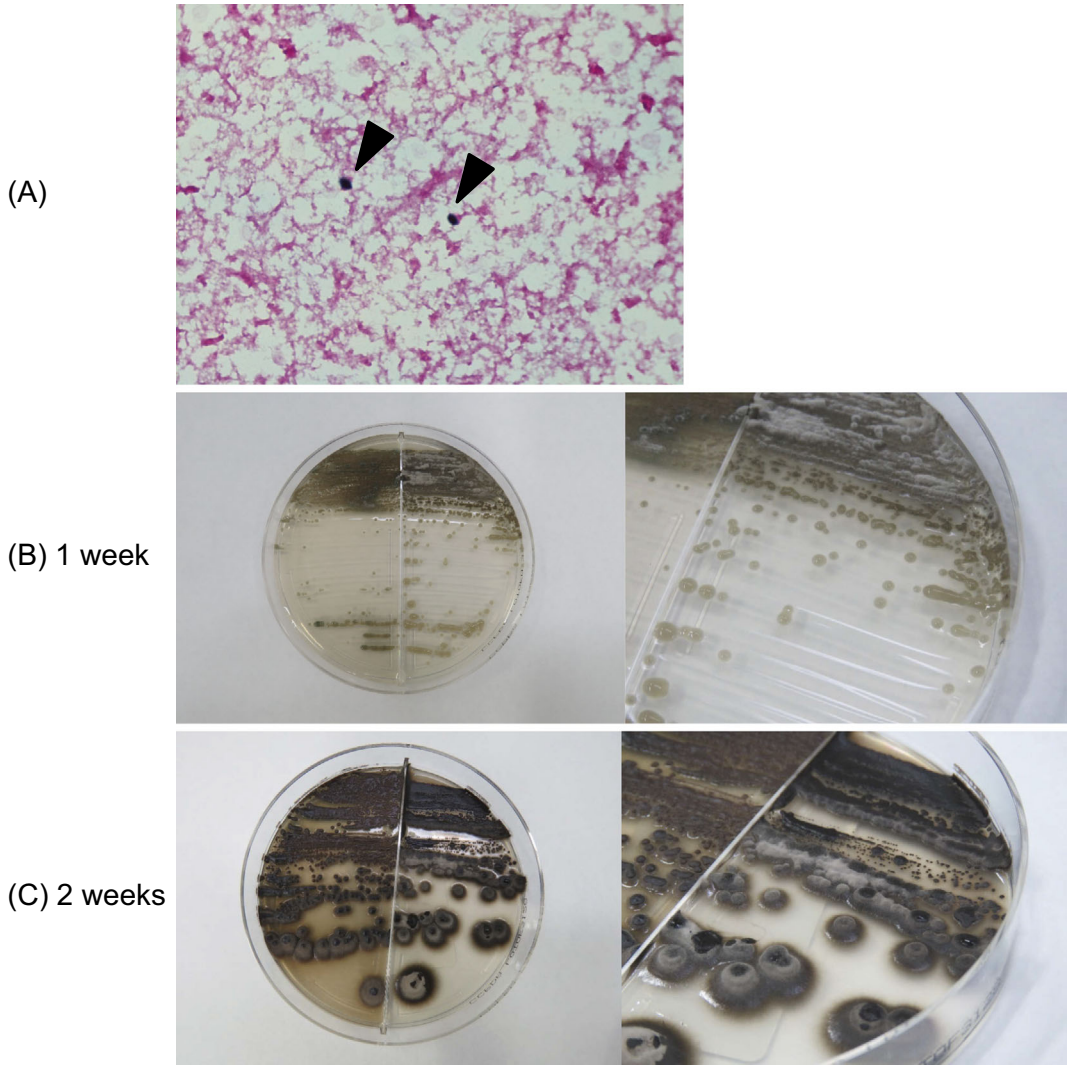

Figure 2. **Appearance of *Exophiala dermatitidis*. (A) *Exophiala dermatitidis* (black arrow) was grown from blood culture and stained by Gram staining. (B, C) Colonies were white in CHROMagar® Candida/potato dextrose base. Colonies turned black after one additional week of incubation.**

Discussion

E. dermatitidis (formerly known as *Wangiella dermatitidis*) is a slow-growing, black dimorphic yeast that is commonly found in damp locations, such as bathrooms and kitchens (7). Originally *E. dermatitidis* was isolated from the skin of a patient in Japan (8). The microorganism grows slowly, forming black colonies over time; infection results in the host becoming immunocompromised. Clinically, *E. dermatitidis* is the cause of skin chromomycosis (7). In addition, pulmonary infection with the pathogen has been reported in bronchiectasis (9) and hematological malignancy (10); outbreaks due to fungal contamination also happen (11). Fungemia caused by *E. dermatitidis* is known to be associated with catheter-related infection (12). The microorganism can form a biofilm over the catheter, although in our case, the penetration site and the PICC appeared to be intact (13). Of further note, an attack of fever occurred the day after PICC removal. While our patient had no respiratory symptoms, CT findings of the lung suggested the possibility of lung infection or thromboembolism, which might have function as the entry site of the pathogen.

Classical biological substrate reaction is an important method for identifying fungal species, although several days are required. Previous cases concerning the clinical manifestation and diagnostic procedure have underscored the importance of considering the patient's immune status and culture test findings (10). The beta-D-glucan test has also shown some utility for detecting fungal infection (11), as 9 out of 12 cases with *E. dermatitidis* infection showed an increase in its level, although our case did not. Identification can also be achieved using MALDI-TOF MS, which has been shown to be a reliable way of distinguishing bacteria in community-based analyses. *E. dermatitidis* can be diagnosed from blood culture samples using MALDI-TOF MS (14). This method is useful for treating the patients with appropriate anti-fungal agents because fungal identification by other

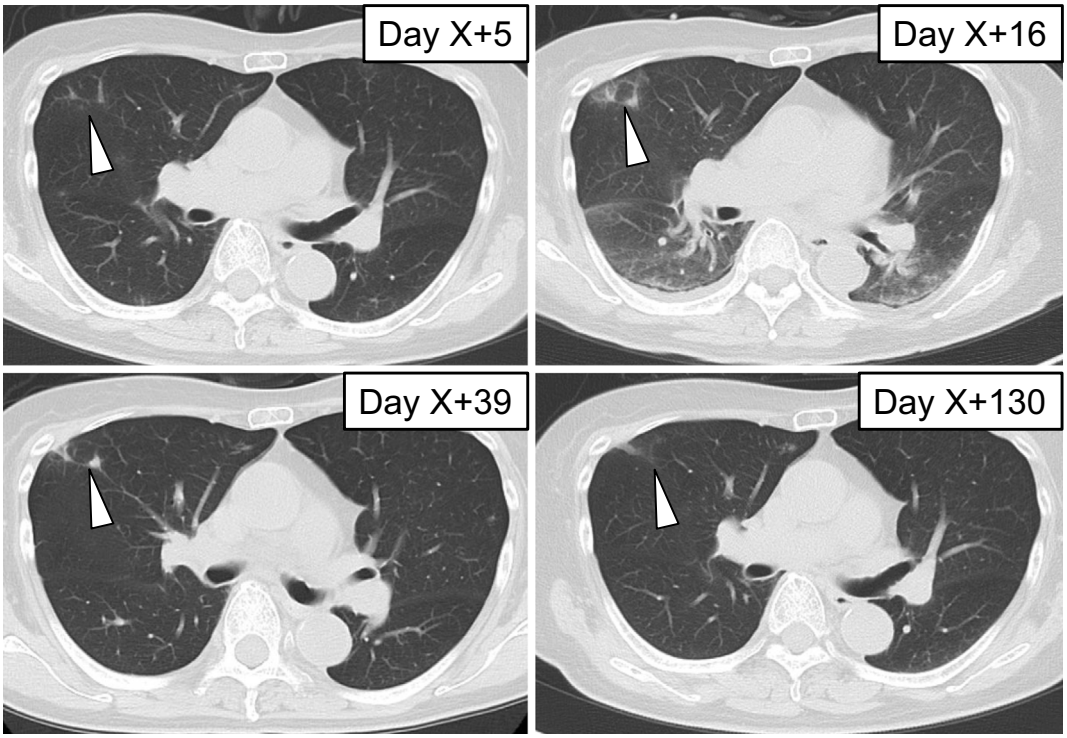

Figure 3. **Lung findings on CT of the chest. Trabecular shadows in the upper lobe of the right lung (white arrow) were observed on day X+5. After temporary deterioration (day X+16), they diminished over time.**

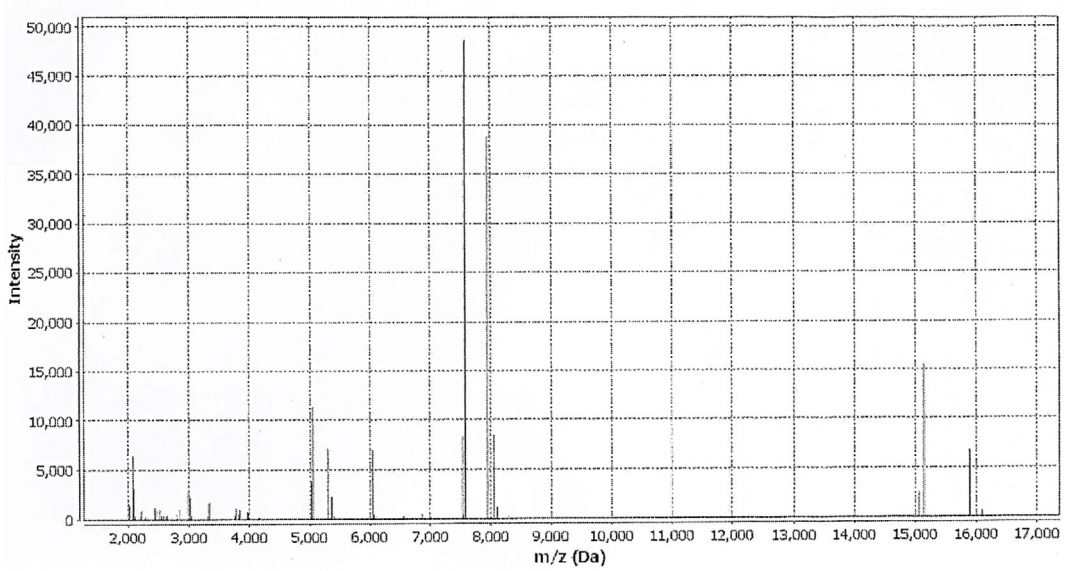

Figure 4. **Mass spectrum of *Exophiala dermatitidis*. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry showed the specific spectrum, which matched the registered pattern of *Exophiala dermatitidis*.**

methods is often difficult and time-consuming. As we experienced, mycosis during chemotherapy is difficult to diagnose and treat. Some fungal infections, such as *Candida* and *Aspergillus* species, are widely recognized and have been described previously in the Infectious Diseases Society of America guidelines (15), Sixth European Conference on Infections in Leukemia guidelines (16), and Japanese domestic guidelines (17). However, *E. dermatitidis* has scarcely been described, with reference to only a few incidences.

Reports of *E. dermatitidis* treatment are limited (9-12). Favorable activities of anti-fungal drugs *in vitro* have been described, such as for voriconazole (18) and micafungin (19). An *in vitro* analysis showed that patient outcomes can be improved using combination therapy with echinocandins and other anti-fungal agents for *E. dermatitidis*; however, no *in vivo* analysis has been performed (20).

Table. **Drug Susceptibility Test of *Exophiala Dermatitidis*.**

	Minimum inhibitory concentration (μg/mL)
Flucytosine	2
Amphotericin B	0.25
Capsofungin	4
Fluconazole	8
Itraconazole	0.25
Micafangin	8
Miconazole	0.5
Voriconazole	0.06

We administered prophylactic oral fluconazole; however, fluconazole breakthrough infections are thought to lead to azole-resistant fungal infection (21, 22). Thus, we were concerned about voriconazole resistance. The actual situation was different; drug susceptibility testing *in vitro* and the clinical course showed that voriconazole was effective. Susceptibility testing is essential for guiding treatment of fungal infections (23). However, it is difficult to decide on the appropriate breakpoint for *Candida* species (24). In practice, voriconazole shows good results when administered at appropriate concentrations, according to therapeutic drug monitoring (25); the appropriate breakpoint for *E. dermatitidis* is still being investigated (26).

FL is an incurable, low-grade B-cell malignancy, and its treatment is determined according to the patient's condition and history of treatment. A prolonged clinical course of FL leads to a greater risk of infection. Consequently, efficacious prophylactic strategies are important during chemotherapy (27).

We herein report our experience concerning *E. dermatitidis* fungemia in a patient with FL. Diagnosing *E. dermatitidis* is difficult, but MALDI-TOF MS can aid in the diagnosis and selection of the appropriate treatment for this pathogen. The prompt diagnosis and treatment of *E. dermatitidis* is critical, especially when yeast-like fungi and black colonies are observed. Further studies are warranted in order to further understand the biology and characteristics of this fungal species.

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

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