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【 CASE REPORT 】

Community-acquired Disseminated *Exophiala dermatitidis* Mycosis with Necrotizing Fasciitis in Chronic Graft-versus-host Disease

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Abstract:

We herein report a case of systemic phaeohyphomycosis by *Exophiala dermatitidis* (*E. dermatitidis*) with chronic graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT). The patient had been taking oral corticosteroids for years to control the GVHD. Yeast-like fungi were identified in a blood culture, so treatment with micafungin (150 mg/day) was begun, with no improvement. The patient passed away on hospital Day 12. A sequence analysis of rRNA revealed the isolate to be *E. dermatitidis*. This report brings attention to an emerging mycosis of community-acquired *Exophiala* species infection in the very-late phase after allogenic HSCT in patients with chronic GVHD.

Key words: *Exophiala dermatitidis*, chronic graft versus host disease (GVHD), phaeohyphomycosis, immunosuppressive drug, acute myeloid leukemia, necrotizing fasciitis

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Introduction

Phaeohyphomycosis is an infection caused by pigmented dematiaceous fungi, such as black fungi (1), and is a clinical challenge, especially in hematologic and hematopoietic stem cell transplant (HSCT) recipients, causing significant morbidity and mortality (2). The disease spectrum is vast, varying from localized cutaneous infection to lethal disseminated phaeohyphomycosis with multiorgan involvement (1). Among the melanized fungi, *Exophiala species* (*E. species*) comprise the most clinically relevant black yeasts, often isolated from environmental substrates, including soil, wood and other plant material (3). In particular, *E. dermatitidis* appears to be associated most commonly with systemic infection as well as with poorer outcomes than other *E. species* (4). However, there are few published reports dedicated to studying the infection in transplant recipients.

We herein report a case of community-acquired disseminated *E. dermatitidis* mycosis in the very-late phase (over 7 years) after allogenic HSCT in a patient with chronic graft-versus-host disease (GVHD).

Case Report

A 21-year-old Japanese woman with acute myeloid leukemia in second remission underwent allogeneic peripheral blood stem cell transplantation from her HLA-identical

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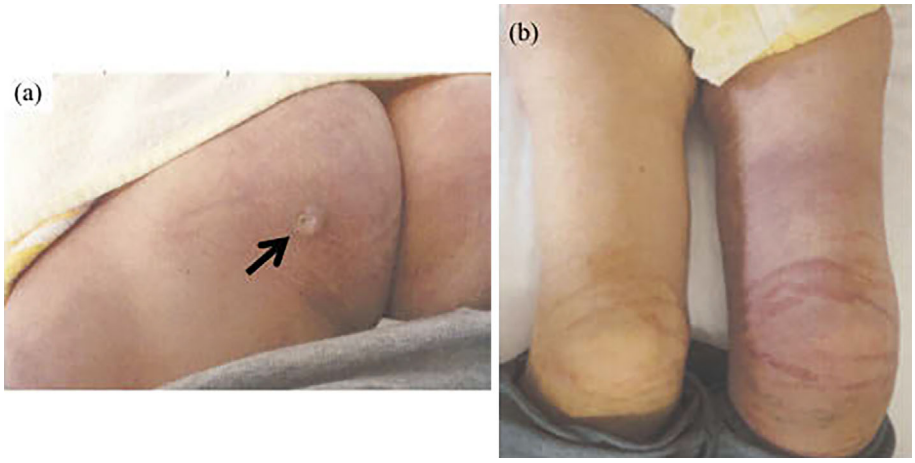

Figure 1. **Macroscopic findings of (a), the left gluteal region (arrow) and (b) left femur on admission.**

Table 1. **Laboratory Examination Findings Obtained on Admission.**

Hematology		Biochemistry	
WBC	2,900 /μL	TP	5.9 mg/dL
myelo	1.5 %	Alb	3.6 mg/dL
meta	4.0 %	BUN	13.0 mg/dL
stab	13 %	Cre	0.39 mg/dL
seg	79.5 %	Na	139 mEq/L
lymph	2.0 %	K	4.3 mEq/L
RBC	376×10^4 /μL	Cl	101 mEq/L
Hb	12.0 g/dL	AST	33 IU/L
Hct	37.3 %	ALT	89 IU/L
Plt	15.1×10^4 /μL	Amy	54 IU/L
		T-Bil	0.8 mg/dL
		LDH	475 IU/L
		ALP	495 IU/L
		γ-GTP	949 IU/L
		CPK	22 IU/L
		CRP	3.53 mg/dL
		IgG	326 mg/dL

brother. She was treated with busulfan and cyclophosphamide as a conditioning therapy and received cyclosporine in combination with methotrexate for the prophylaxis of GVHD. After the transplant, she developed Coombs-negative hemolytic anemia with increased schistocytes in the peripheral blood and an elevated lactate dehydrogenase (LDH) level in serum, in addition to renal dysfunction. She was diagnosed with transplant-associated microangiopathy (TMA).

Although cyclosporine was replaced with tacrolimus, the symptoms became worse. Glucocorticoid was then started. She developed acute GVHD of grade 3 consisting of generalized erythema and liver dysfunction with hyperbilirubinemia and was treated with high-dose glucocorticoid and mycophenolate mofetil. Since she subsequently developed extensive chronic GVHD, including skin lesions, and her liver function fluctuated, she continued oral prednisolone (PSL) at a dose of 8 to 40 mg/day. Accordingly, she suffered from decubitus formation at the left gluteal region, which repeatedly improved and relapsed.

At seven years after the transplant, she was emergently admitted to Fukuoka University Hospital because of exacerbating left buttock pain for two weeks with swelling of the gluteal region at the site of decubitus formation and a low-grade fever despite the administration of oral amoxicillin/clavulanate (750 mg/day) for 3 days. The daily dose of PSL had been increased to 40 mg for 4 months due to the exacerbation of her liver dysfunction. On an examination, her temperature was 36.6℃ blood pressure 142/115 mmHg, pulse rate 95 beats per minute, respiratory rate 16 breaths per minute and oxygen saturation 100% while she was breathing room air. She had an injury (1×1 cm) at the left hip and extensive skin color change at the left knee joint with localized warmth and tenderness (Fig. 1). Otherwise, her physical signs were normal, including her vital signs, and her weight was 60 kg. Laboratory blood tests showed a decreased lymphocyte count (580 cells/μL) and IgG (326 mg/dL) with elevated hepatobiliary enzymes (ALT, ALP, γ-GTP), LDH and C-reactive protein (CRP) (Table 1). Creatine phosphokinase (CPK) was within the normal range.

The administration of ampicillin/sulbactam (12 g/day) and clindamycin (1,800 mg/day) was begun. However, the buttocks and leg lesions were exacerbated, so the antimicrobials were changed to meropenem (3 g/day) and linezolid (2 g/day) from hospital Day 3 (Fig. 2). The blood cultures that had been drawn on admission grew yeast-like fungus (Fig. 3a) on Day 6, and serum (1→3)-β-D-glucan showed a very high titer (>600 pg/mL) with elevated serum lactate and CRP. Micafungin (MCFG) (150 mg/day) was begun, since candidemia was suspected. However, the skin lesions expanded from the waist to the left lower extremities, so relaxation incision surgery was performed from the left buttocks to the left lower leg. Amputation of the left leg was considered, but it was abandoned because the necrotic lesions spread to the hip and leg on the right side just after the incision surgery. On hospital Day 10, the blood culture

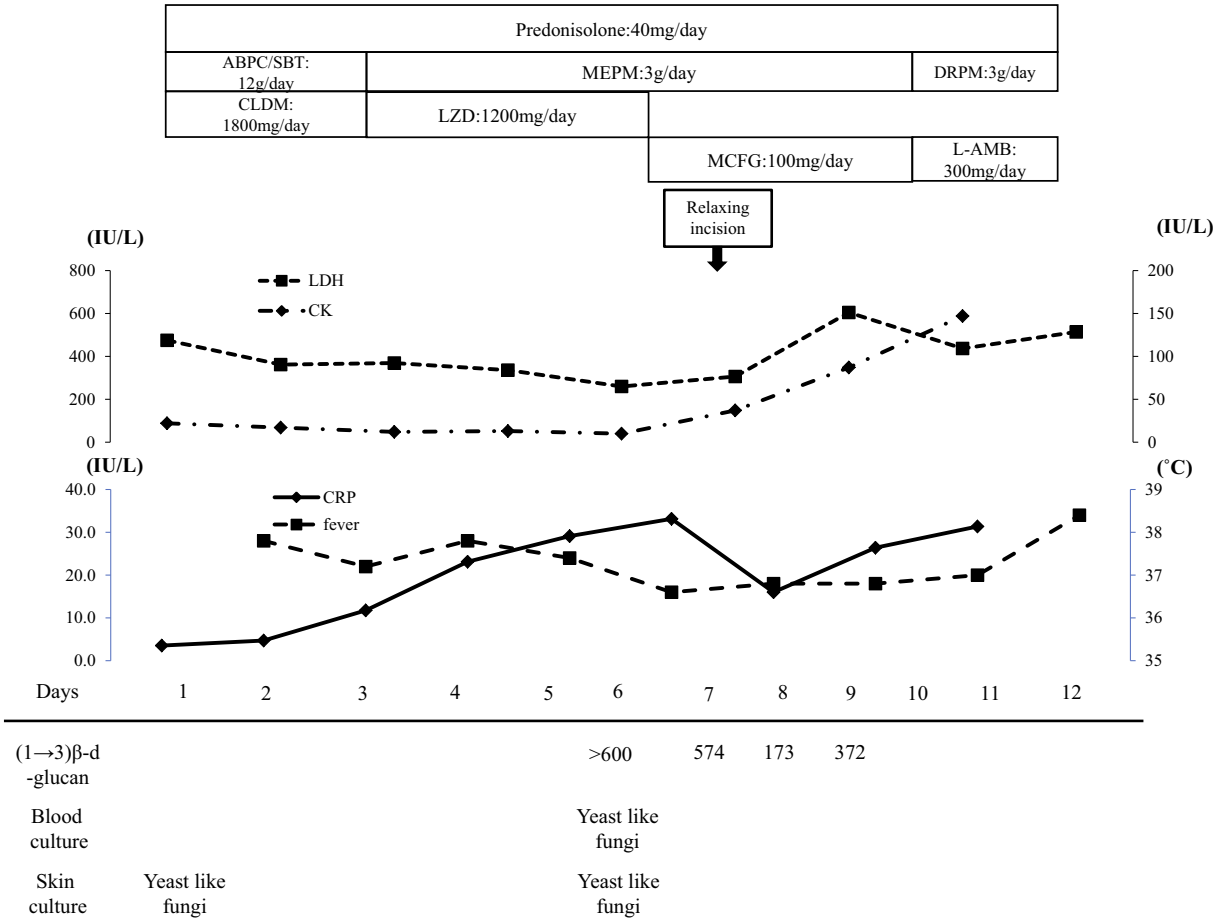

Figure 2. **Clinical course. ABPC/SBT: ampicillin/sulbactam, MEPM: meropenem, DRPM: doripenem, CLDM: clindamycin, LZD: linezolid, MCFG: micafungin, L-AMB: liposomal amphotericin B, LDH: Lactate dehydrogenase, CK: creatine kinase, CRP: C-reactive protein**

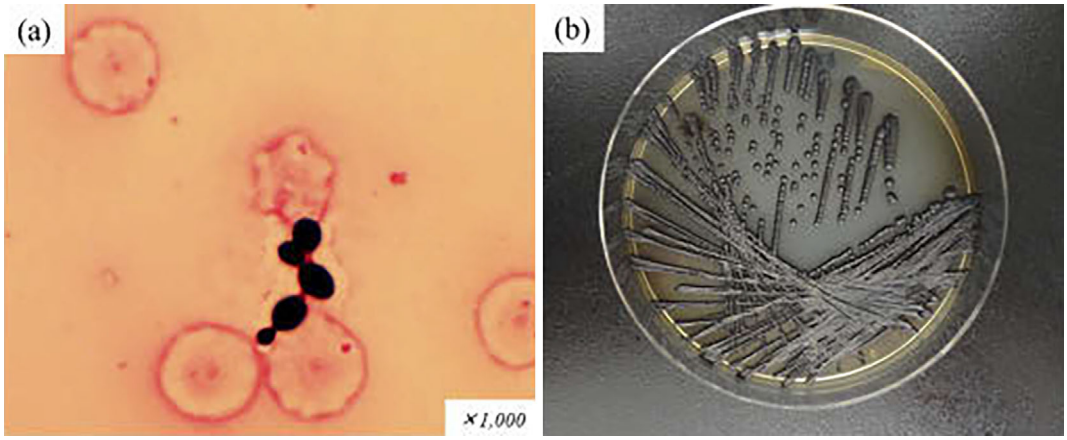

Figure 3. **(a) A high magnification view of a Gram-stain section showed yeast-like fungus (×1,000). (b) Black colonies cultured on potato dextrose agar.**

sample was found to be positive for yeast-like fungus, growing black colonies on potato dextrose agar (Fig. 3b), the antifungal agents were thus switched from MCFG to liposomal amphotericin B (L-AMB) (5 mg/kg). However, the disseminated lesions spread to the upper limbs, upper body and face. On hospital Day 10, the patient became complicated with hemoptysis, and her respiratory failure worsened; she ultimately passed away on hospital Day 12.

A sequence analysis of the internal transcribed spacer region of the rRNA gene revealed the isolate to be *E. dermatitidis*. Postmortem needle biopsies showed disseminated invasion to multiple organs, including the muscle, lung, liver, and intestinal mucosa with massive necrosis, so the final diagnosis was disseminated phaeohyphomycosis caused by *E.*

Table 2. (1→3)β-D-glucan Levels in Previous Case Reports of Invasive *Exophiala* Spp. Infections.

Age/gender	Underlying diseases	Neutropenia	Primary site of infection	Site of detection culture	(1→3)β-D-glucan	Prognosis	Reference
47/F	Recurrent tongue carcinoma	600/μL	Oral	Blood	108pg/mL	Cured	10
54/F	Right middle lobe bronchiectasis	-	Lung	Lung	41pg/mL	Cured	11
53/F	Bronchiectasis	-	Lung	Lung	40.0pg/mL	Cured	12
65/M	Multiple Myeloma	-	Lung	Lung	<3.7pg/mL	Cured	13

dermatitidis.

Discussion

This case was diagnosed as disseminated phaeohyphomycosis caused by *E. dermatitidis* complicating necrotizing fasciitis (NF) because the patient showed multi-system involvement, including the skin and lungs. *E. dermatitidis* infection has been reported in immunocompromised hosts, such as patients with hematologic malignances or allogenic HSCT or patients requiring renal replacement therapy by peritoneal dialysis. Compared to other species, *E. dermatitis* is more frequently associated with disseminated infection in many organs, including the brain, heart valves, lungs and gastrointestinal tract (4). In these cases of disseminated phaeohyphomycosis, a mortality rate of at least 70% has been reported, despite aggressive antifungal therapy (5). *E. dermatitidis* infection has also been observed in seemingly immunocompetent individuals after intravenous drug use or, more recently, after infection with contaminated corticosteroid compounds (6). Interestingly, the clinical cases caused by *E. dermatitidis* have been reported mainly from Asia (3), although its distribution is worldwide.

To our knowledge, this is the second case report of disseminated *E. dermatitidis* infection observed in an allogeneic HSCT recipient. In the case reported by Chalkias et al., *E. dermatitidis* infection occurred 2 years after transplantation (4). In contrast, it occurred 7 years after HSCT in our case, under long-term immunosuppressive therapy with PSL for chronic GVHD. We were unable to identify the invasive entrance of *E. dermatitidis* but suspect it occurred via direct entry through her hip injury by recurrent gluteal decubitus formation due to skin GVHD or through the disrupted gastrointestinal lining due to intestinal GVHD. Of note, a screening of 2,300 samples of feces from humans with and without underlying disease revealed that *E. dermatitidis* is present at a frequency of 5.2 per 1,000 (n=12) (7).

Among 2,191 invasive fungal infections described in the TRANSNET cohort, 56 (2.6%) cases were categorized as phaeohyphomycosis. In the present case, phaeohyphomycosis caused by *E. dermatitidis* developed 7 years after allogenic HSCT, which was a relatively late onset compared with the TRANSNET data (median time: 100 days) (8). It is therefore important to maintain a clinical suspicion for this mycosis in order to make an accurate diagnosis promptly. Our report brings attention to the emerging opportunistic mycosis of community-acquired *E. species* infection, which should be considered in allogeneic HSCT patients with chronic GVHD.

The diagnostic tools and optimization of treatment for *E. dermatitidis* are still being developed. (1→3)-β-D-glucan, which is a representative surrogate marker for the rapid diagnosis of invasive fungal infections (9), showed a very high titer (>600 pg/mL) in this case at the time of the growth of yeast in blood culture on Day 6. The association between invasive *Exophiala* infections and the (1→3)-β-D-glucan level has rarely been described in clinical cases, and we found only 4 previous reports (Table 2) (10-13). Among them (1→3)-β-D-glucan was reported to be elevated in 3 cases. In the present case, whether or not the (1→3)-β-D-glucan level was elevated in earlier stages of the clinical course is unknown; however, further studies to validate (1→3)-β-D-glucan as the modality of invasive *Exophiala* infections may be warranted.

For the treatment of *E. species* systemic infections, a combination of antifungal therapy and surgical intervention is often necessary, in contrast to localized cutaneous disease (4). Regarding antifungal therapy, there are no comparative studies of the efficacy of antifungal agents against phaeohyphomycosis. An evaluation of 66 clinical isolates of *E. dermatitidis* demonstrated good *in vitro* activity of posaconazole (PSZ), ITCZ, VRCZ and AMPH-B (14). Regarding *E. dermatitidis*, there are no established minimum inhibitory concentration (MIC) breakpoints to interpret susceptibility results in a standardized fashion. As antifungal agents, ITCZ, PSZ, VRCZ and AMPH-B are known to exhibit *in vitro* activity, with some variability, while FLCZ has poor activity against many dematiaceous molds (14). In animal models of disseminated infection by *E. dermatitidis*, PSZ was more effective than AMPH-B and ITCZ (15). The species appears to be non-susceptible to echinocandins, probably due to the reduced presence of (1→3)-β-D-glucan in the fungal cell walls (14, 16). Breakthrough fungemia was observed while the patient was being treated with MCFG in the present case.

E. dermatitidis can have a yeast-like appearance with conventional staining, and thus its misidentification as *Candida* or *Cryptococcus* species has been reported. In addition, *E. dermatitidis* takes a long time (typically three days) to develop a black color in Sabouraud dextrose agar, making it

difficult to diagnose the fungus in the early stage of infections. It is therefore important to maintain a clinical suspicion for this mycosis in order to make an accurate diagnosis promptly, and isolation of the yeast under cGVHD should not be dismissed lightly. A recent study showed that matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) allows for the rapid and accurate identification of a wide range of clinically relevant *Exophiala* species; therefore, the introduction of MALDI-TOF MS to laboratory testing may dramatically aid in the early identification of an unknown colony (17).

A number of fungal species can manifest skin lesions as the presentation of their systemic fungal infections in immunocompromised patients with onco-hematological diseases and/or a history of HSCT. These fungi include yeasts, such as *Trichosporon* (18) or *Cryptococcus* (19); molds, such as *Fusarium* (20), *Mucor* (21) or *Pseudallescheria* (*Pseudallescheria/Scedosporium* complex) (22); and other black fungi, such as *Fonsecaea* (23). Importantly, all of these fungi, including *Exophiala*, are non-susceptible to echinocandins, which are one of the most empirically used antifungal drugs for deep-seated mycoses.

In a recently published Japanese guideline (24), prophylaxis with antimold agents mainly targeting Aspergillus (ITCZ, VRCZ, MCFG) is recommended for chronic GVHD after allogenic HSCT. Given the rarity of the disease and the difficulty of effective treatment, in addition to daily life guidance for infection control, such as wound care and avoiding contact with soil, a risk assessment and prophylaxis with oral triazoles should be considered in order to prevent this infection among chronic GVHD patients with immunosuppression.

In summary, *E. dermatitidis* should be kept in mind as a cause of opportunistic invasive mycosis, especially in patients with long immunosuppression after allogeneic HSCT with chronic GVHD. When suspected, the early implementation of debridement and appropriate antifungal therapy should be considered.

Author's disclosure of potential Conflicts of Interest (COI).
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