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Case Report

A fatal case of *Exophiala dermatitidis* disseminated infection in an allogenic hematopoietic stem cell transplant recipient during micafungin therapy☆

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ABSTRACT

Exophiala dermatitidis is a dematiaceous fungus that is increasingly becoming the cause of fungal infection in immunocompromised patients. However, the risk factors and optimal treatment modality for *E. dermatitidis* infection are unknown to date. Herein, we present a fatal case of *E. dermatitidis* infection in an adult patient that developed after allogeneic hematopoietic stem cell transplantation for chronic active Epstein-Barr virus infection. The dematiaceous fungus caused a breakthrough fungemia despite prophylactic administration of micafungin. Although the patient was intensively treated with liposomal-amphotericin B and voriconazole, serum level of beta-D-glucan continuously increased, and the patient eventually died because of cerebral hemorrhage. An autopsy found multiple involvements of the fungal infection at the bilateral lungs, thoracic cavities, diaphragm, and thyroid. To the best of our knowledge, this is the first reported case of *E. dermatitidis* infection involving these tissues as determined via autopsy. This case highlights the importance of attention for *Exophiala* infection in immunocompromised individuals in those given antifungal therapy with echinocandins.

1. Introduction

The *Exophiala* species, which are comprised of dematiaceous fungi, are emerging opportunistic pathogens in individuals with underlying immune dysfunctions [1,2]. Although they generally inhabit in the natural environment [3], they are increasingly reported as a human pathogen causing dermatologic, subcutaneous, and systemic infections [4,5]. Among the various *Exophiala* species, *Exophiala dermatitidis* is associated with deep-seated infection with poor outcomes [2,6]. A literature review in 2012 summarizing 30 published cases between 1993 and 2011 reported that majority of patients with *E. dermatitidis* infections had underlying immunocompromised diseases, and 80% of them had systemic involvements [7]. Recently, severe cases of *E. dermatitidis* infections related to bone marrow transplantation have been described in the literature [1,8]. We report another fatal case of disseminated *E. dermatitidis* infection in a patient with chronic active Epstein-Barr virus (EBV) infection.

2. Case presentation

A 29-year-old man with a history of lymphocyte-predominant leukocytosis has experienced repeated episodes of fever. Due to the persistent symptoms, the patient was admitted to a nearby hospital for systemic examinations. Laboratory tests revealed atypical lymphocyte (46%); low platelet level ($2.4 \times 10^4/\mu L$); impaired liver function; and elevated lactate dehydrogenase (853

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μ/L), ferritin (3296 ng/mL), and soluble interleukin-2 receptor (9100 U/mL). The patient tested negative for human immunodeficiency virus. Radiography demonstrated a massive hepatosplenomegaly. A bone marrow examination showed a slight hemophagocytosis and presence of large granular lymphocyte (12%), and an immunophenotyping via flow cytometry revealed a clonal proliferation of $CD2^+$, sCD3-, cyCD3±, $CD7^+$, $CD19^-$, and $CD56^+$ natural killer (NK) cells. Serum serology for EBV infection showed elevated anti-viral capsid antigen and anti-early antigen, while negative for Epstein-Barr virus-nuclear antibody. Real-time polymerase chain reaction examination for EBV-DNA showed an abnormally high titer (9.5×10^5 copies/μL) in whole blood. The patient was accordingly diagnosed with EBV-associated NK-cell lymphoproliferative disease or chronic active EBV infection.

The patient was first palliatively administered with 0.5 mg/kg prednisolone; however, high fever accompanying progressive thrombocytopenia persisted. He then underwent HLH-2004 protocol; a combination of cyclophosphamide, doxorubicin, vincristine, and prednisolone regimen; a combination of vincristine, prednisolone, and L-asparaginase regimen; and low-dose VP-16 and cytarabine regimen. However, the EBV DNA continued to be detected at high titer in the peripheral blood. Thus, the patient underwent allogeneic peripheral blood stem cell transplantation (PBSCT) from a haploidentical sibling donor. Conditioning regimens included 500 mg/m^2 of etoposide (day −8), 30 mg/m^2 of fludarabine (day −7 to −3), 70 mg/m^2 of melphalan (day −3, −2), 2.5 mg/kg of anti-thymocyte globulin (day −3, −2), and 3Gy total body irradiation which was combined with splenic irradiation (10Gy/5frac). Tacrolimus and methylprednisolone were used for prophylaxis of graft versus host disease.

Due to insufficient EBV suppression after PBSCT, the patient underwent a donor lymphocyte infusion 41 days after PBSCT, followed by cessation of tacrolimus. Two months later, the patient developed severe acute graft-versus-host disease (GVHD) at the gut (stage 4) and skin (stage 1). GVHD treatment with antithymocyte globulin was initiated; however, GVHD was not controlled and thus tacrolimus was added accordingly. Afterward, the patient was further complicated with systemic cytomegalovirus (CMV) infection (viremia, hepatitis, and retinitis) and BK virus infection (viremia and hemorrhagic cystitis) at approximately 4 months and 6 months after PBSCT, respectively.

Nearly 11 months after PBSCT, the patients was still hospitalized and experienced prolonged high fever even with prophylactic administrations of levofloxacin (500 mg/day for one week) followed by cefepime (1 g/day for one week), micafungin (150 mg/day for 3 weeks), acyclovir (200 mg/day since after PBSCT), and ganciclovir (300 mg/day for 2 weeks). The patient underwent immunosuppressive therapy with intravenous prednisolone (100 mg/day) at that time for GVHD management. No skin lesions were noted on physical examination. Laboratory test results showed peripheral white blood cell, neutrophil, and lymphocyte counts to be 1380/μL, 138/μL (10%), and 1145/μL (83%), respectively. C-reactive protein (2.78 mg/dL) and beta-D-glucan (24.8 pg/mL [normal range, <11 pg/mL] levels were slightly elevated (β-glucan test Wako, FUJIFILM Wako Pure Chemical Corporation). Chest and abdominal computed tomography (CT) demonstrated no abnormal findings. Despite the intravenous administration of micafungin, blood culture examination detected a yeast that demonstrated dematiaceous colonies after 4 days of incubation. After ethanol-formic acid extraction, the organism was applied to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a MALDI Biotyper (Bruker Daltonics, Bremen, Germany) that identified it as *E. dermatitidis* with a score of 1.704.

A central venous catheter that was removed and submitted to the microbiology laboratory was negative for the organism. Antimycotic treatment with liposomal-amphotericin B (50 mg/day for 2 weeks) was initiated but was switched to intravenous voriconazole (200 mg/day) because of continuously elevating beta-D-glucan. Despite the antifungal treatment, the amount of pleural effusion bilaterally increased, and *E. dermatitidis* was isolated from the pleural fluid. Then, intrathoracic amphotericin B was additionally administered. Despite these rigorous anti-fungal therapies, serum level of beta-D-glucan increased up to 283.6 pg/mL at peak. Four weeks after the first isolation of *E. dermatitidis* in his blood, he suddenly developed a deep coma with anisocoria. Head CT demonstrated a subdural hematoma and cerebral hemorrhage at the left occipital lobe, and the patient died the next day.

An autopsy showed no dermatologic findings suggesting a fungal involvement. The bilateral intrathoracic wall was massively covered with black debris (Fig. 1A), and a histopathology showed a bilateral invasion of yeast-like forms with hyphae into the parietal pleura where *E. dermatitidis* was isolated (Fig. 1B and C). Furthermore, the fungal invasion extended over the diaphragmatic tissues (Fig. 1D). There was no parenchymal lesion, and lung involvements were observed only in neighboring visceral pleura. Moreover, disseminated fungal lesions were also seen at the thyroid (Fig. 2A and B). Meanwhile, no pathological findings were found in the cerebral tissues damaged by hemorrhage. Additionally, CMV reactivations were immunohistochemically detected at the lungs, liver, intestine, and urinary bladder. Also, BK virus infection was detected at the mucosa of the renal pelvis and urinary bladder.

To confirm the fungal identification, we later performed a molecular identification via sequencing of the internal transcribed spacer (ITS) and D1/D2 regions. The genomic DNA was extracted, and these specific regions were amplified using the following primers; ITS region [ITS1: 5′-TCC GTA GGT GAA CCT GCG G-3′, ITS4: 5′-TCC TCC GCT TAT TGA TAT GC-3'] [9], D1/D2 region [NL-1: 5′-GCATATCAATAAGCGGAGGAAAAG-3′, NL-4: 5′-GGTCCGTGTTTCAAGACGG-3'] [10]. The sequence data were analyzed using BLAST sequence homology search programs (GenBank, DDBJ, and MycoBank). Finally the organism was identified as *E. dermatitidis*, in a comparison of ITS region with the type strain of *E. dermatitidis* (CBS 207.35; accession number, NR_121268.1) with an accuracy of 98.7% (618 bp/626 bp). Also, a comparison of D1/D2 region with another type strain of *E. dermatitidis* (CBS 207.35; accession number, AF050269) suggested the clinical isolate to be *E. dermatitidis* with an accuracy of 99.5% (564 bp/567 bp). We measured the minimum inhibitory concentrations (MIC) of each antifungal agent using the ASTY colorimetric microdilution panel (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo) according to manufacturer instruction; micafungin, 16 μg/mL; caspofungin, 16 μg/mL; flucytosine, >64 μg/mL; fluconazole, 8 μg/mL; miconazole, 0.25 μg/mL; itraconazole, 0.125 μg/mL; voriconazole, 0.06 μg/mL; and amphotericin, 0.25 μg/mL.

Thereafter, we investigated possible ward contamination. Air sampling was performed using Air-sampler System (MAS-100 NT™; Merck KGaA, Darmstadt, Germany) under a sampling condition of 100 L/min for 192 seconds (320 L in total). We also swabbed airconditioners and a sink where we found black color changes. The samples were cultured with Sabouraud Dextrose agar (Kyokuto Pharmaceutical Co., Ltd, Tokyo, Japan) at 35 °C for 24 hours and subsequently 6 days at room temperature. All 3 air samples and 12 swab samples examined tested negative for *Exophiala* species.

3. Discussion

We present a fatal case of *E. dermatitidis* disseminated infection complicated with fungemia and multiple infections at the bilateral thoracic cavities, lungs, diaphragm, and thyroid. This serious

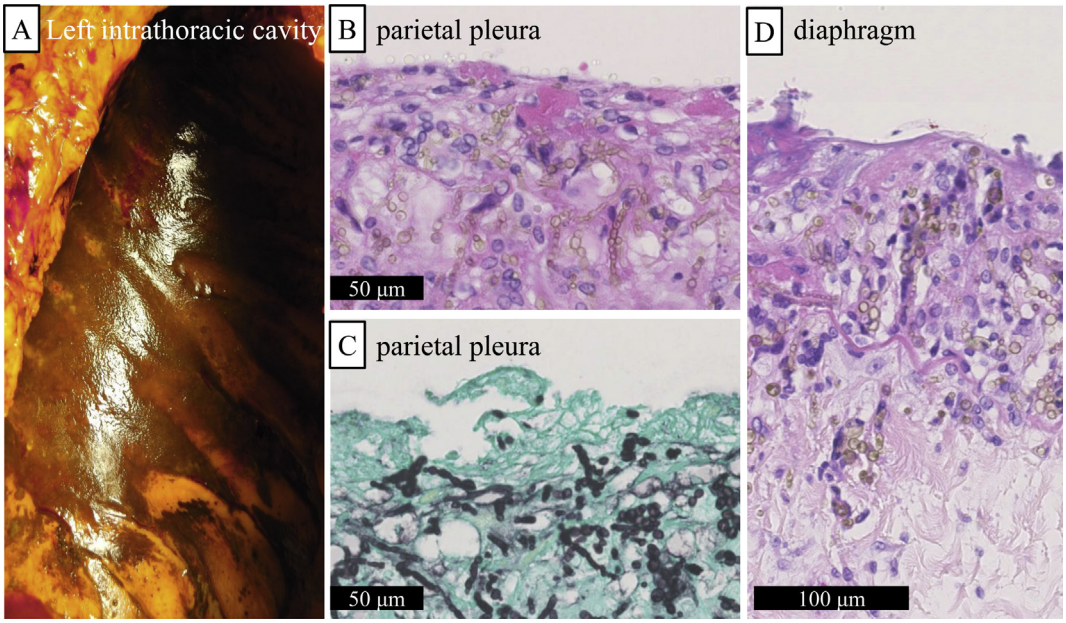

Fig. 1. Pathologic examinations for intrathoracic *Exophiala dermatitidis* infection. Macroscopic appearance of black-pigmented debris predominantly seen inside the left intrathoracic space (A). Hematoxylin-eosin staining (B) and Grocott staining (C) of visceral pleural tissues showing an invasion of yeast-like forms with hyphae into the tissues. Invasion of the fungal infection into diaphragm tissue was observed (D).

infection involved a patient who underwent intensive immunosuppression therapy for GVHD following PBSCT. Notably, the disseminated disease occurred as a breakthrough infection despite prophylactic administration of micafungin. The clinical course of this case indicated that elevated serum beta-D-glucan levels despite anti-fungal treatment suggests a progressive course of the deadly infection. To the best of our knowledge, this is the first case in the literature demonstrating severe chromomycosis in intrathoracic cavities and involvements of the diaphragm and thyroid on autopsy. Although not proven on the autopsy, the sudden intracranial hemorrhage was possibly triggered by neuroinvasive infection of the fungus because *Exophiala* species potentially cause cerebral involvements [11,12], particularly in immunocompromised patients [2,7]. Neurotropism of the pathogen has been previously reported [5], and most patients with disseminated *Exophiala* infections die due to neuroinvolvement of the infection [4]. Similar cases have been described recently [1,8], and the current case highlights the importance of close attention for breakthrough *Exophiala* infections in patients after hematopoietic stem cell transplantation. For early diagnosis, there are no specific markers or examination for the pathogen, and repeated examinations for serum beta-D-glucan and blood culture are necessary.

Among various phaeohyphomycosis, *Exophiala* infections have recently been noted as a clinically important pathogen. According to a previous review of 72 cases of disseminated phaeohyphomycosis, *Exophiala* species accounted for 12.5% (9 cases) [13]. Particularly, while other species only cause local infection, *E. dermatitidis* possibly causes systemic diseases; of the 37 reported cases of *E. dermatitidis* infections, 20 (54%) had evidence of disseminated involvements [4]. Although *E. dermatitidis* rarely causes infections in immunocompetent individuals, it can cause invasive infections such as brain abscess, meningitis, and endocarditis [4,14,15].

The pathway of infection of this lethal fungus should be discussed. *Exophiala* species are ubiquitously found at indoor environments including kitchen sinks, dishwashers, and steam-bath facilities [3,14,16]. Previously, a contaminated hospital water supply induced a nosocomial outbreak of *Exophiala* fungemia [17]. We examined the clinical ward on suspicion of nosocomial infection, but *Exophiala* species were not detected.

Plausible infection routes were unclear in most cases of systemic *Exophiala* infections [6]; however, central venous catheter infections can often be involved. In a recent case series, 23 out of the 29 (79.3%) cases of *Exophiala* fungemia were inserted a central venous catheter when they were diagnosed with the infection

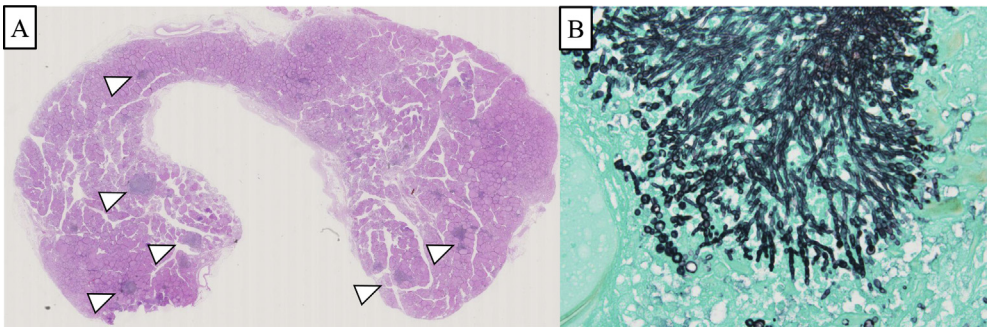

Fig. 2. Multiple thyroidal lesions. *Exophiala dermatitidis* formed multiple fungal lesions in the thyroid (A, magnifying glass; arrow heads). Microscopic examination showing invasions of yeast-like forms with hyphae into the thyroidal tissues (B, Grocott staining).

[18]. A recent report on an outbreak of *E. dermatitidis* bloodstream infections was found to be associated with a contaminated parenteral compound, that caused central venous catheter infections [19]. We speculate that the pathogen infected the patient through the central venous catheter, leading to hematogenous dissemination, rather than via inhalation. This was supported by the pathological findings of predominant involvements of the fungal invasion at the pleural space, but not at the lung parenchyma. Another possible explanation is fungal translocation from his gastrointestinal tract because *Exophiala* species have been known to be gut colonizers [20,21]. Skin lesions can also be a portal of entry, but dermatologic manifestations are usually barely visible [22].

The optimal antifungal therapy for *Exophiala* infection is still unknown. Experimentally, voriconazole, terbinafine, posaconazole, itraconazole, and amphotericin B possess *in vitro* activity against *Exophiala* species [23]. However, there are no established MIC breakpoints, and previous reports referred to the Clinical and Laboratory Standards Institute Document, M38-A2, with some modifications [23], Document M27−A3 [8], or E-test [18] for testing anti-fungal susceptibility. We used the ASTY panel to measure the MIC values of anti-fungal agents in this case; however, the validity of this method for dematiaceous fungus is not warranted. In addition to the difficulty in determining susceptibility, the *in vitro* susceptibility data may not be correlated with clinical efficacy [24], perplexing physicians for appropriate selection for anti-fungal therapy. The overall mortality rate of the infection is as high as 24%−27% [7,18], with higher mortality rates (nearly 40%) in disseminated cases [6]. *E. dermatitidis* forms biofilms that can result in poor efficacy of antifungal agents [25]. However, administration of amphotericin B and/or itraconazole may yield a good prognosis [1,7,8,18]. In this case, the patient eventually died, despite antifungal treatment with liposomal amphotericin B. Under-dosing of the drug could be a possible reason for the refractory course. Additionally, we assume that the fungus had already been disseminated in his body, reaching an untreatable stage when the infection was recognized. Notably, MIC data for echinocandins are insufficient [24], and recent cases [1,8] including the current case suggested a concern about breakthrough infection by *E. dermatitidis* during micafungin administration.

In conclusion, we illustrated a case of disseminated *E. dermatitidis* infection after PBSCT for chronic active EBV infection. To the best of our knowledge, this is only the third reported case in which *E. dermatitidis* breakthrough infection occurred during micafungin therapy. The current case highlights the importance of awareness for *Exophiala* infection in immunocompromised patients after hematopoietic stem cell transplantation.

Conflicts of interest

The authors confirm that there are no conflicts of interests to declare.

Ethical statement

Written informed consent for the publication was obtained.

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