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NOTE

Fungal peritonitis associated with *Curvularia geniculata* and *Pithomyces* species in a patient with vulvar cancer who was successfully treated with oral voriconazole

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Although fungal peritonitis is uncommon, it occurs most often in patients undergoing continuous ambulatory peritoneal dialysis (CAPD) and is associated with significant morbidity and mortality.[1] *Candida albicans* accounts for the majority of fungal peritonitis episodes.[2] However, rare fungal species have become recognized increasingly as important pathogens for the infection. We report a unique case of fungal peritonitis probably associated with *Curvularia geniculata* and *Pithomyces* species developing in a gynecological cancer patient who did not undergo CAPD and was successfully treated with oral voriconazole.

A 61-year-old woman with a 5-month history of vulvar cancer, together with diabetes mellitus complicated by a nephropathy, complaining of health problems was admitted to the Gifu University Hospital on 14 August 2001. Immediately after the admission, her vulvar cancer and diabetes were treated with oral medroxyprogesterone acetate and parentheral insulin, respectively. On day 9 during her hospital stay, the patient became febrile and received antibacterial agents, sulbactam/cefoperazone. After 5 days of antibiotic treatment, she complained of lower abdominal pain and tenderness. Examinations at this time revealed the fever level of 37.3 °C, the white blood cell count of 8900 mm^{-3} and the serum C-reactive protein level of 5.31 g dl^{-1}. Moreover, an extremely high serum level of β-D-glucan (31 750 pg ml^{-1}; the positive cutoff level for systemic mycoses: 20 pg ml^{-1}) was detected. On the basis of the clinical symptoms and laboratory data, we suspected development of an episode of fungal peritonitis and started the antifungal treatment with 300 mg oral voriconazole twice a day (b.i.d.) as a loading dose (following 200 mg b.i.d. for maintaining) in the course of a clinical trial for the marketing application in Japan.[3]

Specimens of ascitic fluid taken before the start of voriconazole therapy were subjected to mycological examinations. According to the standard diagnostic laboratory protocol, Sabouraud dextrose agar plated were inoculated with ascitic fluid, as well as its 10-fold serial dilutions. After 5 days of incubation, pigmented mycelial colonies that were velvety, brownish and flat grew with the yield of 10^8 colony-forming units per ml of ascitic fluid, whereas bacterial culture was negative. The microscopic examination of these colonies picked up arbitrarily revealed that two morphologically different dematiaceous fungi grew on the plates, indicating the presence of two types of fungal isolates in the recovered culture.

Colonies of one of the two types of isolates grown on potato dextrose agar (PDA) at 35 °C for 3 days were found to show greenish brown-to-dark brown surface coloring with a black reverse. Microscopical images of this dematiaceous fungus showed following morphological features: (i) septate with brownish black hyphae; (ii) conidiophores that are septated and bent or knobby at points of conidium formation; and (iii) slightly protruding and curved due to the swelling of a central cell and darkly pigmented conidia that are further characterized by oblong-to-cylindric forms and the presence of mostly three distosepta, 17–30 × 8–11 μm (Figure 1). On the basis of these characteristics, this isolate was phenotypically identified as *C. geniculata*. To confirm the phenotypic identification, sequencing of the internal transcribed spacer (ITS) regions of nuclear rRNA genes was performed. Procedures for DNA extraction and amplification of DNA fragments covering the nuclear ITS regions (ITS1 and ITS4)[4] followed the protocols outlined by Makimura *et al.*[5] DNA sequencing was performed using an Applied Biosystems sequencer (Model 3130; Applied Biosystems, Foster City, CA, USA). A BLAST

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Figure 1 Microscopic image of a *C. geniculata* isolate (lactophenol cotton blue stain).

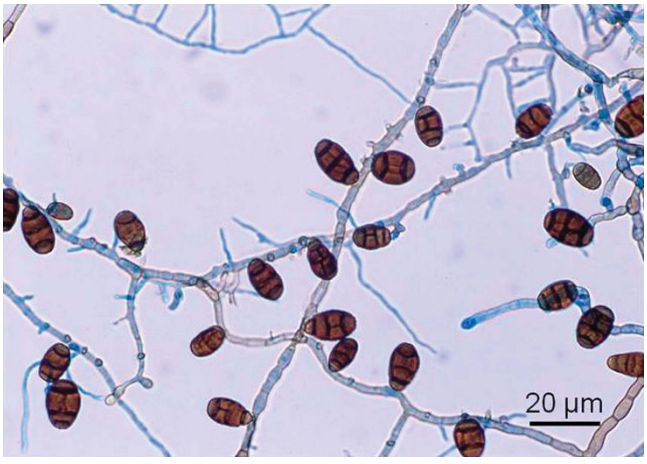

Figure 2 Microscopic image of a *Pithomyces* isolate species (lactophenol cotton blue stain).

(http://blast.ncbi.nlm.nih.gov/Blast.cgi) search using the ITS sequence for this isolate showed it to be 100% similar (891 of 891 bases) to *C. geniculata* NBRC 7407 and MAFF 236895, as well as to *Curvularia inaequalis* AF 313409, and 99% similar (885 of 891 bases) to *C. inaequalis* ZM 020029 and ZW 020028, supporting the phenotypical identification of the isolate as *C. geniculata*. The microscopical examination of cultures of the other types of isolates grown on PDA at 27 °C for 5 days revealed that conidiogenous cells are inconspicuous, short and simple and are produced directly on the side of septate hyphae, and that conidia are muriform, subglobose to broadly elliptical and brown and are produced singly at the apex of each conidiogenous cell (Figure 2). These morphological features appeared to be consistent with those characteristics of the genus *Pithomyces*. We did not perform DNA sequencing of this isolate because it did not retain viability at the time of molecular analysis.

The antifungal susceptibility testing of the two isolates identified as *C. geniculata* and *Pithomyces* species was performed by using a colorimetric microdilution technique for filamentous fungi that had been proposed by the Japanese Society for Medical Mycology.[6] An excellent correlation of the results obtained by this method with those by the NCCL (CLSI) M38 protocol[7] was confirmed in our previous study.[8] The MICs of voriconazole, itraconazole, fluconazole and amphotericin B were 0.25, 0.031, >64, and 0.125 μg ml^{-1}, respectively, for *C. geniculata*, and 0.5, 0.5, >64, and 1 μg ml^{-1}, respectively, for *Pithomyces* species.

From the result of trough level of voriconazole on the 3rd day of the treatment, the dose was decreased from 200 mg b.i.d. to 150 mg b.i.d. on the 5th treatment day. Next day, lower abdominal symptoms were completely resolved, ascitic fluid became culture-negative and the serum β-D-glucan level decreased to 40 pg ml^{-1}. Thus, voriconazole therapy was judged to have achieved both microbiological and clinical cures.

The species of both the genus *Curvularia* and the genus *Pithomyces* are members of a group of dematiaceous fungi. They usually reflect contamination or colonization rather than infection in humans. However, fungal peritonitis due to *Curvularia* species, although very rare, has been reported; in our review of the literature, we found eight cases of *Curvularia* peritonitis.[9–16] The causatives species identified are *Curularia lunata* (four cases),[9–12] *C. inaequalis* (one case)[13] and *C. geniculata* (one case).[14] It should be noted that all of the reported patients with *Curvularia* peritonitis underwent CAPD. Moreover, three cases of CAPD catheter obstruction resulting from *Curvularia* species without evident peritonitis have been reported previously.[17] Unlike *Curvularia* species, *Pithomyces* species have not been reported to be implicated in any human infections including peritonitis. Reports are limited to those of 'facial eczema' or pithomycotoxicosis in sheep and calves developing after ingestion of vegetation bearing *Pithomyces chartarum* that produces a specific family of mycotoxins named sporidesmins.[18] It looks, therefore, likely that in the present case *C. geniculata* may have had a major role, or at least greater role than *Pithomyces* species, in the development of fungal peritonitis.

In our patient with vulvar cancer and diabetes who was considered to be further immunocompromised by receiving anti-cancer and antibiotic therapies, two rare dematiaceous fungi were isolated from the ascitic fluid specimen when an episode of acute lower abdominal symptoms developed. The localized clinical symptoms were accompanied by a marked increase in serum level of β-D-glucan, which is a widely used biomarker of invasive fungal infections.[19,20] These clinical and laboratory findings strongly suggest that the patient had fungal peritonitis probably due to *C. geniculata* as the main pathogen. Our case appears to be unique in that, different from all of the previously reported cases of *Curvularia* peritonitis, the patient did not undergo CAPD. There is the possibility that in our patient with vulva cancer access of dematiaceous fungi in the environment by the genital route to the peritoneal cavity is facilitated through the damaged tissues. Furthermore, the present patient had several risk factors for fungal peritonitis including diabetes mellitus, antibiotic use and cytotoxic immunosuppressive therapy as pointed out previously.[2,13] All these medical conditions and host factors might have contributed to the initiation and establishment of fungal peritonitis.

A literature search revealed that in patients with fungal peritonitis caused by *Curvularia* species, amphotericin B has been the most frequently used agent for treatment.[13–15] However, a case of fungal peritonitis due to the infection with amphotericin B-resistant but voriconazole-susceptible *C. lunata* was reported.[11] We used oral voriconazole for the treatment of the present patient and achieved both clinical and microbiological cures. Thus, voriconazole therapy can be a new option for the management of fungal peritonitis caused by *Curvularia* species.

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