Singapore Med J 2012; 53(5) : e106

Successful treatment of peritonitis by *C. bertholletiae* in a chronic kidney failure patient on continuous ambulatory peritoneal dialysis after kidney rejection

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ABSTRACT Peritonitis is a common problem in patients undergoing peritoneal dialysis. However, peritonitis due to *Cunninghamella* (*C.*) *bertholletiae*, a fungus of the class Zygomycetes, is rare. We present a case of fungal peritonitis in a patient on continuous ambulatory peritoneal dialysis due to kidney rejection. Direct examination of the patient's peritoneal fluid showed fungal hyphae, and the culture was identified as *C. bertholletiae*. A cumulative dose of 1,600 mg fluconazole was given to the patient intraperitoneally over a one-week period. When his condition had stabilised, oral antifungal treatment was administered for two weeks. After removal of the Tenckhoff catheter, the patient was discharged with arteriovenous fistulation for haemodialysis. Zygomycosis due to *C. bertholletiae* is often fatal and non-responsive to systemic antifungal therapy. This case is the first from India with a successful outcome, and highlights the importance of early detection and intervention for successful outcome of peritonitis caused by *C. bertholletiae*.

Keywords: antifungal therapy, Cunninghamella bertholletiae, fungal peritonitis, zygomycosis
Singapore Med J 2012; 53(5): e106–e109

INTRODUCTION

Patients undergoing catheterised continuous ambulatory peritoneal dialysis (CAPD) are at an increased risk of being exposed to several microbial species that cause peritonitis. It is often successfully treated by antibacterial agents. However, these patients may sometimes be infected by invasive fungi, with serious and often fatal complications. The most common fungal agents causing these infections are *Candida* spp., *Aspergillus* spp. and *Cryptococcus neoformans*.[1,2] Similarly, fungal species such as *Trichosporon beigelii*, *Fusarium* spp. and *Paecilomyces variotii* have been reported to cause peritonitis, and some of these pathogens are being isolated with increasing frequency.[3,4]

Peritonitis due to fungi of the class Zygomycetes is very uncommon. *Cunninghamella* (*C.*) *bertholletiae* (class Zygomycetes, order Mucorales) is a saprophytic, ubiquitous fungus found in soil. In recent years, it has emerged as an agent of zygomycoses in immunocompromised patients.[5,6] In the absence of any particular therapy, infection due to *C. bertholletiae* progresses very rapidly and aggressively. Diagnosis of the causative agent is often missed, and despite intervention, the prognosis of disease caused by *C. bertholletiae* is very poor,[7] with an average mortality rate of over 70% and a male-to-female susceptibility ratio of 3:1.[8]

We report the isolation of *C. bertholletiae* from a CAPD patient who was admitted for severe peritonitis. The patient was in a state of chronic kidney failure after kidney graft rejection nearly 30 months prior to admission. We highlight the need for early detection as well as prompt intervention to prevent fatal outcome with *C. bertholletiae* infection.

CASE REPORT

A 52-year-old Indian man was admitted to the hospital with fever, abdominal pain and vomiting in early 2009. The patient had undergone a kidney transplant in 2003. However, the kidney was rejected in late 2006, and he had been on CAPD ever since. Prior to this admission, the patient had oligouria for ten days. At presentation, he appeared ill and pale, with low skin turgor. His pulse was 116 beats/min and blood pressure (BP) was 116/76 mmHg. There was diffuse tenderness around his CAPD catheter. Ultrasonography revealed small kidney size with end-stage renal disease and cholelithiasis. His abdominal cavity was filled with gross free fluid and internal echoes. The peritoneal septation was intact, and his respiratory and cardiovascular systems were also normal. Laboratory investigations of the blood plasma revealed increased levels of sodium (132 mEq/L), potassium (3.6 mEq/L), urea (130 mg/dL) and creatinine (14 mg/dL). The patient was negative for HIV antibody and hepatitis B surface antigen (HBsAg). He was not on steroids or other immunosuppressives. Immediately after his admission, the patient was put on antibacterial treatment with intravenous (IV) cefpirome 1 g and IV linazolid 600 mg every 12 hours, and IV vancomycin 100 mg in 2-litre peritoneal dialysis fluid every eight hours.

To identify the causative agent responsible for the peritonitis, the peritoneal fluid was subjected to microscopic examination and culture. The fluid was found to be turbid, and had a total leukocyte count of 258,000, of which 85% were polymorphs and 15% of cells were lymphocytes. The specific gravity of the fluid

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was 1.075, with 4.8 g total proteins and 34 mg cholesterol. Gram staining did not detect any bacteria, but with wet preparation using 10% potassium hydroxide (KOH), non-septate fungal elements were seen along with pus cells (Fig. 1). The peritoneal fluid was cultured simultaneously for both bacterial and fungal isolates. All three culture plates (blood, chocolate and MacConkey agar) for bacterial isolation did not reveal any bacterial growth, but these as well as the potato dextrose agar (PDA) plates had profuse fungal growth (Fig. 2). The growth was characterised by fluffy, white to gray, globose, rapidly growing colonies on chocolate agar and PDA.

The fungal growth on PDA as well as blood, chocolate and MacConkey agar plates was subjected to microscopic examination after staining with lacto-phenol cotton blue (LPCB). Microscopic examination revealed non-septate hyphal morphology. The fruiting bodies were on sporangiophores, which were found to be wide and straight with single, pear-shaped vesicles bearing oval to pyriform spores on very short stalks (Fig. 3). These features matched well with the description of *Cunninghamella* spp. The genus *Cunninghamella* has about 19 species with a worldwide distribution,[9] and the only member that causes human infections is *C. bertholletiae*, which can be distinguished from *C. elegans* by growth at 45°C.[10,11] To identify the fungus at the species level, fresh inoculations were made on PDA and incubated at 45°C. The organism grew at 45°C. Microscopic examination with LPCB staining revealed exactly the same morphology described above for the growth obtained at 30°C. Thus, taking into account its growth at 45°C, along with a single vesicular sporangiola containing oval to pyriform spores on a terminally swollen conidiophores and non-septate hyphae, the organism was identified as *C. bertholletiae*. Confirmation of the above features was received after the comparison of our isolate with the reference material available at the Department of Microbiology, MGIMS, Sevagram, India.

Soon after the fungal hyphae were detected and demonstrated in the culture, the patient was administered intraperitoneal fluconazole 200 mg daily for eight days. Subsequently, his fever subsided and came under control, and the markers of inflammation were also within normal limits. The patient was moved from the intensive care unit (ICU) to the ward. As the patient's condition had improved, oral antifungal treatment with itraconazole 100 mg twice a day was administered for eight days, to which he responded positively. He became afebrile, and his abdominal irritation and pain subsided and then disappeared totally. The inflammatory markers were also found to be completely normal. No fungal elements were seen in the patient's peritoneal fluid when subjected to microscopic examination and wet preparation using KOH. The culture on PDA also revealed no fungal growth after seven days of incubation at 30°C and 45°C.

As a precautionary measure, the patient's Tenckhoff catheter was removed to prevent further exposure, and maintenance dialysis was carried out through an arteriovenous fistula. He was discharged on oral antifungal therapy for a further seven days. On follow-up seven days later, his condition was found to be under control. At the six-week follow-up, his health parameters had normalised and were under control.

Fig. 1 Photomicrograph shows non-septate hyphae in the peritoneal fluid of the patient after wet preparation in 10% potassium hydroxide (KOH wet preparation, × 400).

Fig. 2 Photographs of culture plates show fluffy, white, graying, globose and rapidly growing colonies of *C. bertholletiae* after 72 hrs of incubation at 30°C. The patient's peritoneal fluid was inoculated on chocolate agar (left) and potato dextrose agar (right).

The fungal isolate was subjected to minimum inhibitory concentration (MIC) determination using E-test for fluconazole, according to the methods described by Koga-Ito et al with the following modifications.[12,13] It was carried out on RPMI 1640 with glutamine (HiMedia) supplemented with 1.5% agar, 2% glucose and 165 mM morpholinopropanesulfonic acid buffer (HiMedia) in 150 mm petri-dishes. The plates were inoculated by spreading fungal spores at 0.5 MacFarland density obtained by vortexing the aged fungal growth in normal saline. The antifungal agent on HiComb (HiMedia) with fluconazole concentrations from 0.016 mcg to 256 mcg was affixed on the inoculated plates. *Candida parapsilosis* (ATCC-22019) was used as a control organism in the test. The plates were incubated at 30°C in a biological oxygen demand incubator for 48 hours. The MIC was found to be 64 mcg.

DISCUSSION

Although microscopic examination of the patient's peritoneal fluid presented cellular markers for inflammation, it did not contain any bacterial agents but showed only aseptate hyphae (Fig. 1). Additionally, all three culture plates for bacterial isolation as well as the PDA plate demonstrated only fungal growth (Fig. 2). Together, these findings confirmed the fungal aetiology of

Fig. 3 Microscopic morphology of *C. bertholletiae* shows a simple sporangiophore forming a swollen, terminal vesicle, around which single-celled, globose-to-ovoid sporangiola developed on swollen denticles (Lactophenol cotton blue staining [LPCB], × 400)

peritonitis in our patient. The growth was rapid and in 24 hours, the colonies had appeared fluffy and white, which turned globose and gray in 48 hours (Fig. 2). Microscopic examination of the fungal culture on LPCB staining revealed non-septate, ribbon-like hyphae. The morphology of the fruiting bodies appeared as globose to ellipsoidal sporangiola on ampoule-shaped, terminally swollen sporangiophores. Some of these appeared broken, indicating that the sporangiospores were exposed for transmission (Fig. 3). This morphology is exhibited by fungi belonging to the genus *Cunninghamella*[10,11] (class Zygomycetes, order Mucorales). They are commonly found in soil and have a worldwide distribution. As of January 2011, the genus contains 19 species.[9] The only known human pathogen in this genera is *C. bertholletiae*.[6,14,15] It is identified by its ability to grow at 45°C.[10,11] The isolate from our patient grew at 45°C and produced fruiting bodies that resembled those grown at 30°C. Further confirmation of the isolate was achieved by comparing the organism with the reference material at MGIMS, Sevagram, India.

Four factors are critical for eradicating mucormycosis: rapidity of diagnosis; reversal of the underlying predisposing factors; appropriate surgical debridement of infected tissue; and appropriate antifungal therapy.[16] There is currently no serologic or polymerase chain reaction (PCR)-based test to allow rapid diagnosis, and up to half the cases of mucormycosis are thus diagnosed postmortem.[16,17] Our patient was treated by intraperitoneal administration of fluconazole for eight days with a cumulative dose of 1,600 mg. This stabilised the patient's condition and controlled his fever at the ICU. When the inflammatory markers were normalised, the patient was moved from the ICU to the ward and administered oral antifungal therapy with itraconazole. Although we used fluconazole as the first-line antifungal agent, the isolate was found to be resistant (MIC > 64 mg/L) by E-test.

Resistance of *C. bertholletiae* to fluconazole is not new. Almyroudis et al studied nearly 13 *Cunninghamella* spp. for susceptibility to various available antifungal agents, and only one *Cunninghamella* spp. was tested with fluconazole and found to be resistant (MIC > 64 mg/L). In the same study, seven isolates were tested against iatraconazole, but only 29% (i.e. two isolates) were found to be susceptible.[18] In another study, two strains of *C. bertholletiae* (ATCC 42115 and CBS 187.84) were found to have MIC > 64 mg/L with fluconazole.[19] Similarly, *C. bertholletiae* isolated from a CAPD patient with peritonitis was found to be resistant to voriconazole (MIC > 16mg/L). Despite this, the use of voriconazole was continued, as the patient had shown good response.[20] The same isolate was also found to be resistant to fluconazole (MIC > 256 mg/L).[20] Almyroudis et al have cautioned about high *in vitro* MIC results, as clinically relevant MIC breakpoints for zygomycetes are lacking.[18] Hence, despite the resistance to fluconazole at MIC > 64 mg/L, our patient responded well to the treatment. Within one week, he was discharged from the ICU with both the body temperature and inflammatory markers in the normal range.

C. bertholletiae is transmitted by inhalation of sporangiospores, and involves mainly the respiratory tract, and in the late stages, other organ systems. It is known to cause mucormycoses in the lungs, upper respiratory tract, sinuses, heart, kidney, spleen, bone marrow, pancreas, stomach, small intestine and vocal cords in immunocompromised patients.[6,15] In addition, patients with haematological malignancies and diabetes mellitus can also be severely affected by the disease.[14] However, such predisposing factors were absent in our patient, who was also HIV negative and not on steroids or other immunosuppressives. Thus, in the absence of any severe form of immunosuppression and non-involvement of systemic organs, the infection in our patient was localised to the peritoneum. He was undergoing CAPD for kidney rejection and had a Tenckhoff catheter. The localised nature of our patient's infection indicates that it may have been acquired through the Tenckhoff catheter for peritoneal dialysis. However, beyond this guess, the precise mode of acquisition could not be confirmed.

Deep invasive infections by *C. bertholletiae* in immunocompromised patients are often fatal despite the use of antifungal agents.[15,21] Quinio et al documented a case of primary cutaneous *C. bertholletiae* zygomycosis in a 54-year-old, insulin-dependent diabetic man who was treated with tacrolimus and steroids after kidney transplantation, and recovered after an early surgical excision of the lesion and daily administration of itraconazole for two months.[6] Pulmonary mucormycosis due to *C. bertholletiae* in a female patient with chronic renal insufficiency secondary to microscopic polyarteritis was successfully treated by administering a cumulative dose of 1,508 mg of amphotericin B, phased reduction of glucocorticoid therapy and chest tube drainage of pneumothorax.[22] Thus, removal of the fungal load either by surgical or other means, coupled with the use of antifungal agents and removal of immunosuppression, appears to be the key therapeutic ingredients responsible for controlling *C. bertholletiae* infections. The non-involvement of respiratory or other organ systems in our patient, in addition to the administration of fluconazole in the peritoneal cavity where the infection was localised, could have been sufficient to control the disease.

Peritoneal fluid from an inflamed peritoneal cavity is a rich source of polymorphonuclear cells (PMN). However, in a recent

study, *C. bertholletiae* was found to be more resistant to human PMN-induced hyphal damage at a low effecter-to-target ratio of up to 10:1, and to produce increased TNF-alpha release.[23] In yet another study, a higher effecter-to-target ratio of 50:1 and 100:1 was demonstrated to favour hyphal damage in a species-specific manner.[24] The recovery of our patient with antifungals suggests that a localised, stagnant, non-reproducing hyphal mass may be cleared readily by innate immune mediators.

Although less common, fungal association with peritonitis in peritoneal dialysis patients is well- known;[1] however, peritonitis due to *C. bertholletiae* has only been described in a single patient.[20] This is the second case of isolation of *C. bertholletiae* from a CAPD patient with peritonitis in the world and the first in India. We would like to highlight the emerging threat that zygomycetes pose to patients with or without an altered immune function. Although *C. bertholletiae* is transmitted most frequently by inhalation of the sporangiospores and established in immunocompromised individuals, people with long-term catheter use must also be screened for bacterial as well as fungal agents for dialysis-associated peritonitis. This report shows that early detection and intervention is crucial for attaining a successful outcome with *C. bertholletiae* infection.

REFERENCES

1. Nannini EC, Paphitou NI and Ostrosky-Zeichner L. Peritonitis due to Aspergillus and zygomycetes in patients undergoing peritoneal dialysis: report of 2 cases and review of the literature. Diagn Microbiol Infect Dis 2003; 46:49-54.
2. Bibashi E, Memmos D, Kokolina E, et al. Fungal peritonitis complicating peritoneal dialysis during an 11-year period: report of 46 cases. Clin Infect Dis 2003; 36:927-31.
3. Madariaga MG, Tenorio A, Proia L. Trichosporon inkin peritonitis treated with caspodungin. J Clin Microbiol 2003; 41:5827-9.
4. Bibashi E, Kokolian F, Sigler L, et al. Three cases of uncommon fungal peritonitis in patients undergoing peritoneal dialysis. Perit Dial Int 2002; 22:523-5.
5. Sugar AM. Mucormycosis. Clin Infect Dis 1992; 14(Suppl 1):S126-9.
6. Quinio D, Karam A, Leroy JP, et al. Zygomycosis caused by Cunninghamella bertholletiae in a kidney transplant recipient. Med Mycol 2004; 42:177-80.
7. Perfect JR and Schell WA. The new fungal opportunists are coming. Clin Infect Dis 1996; 22(Suppl 2):S112-8.
8. Roden MM, Zaoutis TE, Buchanan WL, et al. Epidemiology and Outcome of Zygomycosis: A Review of 929 Reported Cases. Clin Infect Disease 2005; 41:634-53.
9. NCBI taxonomy database [online]. Available at: www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=4827. Accessed January 9, 2011.
10. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. Clin Microbiol Rev 2000; 13:236-301.
11. De Hoog GS, Guarro J, Gene J, Figueras MJ. Atlas of Clinical Fungi, 2nd edn. Utrecht: Centraalbureau voor Schimmelcultures, 2000.
12. Koga-Ito CY, Lyon JP, Resende MA. Comparison between E-test and CLSI broth microdilution method for antifungal susceptibility testing of Candida albicans oral isolates. Rev Inst Med Trop Sao Paulo 2008; 50:7-10.
13. Etest for Antifungal Susceptibility Testing [online]. Available at: www.aspergillus.org.uk/secure/diagnosis/Etest.pdf. Accessed October 24, 2011.
14. Eisenberg ES, Leviton I, Soeiro R. Fungal peritonitis in patients receiving peritoneal dialysis: experience with 11 patients and review of the literature. Rev Infect Dis 1986; 8:309-21.
15. Righi E, Giacomazzi CG, Lindstrom V, et al. A case of Cunninghamella bertholettiae rhinocerebral infection in a leukaemic patient and review of recent published studies. Mycopathologia 2008; 165:407-10.
16. Spellberg B, Edwards J Jr, and Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation and management. Clin Microbiol Rev 2005; 18:556-69.
17. Kontoyianis DP, Vartivarian S, Anaissie EJ, et al. Infections due to Cunninghamella bertholletiae in patients with cancer: report of three cases and review. Clin Infect Dis 1994; 18:925-8.
18. Almyroudis NG, Sutton DA, Fothergill AW, Rinaldi MG, Kusne S. In vitro susceptibili-ties of 217 clinical isolates of zygomycetes to conventional and new antifungal agents. Antimicrob Agents Chemother 2007; 51:2587-90.
19. Singh J, Rimek D and Kappe R. In vitro susceptibility of 15 strains of zygomycetes to nine antifungal agents as determined by the NCCLS M38-A microdilution method. Mycoses 2005; 48:246-50.
20. Pimentel JD, Dreyer G and Lum GD. Peritonitis due to Cunninghamella bertholletiae in a patient undergoing continuous ambulatory peritoneal dialysis. J Med Microbiol 2006; 55:115-8.
21. Passos XS, Sales WS, Maciel PJ, et al. Nosocomial invasive infection caused by Cunninghamella bertholletiae: case report. Mycopathologia 2006; 161:33-5.
22. Koyama N, Nagata M, Hagiwara K, Kanazawa M. Survival of a patient with pulmonary Cunninghamella bertholletiae infection without surgical intervention. Respirology 2008; 13:309-11.
23. Simitsopoulou M, Georgiadou E, Walsh TJ, Roilides E. Cunninghamella bertholletiae exhibits increased resistance to human neutrophils with or without antifungal agents as compared to Rhizopus spp. Med Mycol 2010; 48:720-4.
24. Antachopoulos C Demchok JP, Roilides E, Walsh TJ. Fungal biomass is a key factor affecting polymorphonuclear leucocyte-induced hyphal damage of filamentous fungi. Mycoses 2010; 53:321-8.