Case Report

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Peritonitis due to *Cunninghamella bertholletiae* in a patient undergoing continuous ambulatory peritoneal dialysis

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Peritoneal dialysis-associated peritonitis due to fungi of the class *Zygomycetes* occurs very rarely. A case of fungal continuous ambulatory peritoneal dialysis peritonitis due to *Cunninghamella bertholletiae* is reported in a 39-year-old Aboriginal woman with end-stage renal failure and diabetes mellitus. This isolate was found to be resistant *in vitro* to amphotericin B, 5-fluorocytosine, fluconazole, itraconazole, ketoconazole and voriconazole. However, this patient was successfully treated with voriconazole and removal of the Tenckhoff dialysis catheter. Zygomycoses are an emerging threat among immunocompromised patients, including those with chronic renal failure. Zygomycosis due to *C. bertholletiae* is frequently fatal and is often non-responsive to systemic antifungal therapy. This is believed to be the first reported case of *C. bertholletiae* causing peritonitis in humans and one of the minority of cases involving this organism with a successful outcome.

Introduction

Fungal peritonitis remains a problem in patients undergoing peritoneal dialysis (PD). Peritonitis due to fungi of the class *Zygomycetes* is very uncommon. We present a case of fungal continuous ambulatory peritoneal dialysis (CAPD) peritonitis in an Aboriginal patient from Darwin, Australia, due to the zygomycete *Cunninghamella bertholletiae*. To our knowledge this is the first report of this organism causing peritonitis in humans. The patient was treated with voriconazole and made a full recovery. However, the isolate was found to display *in vitro* resistance to voriconazole. This clinical scenario is followed by a discussion of *C. bertholletiae* and reported strategies for management.

Case report

On 23 January 2005 a 39-year-old Aboriginal woman presented to the Royal Darwin Hospital with a cloudy PD bag, diarrhoea and vomiting. Her background history included type II diabetes and end-stage renal failure. She had received CAPD for the past 2 years. Blood pressure on presentation was 88/60 mmHg, serum white cell count (WCC) was $28.2 \times 10^9 \text{ l}^{-1}$ with a neutrophilia of $27.4 \times 10^9 \text{ l}^{-1}$. Urea was 12.7 mmol l^{-1} and creatinine was $918 \text{ } \mu\text{mol l}^{-1}$. Her temperature was 37.4°C . The PD fluid white cell count was $5600 \times 10^6 \text{ l}^{-1}$ (92% neutrophils) and a Gram stain revealed no organisms. She was admitted and started on intra-peritoneal (IP) ceftazidime 1.0 g daily and cephazolin 1.0 g daily. The initial PD fluid cultures grew one colony of a

Pantoea species on a primary culture plate, and therapy was changed to ceftazidime 1 g IP daily and gentamicin 20 mg IP daily.

During admission several repeat PD fluid specimens were collected. An abdominal CT scan showed free fluid, but no other significant pathology. In spite of IP antibiotic therapy, the clinical picture deteriorated and the PD white cell count remained elevated at $496 \times 10^6 \text{ l}^{-1}$. It was decided to remove the Tenckhoff catheter, which resulted in the resolution of the patient's pain and fever. The catheter, however, was not sent for culture. Maintenance haemodialysis via a native arterio-venous fistula was then commenced.

On 4 February 2005, non-yeast fungal elements were reported in the enrichment culture of PD fluid obtained 2 days earlier. The patient was commenced on voriconazole at 200 mg orally twice daily and followed up as an out-patient over the next 2 weeks. After discharge, her symptoms continued to resolve and her inflammatory markers returned to normal.

Culture of the peritoneal fluid revealed *C. bertholletiae*. Subsequent fungal susceptibility testing showed *in vitro* resistance to a wide range of antifungals, including voriconazole. However, due to the excellent clinical response, the patient was maintained on this therapy. After 6 weeks of antifungal therapy, a repeat abdominal CT identified a few small pockets of fluid, one of which was aspirated. The aspirate was clear, did not contain fungal elements and was culture negative. The patient completed a total of 90 days of voriconazole therapy and remained well at 3 month follow-up. Reinsertion of the PD catheter has been planned.

Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; FP, fungal peritonitis; IP, intra-peritoneal; PD, peritoneal dialysis.

Methods

PD fluid was collected into EDTA tubes (BD Vacutainer, Becton Dickinson) for cell count and sterile containers for Gram's stain and inoculation of primary culture plates. Specimens were also submitted in Aerobic/F and Anaerobic/F BACTEC blood culture bottles (Becton Dickinson) as an enrichment culture. One specimen was submitted in a single Myco/F Lytic bottle (Becton Dickinson). The fluid from EDTA tubes was centrifuged and used to inoculate horse blood agar (HBA) (Oxoid), chocolate blood agar (CBA) (Oxoid) and MacConkey (MAC) (Oxoid) plates as well as a second HBA plate for anaerobic incubation. The BACTEC bottles were placed in a BACTEC 9240 (Becton Dickinson) for incubation. When the BACTEC 9240 detected positive growth, fluid from each bottle was used to inoculate HBA, CBA and MAC plates and another HBA plate for anaerobic incubation. Fluid was obtained from each bottle via a 20-gauge venting needle (BBL, Becton Dickinson). Microscopy was performed with a wet preparation and Gram's staining.

After a fungal isolate was recovered from the enrichment culture, it was subcultured onto Sabouraud agar (SAB) (Oxoid) and incubated at 30 °C in air. Microscopy of the colonies grown on Sabouraud agar was performed using lacto-phenol cotton blue staining.

Results

Aside from one colony of a *Pantoea* species (a probable contaminant), primary culture plates were all culture negative. Additionally, the direct microscopic examination of all PD fluid was negative as well. The initial two BACTEC bottles submitted signalled positive growth in approximately 2 days. However, the wet preparation and Gram's stain from these bottles displayed no organisms. In addition, subcultures from these bottles failed to grow any organisms. A third bottle again had a negative microscopic examination after a positive signal. A close visual inspection of the contents of the bottle revealed clumps of fungal hyphae suspended in the broth. Re-examination of the two bottles submitted earlier showed a similar fungus. Two days later a fourth bottle was found to be positive and fungal colonies were identified in the broth. In total, fungal growth was detected in four separate PD fluid samples.

A wet preparation of the fungal material revealed broad, branching hyphae with very rare septae. On SAB at 30 °C, the organism grew white fluffy colonies within 3 days and began to turn grey by day 4. Microscopically, the organism displayed wide and straight sporangiophores, with single pear-shaped vesicles bearing oval sporangia on very short stalks (Fig. 1). The colonial and microscopic morphology along with the speed of growth was consistent with the genus *Cunninghamella*. This isolate was then referred to the Mycology Unit of the Adelaide Women's and Children's Hospital for identification and antifungal sensitivity testing. Antifungal susceptibilities were determined using broth microdilution. The isolate was confirmed as *C. bertholletiae* and was found to display *in vitro* resistance to amphotericin B (4 ml l⁻¹), 5-fluorocytosine (> 64 ml l⁻¹), fluconazole (> 256 ml l⁻¹), itraconazole (2 ml l⁻¹), ketoconazole (8 ml l⁻¹) and voriconazole (> 16 ml l⁻¹).

Fig. 1. A sporangiophore of *C. bertholletiae* with a pyriform vesicle bearing immature ellipsoidal sporangia on sterigmata. Lacto-phenol cotton blue staining; original magnification $\times 400$.

Discussion

Peritonitis remains a common feature of peritoneal dialysis and complicated the treatment of 64 % of PD patients in a 20 year study by Gloor *et al.* (2003). This study found the most common aetiology was bacterial, with *Staphylococcus epidermidis* (30 %) and *Staphylococcus aureus* (24 %) the most frequently implicated organisms. Catheter replacement or removal was necessary in 63 % of these patients.

Fungal peritonitis (FP) complicates PD less frequently. An 11 year study by Bibashi *et al.* (2003) reported a rate of 5.7 %, with a 15.9 % associated mortality rate. They found the most common cause of FP was *Candida albicans*, while filamentous fungi were found much less frequently. The zygomycetes remain an uncommon cause of fungal peritonitis (Nannini *et al.*, 2003). A recent review of seven cases of PD-related zygomycete peritonitis by Nannini *et al.* (2003) demonstrated a mortality rate of 57 % and that prompt removal of the Tenckhoff catheter was critical for survival. They also noted that most patients were not able to return to PD. Risk factors identified for development of FP include recent bacterial peritonitis, recent exposure to bacterial antibiotics, immunosuppressive therapy and the presence of a bowel perforation (Eisenberg *et al.*, 1986).

C. bertholletiae is a member of the class Zygomycetes in the order Mucorales. The more common pathogens of this order are *Rhizopus* and *Mucor*. *C. bertholletiae* is the only member of the genus identified as a human pathogen and is a resident of soil as well as decaying plant and animal matter (Ribes *et al.*, 2000). It is a rapidly growing organism that initially produces white colonies which usually turn grey with maturity and is thermophilic, growing at 45 °C. Some authors state that a non-pathogenic species, *Cunninghamella elegans*, is distinguished by purely grey colonies and the absence of growth at 45 °C (de Hoog *et al.*, 2000). Others consider *C.*

elegans to be a synonym of *C. bertholletiae* (Richardson *et al.*, 2003). Microscopically, this organism is recognized by the production of sporangiophores that have globose-to-pyriform vesicles which bear globose-to-ellipsoidal sporangia on short stalks called sterigmata (Ribes *et al.*, 2000).

C. bertholletiae is not often reported as a human pathogen. Quinio *et al.* (2004) note that though only 32 cases have been reported, 26 of these have been associated with the death of the patient. Thus it remains a clinically significant pathogen. *C. bertholletiae* usually acts as an opportunistic pathogen in immunocompromised patients, notably those with haematological malignancies, diabetes mellitus or solid organ transplants (Quinio *et al.*, 2004). This organism usually affects the pulmonary system (Kontoyianis *et al.*, 1994; Mazade *et al.*, 1998); however, its manifestations include sinusitis (Ng *et al.*, 1994), primary cutaneous infection (Quinio *et al.*, 2004), endocarditis (Zhang *et al.*, 2002) and disseminated disease (Kontoyianis *et al.*, 1994; Zhang *et al.*, 2002).

C. bertholletiae is often treated with amphotericin B accompanied by aggressive debridement (Mazade *et al.*, 1998). Published reports give varying results of the success of amphotericin B therapy (Ng *et al.*, 1994; Zhang *et al.*, 2002); however, the response to amphotericin B is generally thought to be poor (Ribes *et al.*, 2000). Several reports, including the present case, have documented elevated *in vitro* MICs to amphotericin B (Mazade *et al.*, 1998; Sun *et al.*, 2002; Dannaoui *et al.*, 2002, 2003). Alternatives to the use of amphotericin B include combination antifungal therapy (Dannaoui *et al.*, 2002) and the use of the new triazole posaconazole (Sun *et al.*, 2002). Breakthrough infections during voriconazole therapy have been reported with other zygomycoses (Kontoyiannis *et al.*, 2005) and treatment failure has been described (Ritz *et al.*, 2005). The reported *in vitro* MICs for voriconazole are consistently elevated and the clinical efficacy has not been established (Sun *et al.*, 2002; Dannaoui *et al.*, 2003).

A search of the English literature identified no other cases of peritonitis due to *C. bertholletiae*. Due to its widespread distribution in nature it is often considered a clinical contaminant (Ribes *et al.*, 2000). However, in this case the clinical history and the fact that fungal isolates were found in four separate samples suggest that *C. bertholletiae* was the aetiological agent of this patient's peritonitis. Risk factors included end-stage renal failure, CAPD treatment and diabetes mellitus. The avenue of infection was probably via the Tenckhoff catheter site rather than inhalation, which is the most frequent route. This is supported by the fact that there were no manifestations of dissemination evident in her course or history. Detection of this organism required the use of broth enrichment cultures as all primary culture plates were negative. Though infection by this organism is frequently fatal, our patient experienced a good outcome. The major contribution to her outcome may have been the removal of the PD catheter as this organism displayed *in vitro* resistance to voriconazole.

In conclusion, we would like to highlight the emerging threat that the zygomycetes represent to patients with an altered immune response. Though this is seen most frequently in those with a compromised immune system secondary to haematological malignancies, it must also be considered in those with chronic renal failure. In the case of PD-associated peritonitis, the practice of PD catheter removal along with systemic antifungals appears to be the most appropriate therapy. As treatment options for most zygomycetes are generally quite limited, early intervention remains a crucial element for patient survival.

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