Paecilomyces variotii in Peritoneal Dialysate

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Four cases of peritonitis caused by the filamentous fungus Paecilomyces variotii in patients on continuous ambulatory peritoneal dialysis are reported. Removal of the Tenckhoff catheter and antifungal chemotherapy led to resolution of symptoms in all cases. Possible contaminating events are discussed, and reported infections with P. variotii are reviewed.

Fungal peritonitis in patients on peritoneal dialysis is a more serious complication than bacterial peritonitis. About 75% of reported cases have been caused by yeasts of Candida species. Less frequently, infections with a variety of other yeasts and filamentous fungi have been reported. Since the introduction of continuous ambulatory peritoneal dialysis (CAPD) in 1976 (25), peritonitis has remained the most important complication. The reported incidence of peritonitis was as high as 6.3 episodes per patient-year (29) in the early years of experience with peritoneal dialysis. With further development in methods for connection of dialysate bags and with greater staff experience, the incidence of peritonitis has now decreased to less than 1.3 episodes per patient-year (24, 30). Most series report that about 7% of CAPD-related peritonitis episodes have a fungal etiology (3, 5, 6, 17, 24, 26, 28).

Death from fungal peritonitis occurred in 12 to 44% of reported patients (6, 9, 15, 24, 28). This is in contrast to a mortality rate of 0.6 to 3% for patients with bacterial peritonitis (18, 37). Fungal peritonitis was, after cardiovascular disease, the second most common event associated with death in two Australian studies of patients on CAPD (24, 41). Of 40 deaths reported in those two studies, 15 were associated with cardiovascular disease, 7 were associated with fungal peritonitis, and 4 were due to bacterial peritonitis.

We report here four cases of CAPD-related peritonitis caused by Paecilomyces variotii Bainier.

CASE REPORTS

Patient 1. A 12-year-old boy with end-stage renal failure had been on CAPD since August 1988. In February 1990, he was referred to the Royal Children's Hospital, Melbourne, Victoria, Australia, with a history of intermittent cloudy peritoneal dialysis (PD) fluid. Cultures of the fluid had been carried out in Hobart, Tasmania, Australia, but had not yielded any microorganisms. The patient had been treated with ceftazidime in the dialysate, and during this time he had mild abdominal pain and some suprapubic discomfort. On admission, he was apyrexial and had no frequency or dysuria. P. variotii was grown from each of five bags of PD fluid from the patient. Antifungal susceptibility testing was not performed. He was treated with amphotericin B, 5 mg/liter of PD fluid, for 10 days, but the infection failed to clear. The Tenckhoff catheter was then removed and hemodialysis was commenced. The symptoms of peritonitis rapidly settled without further antifungal therapy and he remained on hemodialysis until a renal transplant from his mother in June 1990. No fungus was isolated from the tip of the dialysis catheter.

Patient 2. A 60-year-old woman with longstanding renal failure had been using CAPD for 4 years. Following several episodes of abdominal pain in May 1990, a Staphylococcus species was cultured from the cloudy PD fluid. She received flucloxacinil and gentamicin and subsequently vancomycin and gentamicin following isolation of Staphylococcus epidermidis and a Pseudomonas species. After fungal growth was seen on a culture from one of the dialysate bags, amphotericin B was introduced and was continued for 5 days. At the time of transfer from Darwin, Northern Territory, Australia, to the Queen Elizabeth Hospital, Adelaide, Australia, the identity of the fungus had not been established.

Upon arrival in Adelaide, the patient was afebrile and the peritoneal fluid showed a moderate number of polymorphs but no fungal elements and no bacteria. Vancomycin was continued for the next week, during which she remained afebrile. Following a report of identification of the fungus as P. variotii, which was isolated from three PD bags, the Tenckhoff catheter was removed. Amphotericin B was given intravenously and was continued for 4 weeks for a total of 1,480 mg. A biopsy specimen taken from a 5-cm white indurated area in the peritoneal wall adjacent to the bladder revealed fungal elements. She did not return to CAPD but continued on hemodialysis. The isolate of P. variotii was reportedly susceptible to amphotericin B, nystatin, natamycin, clotrimazole, micamazole, ketoconazole, fluconosine, and itracanazole but was resistant to fluconazole.

Patient 3. A 56-year-old woman with chronic renal failure had been using CAPD for 4 years. Following a below-knee amputation of the leg in Melbourne in June 1990, she developed abdominal pain and cloudy PD fluid. She was treated with single doses of tobramycin and flucloxacinil intramuscularly; this was followed by treatment with flucloxacinil, 125 mg/liter, in the PD fluid. After an initial improvement, the packs became cloudy again and treatment was changed to cephalothin, 250 mg/liter of PD fluid, plus a further dose of tobramycin intravenously. A Micrococcus sp. was isolated from the PD fluid, and treatment was

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changed to vancomycin, 750 mg/liter of PD fluid. The patient became increasingly unwell. Three specimens of dialysis fluid had leukocyte counts of >500,000/ml. Hyphal elements were observed macroscopically in one of the PD specimens and microscopically in the other two specimens, and P. variotii was isolated from all three specimens. The organism was susceptible to amphotericin B, flucytosine, ketoconazole, and itraconazole but was resistant to miconazole and fluconazole. The Tenc'hkoff catheter was removed, and culture of the catheter tip yielded P. variotii. The patient was treated with ketoconazole, 400 mg orally three times daily for 10 days, and was subsequently discharged from the hospital, having been transferred to hemodialysis.

**Patient 4.** A 39-year-old Australian aboriginal man with chronic renal failure secondary to hypertension and diabetes had been using CAPD since May 1989. In January 1990 and May 1990, he suffered episodes of bacterial peritonitis. In June 1990, he presented to Prince Henry Hospital, Sydney, New South Wales, Australia, because the Tenc'hkoff catheter was draining poorly and he had become overhydrated. At 4 days after admission, he developed peritonitis caused by *S. mitis* and *Acinetobacter* sp. Treatment with imipenem intravenously and vancomycin, 10 mg/liter in the PD fluid, resulted in resolution of most symptoms and clearing of the dialysate, but intermittent abdominal pain continued.

In July 1990, one colony of *P. variotii* was isolated from a specimen of PD, although the fluid did not contain leukocytes or erythrocytes. The fungus was not isolated from four subsequent specimens. Clinical peritonitis then recurred and a *Proteus* sp. was isolated from the PD fluid. Two further specimens grew *P. variotii* (approximately 20 CFU/ml). The organism was susceptible to amphotericin B, flucytosine, ketoconazole, and itraconazole but was resistant to miconazole and fluconazole. The patient was treated orally with ketoconazole, 200 mg daily, the Tenc'hkoff catheter was removed, and hemodialysis was commenced. The patient's abdominal symptoms resolved within a week, and he has remained on hemodialysis. The patient remains well on hemodialysis.

**MATERIALS AND METHODS**

After the needle port of the dialysis bags was disinfected with 70% alcohol, samples of PD effluent were aseptically collected with a sterile syringe and needle. A leukocyte count was performed on the PD fluid by using a hemocytometer. A 25-ml volume of PD fluid was centrifuged, from the pellet a smear was prepared for Gram staining, and blood agar plates were inoculated and incubated aerobically and anaerobically for 5 days. When a fungal etiology was suspected, the pellet was also cultured on brain heart infusion agar, which was incubated at 35°C for 30 days, and on Sabouraud dextrose agar, which was incubated at 26°C for 30 days. A KOH wet mount was also prepared from the pellet for microscopic examination. Saponin lysis of dialysis fluid prior to culturing, in order to increase the microbial yield (39), was routinely carried out in one of the laboratories involved.

After initial identification in the laboratory where the organism was isolated, confirmation of the identity of the isolate and antifungal susceptibility tests were performed at the Royal North Shore Hospital, Sydney, New South Wales (patients 3 and 4) or at the Women's and Children's Hospital, North Adelaide, South Australia (patient 2). The organisms isolated from patients 2, 3, and 4 were tested for susceptibility by the disk diffusion technique by using Neosensitabs (Roscoe Diagnostica, Taastrup, Denmark) (44). For patient 2, the procedure was varied as follows. A standardized light inoculum of the isolate to be tested was streaked onto plates containing either complex Casitone medium (14) or, in the case of flucytosine testing, supplemented yeast nitrogen agar medium (33).

**RESULTS AND DISCUSSION**

The genus *Paecilomyces* is similar to the genus *Penicillium* (20) but is distinguished by the lack of green-colored colonies and by basally swollen phialides tending toward their apex and bearing long, tanged, or divergent chains of smooth-walled, single-celled conidia (31). The phialides are borne in a penicillate arrangement or in verticils, and their necks are often bent away from the main axis. So far, only a few species have been connected to teleomorphs, and these are in the order *Eurotiales*, e.g., the genera *Byssoschlamys*, *Thermoascus*, and *Talaromyces*, or in the order *Clavicipitales*, e.g., the genus *Cordyceps* (31). No telemorph is known for *P. variotii*. This species is a common airborne fungus and is found in soil and decaying organic material. It is well known as a common spoilage organism (21) and has been found to be resistant to preservatives such as benzoic acid, propionic acid, and sorbic acid (32). Optimal growth occurs at 25°C, with a range of growth temperatures from 5 to 50°C (32).

*P. variotii* grows rapidly, with colonies attaining a diameter of 8 cm within 14 days at 25°C on malt agar and having a sweet odor (31). Colonies on Sabouraud dextrose agar are powdery and yellow-brown with a raised velvety center and a brown reverse. *P. variotii* phialides measure approximately 12 to 20 by 2.5 to 5.0 μm (31). Conidia are hyaline to yellow and elliptical in shape and usually measure 3.2 to 5.0 by 2.0 to 4.0 μm, but they are variable in shape and size and may be as large as 15 by 5.0 μm (31). Factors influencing this variation include the age of the colony and the extent of sporulation. Smaller conidia are produced in abundantly sporulating strains, whereas larger conidia can be observed under less suitable conditions (31). Chlamydospores are usually present in old cultures, singly or in short chains, mostly brown with smooth to slightly roughened walls (31).

As defined by Ajello (1), hyalohyphomycosis is an opportunistic infection involving hyphomycetes, such as *Paecilomyces* spp., with hyaline or light-colored cell walls. In contrast, infection with opportunistic hyphomycetes having melanin in the cell wall is called phaeohyphomycosis (27). *P. variotii* has rarely been implicated in human infections. Reports in the medical literature have included a case of peritonitis in a patient on CAPD (7), five fatal cases of prosthetic valve endocarditis (11, 16, 19, 35, 42), a case of phaeohyphomycosis complicating surgery for nephrolithiasis (34), a fatal infection of a venous graft infection (10), and two cases of sinusitis (22, 40), a lacrimal sac infection (13), and a case of pneumonitis complicating hairy cell leukemia (8). A review of human *Paecilomyces* species infections has been presented by Castro et al. (4).

Filamentous fungi are infrequent causes of CAPD peritonitis. The four cases reported here add to an increasing recognition of *P. variotii* as a potential pathogen in patients undergoing CAPD. In the 4 years prior to 1990, isolates of *P. variotii* from five patients undergoing CAPD with peritonitis had been received at the Australian Medical Mycology Reference Laboratory, in the Department of Microbiology at the Royal North Shore Hospital. In addition, there had been
three isolates of *P. variotii* from unused bags of PD fluid. In 1986, at the Prince of Wales Hospital, Sydney, two strains of *P. variotii* were isolated from fluid obtained from unused dialysate bags. Macroscopic fungal elements were noted by a patient as he prepared the dialysate for use. *P. variotii* was also isolated at the Royal North Shore Hospital, Sydney, from an unused bag after macroscopic fungal elements were detected by a patient. This patient subsequently developed chronic peritonitis caused by *P. variotii* and his Tenckhoff catheter had to be removed.

The four cases of *P. variotii* peritonitis reported here occurred between February and July 1990 in Darwin, Hobart, Melbourne, and Sydney. In each case, the fungus was readily isolated from multiple used dialysate bags on several culture media and *P. variotii* was isolated during hospitalization when there had been repeated episodes of known or suspected bacterial peritonitis and repeated antibacterial treatments. Early removal of the catheter and amphotericin B administered intravenously or ketoconazole administered orally was used in successful management. It is important to distinguish among species of *Paecilomyces* (36), because their susceptibility patterns to antifungal agents vary. *P. variotii* is susceptible to amphotericin B and flucytosine, whereas *Paecilomyces lilacinus* (Thom) Samson is highly resistant to these two agents (36).

Reviews of treatment strategies for fungal peritonitis have been published (2, 38). However, the treatment of infections with filamentous fungi was not included. Early removal of the dialysis catheter is an important adjunct to therapy. None of the four patients described here has resumed CAPD. After an episode of bacterial peritonitis, 95% of patients are able to continue CAPD or have the technique reestablished (24), but following fungal peritonitis, no more than 35% of patients are able to continue CAPD or have the technique reestablished (6, 9, 15, 24, 28). Reestablishment of CAPD treatment may not be possible because of peritoneal adhesions, abscess formation, or progressive sclerosing peritonitis (28). The patients continue to be very susceptible to infection, and in one study (18), 50% of cases of fungal peritonitis immediately followed or were superimposed upon gram-negative bacterial peritonitis.

In patients undergoing CAPD, susceptibility to infection is increased because the indwelling catheter acts as a foreign body; there is decreased phagocytic activity in the peritoneal cavity, and opsonic factors in the PD fluid are diluted and lost (12, 29). In most patients with CAPD-related peritonitis, entry of the causative organism is via contamination of the dialysate catheter or PD fluid and additives. Recognized breaks in aseptic technique are a common antecedent to peritonitis in these patients (41). Improvements in aseptic technique, reduction in the number of catheter manipulations, better connections between the Tenckhoff catheter and the dialysate bag, and the use of bacterial filters are important factors in reducing infection (23, 41). When a patient is suffering from repeated episodes of peritonitis, the illness may cause the patient to be unable to properly maintain aseptic techniques, and the use of broad-spectrum antibiotics may allow overgrowth of fungi.

An attempt to find a common link between the four cases of *P. variotii* peritonitis by checking batch numbers and dates of manufacture of dialysate fluids used in these patients was unsuccessful. The spores of *P. variotii* are ubiquitous and abundant. They are likely to be present in the environment at places of manufacture, storage, and use of the bags of PD fluid.

Experiments on PD fluid bags have shown a slow growth rate from small inocula of *P. variotii* (18a), suggesting that contamination of a bag occurs at least 2 to 3 weeks before fungal elements become visible in the fluid. Shortly after the presentation of patient 4, swabs taken from wooden shelves with deteriorating paint work on which the peritoneal dialysis bags were stored grew *P. variotii*. Hence, an observation of fungal filaments in an unused bag of PD fluid suggests an inoculation event during manufacture or storage. *Paecilomyces* spp. are associated with "wood-trimmers' disease" (43), and the finding of *P. variotii* on wooden storage shelves where the peritoneal dialysis bags were stored strengthens the association of *P. variotii* and wood in the environment.

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