Case Report

Neosartorya hiratsukae peritonitis through continuous ambulatory peritoneal dialysis

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Fungal peritonitis is a rare, potentially lethal, complication of continuous ambulatory peritoneal dialysis (CAPD). We report what we believe to be the first confirmed *Neosartorya hiratsukae* CAPD-related peritonitis case in Europe. The patient died, despite early removal of the peritoneal catheter and antifungal therapy. This report highlights the impact of emerging fungal pathogens and the importance of early diagnosis on the outcome in CAPD-related fungal peritonitis.

Introduction

Fungal peritonitis is a rare, yet potentially lethal, complication of continuous ambulatory peritoneal dialysis (CAPD) that accounts for up to 15 % of cases of CAPD-related peritonitis (Bren, 1998; Prasad & Gupta, 2005). The mortality rate of fungal peritonitis varies from 5 % to 53 %, while failure to resume CAPD occurs in up to 40 % of the patients (Prasad & Gupta, 2005). *Candida albicans* has historically been reported as the most common isolate, but in recent reports, a shift has been observed and rare, environmental fungal species, often referred to as 'non-pathogenic', are increasingly being reported as aetiological agents of fungal peritonitis (Bren, 1998; Prasad & Gupta, 2005).

Case report

A 58-year-old male patient was admitted in February 2008 to the Peritoneal Dialysis Unit of Hippokration General Hospital (Athens, Greece) due to abdominal pain and fever of 24 h duration. The patient had been undergoing CAPD therapy for the past 4 years due to end-stage renal disease of unknown aetiology. Past medical history included two episodes of *Staphylococcus epidermidis* CAPD-related peritonitis, multiple CAPD catheter exit-site bacterial infections, coronary heart disease with angioplasty, hypertension and chronic obstructive pulmonary disease.

On admission, his body temperature was 38 °C, his pulse was 110 beats min⁻¹, and his blood pressure was 145/80 mmHg. On examination, his abdomen was tender but soft and the areas of the subcutaneous tunnel and the exit site of the Toronto Western Hospital peritoneal dialysis catheter were clinically unremarkable. His C-reactive protein (CRP) level was 108 mg l⁻¹, blood urea nitrogen and creatinine were elevated (780 mg l⁻¹ and 72 mg l⁻¹, respectively), but the white blood cells (WBCs), haemoglobin and electrolyte levels were within the normal range. The peritoneal fluid was opaque, containing 125 WBCs mm⁻³. As there was evidence of CAPD-related peritonitis, our hospital's standard treatment protocol was commenced, with intraperitoneal vancomycin 2 g q. 5 days and ceftazidime 2 g q. 24 h.

On the fourth hospital day, the CRP increased to 165 mg l⁻¹, while peritoneal fluid WBC counts were 1600 cells mm⁻³. Ciprofloxacin 200 mg q. 12 h i.v. and metronidazole 500 mg q. 8 h i.v. were added to the treatment regimen. Since the patient was not clinically improving, and blood and peritoneal fluid bacterial cultures remained negative, fungal peritonitis was suspected, thus liposomal amphotericin B 3–5 mg kg⁻¹ q. 24 h i.v. and caspofungin 50 mg q. 24 h i.v. were empirically added on day 7 and antibiotics were switched to imipenem/cilastatin 500 mg q. 12 h i.v. On the 8th hospital day, as currently recommended for CAPD-related suspected fungal peritonitis (Piraino *et al.*, 2005; Prasad & Gupta, 2005), the peritoneal catheter was removed to speed up recovery and the patient was transferred to haemodialysis for three times a week. After initiation of antifungal therapy and catheter removal, the patient's clinical status improved, followed by a significant decrease in CRP values. On the ninth hospital day, white fungus balls were observed in the peritoneal

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Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; CRP, C-reactive protein; WBC, white blood cell.

The GenBank/EMBL/DBJ accession numbers for the ITS sequence and β -tubulin sequence of UOA/HCPF9708/CBS124073 are EU714322 and FJ433874, respectively.

fluid aerobic bacterial and mycosis culture bottles (BACTEC Plus Aerobic/F and Myco/F Lytic Culture Vials; Becton Dickinson) that were inoculated on admission day (Fig. 1a). In total, five independently collected peritoneal fluid specimens, inoculated during the first 7 days of hospitalization, were subsequently culture-positive for a fungus. Direct microscopy of all peritoneal fluid specimens was reported as negative, and daily blood and peritoneal fluid cultures were negative for aerobic and anaerobic bacteria and mycobacteria. Bacterial and fungal cultures of catheter components were also negative, indicating that the origin of infection was located in the peritoneal cavity.

On hospital day 21, his temperature rose to 40 °C, with a concurrent increase in CRP value (100 mg l⁻¹). An abdominal CT scan showed sigmoid colon oedema and subcutaneous fluid collection located at the exit point of the original peritoneal catheter. An ultrasound-guided aspiration of the fluid was performed and cultures of the aspirate grew vancomycin-resistant *Enterococcus faecalis*. The imipenem/cilastatin antibiotic regimen was immediately switched to linezolid 600 mg q. 12 h i.v., while amphotericin B/caspofungin was switched to voriconazole

monotherapy 200 mg q. 12 h i.v. Despite antibiotic and antifungal therapy (total administration time: 17 days), the patient's condition deteriorated, and he died due to multiorgan failure 43 days after admission. Autopsy was denied.

Microbiological methods

The fungal balls from the peritoneal fluid specimens were subcultured on standard Sabouraud dextrose agar, Czapek agar and malt extract agar at 25 °C. Macro- and micro-morphology, rate of growth and maximum temperature tolerance were recorded. Final identification to the species level was performed by sequencing of the internal transcribed spacer and partial sequencing of the β -tubulin gene and comparing the derived sequences to published fungal sequences (BLAST; <http://www.ncbi.nlm.nih.gov>) (Samson *et al.*, 2007). The primers used for amplifying and sequencing the above genomic areas were the general fungal primer pairs ITS5/ITS4 and Bt2a/Bt2b (White *et al.*, 1990; Glass & Donaldson, 1995). To further confirm the identification, the derived β -tubulin sequence was aligned (BioEdit; www.mbio.ncsu.edu) with the GenBank *Neosartorya* and *Aspergillus*

Fig. 1. (a) Fungal balls of *Neosartorya hiratsukae* in a BACTEC Myco/F Lytic Culture bottle inoculated with peritoneal fluid, after 2 weeks incubation at 37 °C. (b) Greyish-yellowish green culture of the isolate after 2 weeks incubation on Czapek agar at 25 °C. (c) Cleistothecia (50–150 µm in diameter) after 2 weeks incubation on Czapek agar at 25 °C (stained with lactophenol cotton blue; original magnification $\times 400$). (d) Ascospores displaying two equatorial crests and finely reticulate convex surfaces (fixed with lactophenol; original magnification $\times 1000$).

section *Fumigati* reference homologue sequences deposited by Hong *et al.* (2008). MICs of the major antifungal drugs amphotericin B, flucytosine, itraconazole, posaconazole, voriconazole, anidulafungin, caspofungin and micafungin were tested according to the Clinical Laboratory Standards Institute guidelines M38-A2 (CLSI, 2008).

Results and Discussion

The colonies of the subcultured fungal balls reached diameters of 28 and 42 mm on the selective Czapek and malt extract agars, respectively, after 1 week incubation at 25 °C. Colonies on selective media were greyish-yellowish green and microscopically produced an anamorph with sparse, *Aspergillus fumigatus*-like, conidial heads (Fig. 1b). Ascospores represented by numerous cleistothecia (50–150 µm in diameter) containing spherical eight-spore asci (Fig. 1c) were produced after 2 weeks at 25 °C. Ascospores were hyaline, lenticular, measured up to 5 µm, with two equatorial crests and finely reticulate convex surfaces under $\times 1000$ magnification (oil immersion) (Fig. 1d). Independent multiple tests showed that the isolate's maximum growth temperature was 43 °C. Due to the aforementioned characteristics, the isolate was phenotypically characterized as a *Neosartorya* species, and owing to the reticular nature of the equatorial crests, possibly as a member of the group *Neosartorya fischeri*/*Neosartorya tatenoi*/*Neosartorya hiratsukae* (Samson *et al.*, 2007). The β -tubulin derived sequence (GenBank accession no. FJ433874) showed 100% homology to that for *N. hiratsukae* (AF057324). The isolate was deposited in the Hellenic National Collection of Pathogenic Fungi (UOA/HCPF no. 9708), and in the Centraalbureau voor Schimmelcultures Culture Collection (CBS no. 124073). MICs of the major antifungal drugs were as follows: amphotericin B, 1 µg ml⁻¹; flucytosine, >64 µg ml⁻¹; itraconazole, 2 µg ml⁻¹; voriconazole, 1 µg ml⁻¹; posaconazole, 0.25 µg ml⁻¹; caspofungin, 0.5 µg ml⁻¹; anidulafungin, 0.25 µg ml⁻¹; and micafungin, 2 µg ml⁻¹.

Aspergillus section *Fumigati*, after its recent revision (Samson *et al.*, 2007; Hong *et al.*, 2008), contains 33 taxa: 10 strictly anamorphic *Aspergillus* species and 23 teleomorphic *Neosartorya* species. Out of the 23 *Neosartorya* species, only *N. fischeri*, *Neosartorya fischeri pseudofischeri* (Ghebremedhin *et al.*, 2009) and *N. hiratsukae* (Udagawa *et al.*, 1991; Guarro *et al.*, 2002) have been reported as human pathogens. They are relatively uncommon and as yet only nine cases of *Neosartorya* infections have been well documented in the medical literature (Han & Na, 2008); only one of these was associated with peritonitis (Ghebremedhin *et al.*, 2009). *N. hiratsukae*, the most recently identified member of the three pathogenic *Neosartorya* species, was initially isolated from air and pasteurized aloe juice in Japan in 1992 (Udagawa *et al.*, 1991). Thus far, only one confirmed invasive case, describing a brain abscess in an immunocompromised patient in Brazil, has been published (Guarro *et al.*, 2002). Although its global distribution remains largely undefined, recent retrospective surveys report its isolation from cases of fungal

peritonitis in Argentina (Predari *et al.*, 2007) and New Zealand (Anonymous, 2003). It has also been reported from an allergic (non-invasive) rhinosinusitis case in India (Shivaprakash *et al.*, 2009), and from a hedgehog skin infection in Korea (Han & Na, 2008). In the recent survey on fungal peritonitis in dialysis patients in Argentina (Predari *et al.*, 2007), the identification procedures for the three reported *N. hiratsukae* cases were not described, and therefore the cases can be presently regarded only as tentative.

As it is unclear whether the three isolates from skin and oropharyngeal exudates briefly reported from Spain (Mellado *et al.*, 2006) represent invasive infection or colonization/contamination, the present case should be considered the first documented report of *N. hiratsukae* as an invasive human pathogen in Europe. The organism appears to be an autochthonous member of the fungal flora in our region as we have isolated it from skin specimens as a contaminant (Mycology Laboratory, University of Athens, unpublished observation) and from environmental air sampling in Athens (Dr E. Kapsanaki-Gotsi, Ecology and Systematics, Faculty of Biology, University of Athens, personal communication).

Prompt recognition and correct identification of *Neosartorya* isolates in the clinical laboratory is important for the following reasons. (i) Such isolates are often discarded as contaminants because the white colonies show slow initial growth and sporulation in primary culture (Guarro *et al.*, 2002), thus leading to delayed diagnosis. This may be further complicated by the fixed primary use of bacteriological culture materials in clinical laboratories, but even mycological media may not accelerate the diagnosis in the presence of slow-growing organisms. Finally, the utility of molecular methods for detection of fungi in peritoneal fluid specimens, although interesting, has not been investigated. (ii) The prognosis of such emerging infections may be poor and, as in our case, may worsen by secondary bacterial infection (Bren, 1998). In such cases, the relevant contribution of the bacterial and fungal components, and that of the underlying pathology, to morbidity and mortality may be difficult to assess in the absence of autopsy. (iii) Treatment optimization for *Neosartorya* infections requires records of antifungal susceptibilities, as the susceptibility patterns of the genus are not well known and members of the section *Fumigati*/*Neosartorya* can show elevated antifungal MICs (Alcazar-Fuoli *et al.*, 2008). *N. hiratsukae* MICs for amphotericin B and the azoles are reportedly in the susceptibility range (Guarro *et al.*, 2002; Mellado *et al.*, 2006), with only one strain resistant to itraconazole (MIC=16 µg ml⁻¹) (Shivaprakash *et al.*, 2009); our isolate was similarly susceptible. Susceptibility to the echinocandin class of antifungals remains unknown but our isolate's micafungin MIC was somewhat high (2 µg ml⁻¹). Because data are scant and the reported MIC values are near the recently reported break-points (CLSI, 2008; Verweij *et al.*, 2009), *N. hiratsukae* antifungal susceptibilities in conjunction with clinical efficacy should be further investigated.

Regarding identification procedures, *N. hiratsukae* is considered the only *Neosartorya* species with reticulated ascospores that grows restrictedly on Czapek agar (<14–15 mm after 7 days at 25 °C) (Guarro *et al.*, 2002; Samson *et al.*, 2007), yet, as in this case, growth rate may not be consistent for all isolates. The intraspecies variability, observed in our isolate, also extends to other taxonomic characters such as the size of cleistothecia and the maximum temperature tolerance (reported thus far as 130–220 µm and 45–48 °C, respectively) (Guarro *et al.*, 2002; Samson *et al.*, 2007). In contrast, the macro- and micromorphology of our isolate, with obtusely and finely reticulated convex ascospores, were concordant with those of published descriptions of the species (Udagawa *et al.*, 1991; Guarro *et al.*, 2002; Samson *et al.*, 2007). The ascospore micromorphology may prove a more stable and useful character for identification than either growth rate or temperature tolerance, but its unequivocal appraisal requires scanning electron microscopy (SEM), which is not routinely available in the clinical laboratory. Additionally, since SEM of ascospores has proved unable to distinguish between all *Neosartorya* species (Samson *et al.*, 2007), definite identification to species level in the clinical setting requires sequencing of genes encoding proteins such as β -tubulin or calmodulin (Samson *et al.*, 2007).

In this case, despite the high index of clinical suspicion for mycotic CAPD peritonitis that led to administration of empirical antifungal therapy, laboratory diagnosis was slower than the ideal. This highlights the difficulty of promptly recognizing and characterizing slow-growing and rare fungal pathogens by standard clinical laboratory procedures, although this is of immense importance for early management, which is known to significantly reduce mortality. Thus, registering accurately identified rare and emerging fungal pathogens, and recording their geographical distribution and antifungal susceptibilities, could enhance clinical vigilance, early diagnosis and hence timely initiation of targeted therapy.

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