Diagnosis of aspergillus peritonitis in a renal dialysis patient by PCR and galactomannan detection

J M Scotter, J M Stevens, S T Chambers, K L Lynn, W N Patton

This report describes the use of the polymerase chain reaction (PCR) and galactomannan detection to detect aspergillus in the continuous ambulatory peritoneal dialysis (CAPD) fluid and blood of a patient with multiple myeloma on CAPD and immunosuppressive treatment. Diagnosis of aspergillus was initially made by conventional culture of CAPD fluid, but the PCR and galactomannan assays also detected aspergillus DNA and antigens in the blood, respectively. This suggests that the PCR and galactomannan assays, previously suggested as useful in the management of invasive fungal infections in neutropenic haematological patients, may be suitable for application to a broad range of clinical situations and sample types.

Invasive aspergillusosis (IA) is a major cause of morbidity and mortality in the immune compromised host. An important limiting factor in the successful treatment of IA is the ability to diagnose IA with certainty. Clinical signs can be non-specific, and laboratory diagnosis, based on histology and microbiology, tends to be slow and insensitive. The polymerase chain reaction (PCR) has been shown to improve the laboratory diagnostic precision of IA, primarily in neutropenic haematological patients.

"An important limiting factor in the successful treatment of invasive aspergillusosis is the ability to diagnose it with certainty"

Fungal peritonitis is an uncommon but important complication of peritoneal dialysis. Candida species account for more than 85% of cases; filamentous fungal peritonitis has also been reported. We report a case of Aspergillus fumigatus peritonitis in a patient on continuous ambulatory peritoneal dialysis (CAPD). The diagnosis was made by conventional culture of CAPD fluid. PCR and galactomannan antigen detection assays were also positive for aspergillus, in both the CAPD fluid and the blood.

CASE REPORT
A 60 year old man presented with chronic renal failure secondary to multiple myeloma. He underwent acute haemodialysis and was started on twice weekly cyclophosphamide and an oral dexamethasone regimen of 40 mg for four days, recycling every eight days. Two months later, when stabilised, a Tenckhoff catheter was inserted, CAPD was begun, and further dexamethasone treatment administered.

Three months after the start of CAPD, the patient inadvertently punctured his CAPD bag. Two days later, the CAPD fluid was noted to be cloudy, and the patient felt unwell. He attended the hospital where CAPD fluid was taken for culture and he was given a single dose of vancomycin and gentamicin intraperitoneally. Blood cultures were not taken. Two days later, A fumigatus was isolated from the CAPD fluid, prompting administration of amphotericin B 30 mg intraperitoneally. He remained unwell, with a purulent effluent dialysate. The patient declined removal of the Tenckhoff catheter, CAPD was continued, and he was treated palliatively with oral itraconazole. He died at home 37 days after the initial puncturing incident. A necropsy was not performed.

MICROBIOLOGICAL TESTS
CAPD fluid samples were examined by direct microscopy and were cultured for aerobic, anaerobic, and fastidious bacteria, in addition to fungi.

PCR enzyme linked immunosorbent assay (ELISA) and galactomannan antigen detection were performed on the patient’s blood and CAPD fluid. The PCR methodology was that described by Loeffler et al. Briefly, pelleted fungal cells were recovered from 5 ml EDTA anticoagulated blood, or 10 ml CAPD fluid, followed by enzymatic lysis of fungal cell walls and purification of fungal DNA using the QiaAmp DNA mini kit (Qiagen GmbH, Hilden, Germany). PCR amplification and PCR-ELISA (PCR ELISA DIG detection kit; Roche Molecular Biochemicals, Mannheim, Germany) was performed using an oligonucleotide probe that binds specifically to the DNA of A fumigatus, A flavus, and A versicolor. Negative controls were included at all steps of the assay to control for environmental or laboratory carryover contamination. A further CAPD fluid sample taken from an uninfected patient was also used as a negative control. Galactomannan detection was performed using a commercially available assay kit (Platelia™ Aspergillus; Sanofi Diagnostics, Marnes-La-Coquette, France), according to the manufacturer’s instructions. A 300 μl aliquot of serum was used in each test.

RESULTS
Table 1 summarises the culture, PCR, and galactomannan antigen detection results. No organisms were seen on direct microscopic examination of CAPD fluid samples. One further blood sample taken 13 days before the puncturing of the CAPD bag tested negative for aspergillus DNA and galactomannan.

DISCUSSION
We document the isolation of A fumigatus from the CAPD fluid of a patient with renal failure and multiple myeloma on immunosuppressive treatment. We also describe the rapid detection of aspergillus DNA and galactomannan antigen from blood and CAPD fluid samples. This case shows that PCR and galactomannan detection can successfully detect aspergillus after the start of antifungal treatment.

Both techniques gave positive results in CAPD fluid when routine culture for aspergillus remained negative, implying

Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; ELISA, enzyme linked immunosorbent assay; IA, invasive aspergillosis; PCR, polymerase chain reaction
increased sensitivity for these methods when compared with routine culture. The persistent positive results in CAPD fluid by both PCR and galactomannan testing were consistent with the patient’s progress and suggested the continued presence of aspergillus despite treatment for IA. The positive blood results, by both techniques, are also important. The observation that the second blood sample was positive by PCR and negative by galactomannan detection is consistent with our own observations in other clinical circumstances, in addition to in vitro comparisons, that the PCR assay is more sensitive than the galactomannan antigen test for the diagnosis of IA. There are few reported cases of aspergillus peritonitis in the literature. The disease is associated with considerable mortality, and in survivors, successful return to CAPD is rare because of loss of peritoneal surface area and peritoneal adhesions.

“It is uncertain whether continuing galactomannan antigen or polymerase chain reaction positive results suggest ongoing infection, as opposed to the presence of non-viable organisms”

It is recognised that early, accurate diagnosis of fungal peritonitis is essential to improve patient outcomes. Currently, diagnosis is by a combination of clinical signs and symptoms, and isolation of the causative agent from CAPD fluid. Culture of fungal organisms can be difficult, and considerable delays can occur between the onset of illness and laboratory confirmation of aspergillus infection. One review found a median duration of four days from the onset of symptoms to the first positive culture result for aspergillus. The optimal strategy for the use of these assays in the diagnosis of aspergillus peritonitis remains to be determined, but PCR and galactomannan detection assays both have the potential to decrease the time taken to obtain a laboratory diagnosis of aspergillus infection and may be suitable for application to a broad range of clinical situations and sample types. The disease is associated with considerable mortality, and in survivors, successful return to CAPD is rare because of loss of peritoneal surface area and peritoneal adhesions.

Table 1 Results of aspergillus culture, PCR, and GM antigen detection tests according to sample type and collection time

<table>
<thead>
<tr>
<th>Days after puncture of CAPD bag</th>
<th>A. fumigatus cultured from CAPD fluid</th>
<th>PCR results</th>
<th>GM results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CAPD fluid</td>
<td>Blood</td>
</tr>
<tr>
<td>13</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>19</td>
<td>ND</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

CAPD, continuous ambulatory peritoneal dialysis; GM, galactomannan; ND, not done, (not tested or no specimen available); PCR, polymerase chain reaction; +, positive result; –, negative result.

Take home messages

- We describe the use of the polymerase chain reaction and galactomannan detection to detect aspergillus in the continuous ambulatory peritoneal dialysis (CAPD) fluid and blood of a patient with multiple myeloma on CAPD and immunosuppressive treatment
- These assays have the potential to decrease the time taken to obtain a laboratory diagnosis of aspergillus infection and may be suitable for application to a broad range of clinical situations and sample types

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