

## A case of peritonitis caused by *Rhizopus microsporus*

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### Summary

We report a case of a 62-year-old female patient who developed peritonitis after receiving a renal transplant. *Candida glabrata* was detected and treated with voriconazole. As the patient did not improve under therapy, laparotomy was performed. Mould-like plaques were found on the peritoneum. Using culture as well as pan-fungal polymerase chain reaction (PCR) followed by DNA microarray hybridisation of the amplicon, the causative agent was identified as *Rhizopus microsporus*. Despite aggressive surgical treatment, intravenous therapy with amphotericin B and topical administration of Lavasept (polyhexamethylenbiguanide), the patient died.

**Key words:** *Rhizopus microsporus*, peritonitis, *Candida glabrata*, PCR, hybridisation, Zygomycetes.

### Introduction

*Rhizopus* species and other Zygomycetes are opportunistic pathogens. In patients without underlying condition such as malignancies, immunosuppressive therapy, etc., infections with these fungi are very rare, although severe, requiring early high-dose polyene-based therapy as well as surgical debridement. Postsurgical infections and infections of burns were described previously (for a review see Ref.<sup>1</sup>). Cutaneous zygomycosis usually results from contaminated injuries.<sup>2, 3</sup> Such cases were observed, e.g. after the recent Tsunami disaster in east Asia.<sup>4</sup>

In immunocompromised patients<sup>5</sup> as well as in patients with diabetes<sup>6, 7</sup> Zygomycetes cause life-threatening disease. Because of invasive growth and relative resistance to voriconazole,<sup>8</sup> therapy basically relies on administration of amphotericin B and surgical intervention. However, mortality is still very high. Most cases present with fungal sinusitis, possibly complicated by an invasion of the brain and/or the orbita. There are also rare cases of peritonitis, usually following peritoneal dialysis.<sup>9–11</sup>

We report a case of an immunocompromised patient who developed a massive peritonitis caused by *R. microsporus*.

### Case report

A 62-year-old female patient was admitted to the department of urology for kidney transplantation. She has been dialysed for nearly 5 years because of renal insufficiency caused by poststreptococcal glomerulonephritis. The postoperative course was initially uncomplicated, the kidney transplant immediately began to produce urine. For immunosuppression, ciclosporin 4.5 mg h<sup>-1</sup> and methylprednisolone 75 mg day<sup>-1</sup> were given. Two days after surgery the patient developed symptoms of peritonitis, and a laparotomy was carried out. Peritonitis due to a perforation of the sigmoid colon was found. An ileostoma was performed. Postoperatively, the patient developed a septic shock (hypotonia, tachyarrhythmia, arterial oxygen saturation between 60% and 80%) with multiple organ failure. Laboratory tests revealed leucocytopenia (3.0 GPt l<sup>-1</sup>, normal range: 3.8–9.8), thrombocytopenia (82 GPt l<sup>-1</sup>, normal range: 150–400), decreased red blood cells (2.63 TPt l<sup>-1</sup>, normal range: 4.2–5.4), decreased haemoglobin (Hb) level (5.2 mmol l<sup>-1</sup>, normal range: 7.4–10.7), elevated serum transaminases as well as elevated creatinine (267 µmol l<sup>-1</sup>, normally <97) and urea (17.2 mmol l<sup>-1</sup>, normal range: 3.6–8.9). C-reactive

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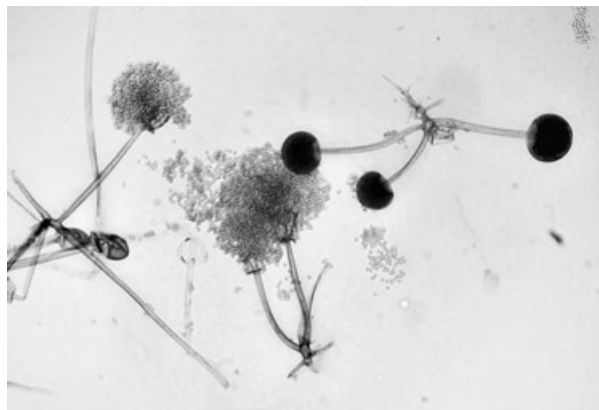
Probe specificity	Probe sequence	Hybridisation result
Fungal generic	GTGAATCATCGAGTCTTTGAACGC	Positive
<i>Aspergillus fumigatus</i>	CCAGCCGACACCCAACTTTATTTT	Negative
<i>Candida albicans</i>	AAACATTGCTTGCGGCGGTAACGTC	Negative
<i>Candida glabrata</i>	GTTTTACCAACTCGGTGTTGATCTAG	Positive
<i>Candida krusei</i>	CTGGCCGAGCGAACTAGACTTTTTT	Negative
<i>Mucor circinelloides</i>	CCAACATTTTTGTTGAATAGGATGAC	Negative
<i>Rhizopus microsporus</i>	AAACATATAATCTAGGGGTTCTGC	Positive
<i>Rhizopus oryzae</i>	ACAAGAGTATAATCCAGCAACTTTCA	Negative
<i>Absidia corymbifera</i>	TTCAGTTGCTGTCATGGCCTTAAATA	Negative

**Table 1** Relevant hybridisation probes and results.

protein (CRP) was elevated (463.3 mg l<sup>-1</sup>, normally <5.0). The patient was admitted to an intensive care unit.

Despite treatment with fluid administration, packed red blood cells, catecholamines, hydrocortison and electrolytes, an acute renal failure occurred. The kidney transplant was removed 14 days after transplantation, and venovenous haemofiltration was started. Immunosuppressive therapy was discontinued, and only a supportive dose of 200 mg hydrocortison daily was given furthermore. Microbiological examination of peritoneal swabs showed a mixed infection with *Escherichia coli*, enterococci, *Bacteroides ovatus*, *Eubacterium limosum* and *Candida* species. Initially, the patient received piperacillin + sulbactam (later substituted by meropenem), metronidazole and fluconazole 400 mg day<sup>-1</sup>. Antifungal treatment was changed to voriconazole 540 mg day<sup>-1</sup> after *C. glabrata* was identified at day 20 after transplantation.

Recurrent second look procedures demonstrated a distinctive gangrene of the bowel and the soft tissue. At day 39 after transplantation, the surgeon described 'mould-like plaques' on the peritoneum. It was decided to leave the abdominal cavity open, but covered with Dacron mesh sutured to fascial margins. Despite repeated replacement of the mesh, abdominal lavages and intravenous therapy with voriconazole, the fungal plaques increased within the next days. From swabs of the gangrenous area taken at day 39 after transplantation, a mould was recovered but identification was delayed as only sterile mycelia were found on culture plates. The fungus was identified by molecular and by morphological means. A pan-fungal polymerase chain reaction (PCR; targeting a ribosomal spacer sequence, forward primer: 5'-GCATCGATGAAGAACGCAGC-3'/reverse primer: 5'-biotin-TCCTCCGCTTATTGATATGC-3') was performed using DNA from bead beater extraction of the culture. For identification, the PCR amplicon was hybridised subsequently against a set of specific probes on a DNA microarray (see <http://www.clondiag.com/products/sys/atssystem/AT-fungi.pdf> and Table 1).

**Figure 1** Microphotograph of cultured moulds revealing morphological features of *Rhizopus* species (original magnification ×100).

Hybridisation and staining were performed as described previously.<sup>12-15</sup> This approach resulted in positive hybridisation signals for both, *R. microsporus* and *C. glabrata* (relevant probe sequences see Table 1). Further subcultures of the mould isolate also revealed morphological features of *Rhizopus* species (Fig. 1). Antifungal therapy was changed at day 41 after transplantation when identification of the pathogen was still pending. The patient received intravenous amphotericin B (10 mg at the first day, then increased to 50 mg day<sup>-1</sup>) as well as irrigation of the peritoneum with amphotericin B and Lavasept. After a retroperitoneal haemorrhage, the patient underwent another laparotomy. She presented with an increasing fungal gangrene with extensive affection of the intestine and musculo-fascio-cutaneous abdominal region as illustrated in Figs 2 and 3, which were taken at day 46 after transplantation. Laboratory tests yielded leucocytosis (25.6 GPt l<sup>-1</sup>), elevated CRP (85.2 mg l<sup>-1</sup>), signs of anaemia (Hb: 3.5 mmol l<sup>-1</sup>, erythrocytes: 2.5 TPt l<sup>-1</sup>), thrombocytopenia and increased liver enzymes (up to the 10-fold of normal serum levels). Despite all therapeutic efforts, the patient died of multiple organ failure caused by septic shock at day 47 after transplantation.



**Figure 2** Mould-like plaques on the peritoneum, aspect after laparotomy and coverage with Dacron mesh (day 46 after transplantation).



**Figure 3** Mould-like plaques on the peritoneum, aspect after laparotomy and coverage with Dacron mesh (day 46 after transplantation; enlarged view).

## Discussion

We present an unusual devastating case of a peritonitis caused by *R. microsporus* in an immunocompromised patient. This species is known to cause peritonitis, but published cases are scarce and refer to patients undergoing peritoneal dialysis only.<sup>9, 10, 11</sup> The patient in the present case was immunocompromised because of a recent renal transplant. The clinical course, however, was determined by a peritonitis due to a perforation of the sigmoid colon. It can be guessed that *Rhizopus* spores have been transmitted via a gastrointestinal route as they are known to be associated with various foodstuffs or even wooden tongue depressors.<sup>16</sup> Zygomycetes can cause intestinal ulcers, which may rupture, causing peritonitis.<sup>18</sup> Another explanation is that a mixed

infection of diverse bacteria and fungi from fecal flora occurred secondary to perforation, and that *Rhizopus* was able to colonise the peritoneum because of its innate resistance after the other agents have been suppressed by therapy with meropenem and voriconazole.

Therapy of a severe infection, such as peritonitis, whether it was caused by Zygomycetes or by other opportunistic moulds (e.g. *Fusarium* species, *Scedosporium* species), is generally problematic due to the invasive growth of the fungus, the underlying conditions of the patients and the side-effects of medication. The development of new antifungal agents such as posaconazole<sup>18</sup> and the additional administration of interferon- $\gamma$ , granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor<sup>18–20</sup> might help to improve outcome of these infections. Clinical experience as well as availability of these substances, however, is still limited and, further studies pending, therapy relies on surgical intervention and amphotericin B. The nephrotoxicity of this compound is a major limitation. Thus, patients with renal transplants will receive rather voriconazole than amphotericin B as first-line antifungal drug although not all fungi are covered by this medication. A major obstacle to such an empiric therapy is that the causative agents are biologically rather unrelated. For that reason, an antifungal drug can be efficient for the treatment of one fungal pathogen, but may fail for another. The present case highlights this difficulty as the patient developed a fatal peritonitis caused by *Rhizopus* species when receiving voriconazole as therapy for another fungal infection with *C. glabrata*. Several other cases of breakthrough zygomycosis in patients receiving voriconazole therapy have already been described within the last few years.<sup>21, 22</sup> The dilemma of choosing the optimal antifungal drug can only be resolved by rapid identification of fungal pathogens. Identification still relies on phenotypic features such as micromorphology, colony morphology and carbohydrate assimilation. This approach is very reliable when performed by experienced staff, but it needs time. For rapid diagnosis, PCR-based methods may help. Unfortunately, it can be necessary to perform several genus- or species-specific PCRs due to the diversity of opportunistic fungi. This can be cumbersome, costly and time-consuming. Another option is the use of 'pan-fungal' PCRs using primers, which target highly conserved regions found alongside with phylogenetically variable segments. In this case, the detection of amplicons by gel electrophoresis is not sufficient, because it does not give information which species of fungus was present in the sample.

Therefore, an amplicon from a 'pan-fungal' PCR needs to be identified subsequently, either by sequencing as in previously described approaches,<sup>23</sup> or by hybridisation to a set of species-specific probes. This technology can be used to identify culture material as it has been described above, but it could be adapted to detect and identify fungal DNA directly from patient samples. DNA microarray technology could provide a convenient platform for DNA probes allowing to identify amplicons from 'pan-fungal' PCRs by sequence-specific hybridisation.

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