Case Report

Fatal pulmonary infection in a leukaemic patient caused by *Hormographiella aspergillata*

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A case of autopsy-proven fungal pneumonia in a relapsed leukaemia patient is reported. The fungus *Hormographiella aspergillata* was cultured from two bronchoalveolar fluid samples and identified through morphological examination and ITS2 sequence analysis. In addition, galactomannan was detected in eight consecutive serum samples, which suggested a co-infection with *Aspergillus* species. The patient was treated with caspofungin.

Case report

In April 2003, a 34-year-old man was diagnosed with acute myeloid leukaemia. He responded favourably to remission-induction chemotherapy followed by two courses of intensive consolidation. In September 2003, the patient underwent an uncomplicated allogeneic peripheral blood stem cell transplantation from an HLA-identical sibling. However, a relapse was diagnosed in December 2003. Reinduction chemotherapy with high-dose cytarabine (3 g m⁻² b.i.d. for 4 consecutive days) was initiated, complicated by serious capillary leak syndrome, hepatic and renal failure and progressive encephalopathy. On day 6 of reinduction, the patient was transferred to the intensive care unit for mechanical ventilation, inotropic support and haemodialysis. On day 13, he experienced a new episode of neutropenic fever while already receiving meropenem therapy empirically. Blood cultures yielded *Staphylococcus epidermidis*; daily radiographs of the chest showed bilateral pulmonary infiltrates that remained unchanged. The patient defervesced after the initiation of vancomycin. On day 28, a diagnosis of probable invasive aspergillosis (according to EORTC/MSG consensus criteria) was made based on a progressive increase in serum galactomannan antigenaemia (Fig. 1), progression of pulmonary infiltrates and development of new consolidations on chest X-ray, dyspnoea and cough, and the microscopic demonstration (Grocott staining) of moulds (compatible with *Aspergillus* species) in a bronchoalveolar fluid (BAL) sample. In addition, cultures taken from two consecutive BAL samples (with an interval of 3 days) yielded 'fungi'. Because of severe hepatic impairment, therapy was started

Fig. 1. Time course of serum galactomannan antigenaemia showing a progressive increase.

with caspofungin (70 mg loading dose, followed by 50 mg daily dose). However, on day 34, while still neutropenic, his clinical condition deteriorated rapidly. A high-resolution CT scan (Fig. 2) of the lungs demonstrated multiple large nodules in both lungs, surrounded by a halo of lower attenuation. The patient died on day 35 from respiratory failure and refractory septic shock.

Autopsy was performed within 6 h of death. Tissue samples were taken from all major organs (except the brain) and from macroscopic abnormalities. On macroscopic examination, multiple intraparenchymal and subpleural haemorrhagic nodules were found in both lungs. Haematoxylin and eosin staining confirmed the presence of septated hyphae (Fig. 3), which had also invaded the surrounding blood vessels. There was no evidence of dissemination of the infection to other

Abbreviations: BAL, bronchoalveolar fluid; ITS2, internal transcribed spacer 2.

Fig. 2. High-resolution CT scan of the lungs demonstrating multiple large nodules in both lungs.

Fig. 3. Haematoxylin and eosin stain of lung tissue taken at autopsy showing the presence of septated hyphae.

organs. Culture of tissue specimens was not performed. In addition, pathologic examination revealed the presence of residual leukaemic myeloblasts in many organs.

A few days after the patient's death, the fungus isolated repeatedly from BAL fluid was identified as *Hormographiella aspergillata*. The fast-growing fungus was cultured within 2 days of incubation at 37 °C on a chocolate agar plate and after 8 days (2 days at 37 °C, followed by 6 days at 22 °C) on Sabouraud glucose (2 %) agar containing chloramphenicol. Macroscopically, multiple white- to slightly cream-coloured dense colonies were observed. Production of conidia could be stimulated by subcultivation on Takashio medium (Sabouraud agar with 0.2 % glucose). Microscopic examination of the fungus revealed conidiophores from which

Fig. 4. Slide culture preparation of *H. aspergillata* showing conidiophores and arthroconidia.

clusters of rectangular arthroconidia were produced (Fig. 4). The arthroconidia were cylindrical with truncate ends but with terminal cells rounded at the tip. No fruit bodies were obtained in culture. These morphological characteristics led to a tentative identification of the fungus as *Hormographiella* species. The fungus was identified as *Coprinus cinereus* (anamorph *Hormographiella aspergillata*) by amplification and sequencing of the internal transcribed spacer 2 (ITS2)

region. Amplification of the ITS2 region and sequence analysis using an ABI Prism 310 automatic sequencer were performed as described by de Baere *et al.* (2002). The obtained ITS2 sequence was compared with all known sequences in GenBank using BLAST 2.0 (<http://www.ncbi.nlm.nih.gov/blast/>). The length of the query sequence was 300 bp. A 99% identity (299/300) with *C. cinereus* genes (AB097562.1) was obtained. Antifungal susceptibility testing was performed by Etest as described previously (Pfaller *et al.*, 2000). The agar formulation used for the Etest (AB Biodisk) was RPMI agar supplemented with 2% glucose. MICs were read at 72 h. The MICs of amphotericin B, voriconazole and itraconazole were 0.5, 0.25 and 2 µg ml⁻¹, respectively.

Discussion

C. cinereus is a basidiomycete with a typical mushroom form normally occurring in compost and sewage. In the laboratory, *C. cinereus* is normally seen as its anamorph, *H. aspergillata* (Gene *et al.*, 1996). *C. cinereus* has previously been implicated in human infections on only a few occasions. A case of endocarditis with a fatal outcome was reported by Speller & MacIver (1971) and an endophthalmitis by Bartz-Schmidt *et al.* (1996). Two strains from skin lesions and one from a catheter were studied by Guarro *et al.* (1992), but no causal relationship with the diseases was proven. Two other pulmonary infections caused by *H. aspergillata* were reported in the last decade: a fatal infection in a profoundly neutropenic patient (Verweij *et al.*, 1997) and an infection with a favourable outcome in a patient recovering from a neutropenic episode (Surmont *et al.*, 2002). Interestingly, all three (including this report) cases of pulmonary infection caused by *H. aspergillata* were from the same geographical region, namely Belgium and the Netherlands.

Identification of filamentous basidiomycetes is difficult because key diagnostic features are often lacking and usually only arthroconidia are formed (de Hoog & Gerrits van den Ende, 1998). Other fungi regularly cultured from clinical samples in which arthroconidia predominate are *Geotrichum candidum*, *Trichosporon* species, *Geomyces pannorum*, *Malbranchea* species, *Arthrographis kalrae* and *Scytalidium* species. Conidiophores are only present in *Arthrographis*, *Geomyces* and *Hormoglyphiella*. Characteristic for *Hormoglyphiella* is the fact that the conidiophore is clearly broader than the conidiogenous hyphae (Guarro *et al.*, 1992). The availability of molecular techniques may facilitate the identification of these fungal species in the future, which may lead to a better estimate of the incidence of invasive infections caused by these fungi.

Screening for circulating galactomannan (Platelia *Aspergillus*; Bio-Rad Laboratories) revealed gradually increasing optical density (OD) indices from 0.2 at day 22 to 1.0 at day 28. The first BAL fluid, from which the basidiomycete was cultured, was obtained at day 28. Galactomannan antigenaemia remained positive (OD index > 1.0) during the next 7 days. However, *Aspergillus* was not cultured from

the nine respiratory samples (including two BAL fluids) that were taken from the patient at the intensive care unit nor from a sample from another body site. The gradual increase in the serum galactomannan antigenaemia in our patient instead of a sudden increase points more in the direction of an *Aspergillus* infection rather than a false-positive reaction. Known causes for false-positive results with the Platelia *Aspergillus* test such as treatment with piperacillin-tazobactam or severe mucositis (Adam *et al.*, 2004; Wheat, 2003) were not present in our patient. ELISA cross-reactivity with *H. aspergillata* is a possibility that cannot be excluded. Unfortunately, DNA extraction was not possible from the paraffin-embedded tissue samples taken at autopsy. The role of *Aspergillus* in this case remains uncertain. Nenoff *et al.* (1997) reported a similar case involving a woman suffering from a relapse of acute lymphoblastic leukaemia who developed a lethal pulmonary infection caused by *Coprinus* species and *Aspergillus flavus*. *Coprinus* species but not *A. flavus* was repeatedly isolated from bronchial secretions and BAL. Post-mortem culture of lung tissue, however, revealed simultaneous growth of *Coprinus* species and *A. flavus*. Galactomannan was detected in this patient's BAL fluid but not in serum samples. The negative serum galactomannan tests may be due to the fact that a latex agglutination test was used (Pastorex *Aspergillus*; Bio-Rad Laboratories) instead of the more sensitive ELISA test (Platelia *Aspergillus*).

Few data on susceptibility tests for *Coprinus* species are available. A variable response to amphotericin B and itraconazole is reported and all strains tested were resistant to fluconazole and flucytosine (Gene *et al.*, 1996; Verweij *et al.*, 1997). We found rather low MIC values for amphotericin B, itraconazole and voriconazole. Our patient did not respond to therapy with caspofungin.

With the continuous expanding spectrum of fungi causing invasive disease, it is important to consider the clinical relevance of every fungus cultured from samples of immunocompromised patients. Contact between the microbiologist and clinician is therefore indispensable. This case also illustrates the difficulty in identifying the aetiological agent(s) of an invasive fungal infection. According to generally accepted consensus criteria (Ascioglu *et al.*, 2002), both *H. aspergillata* and *Aspergillus* species could be considered as probable causes of fungal pneumonia in our case. However, this uncertainty may have significant implications for choosing the most appropriate antifungal therapy.

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