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CASE REPORT

Polymerase chain reaction aids in the diagnosis of an unusual case of *Aspergillus niger* endocarditis in a patient with acute myeloid leukaemia

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Summary Endocarditis secondary to *Aspergillus niger* has not been described in a leukaemic patient. We describe a case of *A. niger* endocarditis in a patient with acute myeloid leukaemia and refractory fever. The microbiological cause of his endocarditis was initially misdiagnosed because he fulfilled the Duke criteria for enterococcal endocarditis. A polymerase chain reaction test utilizing pan-fungal primers detected a product from an *Aspergillus* sp. The DNA was subsequently sequenced and was found to have 100% homology with *A. niger*. A postmortem revealed fungal endocarditis secondary to disseminated aspergillosis, without evidence of bacterial endocarditis. The patient was found to have a lung aspergilloma that was possibly occupationally acquired, and may have been long standing.

Introduction

Invasive aspergillosis is a well recognised opportunistic infection affecting patients with haematological malignancy. However, endocarditis secondary to *Aspergillus* spp. is rarely described in this patient group.[1] Only four cases of endocarditis due to *Aspergillus niger* have been reported.[2–5] All four cases followed cardiac surgery in immunocompetent patients. Microbiological diagnosis of endocarditis relies heavily on isolation of an organism from blood culture. *Aspergillus* spp. are very rarely isolated from blood cultures.[6] Aspergillus antibodies may be raised in aspergilloma and aspergillus endocarditis in immunocompetent patients, but may not prove useful for diagnosis in immunocompromised patients.[6,7] Alternative methods such as antigen detection and polymerase chain reaction (PCR) have been developed to improve the diagnosis of invasive aspergillosis. The case described illustrates how PCR aided in the diagnosis of *A. niger* endocarditis in a patient with haematological malignancy.

Case report

A 56-year-old carpenter presented in May 2000 with

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a three-week history of severe diarrhoea and dehydration, and a 14 kg weight loss over the preceding two months. He had a long past medical history of idiopathic colitis thought to be Crohn's disease. A stool sample grew *Clostridium difficile*- for which he received metronidazole. His total white cell count was 64×10^9/l and a bone marrow aspirate revealed acute myeloid leukaemia (AML, FAB-M1). He was randomised in the Medical Research Council AML XII protocol to receive S-DAT (daunorubicin, Ara-C and 6-TG). Treatment was complicated by further massive weight loss necessitating parenteral nutrition and delaying further chemotherapy.

His second course of S-DAT, at the end of August 2000, was complicated by fever unresponsive to piperacillin/tazobactam and gentamicin. A chest radiograph showed consolidation with possible cavitation in the left mid-zone and a CT scan of the chest showed a left mid-zone soft tissue density consistent with, but not diagnostic of infection. A diagnosis of possible pulmonary aspergillosis was made. The fever settled on intravenous amphotericin B, 1 mg/kg/day. The patient was discharged at the beginning of October 2000 on itraconazole oral solution, 200 mg twice daily, in haematological remission. Although itraconazole levels would have been helpful to ensure a therapeutic trough concentration of 0.5 mg/l, they were not done.

He was readmitted in the middle of November 2000 with a 24 h history of pyrexia and a six-week history of progressive lumbar back pain. On examination he had a previously undetected systolic murmur. He had no neurological signs. A chest radiograph showed a persistent opacity in the left mid-zone. His neutrophil count was 1.45×10^9/l and he was treated initially with piperacillin/tazobactam. A trans-thoracic echocardiogram showed a large vegetation measuring 1.3×0.7 cm on the anterior mitral valve leaflet with severe secondary mitral regurgitation. Six sets of blood cultures over four days grew *Enterococcus faecalis* and *Enterococcus faecium*, both sensitive to vancomycin. The patient, therefore, fitted the Duke criteria for the diagnosis of enterococcal endocarditis. Treatment was changed to vancomycin and gentamicin. Blood cultures remained positive and the Hickman line, which had been inserted six months previously, was removed. The blood cultures subsequently became negative. MRI of the spine showed mild increased signal in the L5/S1 disk and irregularity of the end plates, consistent with partially treated discitis. This was thought to be due to a septic embolus. A bone marrow aspirate showed that he had relapsed and he was given FLA-GCSF (fludarabine, cytarabine, granulocyte-colony stimulating factor) at this time.

The patient remained pyrexial and the size of the vegetation prompted further investigation. Fungal PCR was performed on 4 ml EDTA blood samples using pan-fungal primers as described by Einsele et al.,[8] DNA being extracted by the method of Löffler et al.[9] The product was identified as *Aspergillus* spp. by restriction fragment length polymorphism (in-house method), and sequencing revealed the DNA to have 100% homology with *A. niger*. Positive results were obtained on two occasions, at the beginning of December, separated by one week. Platelia® *Aspergillus* Immunoenzymatic Assay (Bio-Rad Laboratories) for the detection of galactomannan antigen was also performed and was negative on these two occasions. Liposomal amphotericin B 3 mg/kg/day was started for presumed fungal endocarditis. The patient was not considered suitable for cardiothoracic surgery. His cardiac function deteriorated with development of atrial fibrillation and episodes of hypotension. His temperature settled transiently but he became increasingly hypoxic and hypotensive due to a combination of left ventricular failure and sepsis and died in mid-December, pancytopenic.

Post mortem examination revealed that the mitral valve had a $0.8 \times 0.6 \times 0.4$ cm vegetation attached to its anterior leaflet. Attached to the anterior papillary muscle below this valve leaflet there was another area of vegetation, this measuring $1 \times 0.5 \times 0.4$ cm. A second papillary muscle also showed a minute (0.1 cm) similar lesion at its tip. Histological examination showed septate branching hyphae but no bacteria. The cut surfaces of the left lung showed a round cavity measuring 2 cm in diameter located in the mid-part of the upper lobe and occupying that cavity a ball of black-coloured solid material. The histological appearances were consistent with a well-demarcated aspergilloma. A vertical strip through the lumbar spine and sacrum showed collapse of the L5/S1 disk with associated haemorrhage consistent with discitis. Histological examination confirmed disk collapse, but there were no fungal organisms present on Grocott staining. Examination of multiple blocks of cerebrum and cerebellum showed perivascular inflammatory aggregates, and Grocott staining demonstrated perivascular and intravascular septate branching fungi. Microbiological cultures were not done. Fungal PCR was performed on papillary muscle, mitral valve, liver and spleen. Tissue was ground with a pestle and mortar and DNA was extracted by the method of Löffler et al.[9] The product was identified as *Aspergillus* spp. by restriction fragment length polymorphism

(in-house method). Papillary muscle and liver were positive. The DNA from these tissues was sequenced and was also found to have 100% homology with *A. niger*. The diagnosis was aspergilloma with secondary invasion of the heart and brain. The patient died from a combination of continuing sepsis secondary to disseminated aspergillosis and cardiac failure secondary to endocarditis.

Discussion

The organism causing this patient's endocarditis was mistakenly believed to be one or both of the enterococci isolated from blood cultures. The findings of a positive echocardiogram, persistently positive blood cultures with an organism consistent with endocarditis, fever and a peripheral embolus supported a diagnosis of enterococcal endocarditis according to the Duke criteria.[10] It is likely that these organisms were causing a Hickman line infection only, but concurrent bacteraemia delayed the diagnosis of fungal endocarditis. The Duke criteria do not distinguish between bacterial and fungal endocarditis but the application of these criteria to fungal endocarditis has not been evaluated. Fungal endocarditis is characterised by a paucity of classical clinical findings such as finger clubbing, Osler's nodes, splinter haemorrhages and Roth's spots. Fever, changing murmurs and the presence of peripheral emboli—commonly of the large vessels in the brain, mesenteric organs, kidneys, coronory arteries and the limbs—are the most common signs.[1,6]

The two positive PCR results supported the diagnosis of fungal endocarditis, which was suspected clinically based on the large size of the vegetations and persistent fever. Blood cultures are rarely positive in aspergillus endocarditis. The microbiological diagnosis is usually made by culture and histology of the valve or peripheral emboli. The sandwich enzyme-linked immunosorbent assay (ELISA) which uses a monoclonal antibody to galactomannan (Platelia® *Aspergillus*) has been shown to have a sensitivity and specificity of $>$ 90% in the early detection of invasive aspergillosis in leukaemic patients with persistent fever, but was negative in this patient.[11,12]

Immunosuppression is a recognized risk factor for aspergillus endocarditis. In a large review of 270 cases of fungal endocarditis occurring between 1965 and 1995, the major risk factors for fungal endocarditis were previous valvular surgery (54%), antibiotic use (48%), rheumatic heart disease (24%), nonvalvular major surgery (23%), vascular lines (18%), and immunocomprimise (18%). Sixty-six cases (24%) were due to *Aspergillus* spp. The authors commented that remarkably the review included no patient with haematological malignancy.[1] In a review of 61 cases of aspergillus endocarditis in patients without prior cardiac surgery occurring between 1980 and 1999, 17 patients (28%) had an underlying haemotological malignancy.[7]

Combined medical and surgical therapy appears to offer the best outcome in aspergillus endocarditis, although there have been no prospective comparative randomised treatment studies to verify this. The mortality in aspergillus endocarditis is still very high. The overall survival rate is approximately 10%.[1,6] In patients with haematological malignancy the survival rate is even lower. In one series of seven patients diagnosed ante mortem with aspergillus endocarditis and underlying haematological malignancy, four received combined medical and surgical therapy, and three medical therapy alone. None of these patients survived.[7] Missed or delayed diagnosis has been a common occurrence in fungal endocarditis and results in increased mortality.[1]

The most common aspergillus species causing invasive disease are *A. fumigatus* followed by *A. flavus* with *A. niger* accounting for only a small number of cases. Invasive pulmonary aspergillosis is the major manifestation with the development of a mycotic lung sequestrum in many cases. Aspergilloma is a non-invasive condition which is rarely described in immunocompromised patients. The patient's occupation as a carpenter may have predisposed him to developing an *A. niger* aspergilloma, which was probably longstanding.

The patient's second course of chemotherapy was complicated by a possible aspergillus infection, as defined by the revised EORTC/IFISG and NIAID/MSG criteria.[12] He was treated with 1 mg/kg/day amphotericin B for eight days and discharged on itraconazole oral solution, 200 mg bd. When his haematological malignancy relapsed he was at high risk of disseminated aspergillosis. High doses of amphotericin B have been used for secondary prophylaxis of aspergillosis with some success.[13–16] Some authorities also advise surgical excision of a persistent fungal lesion.[14,17,18] For patients for whom surgery or prolonged amphotericin B is contraindicated, itraconazole may be used. Although studies have shown that itraconazole oral solution appears promising as primary prophylaxis against fungal infection in neutropenic patients,[19–21] experience in secondary prophylaxis is limited.[14,22,23] Unfortunately in this patient it was unsuccessful.

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