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CASE REPORT

Disseminated mucormycosis: (almost) fatal!

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Abstract

Mucormycosis is an invasive mould infection, which is associated with haematological malignancies and is often fatal. The keys for successful treatment are focus on early diagnosis, surgical debridement when possible and appropriate administration of appropriate antifungal agents. We report a case of disseminated mucormycosis in a patient with acute myeloid leukaemia diagnosed and identified by polymerase chain reaction analysis. After surgical debulking, the patient was successfully treated with antifungal therapy.

Introduction

Mucormycosis (previously referred to as zygomycosis) is an invasive mould infection caused by members of the order Mucorales. Based on anatomic localisation, mucormycosis can be classified as one of five forms: rhinocerebral, pulmonary, cutaneous, gastrointestinal and disseminated. It most commonly occurs among severely immunocompromised patients such as patients with haematological malignancies.[1] The exact incidence of mucormycosis is unknown and probably underestimated. The reported incidence varies from 1-13% in high-risk patients.[2,3] It appears that the incidence has increased over the last few decades. In a French study the incidence rate of mucormycosis in patients with haematological malignancies increased over time from 0.7 to 1.2 cases/million persons in the period 1997 through 2006 (+24% per year).[4] In most cases mucormycosis is associated with a rapidly progressive clinical course resulting in a fatal outcome. Mortality due to mucormycosis increases significantly if treatment is either inappropriate or delayed, especially in patients with neutropenia. Initiation of a strategic and timely diagnostic process, administration of adequate antifungal agents and surgical debridement when and wherever possible, is essential, especially in the subset of immunocompromised (haematology) patients.[5,6] In contemporary practice the diagnosis of mucormycosis is often challenging. Aspergillosis and mucormycosis share similar clinical and radiological presentations. Since several antifungal agents with activity against Aspergillus are inactive against mucormycosis, a mycological diagnosis is required in order to initiate adequate antimicrobial therapy. Diagnosis traditionally relies on histological examination of the pathological specimen and positive culture of Mucorales species. Obtaining deep tissue samples or bronchoalveolar lavage fluids from patients with suspected mucormycosis may not be feasible, especially in haematological patients because of severe thrombocytopenia. If available, histopathological identification of Mucorales in tissue specimens can be difficult and requires significant expertise. Moreover, Mucorales species observed in tissue samples sometimes fail to grow in fungal culture.[7] In order to avoid these limitations of traditional diagnostic approaches, conducting a deoxyribonucleic acid (DNA) sequence analysis on tissue specimens may contemporarily lead to a more rapid diagnosis and appropriate treatment.

Case report

A 59-year-old man was referred with an elevated white blood cell count, anaemia and thrombocytopenia. He was an occupational vegetable grower and had no prior medical history. The patient was diagnosed with acute myeloid leukaemia (AML), classified as subtype AML-M5 or acute monocytic leukaemia. Combined chemotherapy with idarubicin and cytarabine was immediately started as induction therapy. At day 3 after chemotherapy, pancytopenia and fever occurred. Six days after completion of the chemotherapy regimen the patient was admitted to the intensive care unit (ICU) due to neutropenic enterocolitis

and upper gastrointestinal bleeding with haemodynamic instability. The latter was endoscopically treated and after two days discharge to the haematology ward followed. The patient's neutropenia and fever persisted despite the use of prophylactic antifungal treatment with fluconazole and broad-spectrum antibiotics. Cultures of blood, sputum, urine and stool were negative for fungi and bacteria, and the serum level of galactomannan antigen was not elevated. A chest computed tomography (CT) revealed a small nodular lesion in the lower right lobe of the lung without specific appearance of a halo or air crescent sign. A CT of the abdomen was not performed. Treatment with intravenous voriconazole (6 mg/kg bolus followed by 4 mg/kg twice daily) was initiated under a diagnosis of possible invasive pulmonary aspergillosis based on previously formulated European criteria.[8] At day 22 after completion of the chemotherapy, bone marrow repopulation with clinical recovery occurred and subsequent hospital discharge with continuation of voriconazole maintenance therapy followed. Several days later, high-grade fever and leucocytosis, without an apparent cause, necessitated re-admission to the hospital. The fever gradually subsided under broad-spectrum antibiotics and the second course of induction chemotherapy was started. Five days after completion of this second course, the fever and pancytopenia returned and the antibiotics were switched to piperacillin-tazobactam. Despite treatment the patient suffered from progressive dyspnoea, diarrhoea and diffuse abdominal cramps. A new chest CT revealed progressive pulmonary right lower lobe consolidation and scattered smaller bilateral consolidations. A bronchoalveolar lavage was not performed. Faecal culture and clostridium toxin tests were repeatedly negative. Blood culture yielded Enterococcus faecium supposedly caused by intestinal translocation. Hence vancomycin was added to the antibiotic regime. Over the next six days pulmonary deterioration necessitated a second ICU admission at day 11 after completion of the second course of induction chemotherapy followed by intubation and mechanical ventilation. Empirically piperacillin-tazobactam was switched to imipenem. Physical examination subsequently showed signs of an acute abdomen and an abdominal CT scan revealed a thickened small bowel wall *(figure 1)*. Laparotomy revealed small bowel necrosis, necessitating partial jejunectomy with end-to-end anastomosis. Pathological examination revealed a characteristic appearance of broad nonseptate hyphae with right-angle branches, suspicious for a Zygomycete infection *(figure 2)* and liposomal amphotericin B (L-AmB) was started (5 mg/kg once a day). Unfortunately no tissue samples were obtained for culture. To confirm the diagnosis of Zygomycete infection and to identify the species, DNA was isolated from paraffin embedded material. A polymerase chain reaction (PCR) assay targeting the 18S ribosomal DNA of Zygomycetes was performed in a referral laboratory. Sequencing identified the Zygomycete as *Rhizomucor pusillus*. The *Aspergillus* PCR was negative. After surgical debulking and L-AmB was started, the patient gradually improved. At day 21 in the ICU repopulation of the bone marrow occurred. One week thereafter ICU discharge, and several weeks later hospital discharge followed. One year after admission the patient's AML is still in complete remission and the pulmonary consolidations gradually disappeared.

Discussion

Disseminated mucormycosis is considered to be relatively rare. It should, however, be acknowledged that despite the fact that the diagnosis is difficult to establish, the incidence is increasing, especially in the high-risk population, in which it is associated with high mortality. We present a case here of disseminated mucormycosis with localisations in the small intestines and lung. Our patient was severely immunocompromised (AML treatment) and had an occupational hazard being a vegetable grower (mucormycosis is commonly found in soil and decaying vegetation), both factors promoting colonisation with conidia

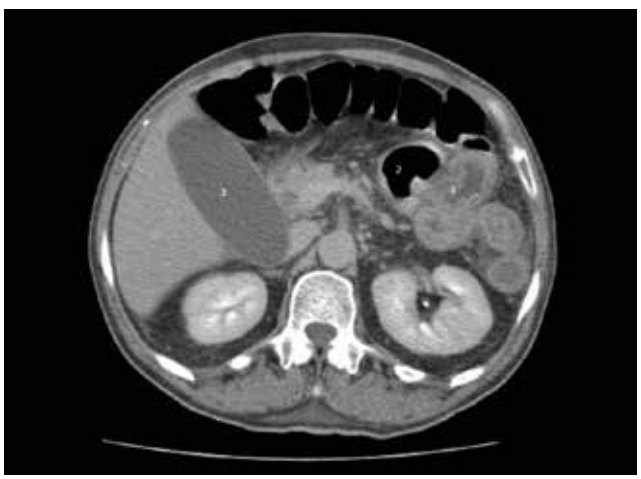

Figure 1. Abdominal computed tomography: 1) Thickened small bowel wall of the ascending duodenum and jejunum; 2) Small bowel dilatation; 3) Large gallbladder

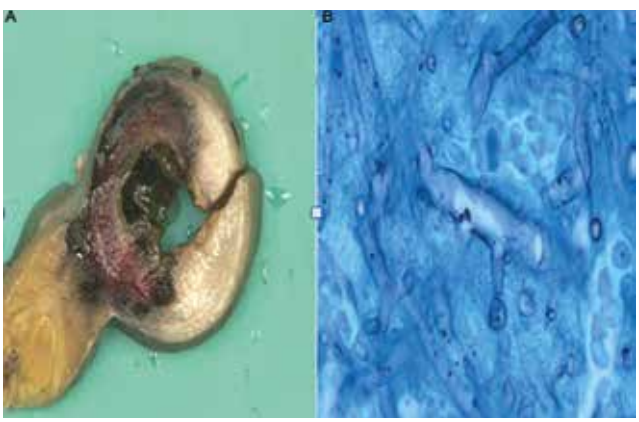

Figure 2A. Macroscopic aspect of the partial jejunectomy shows multifocal haemorrhagic necrosis
Figure 2B. Histopathological examination shows broad-based, ribbon-like, non-septate hyphae with right angle branching and swollen cells

of multiple fungi.[9] Further diagnostic clues were progression of pulmonary infiltrates despite adequate voriconazole therapy with repeatedly negative serum galactomannan-antigen tests (although it could theoretically have been a voriconazole-resistant aspergillosis that responded to the L-AmB therapy, we found it more likely to be a pulmonary localisation of myocormycisis). Moreover, the upper gastrointestinal bleeding, abdominal complaints and deterioration despite empiric broad-spectrum antibiotic treatment were other diagnostic clues, although non-specific. After the jejunectomy, the histopathological examination led us to the diagnosis of mucormycosis. PCR on the paraffin embedded material later confirmed the diagnosis and typed it *Rhizomucor pusillus*. This identification, even in the absence of cultures, made it likely that it was susceptible to L-AmB.[10] We believe that in the presented case, despite the disseminated disease, the combined treatment consisting of the infusion of high-dose L-AmB and surgical excision may have accounted for the positive patient outcome.

Mucormycosis is almost always a lethal opportunistic fungal complication observed in immunocompromised patients, particularly those with haematological malignancies, for which therapeutic options are limited. In patients with localised infection, surgical excision and debridement is fundamental to optimise the chances of cure.[5,11] However, over the past decade, several new antifungal drugs suitable for clinical use and novel strategies for treating invasive fungal infection have been developed. L-AmB, posaconazole and isavuconazole are currently available and exhibit good activity against mucormycosis. In contrast, voriconazole is not effective, as shown in the present case. L-AmB has a less toxic profile compared with conventional amphotericin B (AmB) and can be safely administered at much higher doses (up to 10 mg/kg). L-AmB is the appropriate empirical antifungal for invasive mucormycosis. In cases of L-AmB treatment failure or intolerability, posaconazole or isavuconazole may serve as salvage options.[5,11-13] Nevertheless, unlike invasive aspergillosis, mucormycosis is relatively rare, and the outcome of invasive mucormycosis treatment remains disappointing due to its high mortality rate in immunocompromised patients.[14-16]

Successful treatment of mucormycosis is dependent on timely diagnosis and reversal of predisposing factors.[17] Surgical debridement prior to dissemination of infection to distal organs and tissues has been shown to improve clinical outcomes. When combined with early, high-dose systemic antifungal therapy, studies have shown a greater than 1.5-fold increase in survival rates.[18,19]

So an important key to the successful treatment of mucormycosis is a rapid diagnosis. In general, diagnosing mucormycosis is more difficult than other fungal infections, as there are no specific clinical signs or biomarkers for identifying this fungal species.[5,6] The serum levels of galactomannan antigen are usually not elevated in patients with invasive mucormycosis. Therefore, conducting an early examination under the suspicion of mucormycosis is very important, and tissue biopsies, the gold standard for diagnosis, should be performed in order to diagnose mucormycosis whenever possible. Nevertheless, the rate of positive fungal cultures is usually low, and it is difficult to morphologically classify fungi on the species level.[7,20] In order to appropriately select antifungal agents, differentiating mucormycosis from more common opportunistic moulds, such as *Aspergillus*, is crucial. Voriconazole, which is used as a first-line treatment for invasive pulmonary aspergillosis, does not exhibit good activity against mucormycosis.[12] When cultures are negative, molecular identification from tissue samples can confirm the histological diagnosis. Hammond et al. reported 12 cases that were positive by culture, 10 were also positive by PCR and sequencing was concordant with culture results to the genus level in nine. Among 15 culture-negative cases, PCR was positive and sequencing allowed genus identification in 12.[7] Because molecular techniques are not clinically validated, they should be used cautiously in isolation to make a definitive diagnosis, and thus be used in combination with morphological methods. Several molecular and antigenic assays which detect the presence of Mucorales in laboratory animal models of mucormycosis are now becoming available.[21] Other systems have not been studied in animal model systems but also exhibit analytical sensitivity and specificity for the Mucorales.[22,23] One promising report describes Mucorales DNA in serum samples from nine high-risk patients up to 68 days before mucormycosis diagnosis was confirmed by histopathological examination and/or positive culture. The small number of cases and complexity of the molecular diagnostic platforms limit regulatory reviewing or extrapolation to other laboratories.[24]

Conclusion

An early diagnosis of disseminated mucormycosis and the correct identification of the causative agent are necessary to timely initiate appropriate antifungal therapy, as disseminated mucormycosis frequently has a fatal outcome. However, further validation of the contemporary molecular and antigenic assays is required (so far classifying rather than diagnostic) before they can routinely be used in the clinical setting.

Disclosures

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