

Prompt and definitive diagnosis of acute invasive *Aspergillus* rhinosinusitis in a patient with acute myeloid leukaemia using *in situ* hybridization: a case report

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Introduction

Invasive fungal rhinosinusitis is a life-threatening fungal infection with a reported incidence of around 2% in patients with haematological malignancies (DeShazo RD *et al.*, *New Engl J Med* 1997; **337**: 254–59). The prognosis of patients with invasive fungal rhinosinusitis varies depending on the underlying disease, the stage of infection and antifungal management, showing a case-fatality rate ranging from 28.6% to 60% (Iwen PC *et al.*, *Clin Infect Dis* 1997; **24**:1178–845; Lin SJ *et al.*, *Clin Infect Dis* 2001; **32**: 358–66). Although there is a wide range of causative pathogens, *Aspergillus* species account for most cases of invasive fungal rhinosinusitis (Lin SJ *et al.*, *Clin Infect Dis* 2001; **32**: 358–66). Early diagnosis and therapy is critical to achieve optimal therapeutic results. However, prompt diagnosis of acute invasive *Aspergillus* rhinosinusitis (AIAR) is difficult in immunocompromised patients because clinical symptoms are subtle due to their diminished inflammatory response (Iwen PC *et al.*, *Clin Infect Dis* 1997; **24**: 1178–84). Furthermore, the symptoms are not specific to AIAR and thus must be distinguished from concomitant bacterial infection. In general, the definitive diagnosis of invasive aspergillosis is established by histological evidence of fungal infection and culture confirmation of *Aspergillus* species. Recently, serological examinations of (1–3)- β -D-glucan and of *Aspergillus* galactomannan antigen have been developed and can be applied for

the early detection of invasive aspergillosis (Kawazu M *et al.*, *J Clin Microbiol* 2004; **42**: 2733–41). Furthermore, a molecular technique using *in situ* hybridization in tissue samples has been recognised as a useful method for the prompt and definitive diagnosis of invasive aspergillosis compared with culture confirmation (Myoken Y *et al.*, *J Oral Maxillofac Surg* 2008; **66**: 1905–12; Hayden RT *et al.*, *Diagn Mol Pathol* 2002; **11**:119–26). Treatment must be aggressive, and should include resection of the infected tissue in conjunction with systemic antifungal agents such as the new triazoles, amphotericin B and echinocandins (Iwen PC *et al.*, *Clin Infect Dis* 1997; **24**:1178–84; Vener C *et al.*, *Leukemia & Lymphoma* 2007; **48**:1557–86).

We describe the case of a patient with acute myeloid leukemia (AML) who developed AIAR caused by *Aspergillus fumigatus*. For early and accurate diagnosis of AIAR to manage the disease properly, we highlight the usefulness of a new molecular diagnostic method i.e., *in situ* hybridization in tissue samples.

Case report

On May 31, 2008, a 67-year-old man was admitted to the Department of Hematology with relapse of AML and was promptly administered reinduction chemotherapy with enocitabine and idarubicin. On day 18, the patient was started on itraconazole (ITCZ, 200 mg po q.d.) for antifungal prophylaxis because a dramatically decreased leukocyte count was noted (100 cells μL^{-1} ; 0% neutrophils). On day 22, the patient was febrile and antimicrobial treatment was initiated with panipenem and amikacin. On day 27, due to persistent fever and marked neutropenia, empirical antifungal treatment with micafungin (MCFG, 150 mg iv q.d.) was started in combination with ITCZ. A slightly elevated level of serum (1–3)- β -D-glucan (13.9 pg/ml; normal value

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Accepted for publication 5 July 2011

$<11 \text{ pg ml}^{-1}$) raised suspicion of fungal infection, although serum *Aspergillus* galactomannan antigen (0.3; normal optical density index <0.5) was negative.

On day 30, the patient was referred to the Department of Oral Surgery because he complained of right upper toothache. He was still deeply neutropenic and febrile. On examination, there were no abnormal findings in the oro-facial area, such as facial swelling, nasal congestion, nasal discharge, crusting in the nasal cavity or skin necrosis. The oral mucosa was intact. A positive Waters' view radiograph of the right maxillary sinus was noted, suggesting rhinosinusitis. Computed tomography images demonstrated inflammatory mucosal thickening on the maxillary sinus floor. Although serum *Aspergillus* galactomannan antigen on day 27 was negative, persistently elevated (1-3)- β -D-glucan (20.6 pg ml^{-1}) with radiographic findings suggested rhinosinus involvement by fungal infection. The leukocyte count was $300 \text{ cells } \mu\text{l}^{-1}$ (13% neutrophils), and the platelet count was $22\,000 \text{ cells } \mu\text{l}^{-1}$. Antrotomy of the maxillary sinus was performed under local analgesia with platelet infusion on day 33. Fungal hyphae were found in a 10% KOH preparation of the tissue sample, confirming sinus involvement by fungal infection. The necrotic lateral sinus wall and debris on the sinus floor were removed. A Penrose drain was placed for washing and instilling antifungal drugs into the operated area (Fig. 1). MCFG and ITCZ were discontinued and the patient was started on voriconazole (VRCZ, 200 mg po b.i.d) combined with liposomal amphotericin B (L-AMPH-B, 150 mg iv q.d) for invasive fungal rhinosinusitis. He also underwent maxillary sinus rinses with



Figure 1 Post-surgical photograph showing a Penrose drain (arrow) in the surgical wound.

50 ml of AMPH-B (1 mg ml^{-1} diluted in distilled water) once a day as part of the post-surgical care. Histopathological examination of formalin-fixed, paraffin-embedded tissue sections stained with Grocott's stain demonstrated organisms with hyphae dichotomously branched at acute angles (Fig. 2a). As shown in Fig. 2b, the hyphae in tissue sections were identified as *Aspergillus* species by *in situ* hybridization using a 584-bp DNA probe directed against the alkaline proteinase gene of *A. fumigatus* that is highly specific to *Aspergillus* species (Myoken Y *et al.*, *J Oral Maxillofac Surg* 2008; **66**: 1905–12) on day 36 (3 days after surgery), confirming the diagnosis of AIAR. At this point, we could definitively deny the possibility of non-*Aspergillus* mould infections such as *Fusarium* or *Trichoderma* species, which sometimes demonstrate highly elevated

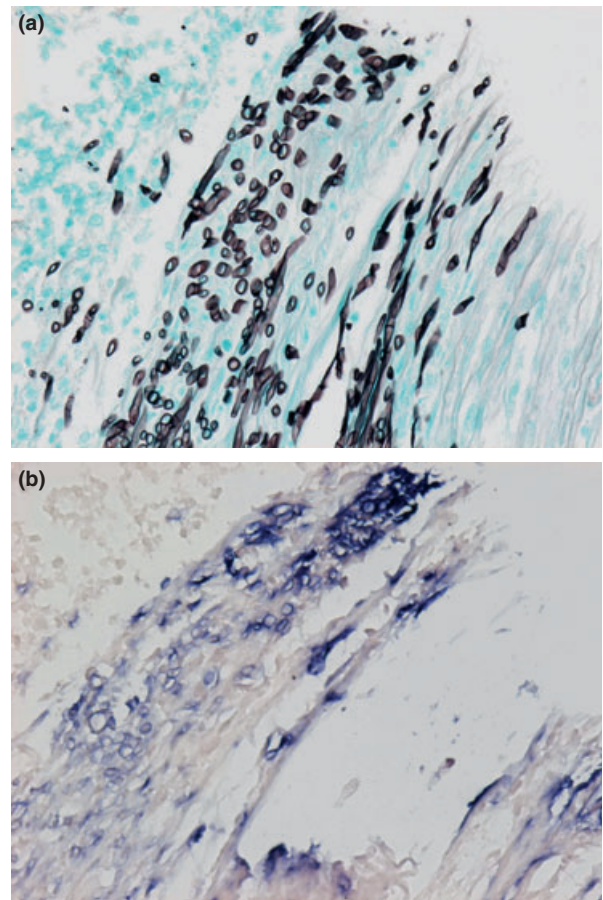


Figure 2 Histopathological findings (a) Tissue preparation stained with Grocott's stain showing septate hyphae branching at acute angles and invading bone (magnification $\times 100$). (b) *In situ* hybridization using an *Aspergillus*-specific DNA probe demonstrating *Aspergillus* hyphae in the same area (original magnification $\times 100$).

MICs for VRCZ *in vitro*. Tissue cultures on Sabouraud's dextrose agar yielded fungi that were morphologically identified as *A. fumigatus* on day 45 (12 days after surgery).

On day 57, the patient became afebrile and the right upper toothache resolved. The serum level of (1-3)- β -D-glucan decreased to a normal value of 8.8 pg ml⁻¹. The leukocyte count increased to 1600 cells μ l⁻¹ (51% neutrophils) after receiving human recombinant granulocyte colony stimulating factor (G-CSF; 2100 μ g total⁻¹). On day 60, there were no further fungal species isolated on culture of sinus samples and the drain was removed to discontinue sinus rinses with AMPH-B. On day 95, the Waters' view radiograph of the right >maxillary sinus showed marked improvement. *In vitro* susceptibility testing done according to NCCLS document M38-P showed that the causative *A. fumigatus* was sensitive to MCFG (MIC = 0.03125 μ g ml⁻¹), ITCZ (MIC = 0.25 μ g ml⁻¹), VRCZ (MIC = 0.125 μ g ml⁻¹) and AMPH-B (MIC = 0.25 μ g ml⁻¹). Intravenous L-AMPH-B therapy was discontinued after the definitive diagnosis of AIAR using *in situ* hybridization, whereas oral VRCZ therapy was administered for 3 months.

Discussion

Invasive rhinosinus aspergillosis is characterised by rapid spread of fungus from the sinus airspace into adjacent structures, especially the brain, with a very high mortality rate. Early diagnosis and aggressive treatment of AIAR are essential for patient survival (Iwen PC *et al.*, *Clin Infect Dis* 1997; **24**:1178–84). Commonly reported symptoms of AIAR are nasal congestion, nasal discharge, abnormal findings in the nasal cavity, buccal swelling with pain, gingival and skin necrosis and high fever (Iwen PC *et al.*, *Clin Infect Dis* 1997; **24**:1178–84; Lin SJ *et al.*, *Clin Infect Dis* 2001; **32**: 358–66). Radiographs and CT scans can disclose rhinosinus involvement. However, these clinico-radiographic signs are not specific to AIAR. In our patient, toothache and a positive sinus X-ray were the first findings, but there were no nasal or facial signs, which hampered diagnosis during the early-stage of AIAR. Serological examination of (1-3)- β -D-glucan is highly sensitive for fungal infections, but not specific for aspergillosis, whereas the detection of *Aspergillus* galactomannan antigen is less sensitive, but more specific (Kawazu M *et al.*, *J Clin Microbiol* 2004; **42**: 2733–41). In our patient, the elevated level of (1-3)- β -D-glucan and with apparent radiographic findings led us to perform antrotomy of the maxillary sinus for further examinations and treatment of AIAR because the

patient was at risk of developing fungal infections due to deep neutropenia. In addition to the usual histopathological examination and fungal culture, we performed *in situ* hybridization using an *Aspergillus*-specific DNA probe constructed in our laboratory to facilitate prompt and accurate diagnosis of aspergillosis (Myoken Y *et al.*, *J Oral Maxillofac Surg* 2008; **66**: 1905–12). Although the standard histopathological examination could be useful to distinguish *Aspergillus* species from *Mucor* species, several filamentous fungi are indistinguishable from each other by this standard method (Hayden RT *et al.*, *Diagn Mol Pathol* 2002; **11**:119–26). In our patient, aspergillosis was clearly diagnosed in 3 days by *in situ* hybridization compared to 12 days by fungal culture. At this point, the possibility of infection with non-*Aspergillus* species could be ruled out, as these non-*Aspergillus* moulds show decreased susceptibilities to VRCZ *in vitro*. Although the probe used in this study is not commercially available in other institutions at present, *in situ* hybridization is a promising technique for the prompt and definitive diagnosis of AIAR compared with culture-based methods, which are often time-consuming and carry the risk of growth failure due to incorrect handling of the tissue samples (DeShazo RD *et al.*, *New Engl J Med* 1997; **337**: 254–59).

Immediate and aggressive systemic antifungal therapy is necessary to manage and eradicate *Aspergillus* infection in immunocompromised patients. Among antifungal agents, the current standard therapy for invasive aspergillosis is VRCZ, which has led to better responses and improved survival (Marr KA *et al.*, *Clin Infect Dis* 2004; **39**: 797–802). Although we initially treated our patient with VRCZ and L-AMPH-B, L-AMPH-B was discontinued after AIAR was diagnosed using *in situ* hybridization. Furthermore, immediate surgical debridement of necrotic tissue is mandatory for pharmacological therapy to reach the infected area, as demonstrated in our patient. Published case series strongly support surgical debridement plus antifungal agents to optimise the outcome, showing a 60% survival rate for patients with invasive fungal rhinosinusitis that would otherwise be as low as 28.6% (Iwen PC *et al.*, *Clin Infect Dis* 1997; **24**:1178–84). We also adopted drainage and AMPH-B instillation into the maxillary sinus after surgical debridement to eradicate aspergillosis (Vener C *et al.*, *Leukemia & Lymphoma* 2007; **48**:1557–86).

In conclusion, although the patient showed some clinico-radiological findings, the positive results of *in situ* hybridization facilitated the prompt diagnosis of AIAR. In addition, surgical treatment and AMPH-B

instillation into the sinus were successfully performed in combination with effective antifungal medication with VRCZ.

Funding

None.

Competing interests

None to declare.

Ethical approval

Not required.