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Case Report

Rhinocerebral mucormycosis in a patient with pre-B cell acute lymphoblastic leukaemia: PCR identifying *Rhizopus oryzae* from culture-negative tissue specimens

Matthew P. Keisling,[1] Ian A. Seemungal,[1] Diana I. Mercado,[1] Spencer Voth,[2] Amanda Michael,[1] Beatriz L. Gomez,[3] Cesar Munoz,[3] Hans P. Schlecht,[1] Beth Carlisle Mapow,[1] Robert T. Ownbey,[1] Suresh G. Joshi[1] and Christopher L. Emery[1]

Correspondence
Christopher L. Emery
cemery@drexelmed.edu

[1]Drexel University College of Medicine and Hahnemann University Hospital, Philadelphia, PA, USA

[2]Philadelphia College of Osteopathic Medicine, Philadelphia, PA, USA

[3]Mycotic Diseases Branch, Center for Disease Control and Prevention, Atlanta, GA, USA

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Introduction: Mucormycosis is an aggressive infection that can cause significant disease in immunocompromised patients.

Case presentation: A case of a diabetic patient who developed rhinocerebral mucormycosis while receiving consolidation chemotherapy for leukaemia is described.

Conclusion: *Rhizopus oryzae* was identified in tissue biopsies by pan-fungal PCR and DNA sequencing, which provided the only means to identify the pathogen.

Introduction

Mucormycosis is an aggressive infection that can cause significant disease in immunocompromised patients. Although many patients with rhinocerebral mucormyosis (RCM) undergo similar treatment, pathogen speciation should not be underappreciated; it may be imperative to guide antifungal drug selection, as some mucoraceous fungi may exhibit variable resistance to conventional therapy (Almyroudis *et al.*, 2007; Alvarez *et al.*, 2009). Speciation is also useful to assess the patient's prognosis. We report a case of a diabetic patient who developed RCM while receiving consolidation chemotherapy for leukaemia. *Rhizopus oryzae* was identified in tissue biopsies by pan-fungal PCR and DNA sequencing, which provided the only means to identify the pathogen.

Case report

A 32-year-old woman presented with afebrile chills, sinusitis and progressive jaw pain of 1 week's duration. Despite treatment with amoxicillin-clavulanate, her jaw pain worsened and she developed facial oedema. The patient had a past medical history of pre-B-cell acute lymphoblastic leukaemia (Philadelphia chromosome negative), which was previously treated with two courses of induction and intensification chemotherapy. She also had a history of diabetes mellitus type 2, β-thalassaemia minor and neutropenia. On examination, the patient had right-sided periorbital oedema and tender, non-bleeding, dark areas on her right palate (Fig. 1a). Forward gaze of the right eye drifted laterally but was correctable with intention. The remaining physical examination was unremarkable. Laboratory data showed a white blood cell count of 200 cells μl^{-1} with 20 % neutrophils and 80 % lymphocytes, and an absolute neutrophil count of 40 cells μl^{-1}. Her haemoglobin level was 8.5 g dl^{-1}, her platelet count was 13 000 cells μl^{-1} and her serum glucose level was 289 mg dl^{-1}. The patient was empirically given broad-spectrum antimicrobials and amphotericin B lipid complex. Computed tomography scans showed right maxillary sinusitis, oedema and fat stranding of the right facial muscles. Magnetic resonance imaging (MRI) of the head (Fig. 1b) revealed extensive right periorbital swelling and proptosis of the right ocular globe.

A right maxillary endoscopic examination and a right anterior ethmoidectomy were performed. Histopathology from the right maxilla, middle turbinate and palatine artery demonstrated broad and thin-walled fungal hyphae with ribbon-like foldings (Fig. 1c) using haematoxylin and eosin, Grocott–Gomori methenamine silver and periodic acid–Schiff stains. The hyphae were both aseptate and sparsely septate, and exhibited variable 90° branching. Invasion and embolization of the blood vessels were noted

Abbreviations: FFPE, formalin-fixed paraffin-embedded; MRI, magnetic resonance imaging; RCM, rhinocerebral mucormyosis

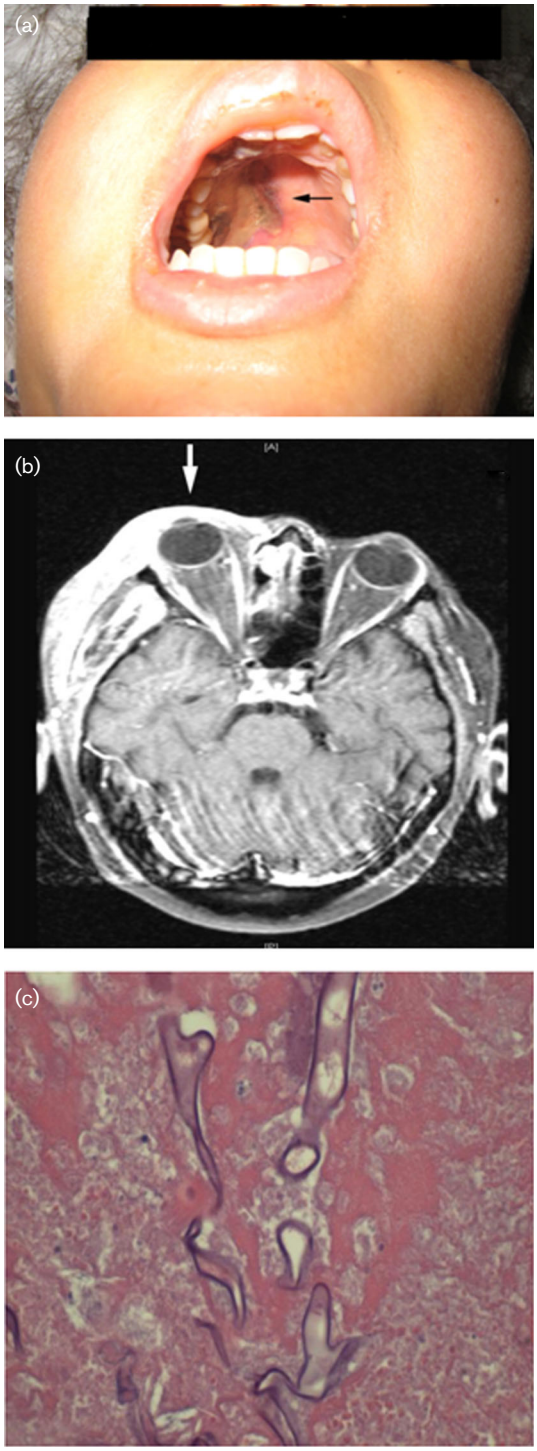

Fig. 1. (a) Right palatal lesion. Non-bleeding, dark grey-black areas were observed (black arrow), adjacent to tan-coloured areas suggestive of tissue necrosis. (b) Magnetic resonance imaging scan. Horizontal (axial) section of patient's head. Extensive right periorbital and right facial soft tissue swelling, and proptosis of the right ocular globe (white arrow) were observed. (c) Histopathology demonstrated broad and thin-walled fungal hyphae with ribbon-like foldings. Haematoxylin and eosin stain; magnification ×40.

consistent with mucormycosis. There was no evidence of concomitant co-infection. The patient subsequently had right malar bone maxillectomy, partial right palatectomy and resection of the right orbital soft tissue. A repeat MRI scan showed meningeal enhancement along the right middle cranial fossa, suggesting intracranial involvement. The patient required extensive debridements showing involvement of the medial wall of the right orbit. A right orbital exenteration was performed.

When the MRI scan suggested meningeal involvement, her antifungal therapy was broadened to include caspofungin, posaconazole and daily maxillary sinus amphotericin irrigation. Her consolidation chemotherapy was held and her blood sugar was aggressively controlled. Iron studies showed elevated ferritin of 7768 ng ml^{-1} and transferrin saturation of 100 %, so iron chelation with deferasirox was initiated. Her white blood cell count improved following granulocyte infusions and filgrastim (a granulocyte colony-stimulating factor analogue). She had intermittent fever that required additional empiric antimicrobial agents. No blood culture test results were positive. She eventually became afebrile, and began to improve clinically. Repeat MRI scans showed stable post-operative changes. She was discharged 3 months after her initial hospitalization and continued to take posaconazole.

Right maxillary sinus, middle turbinate and hard palate biopsies were submitted for fungal culture. Unfortunately, despite the clinical history of RCM, the specimens were homogenized (i.e. by tissue grinding) during processing. None of the fungal cultures yielded growth. Fresh right maxillary sinus and middle turbinate tissue, and shavings of formalin-fixed paraffin-embedded (FFPE) palate tissue were sent to the Mycotic Diseases Branch, Centers for Disease Control (Atlanta, GA, USA) for molecular identification. Initial DNA extraction was performed on fresh tissue using a DNA Mini kit (Qiagen), and on FFPE tissue using a QIAamp DNA FFPE Tissue kit. A gene fragment of the housekeeping human β-globin gene (260 bp) was utilized to assess for amplifiable DNA or the presence of PCR inhibitors (Muñoz-Cadavid *et al.*, 2010). Extracted fungal DNA was detected with pan-fungal PCRs [using internal transcribed spacer 1 (ITS1) and ITS4 primers for fresh tissue, which amplify the ITS1 and ITS2 regions of rRNA genes, and ITS3 and ITS4 primers for FFPE tissue, which amplify the ITS2 region]. The resultant PCR products were sequenced, edited and aligned using Sequencher version 4.9 (Gene Codes Corporation), and species identification was obtained using BLASTN searches of GenBank. The sequence results showed 100 % identity for *Rhizopus oryzae* for both formalin-fixed and fresh tissue specimens.

Discussion

Treatment of patients with RCM should be individualized and should continue until there is resolution of clinical

signs and symptoms, stabilization of residual radiographic signs on serial imaging and/or resolution of underlying immunosuppression (Spellberg *et al.*, 2010). Fungal species identification using PCR testing and product sequencing may be imperative to guide antifungal drug selection, as some mucoraceous fungi may exhibit variable resistance to conventional therapy. For example, *Cunninghamella bertholletiae* (one of the more lethal mucoraceous fungi) is the species least susceptible to amphotericin B, and its identification may help the clinician determine if adjunctive therapies are needed earlier (Almyroudis *et al.*, 2007). Conversely, *Rhizopus* spp. and *Mucor* spp. are highly susceptible to amphotericin B (100 and 94 %, respectively) but only variably so to posaconazole (0–80 %) (Alvarez *et al.*, 2009). Speciation is also useful to assess the patient's prognosis (Roden *et al.*, 2005). In conclusion, we recommend that PCR testing for tissue specimens be routinely performed for patients with RCM, especially as fungal cultures may not yield positive results (50–66 %), and pathogenic mucoraceous fungi can be fastidious and difficult to isolate (Roden *et al.*, 2005). During specimen processing, some of our patient's tissue biopsies were homogenized by tissue grinding, and the fungal cultures yielded no growth (possibly due to damaged hyphae). As this protocol is used in many clinical laboratories, clear communication between physician and laboratory is critical to ensure correct specimen processing when mucormycosis is suspected clinically (i.e. so that tissue is minced with a scalpel and not ground using a tissue homogenizer to maintain fungal viability).

Acknowledgements

This case report was a retrospective study that did not include any experimental work with humans and therefore our institution's Ethical Committee's policies deemed the case report to be exempt from approval, and also the subject's informed consent for the case report was not required. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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