CASE REPORT

Isolated cerebral mucormycosis caused by *Rhizomucor pusillus*

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SUMMARY

A 61-year-old man with relapsing chronic lymphocytic leukaemia, status post allogeneic stem cell transplant and multiple chemotherapy regimens presented to the emergency room after suffering a grand mal seizure. His evaluation revealed a 1.5–2 cm ring-enhancing left temporal lobe brain lesion on the CT scan. This brain lesion was resected and the histopathology revealed an invasive fungal organism resembling mucormycosis. Amplification and sequencing of the 28S ribosomal RNA gene identified the organism as *Rhizomucor pusillus*. The patient was treated with liposomal amphotericin B 5 mg/kg every 24 hours for 4 weeks, and then was transitioned to oral posaconazole. Serial brain imaging at 1 and 3 months, while on therapy, showed significant improvement.

BACKGROUND

We report a rare case of isolated cerebral mucormycosis due to unusual *Mucorales* sp, *Rhizomucor pusillus*, in an immunocompromised host. Diagnosis was established by histopathological examination of the resected mass showing fungal element consistent with mucormycosis. The causative organism was identified with amplification and sequencing gene targets that lead to the final identification of *R. pusillus*. The patient had excellent outcome with combined surgical and medical therapy.

CASE PRESENTATION

A 61-year-old man presented to the emergency department with acute grand mal seizure activity. He had a complicated medical history most remarkable for relapsing chronic lymphocytic leukaemia (CLL), diagnosed 20 years prior to his current presentation, for which he underwent multiple treatments, including an allogeneic peripheral blood stem cell transplant 3 years prior to his presentation. His post-transplant was complicated by chronic cutaneous graft versus host disease (GVHD) for which he was maintained on tacrolimus and prednisone along with photopheresis. After he experienced a CLL relapse, he was started on ibrutinib. He was kept on trimethoprim–sulfamethoxazole for pneumocystis prophylaxis and fluconazole for fungal prophylaxis, and received monthly intravenous immunoglobulin (Ig) for hypogammaglobulinaemia. The patient had no prior history of seizures and was afebrile at presentation. He had no history of intravenous drug use. He lived in the Midwest region of USA and wintered in Arizona state.

On neurological examination, patient displayed expressive aphasia but had no other focal or global neurological deficits. The skin examination had findings consistent with cutaneous GVHD. Respiratory, cardiovascular, gastrointestinal and genitourinary examinations were unremarkable.

INVESTIGATIONS

Initial laboratory evaluations included a complete blood count revealing anaemia (haemoglobin of 8.5 g/dL and haematocrit of 26.8%), lymphopaenia (absolute lymphocyte count of 0.28×10^9/L) and thrombocytopaenia (platelet count of 149/L). Serum creatinine at presentation level was 1 mg/dL. Additional tests included normal chemistries, prostate-specific antigen, lipids and thyroid-stimulating hormone level. Blood PCR for cytomegalovirus was negative. 1–3 Beta-D-glucan testing was performed and was negative (36 pg/mL; ref: <60 pg/mL=negative).

A CT scan of the head revealed a 1.5–2 cm ring enhancing lesion in the left temporal lobe (figure 1). Subsequent MRI showed a 1.8 cm ring-enhancing lesion in the left temporal lobe with vasogenic oedema, which was thought to be an abscess or malignancy. CT of the chest, sinuses and abdomen–pelvis revealed no evidence of extracranial disease. Bacterial and fungal blood cultures remained negative after 5 and 30 days of incubation, respectively, and extensive serological workup for infectious aetiologies was negative, including serum *Cryptococcus* antigen, *Histoplasma* complement fixation and immunodiffusion antibodies, *Histoplasma* urine antigen, *Blastomyces* antibody enzyme immunoassay (EIA), *Coccidioides* antibody EIA and serum galactomannan.

A left temporal craniotomy with mass resection was performed and the initial intraoperative impression was of a metastatic tumour. Consequently, no tissue was sent to microbiology for culture. However, histopathological examination of the tissue showed necrosis with acute and chronic inflammation. Grocott-Gomori methenamine silver (GMS) stain highlighted numerous broad pauciseptate fungal hyphae, suggestive of a *Mucorales* sp (figure 2). To confirm the identity of the causative pathogen, amplification and sequencing of the 28S ribosomal RNA gene and the internal transcribed spacer 1 and 2 (ITS1 and 2) gene regions were performed on the formalin-fixed paraffin-embedded (FFPE) tissue and sequencing results identified the organism as *R. pusillus*.

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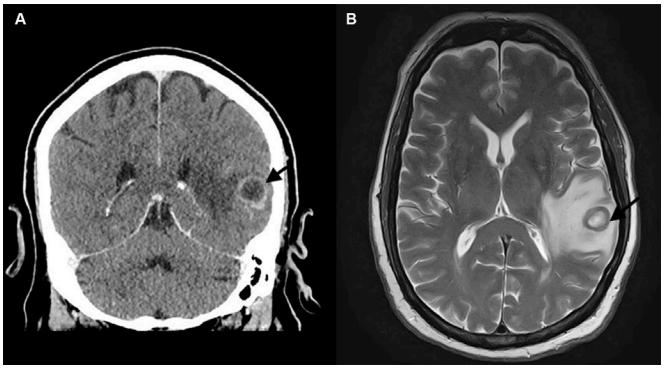

Figure 1 (A) Head CT with contrast, coronal section, showing a left temporal lobe ring-enhancing lesion surrounded by vasogenic oedema (arrow head). (B) Brain MRI, flair sequence, showing left temporal lobe mass surrounded by vasogenic oedema.

TREATMENT

The patient received induction therapy with liposomal amphotericin B 5 mg/kg every 24 hours for 4 weeks, and then was transitioned to oral posaconazole delayed-release tablets. Serial brain imaging at 1 and 3 months, while on therapy, showed significant improvement with decreased oedema and further contraction of the surgical cavity.

OUTCOME AND FOLLOW-UP

Clinically, patient made almost complete recovery and was maintained on oral posaconazole for secondary prophylaxis given the need for ongoing immunosuppression for GVHD. At 1-year follow-up, the patient was doing well and there were no signs of disease recurrence.

DISCUSSION

The term mucormycosis is often used to imply an angioinvasive infection caused by moulds that belong to the order *Mucorales*, a ubiquitous environmental mould found in decaying organic substrates. Members of the *Mucorales* order important in human disease include *Rhizopus* sp, *Mucor* sp, *Rhizomucor* sp, *Lichtheimia* sp and *Cunninghamella* sp, and are characterised by pauciseptate, irregularly branching, ribbon-like hyphae.

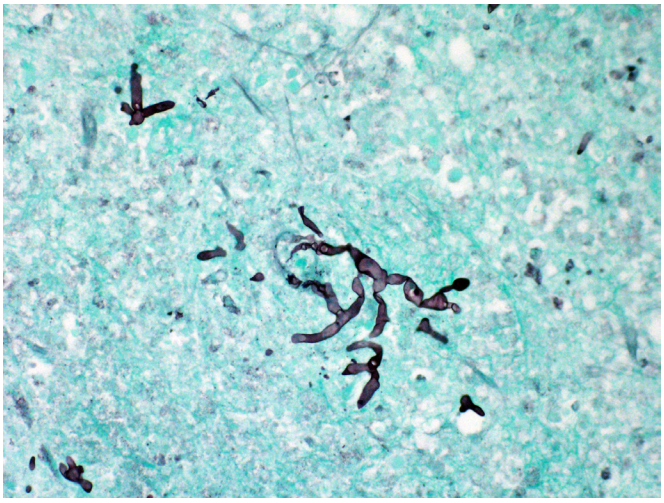

Figure 2 Brain tissue with GMS stain. Numerous broad, ribbon-like, pauciseptate or pauciseptate hyphae are stained, consistent with *Mucorales* sp. GMS, Grocott-Gomori methenamine silver.

In a review of 929 reported cases of confirmed mucormycosis, 47% were attributed to the genus *Rhizopus,* 18% by pathogens belonging to the genus *Mucor,* 7% by the genus *Cunninghamella* and 4% by pathogens in the genus *Rhizomucor*.[1]

Mucormycosis is most often associated with immunocompromised hosts as an opportunistic infection. Risk factors for the development of invasive mucormycosis include diabetic ketoacidosis, neutropaenia, corticosteroid use, haematological malignancy and solid organ or bone marrow transplantation. Invasive mucormycosis in immunocompetent individuals has been reported following trauma associated with natural disasters. Mucormycosis can be fatal in up to 96% of disseminated cases and delays in microbial diagnosis may contribute to high mortality. Site of infection, underlying predisposing factors and the species involved are known predictors for mortality as well.[1]

The genus *Rhizomucor*, first described in 1978, is a thermophilic saprophytic fungus in the order *Mucorales*. *R. pusillus*, *R. miehei* and *R. variabilis* are the pathogenic species associated with human disease. Due to the small size (3–5 µm), the spores of *Rhizomucor* are easily disseminated via the airborne route and aerosolised sporangiospores are readily inhaled. *R. pusillus* has a wide geographic distribution and, although a frequent cause of invasive *Mucorales* infection in animals, has been rarely associated with human disease.[2]

R. pusillus is reported to be less virulent and have lower minimal inhibitory concentrations (MIC) to antifungal agents compared with more common *Mucorales* sp, such as *Cunninghamella*, which is associated with increased mortality.[2] However, infection with *R. pusillus* causes significant morbidity and mortality, and patients have a 30% risk for disseminated infection at presentation and 46% risk of mortality.[2] Individuals with haematological malignancies are at highest risk of acquiring this infection.[2]

Diagnosis of invasive mucormycosis may be clinically challenging. The *Mucorales* are associated with disease presentation involving multiple organ systems, including pulmonary, rhinocerebral, cutaneous, central nervous system (CNS) and the gastrointestinal tract.

Rhinocerebral disease is characterised by rapid invasion into surrounding facial structures and is classically associated with poorly controlled diabetes and ketoacidosis. Pulmonary mucormycosis is more commonly described in patients with neutropaenia. Cutaneous mucormycosis is most often associated with traumatic disruption of the skin barrier. Skin is also a common site of disseminated infection.

Cerebral mucormycosis is most often diagnosed in the context of concomitant sinus disease or disseminated disease. However, isolated cerebral mucormycosis may account for up to 8% of all cases of mucormycosis.[1] Intravenous drug use is the most important risk factor for isolated cerebral disease.[1 3] Radiographic features cannot distinguish between mucormycosis and other pyogenic cerebral infections. However, a rapidly enlarging brain abscess in the basal ganglia in the context of intravenous drug use should raise the suspicion for isolated cerebral mucormycosis.[4 5]

The diagnosis of mucormycosis often relies on histopathological examination of surgically removed tissue and visualisation of broad, ribbon-like, pauciseptate hyphae with wide-angle branching suggests a diagnosis of *Mucorales*. Angioinvasion is common in *Mucorales* infections, and hyphae may be visualised within blood vessels. Acute inflammation with neutrophilic predominance is often present, but may be diminished in patients with neutropaenia.

Distinguishing between *Aspergillus* and *Mucorales* may be complicated when hyphae are not abundant in tissue. Artefactual folds or breakdown of hyphae may also be confused with septations, leading to an incorrect identification. Additionally, histopathological identification of mucormycosis does not provide genus and species information or susceptibility data and has limited ability to detect mixed or dual fungal infections.[6 7]

Culture of *Mucorales* from infected tissue is important for species identification, yet the recovery of *Mucorales* in culture is often difficult. In a large case series of histopathologically confirmed mucormycosis, cultures were positive in only 50% (465/929) of cases.[1]

Processing in the microbiology laboratory may damage the fragile pauciseptate hyphae, decreasing culture yield from tissue sources, and mincing of tissue, rather than grinding, is recommended. Growth is rapid (1–7 days) on routine fungal selective media (eg, Inhibitory mould agar) and isolates often have erect aerial mycelium reaching the lid of the petri dish, leading to the colloquial designation of this group as 'lid lifters'. Despite the angioinvasive nature of *Mucorales,* blood cultures are rarely positive.[8]

Molecular methods are increasingly used for detection and identification of fungi in fresh and FFPE tissue and, most recently, in plasma specimens. Broad-range fungal PCR, using primers targeting the ITS gene regions or the 28S or 18S ribosomal RNA gene regions, followed by sequencing has demonstrated good agreement with culture results, and sensitivity is increased when applied to fresh over FFPE tissue. Molecular detection may allow for the identification of the infecting organism to the genus and species level, a distinct advantage over histopathological diagnosis, which may facilitate the choice of appropriate antimicrobial therapy based on known susceptibility patterns.[8 9]

Advances in the diagnosis of *Mucorales* are needed to accurately and rapidly diagnosis patients with invasive disease. PCR assays targeting the most common species of *Mucorales* have been designed for use in tissue samples or serum/plasma. In one study, frozen plasma specimens from a cohort of 44 patients with proven or probable mucormycosis were retrospectively tested using a set of three *Mucorales* PCR assays targeting *Rhizomucor* sp, *Lichtheimia* sp and *Mucor/Rhizopus* sp. Interestingly, 81% (36/44) of patients had at least one positive PCR, often preceding the date of radiological findings or microbiological confirmation.[10] Clinical use of molecular assays for the detection of *Mucorales* in plasma specimens may allow for earlier diagnosis and initiation of effective therapy in patients. While promising, evaluation of the performance of these assays in larger prospective studies is needed.

Early diagnosis and initiation of *Mucorales* active antifungal therapy in known to improve mortality, and delay in therapy of >1 week has been associated with increased mortality.[11] Antifungal agents active against the *Mucorales* include amphotericin B, isavuconazole and posaconazole, and the two active azoles are commonly used as maintenance therapy after initial treatment with amphotericin B. Surgical resection, if feasible, and modification of immunosuppression are also known to improve mortality.[12]

Learning points

- Diagnosis of mucormycosis may be challenging, and often requires a high index of clinical suspicion and histopathological visualisation of broad, ribbon-like, pauciseptate hyphae with wide-angle branching in biopsy specimens.
- Tissue processing at the microbiology laboratory can affect the yield of cultures. Early communication with laboratory staff regarding clinical suspicion of mucormycosis may permit modified tissue processing steps to optimise culture yield.
- Molecular methods may help to established the diagnosis and identifying causative species, especially when fungal cultures are not sent or are negative.
- Early diagnosis and initiation of antifungal therapy along with surgery, if feasible, are associated with improved patient survival.

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REFERENCES

1 Roden MM, Zaoutis TE, Buchanan WL, *et al*. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis* 2005;41:634–53.
2 Gomes MZ, Lewis RE, Kontoyiannis DP. Mucormycosis caused by unusual mucormycetes, non-Rhizopus, -Mucor, and -Lichtheimia species. *Clin Microbiol Rev* 2011;24:411–45.
3 Verma A, Brozman B, Petito CK. Isolated cerebral mucormycosis: report of a case and review of the literature. *J Neurol Sci* 2006;240:65–9.
4 Hopkins RJ, Rothman M, Fiore A, *et al*. Cerebral mucormycosis associated with intravenous drug use: three case reports and review. *Clin Infect Dis* 1994;19:1133–7.
5 Sundaram C, Mahadevan A, Laxmi V, *et al*. Cerebral zygomycosis. *Mycoses* 2005;48:396–407.
6 Sangoi AR, Rogers WM, Longacre TA, *et al*. Challenges and pitfalls of morphologic identification of fungal infections in histologic and cytologic specimens: a ten-year retrospective review at a single institution. *Am J Clin Pathol* 2009;131:364–75.
7 Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev* 2011;24:247–80.
8 Lackner M, Caramalho R, Lass-Flörl C. Laboratory diagnosis of mucormycosis: current status and future perspectives. *Future Microbiol* 2014;9:683–95.
9 Bialek R, Zelck UE. [PCR-based diagnosis of mucormycosis in tissue samples]. *Pathologe* 2013;34:511–8.
10 Millon L, Herbrecht R, Grenouillet F, *et al*. Early diagnosis and monitoring of mucormycosis by detection of circulating DNA in serum: retrospective analysis of 44 cases collected through the French Surveillance Network of Invasive Fungal Infections (RESSIF). *Clinical Microbiology and Infection* 2016;22:810.e1–810.e8.
11 Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 2008;47:503–9.
12 Farmakiotis D, Kontoyiannis DP. Mucormycoses. *Infect Dis Clin North Am* 2016;30:143–63.