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An Unusual Case of *Actinomucor elegans*: A Challenging Diagnosis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Patient: Male, 70-year-old
Final Diagnosis: Actinomucor elegans
Symptoms: Bleeding • dizziness • eschar
Medication: —
Clinical Procedure: Culture • MALDI • sequencing • tooth extraction
Specialty: Infectious Diseases

Objective: Rare disease

Background: *Actinomucor elegans* is an unusual cause of mucormycosis and can be difficult to identify by conventional methods. Mucormycosis has a very high mortality rate, especially among immunocompromised individuals. Due to the morbid and progressive nature of opportunistic fungal infections, early diagnosis is paramount for effective disease management. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI) and Sanger sequencing are useful methods for rapid diagnosis of unusual fungal pathogens.

Case Report: We report a fatal case of mucormycosis caused by *A. elegans* in an immunocompromised man. The pathogen was isolated from a large nasal septal black eschar that developed rapidly during tooth extraction in a patient with myelodysplastic syndrome and diabetes mellitus. After unsuccessful identification by conventional methods, *A. elegans* was identified using MALDI and Sanger sequencing.

Conclusions: Diagnosing fungal organisms poses many difficulties, but amidst the technological evolution in pathogen identification, there are useful methods for rapid identification, including MALDI and sequencing. With these powerful tools, earlier diagnosis will give health professionals an advantage against potentially fatal fungal infections.

MeSH Keywords: Mucormycosis • Myelodysplastic-Myeloproliferative Diseases • Sequence Analysis, DNA • Spectrometry, Mass, Matrix-Assisted Laser Desorption-Ionization

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Background

The genus *Actinomucor* belongs to the phylum *Zygomycota* and order *Mucorales*. The pathological agent in this case, *A. elegans*, is ubiquitous in nature [1]. Many *Zygomycetes,* including *A. elegans,* are used in soy fermentation as a source of flavor and texture [1]. In a review of *Mucormycetes* from global and regional studies, it was shown that *Rhizopus*, *Mucor*, and *Lichtheimia* accounted for almost 80% of reported cases [2]. However, there are still a large number of cases involving less common *Mucormycetes,* such as *A. elegans*.

There have been 5 previous cases of *A. elegans* reported. These include an 11-year-old female with sinusitis [3], a necrotic foot ulcer in a diabetic male [4], a lethal case from combat trauma confirmed post-mortem [1], a lethal infection from an arm wound in an immune-compromised man [5], and a sinus infection in a patient receiving high-dose corticosteroids after stem cell transplant [6]. All cases received debridement and antifungal therapy for treatment. Dissemination was documented in 2/5, and both cases were lethal. This is consistent with other mucormycoses, in which mortality is greater than 95% for patients with disseminated disease [7]. Early diagnosis and treatment are vital for control of infection and patient survival [8].

Case Report

A 70-year-old white man with a history of myelodysplastic syndrome (MDS) and diabetes mellitus presented with a history of nosebleeds, recent weakness, falls, and tooth pain. He was previously treated for MDS with decitabine therapy and was on azacytidine (VIDAZA) at the time of presentation.

On admission, the patient was found to be hyperglycemic and was started on an insulin drip. An X-ray of the mandible showed a peri-apical abscess at the root of tooth #15, and the left mandibular molar had a large carious erosion involving the crown. Both teeth were extracted on the morning of admission. Although the patient had experienced tooth pain for several months, dental care was delayed due to chemotherapy-induced leukocytosis. During tooth extraction, a black eschar rapidly developed on the hard palate (Figure 1). Although not draining, it was tender and grew in size during the procedure. Right periorbital swelling also developed during the procedure. Further examination identified an eschar extending into the nasal septum. A fungal smear was immediately performed on a swab of the septal eschar, revealing pauciseptate hyphae suggestive of a zygomycete and a budding yeast. The patient was then started on liposomal amphotericin B (Ambiosome), posaconazole (Noxafil), and micafungin (Mycamine). Magnetic resonance imaging of the head with contrast confirmed acute invasive fungal rhino-orbital sinusitis and revealed disseminated multifocal mycotic emboli in the brain (Figure 1).

Two distinct fungal colony morphologies were identified in culture on Sabouraud dextrose agar incubated at 30°C. Smooth colonies with "feet" developed after 24 hours and were quickly identified as *Candida albicans*. This is a common colonizer of the nose and was not thought to contribute to the disease in this patient. On day 3 of culture, a cottony mold with abundant aerial hyphae developed and quickly filled the plate by day 5. The mold was subcultured on day 3 to potato dextrose agar to promote spore production. Lactophenol cotton blue tape preps of the mold and evaluation of sporangia were unable to identify genus or species (Figure 2). Further analysis with MALDI-TOF identified the mold on day 5 as an *Actinomucor* species.

The definitive treatment for rhino-orbital-cerebral mucormycosis is extensive surgery, including enucleation and removal of nose/maxilla, along with combination antifungal therapy [8]. Survival of patients with disseminated disease is less than 5% [7]. Our patient failed to improve with antifungals alone and had significant comorbidities, including poorly controlled DM and decreased immune function related to his MDS. Due to the poor prognosis of disseminated mucormycosis, the patient and family decided to decline extensive and painful surgery and instead transferred the patient to comfort care. The patient died 1 hour later. It had been 14 days since the first symptoms appeared (nosebleeds) and 8 days since hospitalization and tooth extraction.

Images

Figure 1. Photographs of the eschar were taken shortly after tooth extraction. At that time the patient gave consent for the use of the images and related information. Figure 2. Microscopic images of Lactophenol cotton blue-stained *A. elegans*.

Phenotypic identification

Hyphal growth was white and fluffy, filling the plate by day 5. After 6 days of culture, the sample began to turn tan with slight browning at the top. Hyphae were noted to be broad and nearly aseptate. Septa were mostly found near the sporangiophores and as the hyphae aged. Sporangiophores displayed multiple branches (2–10) and formed round sporangia that produced smooth, round sporangiospores. These spores were released when the sac dissolved, revealing the columella (Figure 2). Abundant branching rhizoids were observed, and they were not localized under sporangiophores. Unlike *Lichtheimia*, no apophysis or collaret were observed [9].

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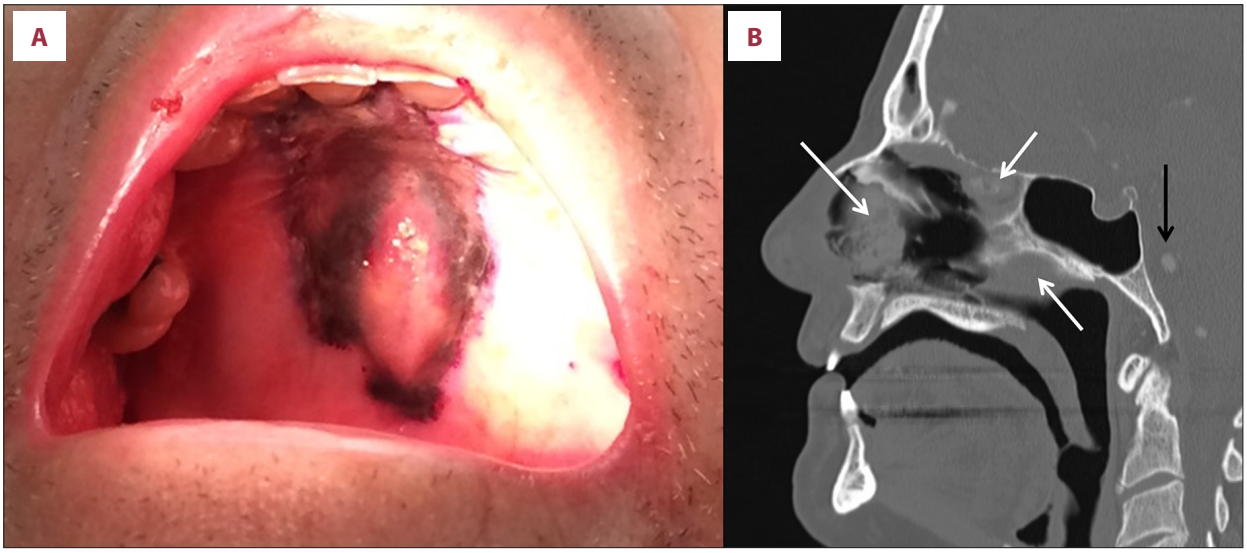

Figure 1. Clinical imaging: (**A**) black eschar on the hard palate shortly after tooth extraction; (**B**) magnetic resonance imaging of the head showing rhino-orbital sinus masses consistent with mucormycosis (white arrows) and mycotic embolus in the brain (black arrow).

Figure 2. Characteristic morphologies of *A. elegans* with Lactophenol cotton blue staining: (**A**) multiple branches of the sporangiophore; (**B**) sac surrounding sporangium, columella with no collaret, and smooth, round sporangiospores from ruptured sporangium; (**C**) an extensive rhizoid system; (**D**) and no apophysis.

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MALDI

The procedure was performed according to the manufacturer's specifications using MALDI Biotyper Compass software (Bruker Daltonik). Extraction of fungal elements followed the protocol outlined by the NIH [10] and the data were compared to the NIH mold (1829014) library. Hyphae were analyzed on day 5 of culture after unsuccessful identification by morphology. At the time of this analysis, there was only a single entry in the fungal database for comparison – *A. elegans*. We applied the standard cutoff values for interpretation of results where identification of species cutoff is ≥2.0 and genus is ≥1.7. With a top score of 1.8, we reported "*Actinomucor* species".

Genotyping

We identified *Actinomucor elegans* to the species level using Sanger sequencing of the internal transcribed spacer (ITS) and D1/D2 divergent ribosomal RNA genes. DNA was extracted using the QIAamp DNA Mini Kit Tissue Protocol (QIAGEN, Hilden Germany). Target genes were amplified using HotStarTaq Master Mix Kit (QIAGEN, Hilden Germany), ITS-2b and ITS-3a, and NL-2b, and NL-3a (IDT, Iowa) primers according to the manufacturer's conditions. PCR products were bidirectionally sequenced according to the manufacturer's instructions on an Applied Biosystems 3500 (Thermo Fisher Scientific, Waltham, MA). Sequencing results were aligned based on ITS and D1/D2 nucleotide-nucleotide searches using the NCBI BLASTn algorithm [11–13].

Genotypic characterization.

BLASTn consensus results demonstrated a 100% match for *A. elegans*. The ITS amplicon showed greatest homology with *A. elegans* accession no. FJ176396, 100% (395/395 bp); and *A. elegans var. meitauzae* accession no. AY492087, 100% (395/395 bp). The D1/D2 amplicon showed greatest homology with *A. elegans var. meitauzae* accession no. JN206492, 100% (389/389 bp) and *A. elegans var. kuwaitiensis* accession no. JN206493, 100% (389/389 bp).

Discussion

Over the years, major advances in healthcare have led to an increase in numbers of immune-compromised individuals and a concomitant increase in life-threatening infections due to opportunistic fungi [14]. Mucormycosis is the second most frequent mold infection in immune-compromised patients and can progress rapidly in both immune-compromised and immune-competent individuals. Mortality rates are very high with 54% overall mortality and 96% for disseminated infections [7]. Unfortunately, high mortality and inadequate treatment persist due to the difficulty of diagnosing mucormycosis [15]. Early diagnosis, surgical debridement, systemic antifungal therapy, and control of underlying conditions are paramount to successful management of disease [8]. While early diagnosis did not save our patient's life, it did allow the care team and family to make an informed decision to withhold painful surgical interventions for a patient with a poor prognosis due to disseminated infection.

This case report is the first to identify *A. elegans* after tooth extraction and the first to address early diagnosis through the use of MALDI, which is a fast, reliable, and cost-effective method for identifying microorganisms [11]. It also highlights the utility of Sanger sequencing for fungal identification. Fungal sequencing is currently underutilized in clinical settings. Diagnosis to the genus level of a lethal case of mucormycosis was obtained from an isolate in under 2 hours by MALDI, and identification to the species level was possible 2 days later by sequencing. Traditional phenotypic methods require 4 or more days for development of conidia, subjective identification of sporangia, and expertise in rare and unusual fungi. Microscopic evaluation alone was not sufficient to identify this pathogen in our institution. Early diagnosis could have aided in getting quicker antifungal susceptibility testing results. In our case, this testing was discussed but declined because it would not affect the family's decision to withdraw care. MALDI and sequencing offer fast and less subjective diagnostic methods to provide clinicians and families with a complete diagnosis in an actionable amount of time. These techniques are broadly applicable to other fungi, which further suggests they should be routinely available in clinical labs.

Conclusions

This case offers multiple learning opportunities. Disease was first recognized in the unusual setting of tooth extraction when the black eschar on the hard palate rapidly developed during the procedure. The patient had a classic history for mucormycosis – sinus infection in a patient with diabetes and immune compromise. However, the etiologic agent was unusual and hard to diagnose. Through the use of MALDI and sequencing, the lab was able to overcome the limitations of subjective phenotypic methods and identified *A. elegans* as the cause of disease. Providing a complete diagnosis assisted the care team and family to make an informed decision regarding patient management. With early detection, decreased delays in treatment could offer patients a greater chance for survival.

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Conflicts of interest

None.

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