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CASE REPORT

Fatal brain infection caused by *Aspergillus glaucus* in an immunocompetent patient identified by sequencing of the ribosomal 18S–28S internal transcribed spacer

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Abstract Cerebral aspergillosis has rarely been reported in immunocompetent patients. We herein describe a unique case of cerebral aspergillosis in a healthy adult that led to his death despite aggressive antifungal therapy. Sequencing of ribosomal 18S–28S internal transcribed spacer identified the organism as *Eurotium herbariorum*, the teleomorph of *Aspergillus glaucus*.

Introduction

Invasive aspergillosis occurs mostly in immunosuppressed patients; however, in recent years, there has been a significant increase in *Aspergillus* infections in immunocompetent hosts where a number of extrapulmonary infections have been noted, among which central nervous system (CNS) aspergillosis [1]. Neuroaspergillosis is an opportunistic fungal infection that usually affects heavily immunocompromised hosts, typically patients with hematological malignancy or bone marrow or solid organ transplantation in whom neutropenia and corticosteroid use are the major risk factors [2]. Commonly, cerebral involvement occurs by direct extension of invasive *Aspergillus* sinusitis or by hematogenous spread from an occult source, usually the lungs [3, 4]. In immunocompetent patients, the disease has been mostly described in the setting of allergic rhinosinusitis caused by *Aspergillus* species, following neurosurgical procedures, and in patients with significant comorbidities such as diabetes mellitus, severe malnutrition, or liver cirrhosis [5, 6]. *Aspergillus fumigatus* is the most frequently reported species in immunosuppressed hosts whereas *Aspergillus flavus* has been mostly recovered from immunocompetent patients. It is the most common species isolated in cultures of invasive aspergillosis of nasal and paranasal origin in patients living in hot and dry environments [5, 7]. Although *Aspergillus glaucus* is ubiquitous in the environment [8, 9], only one case of invasive pulmonary aspergillosis caused by this organism has been reported in a patient with metastatic prostate cancer [10]. Another case of possible hypersensitivity pneumonitis related to *A. glaucus* has been described in a farmer [11]. To our knowledge, there are no previous reports of *A. glaucus* causing brain infection, even in immunocompromised patients. We herein report a unique case of CNS aspergillosis caused by *A. glaucus* in a previously healthy young adult.

Case report

A 26-year-old previously healthy Syrian man presented in February 2004 to an outside hospital with sudden-onset headache and right orbital pain. Computed tomography

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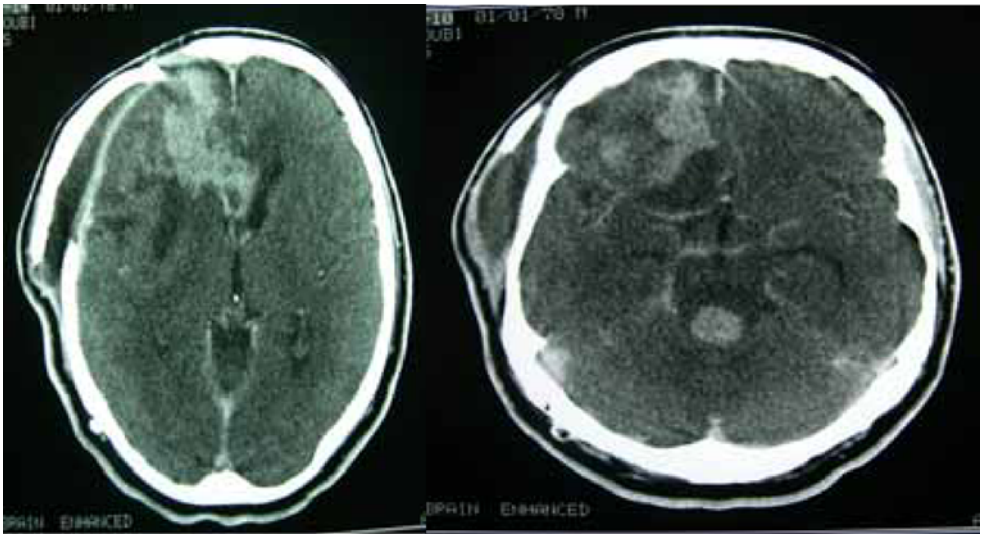

Fig. 1 CT scan of the brain showing a large ill-defined enhancing lesion involving the anterior and inferior aspects of the right frontal lobe with significant surrounding edema. A globular 2-cm enhancing lesion was seen at the level of the quadrigeminal plate cistern

(CT) of the brain showed a right frontal mass for which he underwent total resection. Pathological examination showed numerous branching fungal hyphae associated with necrotizing granulomas. The patient received 4 g of amphotericin B along with four antituberculous drugs for 6 months. Nine months later, he developed seizures and drowsiness. A repeated CT scan of the brain showed recurrent brain abscesses. The patient was restarted on antituberculous therapy and itraconazole. He was then transferred in September 2005 to the American University of Beirut Medical Center (AUBMC) for further management.

On admission, the patient was somnolent. His laboratory data including human immunodeficiency virus (HIV) serology were unrevealing. The patient had no known predisposing factors to invasive aspergillosis. He was not diabetic and had no history of intake of steroids or prolonged antibiotic therapy. A CT scan of the brain showed an irregularly enhancing lesion involving the right frontal lobe with significant edema (Fig. 1). The patient worked in the United Arab Emirates as a comptroller in a financial institution. He had no other travel history. One week before the onset of the illness, he swam in a lake. His family denied any exposure to damaged or rotting food that could potentially be harboring molds. Pathological examination of a repeated brain biopsy revealed numerous septated hyaline hyphae associated with florid noncaseating granulomatous and lymphoplasmacytic inflammation with necrosis and fibrosis in the brain parenchyma. The patient was started on voriconazole for the treatment of suspected *Aspergillus* brain abscess. The patient's condition deteriorated despite treatment with voriconazole. He later developed aspiration pneumonia and died.

The brain tissue culture grew cottony mold colonies after 26 days of incubation on Sabouraud dextrose agar. These colonies displayed a white periphery and a yellow-brown center (Fig. 2). The reverse of the colonies was yellowish to brownish. Tease preparations with lactophenol cotton blue staining showed asexual structures consistent with *Aspergillus* species. These included smooth-walled septated conidiophores with uniseriate phialides covering the entire head, topped by rows of conidia. No asci or cleistothecia were identified. The microscopic morphology did not allow species identification. Species identification was achieved by sequencing of the 18S–28S ribosomal internal transcribed spacer regions 1 (ITS1) and 2 (ITS2) as previously described [12, 13]. The combined ITS1 and ITS2 sequence was compared to sequences in GenBank and the University of Washington Fungal Project sequence database [14]. The sequence of this organism showed 99.6% homology to that of *Eurotium herbariorum*, the teleomorph of *Aspergillus glaucus*.

Fig. 2 Gross morphology of *Aspergillus glaucus* colonies on Sabouraud dextrose agar

Discussion

We describe a case of invasive brain infection due to *A. glaucus* in an immunocompetent patient. *A. glaucus*, a member of the genus *Eurotium*, is a mold that belongs to the *Aspergillus glaucus* group. The three most common species of this group are *A. glaucus* (teleomorph *Eurotium herbariorum*) characterized by large conidia, *Aspergillus hollandicus* (*Eurotium amstelodami*), and *Aspergillus chevalieri* (*Eurotium chevalieri*) with small conidia [15]. Members of the *A. glaucus* group can generally be distinguished from other *Aspergillus* spp. by the green and yellow coloration of their colonies with the prominence of numerous yellow sectors of cleistothecia, which could not be identified in our case. In contrast to *A. fumigatus*, *A. glaucus* grows slowly on potato dextrose agar with 20% sucrose at 25°C and its growth is inhibited at 35°C. The fungus is widely distributed in the environment and is often isolated from soil, plants, house dust, and dried food [15, 16].

The route of infection in our patient is unclear. Despite the frontal location of the lesion, he had no evidence of sinus disease on CT scan. No other sites of aspergillosis were detected. Moreover, no factors predisposing the patient to invasive aspergillosis such as diabetes mellitus, recent intake of corticosteroids, or antibiotics could be found. In addition, his neutrophil count was normal and serological testing for HIV antibodies was negative. The patient denied any history of drug abuse including sniffing drugs and denied any unusual practices such as inhalation of incense, a well-known practice in the region. It is highly likely that infection may have resulted from inhalation of spores while swimming in a lake 1 week before the onset of his symptoms. *A. glaucus* has been previously isolated from dental waterlines and hospital ducts [8, 9]. The mechanism by which it causes invasive disease in immunologically competent patients is unknown. Case series of craniocerebral aspergillosis in immunocompetent hosts have been reported mainly from Pakistan, India, Saudi Arabia, Sudan, and other African countries [7, 17]. Such increased prevalence is thought to be due in part to tropical environmental conditions, bad hygiene, and poor socioeconomic status. Our patient was Syrian but lived in the United Arab Emirates for several years. Whether the dry and hot climatic conditions contributed to his illness is unclear.

Culture of *Aspergillus* species from brain tissue is often difficult [6]. Of 25 immunocompetent patients with cerebral aspergillosis, only 15 had positive cultures [5]. The culture of *A. glaucus* is more difficult since the organism requires at least 7 days to grow on Sabouraud dextrose agar unless the media is enriched with sucrose. Such a characteristic makes the diagnosis of *A. glaucus* infection more complex in the absence of a high index of clinical suspicion. In our case, *A. glaucus* grew after 26 days of incubation. The diagnosis of cerebral aspergillosis is made by histopathological examination showing fungal hyphae with dichotomous branching at narrow angles [5]. Other features described in immunocompetent patients include the presence of noncaseating granulomatous inflammation with epithelioid and multinucleated giant cells as seen in our case.

Treatment of cerebral aspergillosis is disappointing with a mortality rate reaching 99% [18]. The new triazole, voriconazole, has been shown to be more potent than amphotericin B deoxycholate in the treatment of CNS aspergillosis due to excellent drug concentration in the CSF [19]. The poor outcome in our patient is likely related to a low CSF level of amphotericin B or itraconazole. Response to amphotericin B and itraconazole occurred in only 3 of 34 patients with CNS aspergillosis [20]. In vitro susceptibility of *A. glaucus* to various antifungal agents has not been well studied. The organism appears to be susceptible to amphotericin B, itraconazole, and voriconazole with low minimal inhibitory concentration compared to other *Aspergillus* species. However, there is no clear definition of the breakpoints for the different antifungal agents [21]. Despite the fact that the new antifungal class of echinocandin drugs such as caspofungin, micafungin, and anidulafungin has excellent in vitro activity against most *Aspergillus* species and is useful in treating pulmonary *Aspergillus* infections, these drugs typically lack good CNS penetration and should not be used as the sole antifungal drug to treat CNS aspergillosis [22].

We report the first case of a rare emerging pathogen, *Aspergillus glaucus*, causing brain infection in an immunocompetent patient identified by sequencing of the ribosomal 18S–28S internal transcribed spacer. This case highlights the diagnostic and management challenges presented by CNS aspergillosis and underscores its grave prognosis.

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