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CONCISE ARTICLE

# *Alternaria infectoria* brain abscess in a child with chronic granulomatous disease

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**Abstract** In the present report, we describe the first case of a phaeohyphomycotic brain abscess in a 5-year-old boy with chronic granulomatous disease (CGD) admitted to hospital with seizures. A computed tomography (CT) scan revealed a cerebral abscess and the microbiology study showed a dark, melanin-pigmented fungus, exhibiting only sterile hyphae. This fungus was identified by the amplification and sequencing of the 5.8S RNA gene and of the adjacent internal transcriber spacer domains, ITS1 and ITS2, as *Alternaria infectoria*. Due to the impossibility of a surgical excision, and although several therapeutic strategies were attempted, the patient died. Limitations in the routine identification procedures and therapeutic options of this emerging opportunistic agent are highlighted in this report.

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## Introduction

Chronic granulomatous disease (CGD) is the most common inherited disorder of neutrophil function. The most frequent form of the disease is a mutation in the X chromosome, known as the X-linked form, representing 65% of cases, with the autosomal recessive form representing 35% [1]. Phagocytes from patients with CGD are unable to generate superoxide anions and other microbicidal oxygen metabolites, resulting in severely impaired intracellular killing of catalase-positive micro-organisms. As a result, patients with CGD have an increased susceptibility to bacterial and fungal infections [1, 2]. Common bacterial pathogens include *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcescens* and *Nocardia* species. Fungal pathogens include, particularly, *Aspergillus fumigatus* and *Candida albicans* [2]. These infections usually involve the skin, soft tissues, lung, nodes, liver, spleen or bones. Brain abscesses are exceptional in patients with CGD [3].

The genus *Alternaria* includes numerous species of melanised dematiaceous fungi causing diseases in plants. Two saprobic species are commonly occurring on plants, in soil, food and indoor air environments, and these are increasingly found as aetiologic agents of human disease [4]. Initially, *A. alternata* was frequently reported [5]. *A. infectoria* is a rare opportunistic agent of phaeohyphomycosis [6], although the number of reported cases increased with immunocompromised patients. The case reported here describes a rare pathology, cerebral alternariosis due to *A.*

*infectoria*, in an immunocompromised child and outlines the clinical and laboratory difficulties that we encountered during the course of this case.

## Case report

In August 2003, a 5-year-old boy with CGD was admitted to the hospital after a short-time focal seizure involving the right upper limb. The patient was clinically well, except for sinusitis. A computed tomography (CT) scan revealed a cerebral abscess in the left parietal dorsally frontal cortex, with a vasogenic oedema (Fig. 1). Eighteen months earlier, he had successfully been treated for a pulmonary aspergillosis and had remained under daily prophylaxis with trimethoprim/sulphamethoxazole (400 mg per day) and itraconazole (10 mg/kg of body weight per day). After the seizure, the clinical examination was normal, except for a giant lymphadenopathy at the left axilla. This lymphadenopathy had first been observed at the age of 6 months, when it had the same volume, and it was never drained on the assumption that a reaction to BCG was concerned, as is known for other CGD patients [7]. The CT scan also revealed a sinusitis, confirming the clinical observation.

Therapy was started with liposomal amphotericin B, 3 mg/kg, intravenously every day, interferon gamma (IFN-γ; 10 subcutaneous administrations; 75 μg/m$^2$/day, three times a week), methylprednisolone (day 26, 1.5 mg/kg/day) and intravenously administered antibiotics (vancomycin, 50 mg/kg/day, twice a day; ceftazidime, 100 mg/kg/day, every 8 h). Microbiological parameters included blood culture, culture of cerebrospinal fluid (CSF) and blood aspergillosis serology (precipitin test). The microbiological analysis yielded a negative result. On hospital day 3, a brain magnetic resonance imaging (MRI) scan confirmed the previous diagnosis and showed a left brain lesion with a focal region surrounded by a vasogenic oedema (Fig. 2).

One month later, the purulent material of the brain abscess was aspirated and sent to the microbiology laboratory. Culture from this material revealed a dark

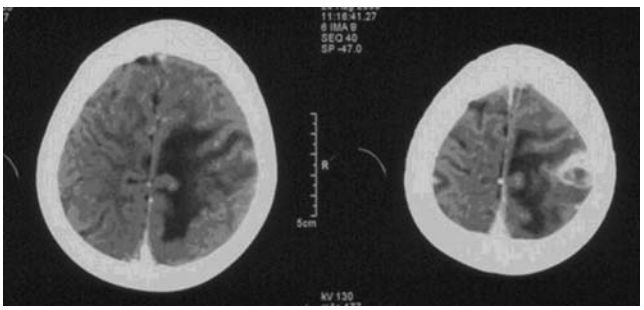

**Fig. 1** Brain computed tomography (CT) scan of the 5-year-old boy with chronic granulomatous disease (CGD), taken on hospital admission

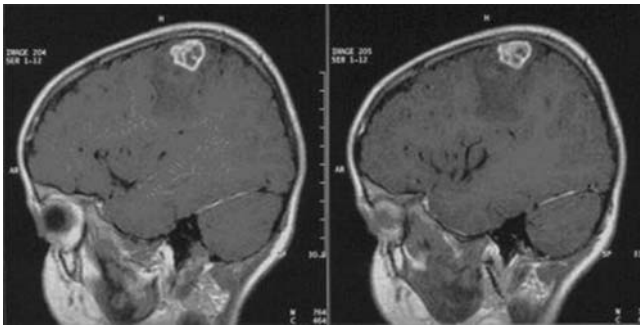

**Fig. 2** Brain magnetic resonance imaging (MRI) scan taken at day 3, showing a unifocal infection surrounded by a vasogenic oedema

pigmented fungus, exhibiting sterile hyphae only. At that time, it was impossible to identify the fungus, but judging from the melanised character, a recurrence of invasive aspergillosis was excluded. This result prompted a change in therapy. Amphotericin B and antibiotics were replaced by voriconazole (8 mg/kg, intravenously, twice a day) and caspofungin (50 mg per day). In the course of this therapeutic strategy, the patient's condition gradually improved. Nevertheless, his condition deteriorated after 117 days of voriconazole and 97 days of caspofungin therapy. Upon the diagnosis of a hydrocephaly, a ventricular reservoir was placed, and some liquid was removed daily, in order to decrease the intracranial pressure. Therapy with intrathecal 5 mg of caspofungin was started on a daily basis. However, the child became critically ill and died after 144 days of voriconazole, 124 days of caspofungin, 60 days of IFN-γ and 12 days of intrathecal caspofungin.

Several attempts were made to identify the dematiaceous fungus on the basis of classical morphological features. This approach was unsuccessful, since, in our routine setting, it was not possible to optimise the conditions for

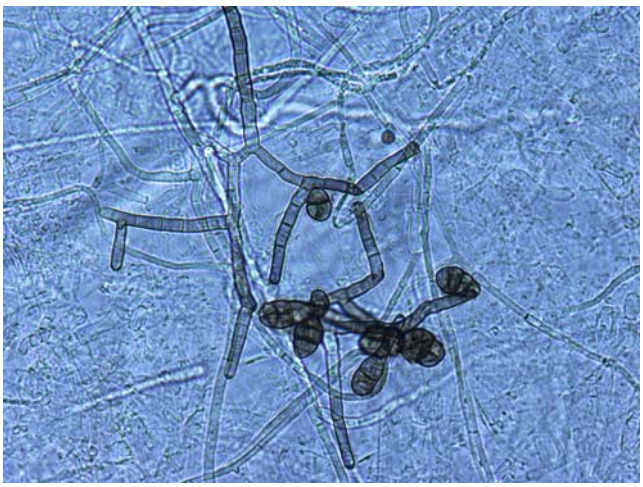

**Fig. 3** Morphology of the *Alternaria infectoria* isolate obtained from the purulent material of the brain abscess. The fungus was grown during 12-h alternating light–dark incubation on corn meal agar

sporulation, and, hence, morphological key features for identification remained absent. Amplification of the rDNA ITS region was performed using the primers ITS1 5′-TCC GTAGGTGAACCTGCGG-3′ and ITS4 5′-TCCTCCGCTT ATTGATATGC-3′. The fragment obtained was sequenced in an ABI Prism 310. Using comparable sequences available in GenBank and a dedicated database maintained at CBS, the fungus was identified as *A. infectoria*. Following this identification, the fungus was grown in 12-h alternating light–dark incubation on corn meal agar, and scarce conidia, characteristic of *A. infectoria*, were obtained (Fig. 3). The fungus also proved to be catalase-positive.

## Discussion

The 5-year-old patient with CGD reported here developed a brain abscess due to a melanised, catalase-positive fungus, *A. infectoria*. The genus *Alternaria* belongs to the order *Pleosporales*; their rapidly growing anamorphs are classically referred to as *Dematiaceae* [8]. A PubMed search revealed 11 human clinical cases by *A. infectoria* to date [6, 9–18]. With the growing number of immunocompromised patients, cases of phaeohyphomycosis are increasingly being reported, and among these are cerebral phaeohyphomycosis. In the majority of such infections, *Cladophialophora bantiana* and related *Chaetothyriales* are causative agents [19]. *Alternaria* is otherwise predominantly involved in traumatic, (sub)cutaneous infections [5].

Brain abscesses are among the most life-threatening pathophysiological processes. The origin of the infection can either be located in a neighbouring focus or it may be spread via haematogenous dissemination [20]. Rhinocerebral phaeohyphomycosis is mostly restricted to the environmental genera *Bipolaris* and *Exserohilum* as a secondary extension from the sinus [19]. In the present case, one of the clinical complaints at admission was a sinusitis, confirmed by CT, leading us to the conclusion that the focus of infection was in the sinuses. Sinusitis has been reported as an underlying predisposing condition for the development of a brain abscess, especially in young patients [20]. Hence, the most likely route of infection in the case of cerebral phaeohyphomycosis described here is inhalation rather than trauma [21], caused by *A. infectoria*. Another relevant aspect about this case is the fact that CGD patients are more vulnerable to catalase-positive microorganisms [2] and, in fact, the aetiological agent of the brain abscess described here is catalase-positive.

The species identification of *Dematiaceae* is somewhat difficult because of their special conditions required for sporulation and morphological variability. Methods based on the analysis of the internal transcriber spacer (ITS) rDNA region [13, 14] are very useful in the identification of dematiaceous fungi, particularly because clinical strains of this group tend to be morphologically degenerate. The strain of *A. infectoria* isolated from this patient showed only very few conidia, a characteristic of this species of the genus *Alternaria* [18], and could only be recognised by rDNA ITS domain sequencing.

In a review published during 2004 [21], amphotericin B was listed as the drug of choice for the therapy treatment of melanised fungi, combined with itraconazole. The overall mortality was, nevertheless, 73%, although surgical intervention showed improved outcomes, but only when the brain lesion could be excised completely [21]. In our case, the initial therapy with liposomal amphotericin B combined with voriconazole proved to be unsuccessful. Given the fact that the patient had CGD as an underlying disease, and surgery would have a high risk of infection with catalase-positive bacteria such as *S. aureus*, the decision was taken to not operate. Interferon gamma (IFN-γ) has been reported to be useful in the treatment and prophylaxis of patients with CGD, reducing the incidence of infection and mortality by a mechanism that is not yet well understood [1]. It has been combined successfully with antifungal therapy against infections of the central nervous system by filamentous fungi [3]. The combined therapy used as the last resource, caspofungin and voriconazole, was proven to be effective in the eradication of fungal pneumonia [22] and of the brain abscess due to fungi [23]. The immunological impairment of the patient reported here has probably accounted for the unsuccessful therapy.

This case emphasises the inherent difficulty of managing infections caused by unusual fungal opportunistic agents, which are difficult to identify by routine laboratory techniques, and having poor prognosis when innate immunity is severely impaired.

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