

Aspergillus terreus Causing Probable Invasive Aspergillosis in a Patient with Cystic Fibrosis

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Abstract *Aspergillus terreus* may colonize the airways of patients with cystic fibrosis (CF). Whether this merits antifungal treatment is still unclear due to heterogeneous reports regarding its pathogenic potential. Although allergic manifestations are documented, invasive aspergillosis (IA) caused by *A. terreus* has not been described. We present here one case of probable IA caused by *A. terreus*, highlighting its role as an emerging agent of IA in CF patients. Voriconazole was effective, and the patient was healthy after 12 weeks of treatment. Serial determination of serum galactomannan level and periodic cultures from respiratory specimens may help in monitoring CF patients by identifying such pathogens. Further studies on the fungal species colonizing the CF airways are warranted, especially in India. This case report suggests *A. terreus* as a potential agent of IA which should not be ignored, particularly in this patient group.

Keywords *Aspergillus terreus* · Cystic fibrosis · Invasive aspergillosis · Emerging

Introduction

Cystic fibrosis (CF) is a multi-system disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene [1]. Defective ion transport causes mucociliary clearance defects, and viscid secretions promote fungal spore entrapment. Highly diverse fungi can colonize the CF airway, and among *Aspergillus* species, the most common is *A. fumigatus* [2]. The most relevant clinical presentation of *Aspergillus* species in CF is allergic bronchopulmonary aspergillosis (ABPA), which results from an immunological hypersensitivity to the fungus [3]. However, there are few reports of invasive aspergillosis (IA) in these patients, predominantly due to *A. fumigatus* [4–6]. Although *A. terreus* is reported to cause IA in immunocompromised patients including lung transplant recipients [7], its role in causing invasive disease in CF patients is still unclear. In the Indian scenario, *A. terreus* is predominantly reported from environmental samples; however, infections in humans are also described infrequently. To the best of our knowledge, the case reported herein is the first report of *A. terreus* causing IA in CF patients from India.

Case: An 8-year-old male presented to our center with fever and cough with mucopurulent sputum.

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Preliminary investigations showed eosinophilia (23%), while hemoglobin, leukocyte count, serum electrolytes, liver and renal function tests were normal. Bacteriological examination of sputum was not significant. Contrast-enhanced computed tomography (CECT) and magnetic resonance imaging (MRI) of chest revealed cystic bronchiectasis with perihilar lymphadenopathy.

The child was compound heterozygote for *CFTR* gene mutation (F508del mutation/R177H) and had mildly elevated sweat chloride levels (40–50 meq/L) fitting into the category of possible CF as per the Cystic Fibrosis Foundation consensus report [8]. He received inhalational steroids for 6 months at another center where he was misdiagnosed as a case of asthma.

In view of an elevated total serum IgE (> 500 IU/mL), positive (Type 1) skin test for *A. fumigatus* and the presence of infiltrates on chest X ray a diagnosis of CF with allergic bronchopulmonary aspergillosis (ABPA) was made as per the Consensus Conference proposed minimal diagnostic criteria, instead of the classical criteria by Stevens et al. [9]. It may be referred to as an incomplete form of ABPA. Serial IgE levels showed a decrease from > 10,000 to 3750 kIU/L, suggesting response to treatment with low-dose inhaled steroids.

Presently, total serum IgE was elevated at 7997 kIU/L, *A. fumigatus* specific IgE was 52.8 kUA/L, and immunodiffusion test was negative for *Candida albicans*, *A. flavus*, *A. fumigatus* and *A. niger*. The serum galactomannan index (GMI) was 0.46, and in view of clinical worsening, oral voriconazole was advised. Fungal culture of sputum grew *A. terreus* after 1 week which was confirmed by DNA sequencing using primers targeting the ribosomal internal transcribed spacer (ITS) regions (National culture collection of pathogenic fungi; NCCPF ID 80054). Subsequent evaluation revealed a rise in serum GMI to 2.15 with clinical worsening; thus, the patient was admitted to our hospital and intravenous voriconazole was initiated. Bronchoalveolar lavage (BAL) fluid microscopy showed septate and hyaline hyphae, and BAL culture also grew *A. terreus*. Antifungal susceptibility testing (AFST) of both isolates was performed by microbroth dilution method as per Clinical laboratory standards institute (CLSI)-M38A2 guidelines [10]. Briefly, the working solutions of antifungal drugs were prepared in RPMI 1640 medium with L-glutamine. The conidial suspensions obtained from

sporulated *A. terreus* cultures were adjusted, 100 µL of the final conidial suspension was added to 100 µL of each antifungal drug, and the microplates were incubated at 35 °C. The isolate was susceptible to all tested antifungals with minimum inhibitory concentrations (MICs) of 1 µg/mL for amphotericin B, 0.062 µg/mL for fluconazole, voriconazole, itraconazole and posaconazole, and of 0.5 µg/mL for caspofungin, anidulafungin and micafungin.

Intravenous voriconazole was continued, after 15 days, clinical improvement was noted, and he was discharged on intravenous voriconazole. Repeat sputum evaluation after another 2 weeks was sterile, and the child was healthy after 12 weeks of antifungal treatment.

Discussion

Although colonization of CF lung by *Aspergillus* species causes reduced lung function and frequent hospitalizations, studies on the requirement of antifungal intervention in such cases show heterogeneous results [11]. Due to side effects of giving antifungals and potential drug resistance, further studies are required to ascertain if fungal colonization in this group merits treatment.

Usually *Aspergillus* species do not cause IA in CF patients as seen in immunocompromised patients such as those with hematological malignancies, transplant recipients, etc. In developing countries such as ours, *A. fumigatus* is the most common causative agent of IA in immunocompromised patients while *A. flavus* is frequently isolated from cases of sino-orbital aspergillosis [12]. *Aspergillus terreus*, which is a common environmental fungus in India, is emerging as an agent causing progressive disease in immunocompetent and immunocompromised patients [13]. Various studies have reported *A. terreus* to colonize to CF airways [14, 15]; however, no data are available regarding its ability to cause airway colonization or pulmonary invasion in CF patients from India.

The patient described herein was not immunocompromised per se except prolonged use of steroids; however, CFTR mutations have been suggested to cause mucosal immunodeficiency by affecting innate immune functions and contributing to oxidative stress in the airways [16]. This local dysregulation of immune function could allow colonization and

subsequent invasion by fungal pathogens. This possibility was seen in another 5-year-old male at our center, with homozygosity for the F508del mutation but no immunodeficiency, who presented with productive cough and respiratory distress. His chest radiograph showed multiple fibronodular opacities bilaterally, and two sputum samples grew *A. terreus* (NCCPF number: 860055) confirmed by DNA sequencing. He also had an elevated serum GMI of 0.63 and 0.83 recorded on two occasions and showed clinical improvement on treatment with intravenous voriconazole. Despite the lack of any obvious immunodeficiency, this could be a case of IA caused by *A. terreus* due to mucosal immunodeficiency resulting from the *CFTR* mutation. The clinical relevance of this argument, however, is debatable since *CFTR* mutations are seen in all patients with CF, but IA is rarely reported.

CF heterozygote carriers are generally disease-free although links have been suggested between F508del heterozygosity and increased bronchial reactivity or asthma. Our case being a F508del heterozygote did not have the typical manifestations of CF, and sweat chloride levels were < 60 meq/L throughout the observation period. Compromised local immunity due to prolonged inhaled steroids may have contributed to the airway colonization by *A. terreus* in our patient. Steroids are known to promote *A. fumigatus* growth, and an increased risk of *A. fumigatus* isolation in CF patients treated with inhaled steroids has been previously reported [17]. A study by Warren et al. [18] evaluating the levels of serum galactomannan in CF patients revealed negative serum galactomannan in CF patients who were merely colonized by *Aspergillus*. The elevation of GMI in the patients herein is therefore suggestive of endothelial invasion. Thus, host factor of immune deficiency, chest radiography findings, microbiological evidence (positive cultures and elevated GMI) and clinical response to voriconazole indicate probable invasive aspergillosis in this case.

Aspergillus terreus may exhibit intrinsic or acquired resistance to amphotericin B, and a lower response to antifungals is observed with higher mortality compared to non-*terreus Aspergillus* species [19]. However, voriconazole administration for 12 weeks was found beneficial in our patient. *Aspergillus terreus* is therefore a cause of concern in patients with CF, particularly those with ABPA or on prolonged corticosteroid therapy. Serum GMI can also

serve as a valuable test for the evaluation of such cases.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Standards This study was based on retrospective data analysis and did not include any intervention besides standard of care during hospitalization.

Informed Consent Informed consent was obtained from the parents of the patient included in this study.

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