A rare case of allergic bronchopulmonary mycosis caused by *Alternaria alternata*

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A rare case of allergic bronchopulmonary mycosis (ABPM), caused by *Alternaria alternata*, is reported in an immunocompetent resident of Delhi. Her complaints included a generalized, urticarial skin rash and occasional pain in the right lower chest. Her differential count showed eosinophils, 22%; absolute eosinophil count (AEC), 2400 cells/μl; and total IgE, 4007 IU/ml. The computerised tomogram (CT) scan of her thorax showed an enhancing lesion with surrounding ground glass haziness in the right lower lobe. Histopathologic examination of the resected lung revealed a necrotizing granulomatous inflammation, parenchymal infiltration by eosinophils, lymphocytes, neutrophils, plasma cells and some exudative bronchiolitis suggestive of ABPM. Observation of KOH wet mounts of repeat sputum and BAL samples demonstrated the presence of septate, brownish hyphae and cultures of these specimens yielded *A. alternata* (identified by sequencing of the ITS region). Her serum showed a three-fold higher specific IgE to *A. alternata* antigens than control levels, and the type I cutaneous hypersensitivity response to antigens of *A. alternata* was strongly positive. She was treated successfully with oral glucocorticoids and itraconazole. To our knowledge, ABPM due to *Alternaria alternata* has not been reported previously.

**Keywords** allergic bronchopulmonary mycosis, *Alternaria alternata*, India

**Introduction**

Allergic bronchopulmonary mycosis (ABPM) is a hypersensitivity mediated disease of the lower airways with a worldwide distribution. Although *Aspergillus fumigatus* is the most common etiologic agent [1], it may be sporadically caused by members of the genera *Candida, Curvularia, Bipolaris, Drechslera, Helminthosporium, Scedosporium, Schizophyllum* and *Stemphyllium* [2–10]. The criteria for diagnosis of ABPM are essentially the same as proposed for allergic bronchopulmonary aspergillosis (ABPA) by Rosenberg *et al.* [11]. The primary criteria include: episodes of bronchial obstruction (asthma), blood eosinophilia (>1000/mm³), type I cutaneous hypersensitivity to antigens of the etiologic fungus, serum precipitins against the offending fungal antigens, elevated total serum IgE (>1000 IU/ml), elevated serum IgE/IgG antibodies specific to the etiologic fungus and a history of pulmonary infiltrates (transient or fixed) on chest radiographs or CT scans or central bronchiectasis on chest CT scans. The secondary diagnostic criteria include: demonstration of the etiologic fungus in sputum/bronchial aspirate by microscopy and culture, history of expecoration of mucus plugs or flecks and an Arthus reaction (type III hypersensitivity).
to the fungal antigens. As in ABPA, the minimal essential diagnostic criteria used for ABPM are the presence of asthma, a type I skin reaction to the etiologic fungus, total serum IgE levels greater than 1000 IU/ml, elevated serum IgE and IgG levels to the offending fungal antigens and central bronchiectasis. However, since ABPA can occur in asymptomatic patients or can be masked by steroid therapy, it has been proposed that in the absence of central bronchiectasis, the demonstration of total serum IgE, specific IgE/IgG and a type I cutaneous hypersensitivity should suffice to establish a diagnosis of ABPA [12], which holds equally true for ABPM in such cases.

There are a number of reports implicating *Alternaria alternata* in the etiology of allergic rhinosinusitis [13,14], bronchial asthma [15], hypersensitivity pneumonitis [16], ocular mucositis [17], onychomycosis [18] and skin infections [19,20]. Besides, in immunocompromised patients the fungus has been frequently reported as the etiologic agent of cutaneous infections in lung transplant recipients [21,22], granulomatous pulmonary disease [23], cerebral and disseminated mycosis [24]. Recently, we reported a rare case of ABPM due to *Bipolaris hawaiensis* [5]. Herein, we report the first case of ABPM, in an immunocompetent host which was caused by *Alternaria alternata*, another dematiaceous fungus.

**Case report**

A 36-year-old housewife from suburban Delhi, India, was referred to Vallabhbhai Patel Chest Institute in March 2010, with complaints of a generalized, urticarial skin rash recurring periodically for the last five years and occasional pain in the right lower chest and flank of a month’s duration. She gave no history of fever, myalgia, arthralgia, rhinorrhoea or other prodromal features suggestive of a viral illness. She had been taking oral H1 anti-histamines during episodes of urticaria but no systemic steroids or any other immunosuppressive drugs. Upon examination, she was found to have pallor, tenderness of the right renal angle and bilateral vesicular breath sounds with reduced intensity over the right infrascapular area, associated with crepitations. Examination of her previous records showed haemoglobin – 11.9 g/dl, differential count – 22% eosinophils, absolute eosinophil count (AEC) – 2400 cells/μl and a total IgE level of 4007 IU/ml. The patient had normal blood sugars and tested negative for human immunodeficiency virus (HIV) 1 and 2. Her CD4:CD8 cell ratio was normal. Stool test for parasite/ova and blood cultures were negative as were c-ANCA and p-ANCA done to investigate vasculitides. Her chest X-ray was unremarkable but CT studies of the thorax revealed a non-homogenously enhancing lesion with surrounding ground glass haziness in the right posterior and lateral basal segments of the right lower lobe (Fig. 1).

Bacteriological cultures of broncho-alveolar lavage (BAL) and transbronchial lung biopsy performed on 4 February 2010 were negative for pyogenic organisms and acid fast bacilli. Histopathologically, the biopsy showed a moderate interstitial inflammatory infiltrate, comprising lymphocytes, plasma cells and eosinophils, with focal bronchiolar and interstitial fibrosis, an ill-formed giant-cell granuloma with foamy cells and type II pneumocyte hyperplasia. The patient underwent a video-assisted thoracoscopy on 5 March 2010, which showed her right lung adherent to the underlying diaphragm. She was subjected to right posterior-basal segmentectomy. Histopathological examination of resected lung revealed replacement of bronchi/bronchioles by a necrotizing granulomatous inflammation. Adjacent pulmonary arteries were seen to have chronic inflammation while pulmonary parenchyma showed mixed inflammatory infiltrate consisting of eosinophils, lymphocytes, neutrophils and plasma cells. Some of the bronchioles had exudative bronchiolitis. These features were suggestive of ABPM (Fig. 2A). Post-operatively, the patient was discharged six days later with supportive treatment and referred to the Medical Mycology Department, VPCI, for myco-serological investigations to rule out a possible fungal etiology. When queried about the condition of her house, she reported the occurrence of dampness and moldy patches on the walls due to leakage of water pipes. However, no mycological sampling of the house was possible because it had been demolished in the interim.

**Mycoso-serologic investigations**

Direct microscopy of KOH wet mounts of serial sputum and BAL specimens collected on 29, 30 and 31 March 2010 consistently revealed septate hyphae with a brownish tinge (Fig. 2B). Sabouraud dextrose agar (SDA) plate cultures inoculated with portions of sputum and BAL yielded
multiple, powdery, olivaceous, mould colonies after three days of incubation at 28°C and 37°C (Fig. 2C). Observations of slide cultures of the isolate on potato dextrose agar (PDA) revealed unbranched, septate, conidiophores, bearing brown, multisepalate, muriform conidia with a characteristic beak (Fig. 2D) compatible with *Alternaria alternata*. Specific IgE binding of the patient’s serum collected on 30 March 2011 against the culture extract antigen from the patient’s *A. alternata* isolate showed an elevated IgE level which was >3-fold higher than that of the normal control.

**Specific IgE estimation by Enzyme-linked Immunosorbent assay (ELISA)**

*Alternaria alternata* extract was prepared and standardized as described earlier [25]. Specific IgE in the patient’s sera was determined by ELISA as previously reported [26]. Briefly, a microtitre plate (Nunc-ImmunoTM modules, Roskilde, Denmark) was coated with *A. alternata* extract (1 µg/100 µl/well) in carbonate buffer, pH 9.6, blocked with 3% defatted milk, washed with PBST (0.1 M PBS containing 0.2% Tween 20) and incubated with the patient’s serum or control sera (1:10 v/v) at 4°C overnight. The plate was then incubated with 1:1000 v/v anti-human-IgE peroxidase (Sigma, St. Louis, USA), the reaction was stopped by 3 M H₂SO₄ and color developed with o-phenylenediamine and read at 492 nm in an ELISA reader. ELISA was performed in triplicate and the mean of three readings taken for analysis. Normal human sera (pooled) were used as negative control.

**Skin testing**

The patient underwent an intradermal skin test with the culture filtrate antigens (concentration 0.02 ml, 1/100 w/v) of her *A. alternata* isolate. A strong, type I hypersensitivity response (induration diameter, 12 × 10 mm) was observed.
but there was no Arthus reaction (type III) or delayed hypersensitivity response. The positive control included in the test was histamine phosphate (base; 100 μg/ml) which gave an induration of 4×5 mm, while a 1×1 mm induration was seen with the negative control (buffered saline). The patient also underwent a skin prick test (SPT) with other fungal antigens available to us, i.e., Aspergillus fumigatus, A. flavus, A. niger, A. terreus, Curvularia lunata, Bipolaris hawaiiensis, Schizophyllum commune and Scedosporium apiospermum (Allcare Pharma Pvt. Ltd, Delhi, India) but with negative results.

Based upon the aforementioned investigations, the patient was diagnosed as a case of ABPM and treated with steroids and antifungals. The number of A. alternata colonies growing in sputum cultures progressively declined and finally disappeared during therapy which was discontinued on 4 August 2010.

**Molecular identification of A. alternata**

The DNA extraction and amplification procedures for the ITS region (ITS-1, 5.8S rRNA and ITS-2 of rDNA) of the A. alternata isolate, were the same as described previously [5]. The amplicons were purified and both strands of amplified DNA fragments were sequenced. The sequencing reactions were carried out by using the cycle DNA sequencing kit (Big Dye Terminator v3.1 cycle sequencing kit RR100, Foster City, CA, USA) with ITS1, ITS4, ITS1FS, ITS2, ITS3 or ITS4RS as sequencing primers [5]. GenBank basic local alignment search tool (BLAST) searches (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi) were performed for species identification. The entire ITS region sequence (495 nucleotides) of our isolate (Gene Bank accession no. JN880415) exhibited 99% identity with the corresponding sequences of strain ATCC 66981 with accession numbers D38763.1 and several other strains of A. alternata with accession numbers JF835810.1, JF835811.1, and JF835812.1.

**Antifungal susceptibility testing**

Antifungal susceptibility testing (AFST) of three serial isolates of A. alternata originating from sputum and BAL specimens of the patient during her therapy was performed following the European Committee for Antimicrobial Susceptibility Testing (EUCAST) methodology [27]. The antifungals tested were amphotericin B (Sigma, St Louis, MO, USA), fluconazole (Pfizer, Groton, CT, USA), itraconazole (Lee Pharma, Hyderabad, India), voriconazole (Pfizer), posaconazole (Schering-Plough Corp., Kenilworth, NJ, USA now Astellas), isavuconazole (Basilea Pharmaceutica International AG, Basel, Switzerland), 5-flucytosine (Sigma), caspofungin (Merck & Co. Inc., Whitehouse Station, NJ, USA), micafungin (Astellas Toyama Co. Ltd, Toyama, Japan) and anidulafungin (Pfizer). For the EUCAST broth microdilution test, RPMI 1640 medium with glucose and 2% glucose but without bicarbonate (Sigma) buffered to pH 7.0 with 0.165M 3-N-morpholinepropanesulfonic acid (Sigma) was used. Final inoculum was adjusted to 1–2.5×10⁵ spores/ml by counting spores in a haemocytometer chamber. Drug-free and mold-free controls were included and microtiter plates were incubated at 28°C and 35°C for 48 h. The final concentrations of the drugs were 0.12–64 μg/ml for fluconazole and 5-flucytosine and 0.03–16 μg/ml for amphotericin B, itraconazole, voriconazole and 0.015–8 μg/ml for posaconazole, isavuconazole and echinocandins. Quality control strains, Candida krusei, ATCC 6258, and Candida parapsilosis, ATCC 22019, and reference strains, Aspergillus fumigatus ATCC 204305 and A. flavus ATCC 204304 were included with each test. The MIC end points were read visually which, for azoles and amphotericin B, were defined as the lowest concentration at which there was 100% inhibition of growth compared with the drug-free control wells. For echinocandins, minimal effective concentration (MEC) was defined as the lowest concentration of drug that led to the growth of small, rounded and compact hyphal forms. The results of susceptibility testing of the three isolates to amphotericin B, azoles and echinocandins are presented in Table 1.

**Therapy**

Prior to her referral to our Institute, the patient had been treated with oral levofloxacin in daily doses of 750 mg and amoxicillin and clavulanic acid combination in daily doses of 625 mg, three times a day, but her symptoms persisted. A presumptive diagnosis of pulmonary mycosis was made and the patient treated with oral itraconazole (200 mg daily in divided doses) and discharged on 14 April 2010. Her AEC and total IgE at that time were 1870 cells/μl and 3609. IU/ml, respectively. After the diagnosis of ABPM was established on 20 April 2010, the patient was put on oral glucocorticoids (prednisolone 25 mg daily for 15 days and thereafter on alternate days) in addition to itraconazole. A month after the initiation of therapy, the patient’s total IgE level showed a modest decline from 3609 IU/ml to 2743.5 IU/ml and went down further to 1290 IU/ml, two months later. However, her sputum cultures remained persistently positive for A. alternata. The patient was advised to continue prednisolone 25 mg on alternate days in addition to itraconazole. A month after the initiation of therapy, the patient’s total IgE level showed a modest decline from 3609 IU/ml to 2743.5 IU/ml and went down further to 1290 IU/ml, two months later. However, her sputum cultures remained persistently positive for A. alternata. The patient was advised to continue prednisolone 25 mg on alternate days in addition to itraconazole 200 mg in daily divided doses for another one month. When reviewed at the end of four months of therapy, she was found to be symptom-free and thus advised to discontinue all medication. Investigations carried out at this stage showed a total IgE level of 513.3 IU/ml and sputum cultures were negative for fungi.
Table 1  *In vitro* antifungal susceptibility of three isolates of *Alternaria alternata* by EUCAST at 28°C and 35°C.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>GM</th>
<th>Range</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>GM</th>
<th>Range</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
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<tr>
<td>AMB</td>
<td>0.79</td>
<td>0.25–2</td>
<td>1</td>
<td>2</td>
<td>0.79</td>
<td>0.25–2</td>
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<td>ITC</td>
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<td>0.25</td>
<td>0.39</td>
<td>0.25–0.5</td>
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<td>VRC</td>
<td>0.79</td>
<td>0.5–1</td>
<td>1</td>
<td>1</td>
<td>0.79</td>
<td>0.5–1</td>
<td>1</td>
<td>1</td>
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<td>4</td>
<td>4</td>
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<td>FLU</td>
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<td>64</td>
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<td>32–64</td>
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<td>32</td>
<td>64</td>
<td>40.3</td>
<td>32–64</td>
<td>32</td>
<td>64</td>
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<tr>
<td>CAS</td>
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<td>0.25–0.25</td>
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<td>0.25</td>
<td>0.123</td>
<td>0.06–0.25</td>
<td>0.125</td>
<td>0.25</td>
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<tr>
<td>MFG</td>
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<td>0.003–0.125</td>
<td>0.06</td>
<td>0.125</td>
<td>0.097</td>
<td>0.06–0.125</td>
<td>0.125</td>
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<tr>
<td>AFG</td>
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<td>0.06–0.25</td>
<td>0.125</td>
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<td>0.097</td>
<td>0.06–0.125</td>
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AMB, amphotericin B; ITC, itraconazole; VRC, voriconazole; ISA, isavuconazole; POS, posaconazole; FLU, fluconazole; 5-FC, 5-flucytosine; CAS, caspofungin; MFG, micafungin; AFG, anidulafungin

There were no adverse effects of medications as monitored by serial blood counts, liver function tests and tests for blood sugars. In addition, a bone densitometry performed at the outset and after the completion of treatment did not show osteoporosis. At the time of her last review on 30 March 2011, she was still asymptomatic and was carrying out her routine daily activities. Furthermore, her specific IgE levels against *A. alternata* had declined by 1.5-fold compared to the pre-treatment titers.

**Discussion**

To the best of our knowledge, this is the first case of ABPM due to *A. alternata* in an immunocompetent patient. However, another species of *Alternaria, A. infectoria*, has been implicated in a case of pulmonary phaeohyphomycosis in an immunosuppressed patient [28]. Our case is particularly noteworthy because the patient was not an asthmatic. Notwithstanding the fact that the diagnostic criteria required for ABPA/ABPM [11,29] include that the patient be an asthmatic, there are numerous cases on record of ABPA that were diagnosed in non-asthmatic subjects [30–35]. In this context, it is important to emphasize the relevance of ABPM in the differential diagnosis of hypersensitivity respiratory diseases in non-asthmatic subjects, especially in countries with high prevalence of pulmonary tuberculosis. It is very likely for such patients to be misdiagnosed and treated for tuberculosis. Lastly, one can hardly over-emphasize the importance of correct early recognition of this disease as that alone will guard against unwarranted clinical complications and potentially disastrous interventions.

The patient investigated was healthy, without any signs of immunosuppression. Given her presentation of chest pain and cough, she was initially suspected of having a malignancy, subjected to a video-assisted thoracoscopy and subsequently an unwarranted segmentectomy of the right posterior basal segment lobe. Histopathological examination of the resected specimen was consistent with the diagnosis of ABPM, and subsequently *A. alternata* was isolated in culture. The diagnosis of ABPM caused by *A. alternata* was based on her elevated levels of total IgE, peripheral eosinophilia, raised IgE antibodies specific to *A. alternata* which showed a three-fold rise in titre, a strongly positive type I cutaneous response to *A. alternata* and repeated culture of *A. alternata* from sputum and BAL. Her Ouchterlony’s immunodiffusion test was negative for *A. alternata*, as well as *A. fumigatus, A. flavus, A. terreus* and *A. niger*, possibly because precipitin assays are relatively insensitive, and might have been suppressed due to corticosteroid therapy [36]. Results of SPT were also negative with other fungal antigens available to us and likely to cross-react, i.e., *A. fumigatus, A. flavus, A. terreus, A. niger, C. lunata* and *B. hawaiensis*. The identification of the *Alternaria* isolate in the present study was based on phenotypic morphological characteristics and ITS sequencing. It seems pertinent to mention that the ITS variability within the genus is relatively limited and, therefore, it may be necessary to use a gene for the *Alternaria* major allergen, Alt a 1 sequences for higher resolution [37,38].

Our patient responded very well to combined steroid and itraconazole treatment. The three serial isolates of *A. alternata* had low MICs of itraconazole and posaconazole which is not always the case. Previous *in vitro* studies with a number of clinical *A. alternata* isolates showed lower MICs ranging up to 16 μg/ml for itraconazole and posaconazole [39]. The MIC ranges of our isolates against voriconazole (0.5–1 μg/ml) and posaconazole (0.125–0.5 μg/ml) are compatible with the results of other investigators [40,41]. In comparison to itraconazole, voriconazole is apparently not a good candidate for treatment of ABPM due to *Alternaria*.

*A. alternata* and other species of the genus are ubiquitous molds occurring in soil, air and decaying vegetable...
matter. Exposure of the population to this fungus is therefore likely to be a common occurrence. Considering that A. alternata has widespread environmental prevalence, hypersensitivity to Alternaria spp. among asthmatics is well known. It seems enigmatic that ABPM due to A. alternata has not been reported so far. Systematic studies on the role of Alternaria spp. as etiologic agents of ABPM are suggested to determine the extent of prevalence of this as yet little known clinical entity.

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