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Aspergillus bronchitis without significant immunocompromise

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***Aspergillus* bronchitis is poorly understood and described. We extracted clinical data from more than 400 referred patients with persistent chest symptoms who did not fulfill criteria for allergic, chronic, or invasive aspergillosis. Symptomatic patients with a positive culture or real-time PCR for *Aspergillus* spp. were reviewed. Seventeen patients fulfilled the selected criteria. Fourteen were women, with a mean age of 57 years (range 39–76). Sixteen of the patients had productive cough, eight had voluminous tenacious sputum, and seven had recurrent chest infections. Eight patients had Medical Research Council dyspnea scores of 4–5; 12 had bronchiectasis; and 13 patients grew *A. fumigatus*, 3 *A. niger*, and 1 *A. terreus*. Twelve of the 17 patients (71%) had elevated *Aspergillus* IgG (47–137 mg/L, mean 89.2) and 5 (29%) had elevated *Aspergillus* precipitins. Six of 12 (50%) had a major response to antifungal therapy and five of 12 (42%) patients relapsed, requiring long-term therapy. *Aspergillus* bronchitis is a discrete clinical entity in patients with structural lung disease but who are not significantly immunocompromised. It is distinct from asymptomatic fungal colonization and other forms of aspergillosis, and may respond to antifungal therapy.**

Keywords: mannose binding lectin; fumigatus; niger; precipitins; bronchiectasis; aspergillary

Introduction

Aspergillus spp. exhibit a range of interactions with the human airway, including colonization, mucosal invasion, and provoking an allergic response.[1–3] *Aspergillus* infections that are limited entirely or predominantly confined to the tracheobronchial tree are currently termed *Aspergillus* tracheobronchitis[4] and usually occur in immunocompromised patients. Manifestations of *Aspergillus* tracheobronchitis include pseudomembranous tracheobronchitis, ulcerative tracheobronchitis (in lung transplant recipients), mucoid impaction of the bronchi (often in allergic bronchopulmonary aspergillosis [ABPA]), and obstructing bronchial aspergillosis.[5–7] Superficial tracheobronchitis is also recognized in lung transplant recipients.[6] Persistent airway colonization is characteristic of ABPA[8] and is sometimes seen in patients with chronic obstructive pulmonary disease (COPD)[9] and asthma.[10] Nearly forgotten is the entity *Aspergillus* bronchitis in non- or mildly immunocompromised patients.

The first description of *Aspergillus* tracheobronchitis was in 1890, in a nonimmunocompromised three-year-old girl who died[11] (Table 1) (Fig. 1). The first report, akin to the reports we offer here, was from Hoxie and Lamar in Kansas City writing in 1912 and described extreme coughing and husky voice affecting two patients with airways containing material consistent with *Aspergillus* spp.[12] Other notable descriptions include those of Wahl, Schneider, Von Ordstrand, and Riddell and Clayton (Table 1).[14,16,18,20] In 1962, Symmers described *Aspergillus* bronchitis from a pathological

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Table 1. Landmark papers with regard to *Aspergillus* tracheobronchitis and bronchitis

Date	No of cases	Description	Current terminology	Reference
1890	1	Autopsy description of a 3-year-old who died after a 10-day pneumonic illness of white adherent patches on the trachea and bronchi that showed microscopic features most consistent with *Aspergillus* spp.	Invasive *Aspergillus* tracheobronchitis	Wheaton[11]
1912	2	Two adults with cough and either breathlessness or husky voice had multiple sputa showing hyphae, one culture negative the other non-sporulating mold. Fungous tracheobronchitis.	*Aspergillus* bronchitis	Hoxie & Lamar[12]
1926	1	Presence of mucous membranes of the bronchi with ulceration; bronchitic aspergillosis	Pseudomembranous *Aspergillus* tracheobronchitis	Lapham[13]
1928	1	Severe symptoms of cough and wheeze following environmental exposure, with *A. flavus* cultured from sputum, which remitted after some months and NaI treatment	*Aspergillus* bronchitis	Wahl[14]
1928	0	Complete classification of all bronchopulmonary fungal infections.	Many	Castellani[15]
1930	1	Single case without underlying disease of cough and mild haemoptysis with low grade fever over 11 years with 5-year remission	*Aspergillus* bronchitis	Schneider[16]
1936	0	Review of the earlier work		Fawcitt[17]
1940	1	Three week illness with cough, fever, night sweats, weight loss in a nurseryman, who had no prior history. Sporulating *A. fumigatus* in sputum and positive skin prick test. Parenchymal infiltrates from both hilum.	Acute *Aspergillus* bronchitis	Van Ordstrand[18]
1952	0	Description of aspergilloma (including one bronchial aspergilloma) and probably ABPA. Detailed review of prior literature.	Bronchial aspergilloma and ABPA	Hinson[19]
1958	12	Patients with bronchitis who grew *Aspergillus* (usually *fumigatus*) in their sputum	*Aspergillus* bronchitis	Riddell & Clayton[20]
1962	>5	The first real description of the anatopathological features of *Aspergillus* bronchitis. Virtually saprophytic growth in the mixture of bronchial secretion, low grade inflammatory exudate and desquamated epithelial cells. Often conidiophores are found growing in the airways. Specially stained preparations "show very clearly that the fungus sometimes has a distinct attachment to the basement membrane (BM) of the chronically inflamed mucosa." The BM may become greatly thickened. Some of the hyphae end in single, vesicle-like expansions situated in contact with the thickened BM.	*Aspergillus* bronchitis	Symmers[21]
1964	35	All with *A. fumigatus* in their sputum, often heavy growths on many occasions. Long history of "bronchitis" with a productive cough. 25/35 (71%) had asthma. 0/28 were skin prick test positive. 11/35 (31%) had detectable *Aspergillus* precipitins. Aspergillary bronchitis.	*Aspergillus* bronchitis	Campbell & Clayton[22]

Continued

Table 1. ***Continued***

Date	No of cases	Description	Current terminology	Reference
1970	8	Autopsy series showing *Aspergillus* bronchitis in 8 mildly immunocompromised patients described as a "localized form of aspergillosis characterized by bronchial casts containing mucus and mycelia." Invasion of mucosa is uncommon but there may be superficial erosion. Aspergillary bronchitis.	*Aspergillus* bronchitis and invasive *Aspergillus* tracheobronchitis	Young[23]
1989	2	Neutropenic patients with bronchial mucosa showing superficial erosions and ulcerations	Invasive *Aspergillus* tracheobronchitis	Berlinger & Freeman[24]
1991	6	Ulceration and invasion of cartilage in lung transplant recipients, especially around the anastomosis	Ulcerative *Aspergillus* tracheobronchitis	Kramer[5]
1991	3	HIV infected patients with obstruction of their airway with mucous containing abundant mucous, without ulceration or invasion	Obstructing (obstructive) *Aspergillus* tracheobronchitis	Denning[25]
1993	4	Severely immunocompromised AIDS, 3 with diffuse tracheobronchitis, multiple ulcerative or "plaque-like" inflammatory lesions, and occasionally nodules involving the mainstem and segmental bronchi. One patient had a single deep ulceration of the proximal trachea. There was variable invasion of the mucosa, submucosa, and cartilage and one had evidence of disseminated aspergillosis.	Invasive *Aspergillus* tracheobronchitis	Kemper[26]
1995	0	Review and classification with ulcerative tracheobronchitis, pseudomembranous tracheobronchitis, invasive tracheobronchitis, obstructive tracheobronchitis, and *Aspergillus* tracheobronchitis	All	Denning[4]
2005	1	Invasive pulmonary aspergillosis in AIDS, transformed by antifungal therapy and immune reconstitution into fatal obstructing bronchial aspergillosis	Obstructing *Aspergillus* tracheobrochitis	Sambatakou[27]
2006	6	*Aspergillus* bronchitis in cystic fibrosis patients growing *A. fumigatus* in sputum, who did not fulfill the criteria for ABPA, or respond to antibiotics, who made a good response to antifungal therapy	*Aspergillus* bronchitis	Shoseyov[28]

perspective.[21] He described *Aspergillus* growing as sporing mycelium with mild inflammation of the mucosa and/or excess production of mucus without invasion of the mucosa. Campbell and Clayton described the clinical features of "aspergillary" bronchitis as repetitive isolation of *Aspergillus* spp., often heavy growth, without skin test reactivity, and sometimes positive *Aspergillus* precipitins (11/35 [31%]).[22] In an autopsy series, Young *et al*. found that 8 out of 98 (8%) patients had localized *Aspergillus* bronchitis confirmed histologically and characterized by bronchial casts containing mucus and mycelia.[23] All had superficial erosions and ulcerations and occurred in patients with less neutropenia and less exposure to corticosteroids or antineoplastic agents. Recently Shoseyov *et al.* described six cystic fibrosis (CF) patients with repeat positive sputum cultures of *A. fumigatus* and clinical deterioration, without strong evidence of sensitization to *A. fumigatus*,[28] who responded to antifungal therapy.

We noticed several patients with chronic symptoms who did not fulfill current diagnostic criteria for aspergillosis. Here, we describe the clinical

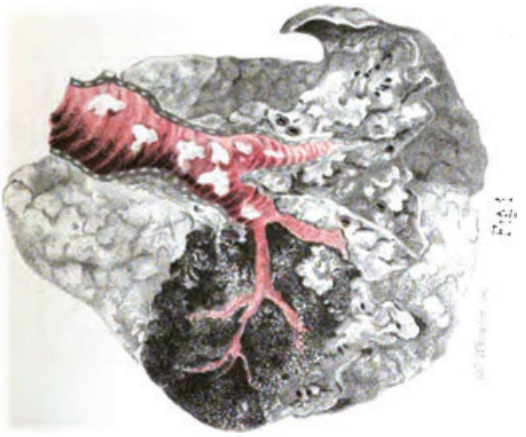

Figure 1. Drawing of the appearance of the airways from the autopsy of a 3-year-old girl with *Aspergillus* tracheobronchitis who was presumably not immunocompromised.

manifestations, laboratory findings, and treatment outcome of *Aspergillus* bronchitis. We propose criteria for the diagnosis of *Aspergillus* bronchitis.

We recognize that there may be an overlap with invasive *Aspergillus* tracheobronchitis in patients with no preexisting immunocomprise (such as those with severe influenza or given corticosteroids for asthma) and possible confusion with terminology, hence we suggest using bronchitis rather than tracheobronchitis to minimize the potential for confusion.

Methods

Study design

We reviewed the literature for all forms of *Aspergillus* infection of the airway, with a particular emphasis on literature before 1970. A retrospective, observational cohort study was performed. Chart reviews were undertaken of patients who did not fit current diagnostic criteria for allergic, chronic, and invasive aspergillosis identified over seven years from over 400 patients referred to the National Aspergillosis Center until May 2011. Demographic data, comorbidity, clinical presentations, radiological, bronchoscopic, microbiological, and serological findings were collected on patients identified to have *Aspergillus* bronchitis. One patient has previously been reported.[29]

Definitions

Aspergillus bronchitis was defined as (i) symptomatic chronic lower airway disease (symptoms of "chronic bronchitis"[30]), (ii) detection of *Aspergillus* spp. in sputum or BAL by culture or real-time validated PCR, and (iii) detection of IgG antibodies to *Aspergillus* spp. Patients were excluded if they fulfilled the diagnostic criteria for an established fungal-related disease (i.e., chronic pulmonary aspergillosis with or without aspergilloma (CPA), allergic bronchopulmonary aspergillosis (APBA), severe asthma with fungal sensitization (SAFS), invasive aspergillosis (IA)). Some patients with dual or triple *Aspergillus* diseases were excluded. Patients with known CF, neutropenia, or other established profound immune deficit (HIV/AIDS, systemic immunosuppressive agents apart from low-dose steroids to treat respiratory disease exacerbations) were also excluded. Bronchiectasis was defined as bronchial dilatation with respect to the accompanying pulmonary artery (signet ring sign), lack of tapering of bronchi, and identification of bronchi within 1 cm of the pleural surface.[31]

Data collection

Demographic data, underlying diseases, risk factors, and clinical presentations along with radiological, bronchoscopic, microbiological, and serological findings were correlated. Comorbidities were reviewed, with emphasis on immune status. Patients' breathlessness was assessed on the Medical Research Council (MRC) dyspnea scale.[32] Based on this scale, subjects are scored from 1 to 5 on a worsening scale of perceived breathlessness (1 is normal; 5 is severely breathless with trivial activity).

Laboratory methods

Aspergillus precipitin titers were measured using a long-established in-house precipitins IgG assay, as described elsewhere.[33] *Aspergillus* IgG and IgE and total IgE were tested by ImmunoCap® (Phadia, Sweden). Fungal culture of sputum was according to standard UK methods.[34] Sputum was digested with Sputasol (ratio 1:1), then vortexed and 10 μL streaked on two Sabouraud dextrose plates. Plates were incubated for seven days at 30 °C and 37 °C. DNA extraction from sputum was performed from 0.5 mL to 3 mL of sample immediately after the samples were received according to the MycXtra® fungal DNA extraction kit manual (Myconostica, Manchester, UK) using the BBL® Mycoprep™ Specimen Digestion/Decontamination kit (Becton Dickinson, Oxford, UK).[8] Real-time PCR was done with the commercially available MycAssay™ *Aspergillus* (Myconostica) assay.[8] Minimum inhibitory concentrations (MICs) to triazoles were

determined by EUCAST methodology as described previously.[35] Antifungal therapeutic drug monitoring was routine for all azole therapies as described elsewhere.[36,37] Serum MBL concentrations were determined by enzyme-linked immunosorbent assay (ELISA) (MBL Oligomer ELISA Kit, BioPorto Diagnostics, DK) with an upper and lower reported detection limit of 4.00 and 0.05 mg/L, respectively.[38]

Response to therapy

Clinical response to antifungal treatment was assessed by course after two months of therapy and at the end of treatment. We excluded patients who received less than four weeks of therapy from response analysis. Duration of treatment along with relapse rate and the need of retreatment were noted. Reasons for the end of therapy, including planned end of therapy, convincing good clinical response or discontinuation due to drug-related adverse events were reviewed.

Results

We identified 17 patients, 14 women, ranging in age from 30 to 76 years (mean 57) who fulfilled our criteria for *Aspergillus* bronchitis. Nearly all patients had concurrent pulmonary or airways disease, most commonly bronchiectasis ($n = 12/14$ (86%)). Six patients were on long-term oral prednisolone, three at $\geq$10 mg daily, one was receiving infliximab, and 12 (70%) were taking inhaled corticosteroids (Table 2). Numerous other comorbidities were seen; two patients had no evidence of underlying disease or were receiving immunosuppressive agents. Mannose binding lectin deficiency was found in 9/16 patients (56%) (Tables 2 and 3).

The clinical presentation was mainly that of persistent cough with sputum production, with frequently recurring exacerbations. Patients initially reported main complaints to be productive cough ($n = 16$, 94%), excessive tenacious sputum ($n = 8$, 47%), severely limiting shortness of breath (MRC dyspnea score $\geq$ 4) ($n = 8$, 47%), recurrent chest infections ($n = 7$, 41%), extreme fatigue/malaise ($n = 4$, 23%), weight loss more than 3 kg ($n = 3$, 17%), hemoptysis ($n = 1$, 6%), and mucoid impaction requiring urgent bronchoscopy ($n = 1$, 6%).

Bronchoscopy was undertaken in seven patients (Table 4). The bronchoscopic appearances varied widely, from localized areas of inflammation and contact bleeding through marked mucus plugging to near normality. Patient 6 had four bronchoscopies to remove tenacious sputum. The histological features of transbronchial biopsy of patient 2 are seen in Figure 2. Chest imaging including CT scanning was noncontributory other than demonstrating bronchiectasis and underlying lung pathology.

At least one, and usually multiple, respiratory cultures were positive in all 17 patients. *A. fumigatus* was grown in 13 patients, *A. niger* in three and *A. terreus* in one. Two patients grew three different species at different times. One isolate was itraconazole and voriconazole resistant, but not all were susceptibility

Table 2. Underlying pulmonary disease, comorbid conditions, and mannose binding lectin levels in patients with *Aspergillus* bronchitis

Underlying diseases	Number affected (%)
Pulmonary disease	$n = 17$
COPD[a]	6 (35)
Asthma[a]	4 (23)
Bronchiectasis[b]	12/14 (86)
Mucus impaction[b]	2 (12)
Lung cancer	1 (6)
Oral corticosteroids >10 mg/day	3 (18%)
Oral corticosteroids <10 mg/day	3 (18%)
Infliximab	1 (6%)
Inhaled corticosteroids	12 (70%)
Breast cancer radiotherapy	2 (12%)
Hyperthyroidism	2 (12%)
Gamma-IFN production low	1 (6%)
Alpha1 antitrypsin deficiency	1 (6%)
Type II diabetes mellitus	1 (6%)
Hypogammaglobulinemia	1 (6%)
Fibromyalgia	1 (6%)
Irritable bowel syndrome	1 (6%)
No comorbidity	2 (12%)
Mannose binding lectin levels (mg/L)	$N = 16$
>1 (normal)	7 (44%)
>0.5–< 1 (possibly low)	3 (18%)
>0.1–< 0.5 (low)	4 (24%)
<0.1 (undetectable)	2 (12%)

[a]COPD and asthma noted if severe and long established, otherwise probably underestimated.
[b]Bronchiectasis and mucus impaction as determined by HRCT.
COPD, chronic obstructive pulmonary disease; IFN, interferon.

Table 3. Characteristics of each patient with *Aspergillus* bronchitis

No.	Demographics	Presentation	Comorbidities	Therapies	Bacterial culture	*Aspergillus* culture	*Aspergillus* PCR	IgG/ Precipitins[a]	Itraconazole weeks
1	M/72	Weight loss, productive cough, severe SOB	COPD, right lung carcinoma, right pneumonectomy	Inhaled steroids, systemic steroids	*S. liquefi-cans, C. parapsilo-sis*	*A. fumigatus* BAL	Positive	IgG 25, precipitins 1/2	12
2	M/30	Cough, tenacious phlegm, recurring chest infections, extreme fatigue	Asthma	Inhaled steroids	*H. influen-zae, P. aerugi-nosa*	*A. fumigatus* BAL + sputum	Positive	IgG 90	52
3	F/30	Low grade fever, cough, excessive sputum, malaise	Asthma, frequent chest infections	Inhaled steroids, systemic steroids	Negative	Sputum – repeatedly *A. niger*	ND	IgG 19	1
4	F/62	SOB, cough, yellow, thick sputum, 8–9 chest infections/year, weight loss 15 kg/year	Previous breast cancer	Inhaled steroids	*P. aerugi-nosa, S. pneumo-niae*	*A. fumigatus* sputum	Positive	IgG 4	9
5	F/58	SOB, exercise tolerance 30 yards, thick green sputum	COPD, fibromyalgia	Inhaled steroids	*S. aureus, H. influenzae*	*A. fumiga-tus*, *A. niger*, and *A. terreus*	Positive	IgG 82	20
6	F/58	Recurrent chest infections, thick phlegm, occasional night sweats, persistent cough	Hyperthyroidism, asthma	Inhaled steroids	Negative	*A. fumigatus* sputum + BAL	Sputum posit, BAL neg	IgG 82	26
7	F/58	SOB, MRC 4, frequent chest infections, massive sputum production	Irritable bowel syndrome	No steroids noted	*P. aeruginosa*	*A. fumigatus*	Positive	IgG 72, precipitins 1/1	52
8	F/46	Recurrent chest infections, severe SOB MRC 4–5, no sputum, no cough, no haemoptysis, weight loss 15 kg	Previous breast cancer with radiotherapy, COPD	Periodic systemic steroids for chest symptoms	Negative	*A. fumigatus* BAL	Positive sputum + BAL	IgG 144	0
9	M/58	Recurrent chest infections and hospital admissions, MRC 5, home oxygen therapy	Severe COPD, type 2 diabetes, ischemic heart disease	Systemic steroids long term, inhaled steroids	*P. aeruginosa*	Negative	Positive sputum	IgG 65	3
10	F/68	Recurrent chest infections, severe cough, voluminous sputum, SOB MRC 5, exercise tolerance 20 yards, weight loss	Severe COPD, osteoporosis, vertebral fractures	Short systemic steroids, inhaled steroids	*P. aeruginosa* + coliforms	*A. fumigatus*	Unresolved – inhibition	IgG 100	20

Continued

Table 3. ***Continued***

No.	Demographics	Presentation	Comorbidities	Therapies	Bacterial culture	*Aspergillus* culture	*Aspergillus* PCR	IgG/ Precipitins[a]	Itraconazole weeks
11	F/73	Recurrent chest infections, voluminous green sputum, SOB MRC 3, wheeze	Previous cavitating pulmonary tuberculosis, asthma, hypertension, hypercholesterolemia	Inhaled steroids	*P. aeruginosa, H. influenzae*	*A. fumigatus*	ND	Precipitins 1/4	52
12	F/42	Frequent chest infections, recurring hemoptysis	Bronchiectasis, alpha1 antitrypsin deficiency	Probably no steroids	*S. aureus, H. influenzae*	*A. fumigatus*	Positive	IgG 47, precipitins 1/4	4
13	F/59	Low-grade fever, frequent chest infections, green sputum, normal exercise tolerance	Asthma, MAI infection, latent TB	Inhaled steroids	MAI, *S. aureus, P. aeruginosa, Candida*	*A. fumigatus*	Negative	IgG 57	26
14	F/73	Bronchial mucoid impaction, lobe collapse, tenacious thick sputum	Nil	Inhaled steroids	Negative	*A. fumigatus* BAL	ND	IgG 137, precipitins 1/1	0
15	F/76	SOB, cough, sputum hyperproduction	Rheumatoid arthritis	Infliximab	*H. influenzae*	Negative	Positive	IgG 105	0
16	F/48	SOB, cough, fatigue, low-grade fever, fatigue, sticky mucous sputum	Gamma-interferon deficiency, adrenal insufficiency	Inhaled steroids, cortisole replacement	Negative	BAL *A. fumigatus*, sputum *A. fumigatus*, and *A. niger*	Positive	*A. niger* IgG positive, *A. fumigatus* IgG 16, negative precipitins	38
17	F/67	SOB, exercise tolerance 30 yards, white sputum	COPD	Hyperthyroidism, hypogammaglobulinaemia, steroid inhalers + short course of oral steroids	*P. aeruginosa, S. maltophilia*	*A. fumigatus*	Positive	IgG 86, precipitins 1/2	2

[a]The figures after precipitins refers to the dilution titre that remains positive. COPD, chronic obstructive pulmonary diseases; SOB, shortness of breath; MRC refers to the Medical Research Council dysnea score (range 1 to 5; 1 = normal, 5 = breathless eating, talking, etc.) MAI, *Mycobacterium avium intracellulare*; ND, not done.

tested. Real-time PCR was positive in 12/14 (86%) samples taken in Manchester.

Elevated *A. fumigatus*-specific IgG antibody (ImmunoCap) was detected in 12 (range 47–144 mg/L (mean 88.9 mg/L)) and precipitins in six patients (range 1:1–1:4 titers), in three cases without positive ImmunoCap antibodies. Three patients had detectable *A. niger* IgG (done in three different external laboratories) and one had detectable *A. terreus* IgG antibody. In two of those with positive *A. niger* IgG antibodies, *A. niger* complex was the most frequent isolate from respiratory samples. Total serum IgE was $\leq$ 100 in 11 (65%) patients and ranged up to 760 KIU/L. This last patient did not fulfill criteria for ABPA and had a rapid and dramatic response to itraconazole. *Aspergillus*-specific IgE was $\leq$ 0.4

Table 4. Findings in the seven patients who underwent bronchoscopy

Patient	Bronchoscopic findings	Biopsy	Mycology and bacteriology
1	Inflamed left main bronchus, with several areas of contact bleeding; distal airways normal	Hyphae invading bronchial mucosa	BAL *A. fumigatus, Candida parapsilosis*, and *Serratia liqueficans* cultured; *Aspergillus* PCR sputum positive
2	Cream-colored mucosal irregularity LUL, sticky pale secretions throughout, mucosa friable and easy bleeding, neutrophil inflammation, plaque-like lesions with ulcerations due to *Aspergillus*	Focal squamous metaplasia. Area of ulceration covered by granulation tissue, with a superficial layer of fungal hyphae. Chronic inflammatory cell infiltrate without eosinophilia.	BAL *A. fumigatus*. Also grew *H. influenzae* and *P. aeruginosa* from sputum
6	Edematous narrowed airways—suggestive of bronchitis/asthma	ND	BAL *A. fumigatus*; *Aspergillus* PCR negative. Sputum culture *A. fumigatus*, *Aspergillus* PCR sputum positive
8	Normal bronchoscopy	ND	BAL *A. fumigatus*; *Aspergillus* PCR positive. Sputum *Aspergillus* PCR sputum positive
12	Normal apart from tracheobronchopathy chondroplasitica, Bronchoscopy having stopped posaconazole.	ND	Sputum culture *A. fumigatus*, *Aspergillus* PCR sputum positive. Azole resistant. Also grew *S. aureus* and *H. influenzae*
14	Thick tenacious sputum, plugging the LLL bronchus	ND	BAL fungal culture negative; *Aspergillus* PCR positive
16	Normal with three tiny black spots visible on subsequent bronchoscopy (on treatment)	ND	BAL *A. niger* cultured and *Aspergillus* PCR positive

KIU/L in 12 (71%) and 8.7 KIU/L was the highest value.

Patients ($n = 14$) were started on oral itraconazole, 200 mg twice a day or voriconazole ($n = 1$), and dose was adjusted in five patients according to drug levels. The intended duration of the first course of antifungal therapy was arbitrarily predetermined to be four months. For relapse, longer courses for up to 52 weeks were given, or longer in the case of further relapses. In the case of itraconazole intolerance ($n = 7$, 50%), voriconazole was substituted in three patients but had to be discontinued due to side effects in all three after 16, 6, and 36 weeks. Posaconazole was started in two patients, one with itraconazole and voriconazole resistance, the other with significant intolerance to both agents. Both patients responded to posaconazole, but discontinuation in one patient after four months resulted in relapse, which responded to reinstitution of long-term posaconazole.[29]

Antifungal response as initial treatment was assessable in 12 patients (11 with itraconazole). After two months of antifungal therapy, major symptomatic improvement was reported by six patients (50%), mild-to-moderate improvement was reported by five (42%) patients, and no improvement was reported in one who had itraconazole resistance. Seven patients maintained the improvement gained during therapy after discontinuing itraconazole and without relapse after 9–52 weeks of therapy (median 26 weeks). Five patients relapsed, two after itraconazole, two

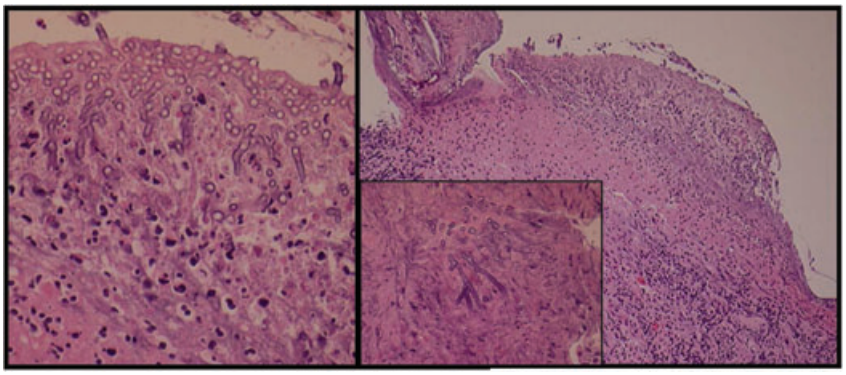

Figure 2. **Transbronchial biopsy of patient 2 stained with H&E showing focal squamous metaplasia in the superficial layers of the bronchial epithelium (left panel). An area of ulceration covered by granulation tissue, with a superficial layer of fungal hyphae is seen from 12–3:00 o'clock. Deeper in the biopsy there is a chronic inflammatory cell infiltrate without eosinophilia. The inset shows septate branching hyphae typical of Aspergillus superficially within bronchial tissue.**

after voriconazole, and one after posaconazole and then reresponded (major response) after restarting antifungal therapy. Three patients had a full recovery, one having relapsed after itraconazole and responded to voriconazole. At the time of analysis six patients continue on antifungal therapy with good control of their symptoms.

There were seven patients whose main complaint upon presentation included frequent lower respiratory tract infections, poor response to antibiotics, and rapid relapse after antibiotic discontinuation. Four of these patients showed major response to antifungal therapy with resolution of respiratory symptoms, and partial response was seen in two cases; one did not tolerate antifungal therapy. Two of those with major responses relapsed after discontinuing antifungal therapy with rapid resolution of symptoms after restarting antifungals. Of these seven patients, five were chronically colonized with *Pseudomonas aeruginosa*, one with *Staphylococcus aureus,* and one had no evidence of bacterial colonization of the bronchial tree. Six of the patients had bronchiectasis.

Discussion

There were two common presentations of *Aspergillus* bronchitis: recurrent chest infections that were treated with repeated unsuccessful courses of antibiotic treatment, and significant breathlessness with mucoid impaction. In occasional instances in the literature, symptoms followed a significant presumptive exposure to airborne fungi, but not in our patients. In several of these patients, antifungal therapy was given as a therapeutic trial after no response to multiple antibacterial regimens and their clinical response was surprisingly prompt, some with subsequent relapse. Before itraconazole availability, some responses were noted to oral iodide therapy.[12,18]

A key finding in all the cases was repetitive identification of *Aspergillus* spp. in respiratory samples by culture or real-time PCR. In order to infer a persistent disease process of the airways, repeated detection of that pathogen from the airways is essential. In the early literature, microscopy alone or combined with culture was sometimes used for diagnosis. The significance of a single isolation of *Aspergillus* from sputum can be difficult to assess, as it may be normal flora for some people[8,39] and/or represent sample or agar plate contamination. Real-time PCR is much more sensitive than culture and is quantitative.[8,40–42] High signals were characteristic of these patients, consistent with heavy *Aspergillus* loads in the airways. The line between long-term colonization and airway infection in the context of structural airway damage and immunological deficit is often difficult to draw and may not be static, with excursions to a more invasive or superficially invasive form at periods of increasing immunosuppression. Therein lies the clinical challenge: to distinguish *Aspergillus* colonization from airway infection in patients with persistent symptoms.

Evidence in favor of *Aspergillus* bronchitis as a discrete clinical entity includes finding overt disease on bronchoscopy (Fig. 1). Two of our patients had bronchial biopsy that showed localized invasion of hyphae. Both patients had months

of symptoms, which is not consistent with the clinical course of invasive *Aspergillus* tracheobronchitis. In pathological specimens, Symmers described mild bronchial inflammation and/or excess production of mucus without invasion,[21] whereas Young *et al.*[23] found bronchial casts containing mucus and mycelia with superficial mucosal erosion and ulceration. Bronchial erythema and excess mucus is also well recognized in orthoptic lung transplant recipients[6] and may precede invasive *Aspergillus* tracheobronchitis[7] or ulcerative *Aspergillus* tracheobronchitis.

Other potentially supportive data include a humoral antibody response. *Aspergillus* precipitating IgG antibodies were detected in six patients, while specific IgG were raised in 12 patients out of 14 with *A. fumigatus* bronchitis. Only four patients had both raised *Aspergillus*-specific IgG and positive precipitins. Three of our patients were infected with non-*fumigatus Aspergilli* (*A. niger* and *A. terreus*), which may not reliably induce cross-reacting antibodies and would require alternative (and less well studied) IgG testing. These antibody tests were introduced in the 1960s and so cases prior to this were not tested.

On the basis of our observations, we propose criteria for the diagnosis of *Aspergillus* bronchitis in patients without significant immunocompromise

Table 5. Proposed criteria for the diagnosis of *Aspergillus* bronchitis

	Aspergillus colonization	*Aspergillus* bronchitis
Essential criteria		
1. Microbiology	Single sputum or PCR positive	Repeat sputum culture or PCR positive for *Aspergillus* sp.
2. Symptoms	Lack of new substantial symptoms	Chronic (> 4 weeks) pulmonary symptoms Possibly systemic symptoms
3. Other forms of aspergillosis	Patient does not fulfill the criteria for invasive, allergic, or chronic aspergillosis (appropriate tests done)	
4. Immune system deficiency[a]	No overt immunocompromise, such as recent chemotherapy, transplantation or AIDS, where a more severe and invasive form of *Aspergillus* tracheobronchitis usually develops	
Supportive criteria		
5. Serology[b] (IgG or precipitins)	*Aspergillus* antibody negative in serum	*Aspergillus* IgG antibody detectable in serum
6. Bronchoscopy findings	Normal or fixed structural abnormality	Mucoid impaction, thick tenacious sputum with bronchial plugging, bronchial erythema (touch bleeding) and/or ulceration Superficial invasion of mucosa by *Aspergillus* hyphae
7. Response to therapy	Equivocal or no response in given	Good response to an eight-week course of antifungal therapy

[a]If the patient otherwise fulfills the criteria for *Aspergillus* bronchitis but is significantly immunocompromised, even temporarily such as receiving high-dose corticosteroids for an asthma exacerbation, the term invasive *Aspergillus* tracheobronchitis should be applied.

[b]This could be regarded as an essential criterion, if superficial mucosal invasion is not demonstrated, but the lack of study of *Aspergillus* IgG antibody testing without a cutoff prevents this from being adopted in the current state of knowledge.

(Table 5). Persistent respiratory symptoms unresponsive to antibiotics in either nonimmunocompromised patients or patients with minor immunocompromising factors with *Aspergillus* spp. detectable in sputum (culture or real-time PCR) could indicate *Aspergillus* bronchitis. Bronchoscopy findings of mucoid impaction and/or localized ulceration with superficial invasion by *Aspergillus* hyphae are characteristic. Detectable *Aspergillus* IgG antibodies is a supportive criterion. Further studies are needed to define a cut-off for *Aspergillus* IgG antibody. Coinfection with bacterial pathogens appears to be common in *Aspergillus* bronchitis.

The precise nature of the local immune deficit in *Aspergillus* bronchitis requires better definition. Variable presentations of human encounters with pathogens that are abundantly present in the environment is to be expected. *Aspergillus* bronchitis occurs primarily in patients with bronchiectasis who are not overtly immunocompromised. Yet, some impairment of immune defense is likely. After inhalation, alveolar macrophages eliminate *Aspergillus* spores by phagocytosis.[43] Epithelial impairment in emphysema and the use of corticosteroids, especially in the inhaled form, have been suggested as instrumental in the increase of IA in the immunocompetent population.[44] Guinea noted systemic corticosteroid use to be an independent risk factor for invasive aspergillosis in COPD in critical care settings.[9] The TORCH study noted an increase in pneumonia related to high-dose inhaled corticosteroids, but not opportunistic lung infection.[45] The Th1/Th2 balance and IL-17/IL-23 immunotolerance regulation appear also to be pivotal in the defense against *Aspergillus*,[46] with mucosal immunity particularly dependent on Th17 responses. We found over 50% of our patients to have MBL deficiency. MBL could be a disease modifier in the context of *Aspergillus* bronchitis, as has been suggested for CPA and/or allergic aspergillosis in cystic fibrosis.[38,47] *A. fumigatus* also appears to have potent capacity to induce hyperproduction of mucus in the bronchial epithelium via the activation of the TACE/TGF-α/epidermal growth factor receptor pathway.[48] Biofilm formation could also be relevant to pathogenesis.

Thus, we have described a group of patients with underlying structural and immunological compromise of varying severity, who present with difficult-to-treat and readily relapsing symptoms of chronic bronchitis combined with microbiological and immunological evidence of *Aspergillus* infection. While not immunocompromised in the sense of common clinical parlance, these patients usually have one or more minor immune deficits. Most of these patients benefit from a course of oral antifungal therapy, though many relapse. Additional work is necessary to optimize therapy, establish an appropriate duration, and define groups of patients who need long-term therapy. *Aspergillus* bronchitis should be regarded as a chronic superficial airway infection similar to bacterial bronchitis. Introducing this category of disease enables a distinction between asymptomatic fungal colonization and allergic bronchopulmonary aspergillosis, facilitating treatment of those afflicted. Further research into the category of subtle underlying defects of immune response and patterns of interactions with opportunistic pathogens is needed.

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Conflicts of interest

The authors declare no conflicts of interest.

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