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Nontuberculous mycobacteria in cystic fibrosis associated with allergic bronchopulmonary aspergillosis and steroid therapy

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ABSTRACT: Nontuberculous mycobacterial (NTM) infection, particularly due to *Mycobacterium abscessus*, is an emerging disease that can be relentlessly progressive, particularly in cystic fibrosis (CF) patients. The risk factors that were associated with this increasingly symptomatic infection in a group of CF patients were investigated.

A total of 139 CF patients aged 2–52 yrs were reviewed. Sputum was cultured for NTM annually or whenever clinical deterioration was unexplained.

In total, 12 patients (8.6%) had positive cultures and six (4.3%) met the criteria for NTM pulmonary disease (five with *M. abscessus*). Five had allergic bronchopulmonary aspergillosis (ABPA) compared with one out of 133 patients without NTM disease. Five had received systemic steroids (four as a treatment for ABPA) compared with only one out of 133 without NTM lung disease. All six NTM patients deteriorated markedly following mycobacterial infection, and forced expiratory volume in one second dropped 18–46%. Despite prolonged triple antibiotic therapy, *M. abscessus* was not eradicated, and four out of six did not return to baseline clinically.

In conclusion, severe nontuberculous mycobacterial lung disease, particularly with *Mycobacterium abscessus*, is becoming a perplexing challenge in cystic fibrosis patients. Allergic bronchopulmonary aspergillosis and systemic steroids appear to be risk factors, although small patient numbers limit this to a descriptive observation. When pulmonary condition deteriorates, increased surveillance for mycobacteria would enable prompt diagnosis and treatment.

KEYWORDS: Allergic bronchopulmonary aspergillosis, cystic fibrosis, *Mycobacterium abscessus*, nontuberculous mycobacteria, pulmonary infection, steroid therapy

Life expectancy and quality of life have been improving steadily in cystic fibrosis (CF), as treatment regimens become more aggressive. Concomitantly, reports describe the rising incidence of unusual pulmonary pathogens in CF, including *Stenotrophomonas maltophilia*, *Alkaligenes xyloxidans*, Nocardia and nontuberculous mycobacteria (NTM) [1]. Recently, there has been increasing concern about the potential pulmonary pathogenicity of NTM in CF [2]. At least 12 single-site studies described a total population of ~1,300 CF patients, with an NTM prevalence varying 2–28% [3–9].

A USA multicentre study of 986 CF patients aged >10 yrs showed a 13% NTM prevalence [10], and 16% of these had *Mycobacterium abscessus* (previously *M. chelonae*, subspecies *abscessus*). Although the clinical course appeared relatively benign [11], an editorial comment [12] emphasised that all NTM isolates were considered together as potential pathogens, and the majority were *M. avium* complex. Focusing on more virulent pathogens, such as *M. abscessus*, might accentuate important differences in prognosis, but small numbers probably precluded this. In contrast, a French study [7] described *M. abscessus* as the most prevalent NTM by far, particularly in younger CF patients.

Recently, the current authors have encountered CF patients with severe unexplained pulmonary deterioration, which proved to be related to NTM infection, particularly that due to *M. abscessus*. In this study, the apparent associated risk factors in this group are reported, and the challenges involved in the management of this difficult emerging disease are described.

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METHODS

Between 1997 and 2002, the charts of 139 Israeli CF patients, followed at the Graub Cystic Fibrosis Center and Pulmonary Unit, Schneider Children's Medical Center of Israel, Petah Tikva, Israel, and the Cystic Fibrosis Center, Carmel Medical Center Haifa, Israel, were reviewed. Sputum was cultured for NTM annually or whenever there was unexplained clinical deterioration.

CF patients with NTM disease were characterised by cystic fibrosis transmembrane regulator (CFTR) mutations, sweat test, clinical status and treatment regimens. Pulmonary disease was evaluated by lung function, chest radiograph, and sputum culture for bacteria and fungi, as well as mycobacteria. Chest computed tomography (CT) was performed when indicated clinically. Exocrine pancreatic function and diabetes were also assessed.

Culture for NTM was performed using a modification of the technique recommended for CF [13]. Sputum specimens were rapidly transferred to the laboratory, homogenised in *N*-acetyl cysteine and decontaminated with 2% NaOH. Positive cultures were sent to the Israeli Mycobacteria Reference Laboratory at the Public Health Laboratory of the Ministry of Health, Tel Aviv, Israel, for species identification and initial susceptibility testing using a nonvalidated E-test with control specimens. Isolates of *M. abscessus* in patients with signs of clinical deterioration were referred for further susceptibility testing to the Mycobacterial Consultation Service, Denver, CO, or to the Mycobacteria/Nocardia Research Laboratory, Tyler, TX, USA. Although susceptibility testing has not been validated for NTM infections, in the USA, these are national reference laboratories for susceptibility testing of mycobacteria. The antibiotic panel included amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, ofloxacin, linezolid, kanamycin, cycloserine and, in certain highly resistant specimens, tigecycline. The method of broth microdilution minimum inhibitory concentrations was used [14], according to the protocol and tentative standard of the National Committee for Clinical Laboratory Standards, Wayne, PA, USA [15].

Statistical analysis

Statistics were calculated using Fisher's exact test (two-tailed) to compare the incidence of NTM lung disease in patients who developed allergic bronchopulmonary aspergillosis (ABPA) and in patients who had received prolonged steroid therapy; each was compared with the rest of the clinic population.

RESULTS

Out of 139 CF patients aged 2–52 yrs followed at two Israeli CF centres, 12 patients had positive NTM sputum cultures. Six of these (50%) met the American Thoracic Society criteria for NTM pulmonary disease [16]: all had at least three positive smears for acid-fast bacilli by Ziehl-Neelsen staining, five of these had more than three positive cultures for *M. abscessus* and, in one, *M. simiae* was repeatedly cultured. This represents 4.3% of the study population. Six additional patients had intermittently positive NTM cultures and did not fulfil the criteria for disease: three had *M. abscessus*, and one each had *M. gordonae*, *M. fortuitum* and *M. avium* complex.

All six patients with NTM pulmonary disease had severe CFTR mutations. Clinical and microbiological details of these patients are presented in table 1. They were all pancreatic insufficient, but none had diabetes. Patient No. 6 had severe CF-related cirrhosis and portal hypertension, which led to his death 1 yr after diagnosis of NTM infection.

In total, five out of the six patients with NTM lung disease also had ABPA as diagnosed by accepted criteria [17]. In four of these, ABPA preceded NTM infection, as described previously. The fifth patient subsequently developed ABPA. In comparison, only one patient out of the 133 without NTM disease had ABPA ($p<0.001$). Five of the six patients with NTM disease had received prolonged systemic steroid treatment, four of which were for ABPA. The fifth received steroids for a prolonged allergic reaction to chemotherapy for disseminated oesophageal carcinoma. This compared with only one of the 133 patients who received steroids (for ABPA), but did not develop NTM disease ($p<0.001$).

Clinically, all six patients had been stable previously, but deteriorated following the onset of NTM lung disease. Forced expiratory volume in one second (FEV_1) decreased by 18–46%, and oxygen saturation, which was normal prior to infection, fell significantly in three out of six patients (table 2). One patient required ventilation with bi-level positive airway pressure due to respiratory failure and, despite a partial response to therapy, remained oxygen dependent.

TABLE 1 Clinical and laboratory features at diagnosis of nontuberculous mycobacteria (NTM) lung disease

Case	Age yrs	CFTR mutation	*P. aeruginosa* infection	ABPA	Steroid therapy months	AFB	NTM	NTM-positive cultures
1	17	ΔF508/W1282X	-	+	40	+++	*M. abscessus*	>5
2	7	W1282X/G542X	+	+	48	+++	*M. abscessus*	>5
3	33	W1282X/N1303K	+	-	2[#]	+++	*M. abscessus*	>5
4	8	N1303K/1717-1-G->A	+	+	24	+++	*M. abscessus*	>5
5	11	ΔF508/ΔF508	+	+	6	+++	*M. simiae*	>3
6	18	G542X/N1303K	+	+[¶]	0	++	*M. abscessus*	>3

CFTR: cystic fibrosis transmembrane regulator; *P. aeruginosa*: *Pseudomonas aeruginosa*; ABPA: allergic bronchopulmonary aspergillosis; AFB: acid-fast bacilli; -: negative; +: positive; +++: positive NTM culture, with high density on smear; ++: positive NTM culture, with low density on smear; *M. abscessus*: *Mycobacterium abscessus*; *M. simiae*: *Mycobacterium simiae*. [#]: steroid therapy for drug allergy during oesophageal carcinoma treatment; [¶]: ABPA was diagnosed after NTM infection.

TABLE 2 Progression of nontuberculous mycobacteria (NTM) pulmonary disease

Patient No.	Oxygen saturation %		FEV_1 % pred		CR/CT
	Pre-NTM	Post-NTM	Pre-NTM	Post-NTM	
1	98	87	70	30	B and N (CT)
2	98	98	104	110	B (CT)
3	98	88	33	ND	B and N (CR)
4	98	96	77	60	B (CT)
5	96	96	67	54	B (CT)
6	95	93	42	35	B (CR)

FEV_1: forced expiratory volume in one second; % pred: % predicted; CR: chest radiograph; CT: computed tomography; ND: not done, as patient deteriorated, required bi-level positive airway pressure and could not perform spirometry; B: bronchiectases; N: peripheral nodular disease.

Chest radiographs and CT demonstrated widespread bronchiectatic changes that were known to be associated with NTM lung disease (table 2). However, these could not be differentiated from CF-related bronchiectases. Only two cases had peripheral nodules consistent with granulomata, which are specifically associated with NTM and not typical of CF [18].

All six cases of CF and mycobacterial lung disease had multiresistant strains of NTM even before therapy was instituted. Even when the NTM was susceptible initially, resistance developed rapidly after the institution of therapy, both clinically and as reported by reference laboratory susceptibility testing in the USA. Nevertheless, triple therapy was used whenever possible, while rotating drugs as tolerated and until clinical improvement occurred, as assessed by symptoms, FEV_1 and oxygen saturation. Examples of clinical progression, details of antibiotic therapy and mycobacterial response are presented in figure 1. All patients in the current study received 3–12 months of continuous *i.v.* antibiotics, and improvement was usually very gradual. Despite this aggressive therapeutic approach, all five patients with *M. abscessus* had persistently positive sputum cultures and four did not return to clinical baseline. Microbiological eradication was achieved only in the patient with *M. simiae*.

DISCUSSION

In the current study, six out of 139 CF patients, suffering from NTM disease, mainly due to *M. abscessus*, are described. It was found that ABPA and systemic steroid therapy were associated with this complication. As this is a rare condition, the series of patients with NTM reported in this study is small, and, therefore, the statistical analysis must remain weak at best. Nevertheless, it is believed that this experience underlines an important and challenging clinical aspect in CF.

NTM infection appears to be an emerging disease. A clinical microbiology laboratory in Israel reported that NTM and, in particular, the species *M. chelonae* (of which *M. abscessus* was previously considered a subspecies), *M. fortuitum* and *M. simiae* are increasing in incidence [19]. During 1996–1999, 135 specimens from 9,391 patients cultured positive for NTM. Of these, five patients had *M. chelonae*. Between 2000 and 2003, 364 out of 9,031 patients were culture positive for NTM ($p<0.0001$) and, of these, 51 had *M. chelonae* ($p<0.005$). It is not known what the causes of this changing epidemiology are, or whether this regional clustering found in Israel is a more general phenomenon. However, it does affect all populations, including those without CF.

The present study shows that NTM lung disease due to *M. abscessus* can be relentlessly progressive in CF patients. Initial clinical and microbiological response to therapy was often good, but disease recurred within weeks of the cessation of therapy (fig. 1). As others have described in CF [20], the current authors found that *M. abscessus* infection was impossible to eradicate. Disease is diffuse and is, therefore, not amenable to surgery. There are no clear guidelines for the optimal duration of therapy, which includes parenteral drugs and is usually limited by patient intolerance [16]. According to the tentative standards of the USA reference laboratories that analysed the specimens, the current CF patients have multiresistant strains of *M. abscessus*, making the selection of appropriate triple antibiotic therapy even more difficult. Curative end-points are rarely attained. The only course with presently available agents appears to be intermittent palliation when clinical signs reappear.

In the current study, the CF patients with NTM lung disease were mainly <18 yrs of age, had severe mutations and five out of six had chronic Pseudomonas infection (table 1). Other recent studies confirm the current authors' experience and describe *M. abscessus* and *M. chelonae* lung disease in children >2.5 yrs old [4, 7, 9]. In contrast, the USA epidemiological study [10] found CF patients with NTM to be older, with milder lung disease and less *Pseudomonas aeruginosa* infection. However, only patients aged >10 yrs were included. It is certainly recommend that sputum should be cultured for NTM in any CF child with unexplained pulmonary deterioration.

The association with ABPA, which is described in five out of six patients with NTM pulmonary disease, may be due to specific host immune factors in this subgroup of CF patients. An association with altered immune balance, such as that found in ABPA, has not been described in previous studies. However, a possible relationship between ABPA and mycobacterial infection has been mentioned in one study, in three out of 15 CF patients with NTM infection [7]. Two of these 15 patients had taken systemic steroids. ABPA is associated

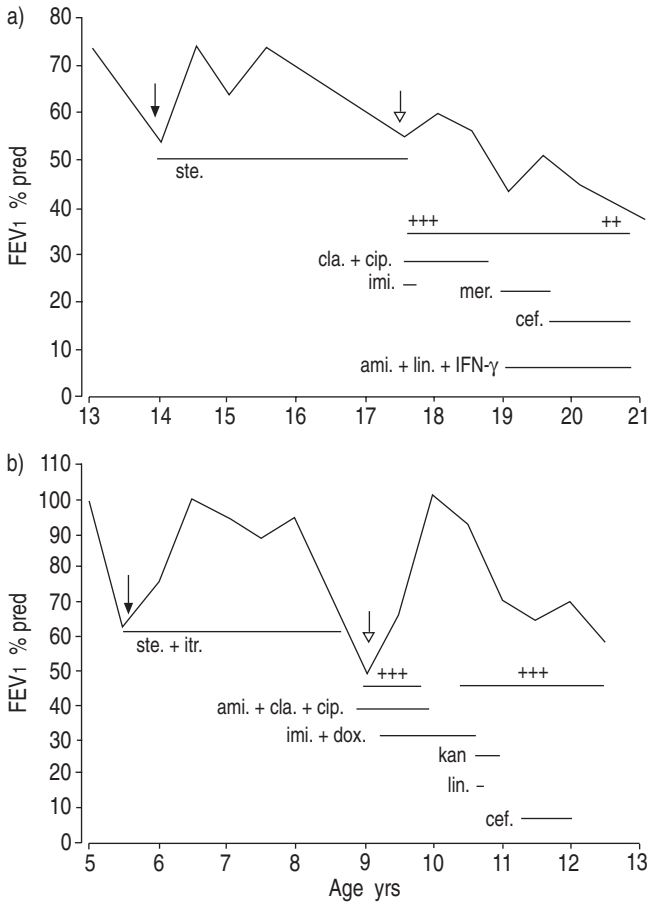

FIGURE 1. Clinical course of two patients with *Mycobacterium abscessus* lung disease. a) Steroid therapy (ste.) for allergic bronchopulmonary aspergillosis (ABPA; arrow) stabilised forced expiratory volume in one second (FEV_1) for 2 yrs. Subsequent deterioration led to diagnosis of *M. abscessus* (open arrow) with high-density acid-fast bacilli. Triple antibiotic regimes and inhaled interferon (IFN)-γ were of transient benefit. The patient now awaits lung transplant. b) ABPA (arrow) was diagnosed at age 5.5 yrs and treated successfully with steroids and itraconazole (ste. + itr.). Deterioration from age 8 yrs led to diagnosis of *M. abscessus* (open arrow). Improvement followed triple antibiotic regimes but was transient, without the eradication of nontuberculous mycobacteria (NTM). +++: positive NTM culture, with high density on smear; ++: positive NTM culture, with low density on smear; cla.: clarithromycin; cip.: ciprofloxacin; imi.: imipenem; mer.: meropenem; cef.: cefoxitin; ami.: amikacin; lin.: linezolid; dox.: doxycycline; kan: kanamycin.

with an increased T-helper (Th)2 CD4+ T-cell response to Aspergillus and high levels of secretion of interleukin (IL)-4, IL-5 and IL-10 by peripheral blood mononuclear cells [21, 22]. IL-10 downregulates Th1 CD4+ T-cells and related cytokines, such as interferon (IFN)-γ, IL-2 and tumour necrosis factor. These cytokines enhance cytotoxic and macrophage functions that are crucial for NTM eradication [23, 24]. Thus, an immune system geared towards Th2 responses in CF patients with ABPA may favour the persistence and increase the virulence of NTM infection. Indeed, a trial of systemic IFN-γ added to anti-mycobacterial therapy showed clinical improvement in seven patients with disseminated *M. avium* complex infection [25]. In the current study, IFN-γ to triple antibiotic therapy in the first patient (fig. 1a), but long-term clinical improvement or NTM eradication was not achieved. Nevertheless, this treatment may be studied in prospective trials as a future adjunct to the therapy of CF patients with severe NTM lung disease.

Although steroids have not been shown to be a predisposing factor for aggressive NTM lung disease in healthy subjects, they may play a role in CF patients by further suppressing innate lung defence, including IFN-γ signalling and nitric oxide production, which are already impaired [26]. This could be particularly crucial in patients with immune modulation due to ABPA. A small retrospective study suggested a link between NTM lung disease in CF patients and steroid therapy [6], although this was not shown in the large USA study [10]. Progressive *M. abscessus* disease was described in other CF patients with immune modulation, including those post-lung transplantation [27] and following ibuprofen treatment [28]. In non-CF patients, severe NTM lung infection has been associated with HIV [29] and malignancy [30]. Finally, steroid treatment has been described as predisposing for *M. chelonae* cutaneous infection and osteomyelitis [31].

Mycobacterium abscessus, part of a changing microbiological flora in cystic fibrosis patients, poses a perplexing therapeutic dilemma. The current authors recommend close surveillance for nontuberculous mycobacterial infection in all cystic fibrosis patients, including young children with unexplained pulmonary deterioration, especially those suffering from allergic bronchopulmonary aspergillosis and/or receiving systemic steroids.

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