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Original Article

Increasing nontuberculous mycobacteria infection in cystic fibrosis

Ophir Bar-On [a], Huda Mussaffi [a,b], Meir Mei-Zahav [a,b], Dario Prais [a,b], Guy Steuer [a], Patrick Stafler [a,b], Shai Hananya [a], Hannah Blau [a,b,*]

[a] Graub CF Center, Pulmonary Institute, Schneider Children's Medical Center of Israel, Israel
[b] Sackler Faculty of Medicine, Tel-Aviv University, Ramat Aviv, Tel Aviv, Israel

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Abstract

Background: Nontuberculous mycobacteria (NTM) are emerging infections in the CF population.
Aims: To assess NTM infection prevalence and associated features in our CF clinic population.
Methods: Patient records, 2002–2011, were reviewed for NTM infection. FEV_1, pancreatic function, sputum microbiology, and serum cytokines were compared in patients with and without NTM infection.
Results: Incidence rate of NTM infection increased from 0 in 2002 to 8.7% in 2011 ($p < 0.001$). NTM infection prevalence increased 3-fold from 5% (4/79) in 2003 to 14.5% (16/110) in 2011 ($p = 0.05$). Prevalence of chronic NTM lung disease has decreased somewhat since a peak in 2009, with institution of aggressive triple therapy. Of NTM-infected compared to uninfected patients, 88.2% vs. 60.3% had a known 'severe' CFTR genotype ($p = 0.04$), 88.2% vs. 58.9% were pancreatic insufficient ($p = 0.02$); 70.6% vs. 43.8% had chronic *Pseudomonas aeruginosa* ($p = 0.06$); 75% vs. 32% had *Aspergillus* infection ($p = 0.007$) and 23.5% vs 2.7% had allergic bronchopulmonary aspergillosis ($p = 0.01$). Patients infected with *Mycobacterium abscessus* had increased TGF-β, TNF-α, IL-1β, IL-2, IL-4 and IL-5 levels ($p < 0.05$). There was no difference in cytokine levels for all NTM infected compared to uninfected patients. *M. abscessus* comprised 46% of all NTM infections. Comparing *M. abscessus* versus other NTM, duration was 10.5 (1–118) months versus 1 (1–70) month, median (range) ($p = 0.004$); lung disease occurred in 69% versus 17% ($p = 0.0004$), with sputum conversion in 4/11 versus 5/6, respectively (NS).
Conclusions: NTM incidence and prevalence have increased dramatically in our CF clinic, associated with a severe CF genotype and phenotype. *M. abscessus*, the most prevalent NTM, caused prolonged infection despite therapy. There has been some decrease in the prevalence of NTM lung disease since 2009.

Keywords: Cystic fibrosis; Nontuberculous mycobacteria; *Mycobacterium abscessus*; Allergic bronchopulmonary aspergillosis

1. Introduction

As survival in cystic fibrosis (CF) increases, the emergence of new and resistant bacterial infections, including nontuberculous mycobacteria (NTM) is an increasing concern [1]. NTM infection was first described in CF patients in the 1980s [2,3] but was considered rare and of unknown pathogenicity. In the 1990s an increasing number of CF centers reported NTM infection, with various single-site studies describing about 1300 CF patients, and an estimated NTM prevalence of 2–28% [4]. In 2002, a cross-sectional multi-center study of CF patients in the United States, reported an overall NTM prevalence of 13% [4], but no data was given regarding changing prevalence over time.

Indeed, the prevalence of NTM as a cause of significant pulmonary disease has been increasing globally [5]. Although variable, prevalence within the CF population is also rising and could be associated with increasing survival as well as prolonged antibiotic therapy [6–8]. In addition, the infection may be

* Corresponding author at: Graub CF Center and Pulmonary Institute, Schneider Children's Medical Center of Israel, 14 Kaplan Street, Petah, Tikva 49202, Israel.
E-mail address: hblau@post.tau.ac.il (H. Blau).

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diagnosed more often due to increasing awareness by both clinicians and microbiologists of the consequences of NTM lung disease. A previous study of NTM in Israeli CF patients showed that the prevalence was highest (up to 29%) in the center and south of the country and in the coastal cities where the weather is hot and humid, rather than the dry mountains of Jerusalem [9].

Species diversification of NTM within the CF population appears to vary with geographical distribution. In the United States, *Mycobacterium avium* complex (MAC), followed by *Mycobacterium kansasii* and *Mycobacterium abscessus* are the most frequently recognized pulmonary pathogens [4]. In Europe, however, *M. abscessus* appears to be the major pathogen in CF [6].

NTM are ubiquitous and are readily recovered from environmental sources, such as soil, water, plants and animals. Tap-water is considered the major reservoir for most NTM species pathogenic to humans, and bacteria can be isolated from the solid–liquid interface biofilm, especially within piping systems [10]. This renders the mycobacteria less susceptible to disinfectants and antimicrobial therapy.

Although in the past NTM was not considered a major pathogen, descriptions of fulminant NTM infections, particularly with *M. abscessus*, are increasingly evident in the CF population [5,11,12]. Other NTM species are of undetermined and variable clinical importance [13]. Nosocomial spread of NTM infection in CF was previously considered unlikely [4]. Recently however, whole-genome sequencing revealed frequent transmission of multidrug resistant *M. abscessus*, subspecies *massiliense* [14]. This may have been by indirect means, and occurred despite conventional cross-infection measures.

Normal host defenses against NTM include a well-orchestrated inflammatory response. Initially, mycobacteria bind Toll-like-Receptor (TLR)2 on macrophages which produce TNF-α and IL-12, up-regulating a TH1 response and IFN-gamma production, activating NK-cells and resulting in intra-cellular mycobacteria killing. Multiple cytokines are involved, including GM-CSF, IL1β, IL2 and IL8. Deficiency of leptin as in malnutrition increases susceptibility to rapidly growing mycobacteria, as does increased IL10. Similarly, immune dysregulation, with increased TH2 or decreased TH1 response, may enhance NTM infection [15–17].

We have had the impression that NTM lung disease has been increasing steadily in our CF clinic. As a result, aggressive, prolonged triple therapy has been instituted in recent years. We therefore decided to review and analyze the incidence and prevalence of NTM infection and lung disease in the past decade, and to correlate this with demographic, clinical and immunologic patient data.

2. Methods

This was an observational, longitudinal, retrospective study conducted at a single CF center in Israel. The study was approved by the ethics committee of SCMCI, approval no. 7043 0295-12.

The study population included CF patients attending the Graub CF Center at Schneider Children's Medical Center of Israel (SCMCI) from 2002 till 2011, and diagnosed with CF according to accepted criteria [18].

As part of the routine protocol, sputum was cultured for nontuberculous mycobacteria (NTM) as well as other bacteria and fungi, every 3–6 months. In patients previously diagnosed with NTM infection, sputum was sent for NTM culture at every clinic visit (every 1–2 months). In addition, sputum culture for NTM was performed when clinical deterioration in pulmonary disease was not clearly explained by the presence of other bacteria or fungi.

3. NTM laboratory diagnostic protocol

Expectorated sputum was transferred immediately and analyzed at the mycobacteria laboratory within the Department of Microbiology, Rabin Medical Center, adjacent to SCMCI. Specimens underwent mucolysis using AlphaTec NAC-PAC-Red (N-Acetyl-Cysteine) and then decontamination using 3% NaOH for 15 min followed by addition of buffer (AlphaTec NPC67, Vancouver, Washington, USA) to neutralize the NaOH. Several drops of the resultant fluid were used for Ziehl–Neelsen (ZN) staining, and the rest was inoculated onto Loewenstein–Jensen (LJ) slanted agar (Loewenstein–Jensen + Glycerol + PACT, Heipha, Germany) and BD BACTEC MGIT incubator tubes. LJ tubes were placed in a 37 °C incubator and inspected weekly for growth till 8 weeks. MGIT tubes were placed in computerized incubators at 37 °C. Once growth was seen in either media, a repeat ZN stain was performed and further identification was performed by biochemical PCR using Mycobacteria Genotype kits (Hain Life Science, Germany).

Susceptibility testing was performed at the Mycobacterium Reference Laboratory, the Public Health Laboratory of the Ministry of Health, Abu-Kabir, Israel.

Both the clinical and the laboratory diagnostic protocol were consistent and did not change throughout the study period.

4. Patient data collection

CF patient charts were reviewed and data recorded from January 2002 to December 2011. Microbiologic data included results of NTM culture, ZN staining, species of NTM and results of culture for other bacteria and fungi.

Annual incidence rate and prevalence were assessed throughout this period as was the use of azithromycin.

In 2008 a cross-sectional assay for cytokines was performed for all CF patients aged >2 years at the Graub center, while in a stable pulmonary state. We now reviewed levels of cytokines considered to have a role in host defense against NTM (IL1β, IL2, IL-4, IL-5, IL-10, IL-12, IL-17, TNF-α, INF-γ, GM-CSF and leptin). Demographic, genetic and clinical data for all clinic patients are presented at the time of cytokine testing in 2008, including gender, age, height, weight, CFTR mutations, sweat chloride, FEV_1 (best value measured during that year), pancreatic enzyme therapy, azithromycin therapy (number of years with at least 3 months of therapy), presence of CF related diabetes treated with insulin, and 25-OH vitamin D levels and were compared between patients with at least one positive

culture for NTM from 2006 to 2009 and in patients negative for NTM during this period. Genotype and cytokines measured in 2008 were also compared for patients with or without *M. abscessus* infection during these years.

5. Definitions

5.1. NTM lung disease versus presence of NTM infection

We defined patients as having 'NTM lung disease' if they had clinical symptoms as well as radiological signs and microbiologic criteria as by the ATS consensus statement (at least 2 positive NTM cultures from separate expectorated sputum samples) [19]. In addition, we required that repeat cultures grew the same mycobacteria species, with 12 months or less between two positive NTM cultures. Patients were defined as having a presence of NTM infection if they had only one positive NTM sputum culture during the observation period, and no unexplained clinical deterioration. The overlap between clinical signs of CF and NTM lung disease made 'the absence of clinical symptoms' impossible to include in defining the presence of NTM infection in this group [19]. We chose the term 'presence of infection' rather than 'colonization' for this group, as airway and lung parenchyma pathologic involvement have been demonstrated even in this situation [20].

5.2. Sputum conversion

Patients were considered to have NTM sputum conversion if cultures were negative for one year after being diagnosed with NTM lung disease [21]. Spontaneous conversion implies conversion without receiving specific anti-mycobacterial therapy.

5.3. NTM incidence and prevalence

Annual NTM incidence from 2002 till 2011 refers to the total number of patients with the presence of a NTM positive sputum culture for the first time during each year. Annual incidence rate refers to patients with a new NTM positive sputum culture as a percentage of all clinic patients at the end of that year ("new" culture includes patients already infected with one strain of NTM who acquired a different strain).

Annual NTM prevalence refers to the percentage of clinic patients at the end of each year with at least 1 positive NTM culture during that year. Annual NTM lung disease prevalence refers to the percentage of clinic patients at the end of each year with at least 2 positive NTM cultures during that year.

5.4. Chronic Pseudomonas aeruginosa infection

At least 2 sputum cultures in a year, of which >50% were positive for *P. aeruginosa* [22].

5.5. Years of azithromycin therapy

This refers to the number of years with >3 months of continuous therapy in that year, between 2006 and 2009, compared for NTM positive and negative patients in 2008.

5.6. CFTR genotype severity

We classified patients as having a severe CFTR genotype if they had 2 mutations from class I or class II, known to be associated with minimal CFTR function and a mild CFTR genotype if they had at least one mutation from class IV or V, known to be associated with residual CFTR function [23]. If patients had 1 unknown and 1 severe mutation or 2 unknown mutations we classified them as of unknown genotype severity and did not include them in the analysis of the effect of genotype on NTM infection. Our cohort did not include any patients with class III mutations.

5.7. Statistical analysis

We summarized patient demographic, genetic and clinical characteristics using median and range or mean and standard deviation as appropriate. We calculated proportions for categorical variables. These parameters were compared between NTM-positive and NTM-negative subjects by unpaired non-parametric (Wilcoxon) tests, or χ^2 test, as appropriate. Duration of NTM positivity for different NTM species was compared using unpaired non-parametric (Wilcoxon) test, while the prevalence of NTM infection versus lung disease for different NTM species was compared using χ^2 test. Fisher's exact test was used to compare CFTR allele frequency and genotype severity in *M. abscessus* positive and NTM positive patients compared to uninfected patients. The coefficient of determination (R^2) for the time dependent linear trend of the incidence and prevalence of NTM-positive patients was calculated. All analyses were 2-tailed, and a p-value <0.05 was considered significant. Statistical analyses were performed using SPSS software (v. 21, IBM® SPSS® Inc., Chicago, IL).

6. Results

6.1. Incidence and prevalence of NTM infection

The annual incidence rate of NTM infection increased between 2002 and 2011 from 0 to 9%, $R^2 = 0.8776$, $p < 0.001$ (Fig. 1). This was despite a constant rate of 4–5 NTM cultures/patient/year over the decade reviewed (E-Table 1, online data supplement).

Prevalence of patients with NTM positive cultures was 5% (4/79 patients) in 2003 but subsequently increased almost 3-fold, to 14.5% (16/110) by 2011, $R^2 = 0.9103$, $p = 0.05$ (Fig. 2). The prevalence of NTM lung disease appears to have peaked in 2009 and be decreasing somewhat since then (Fig. 2).

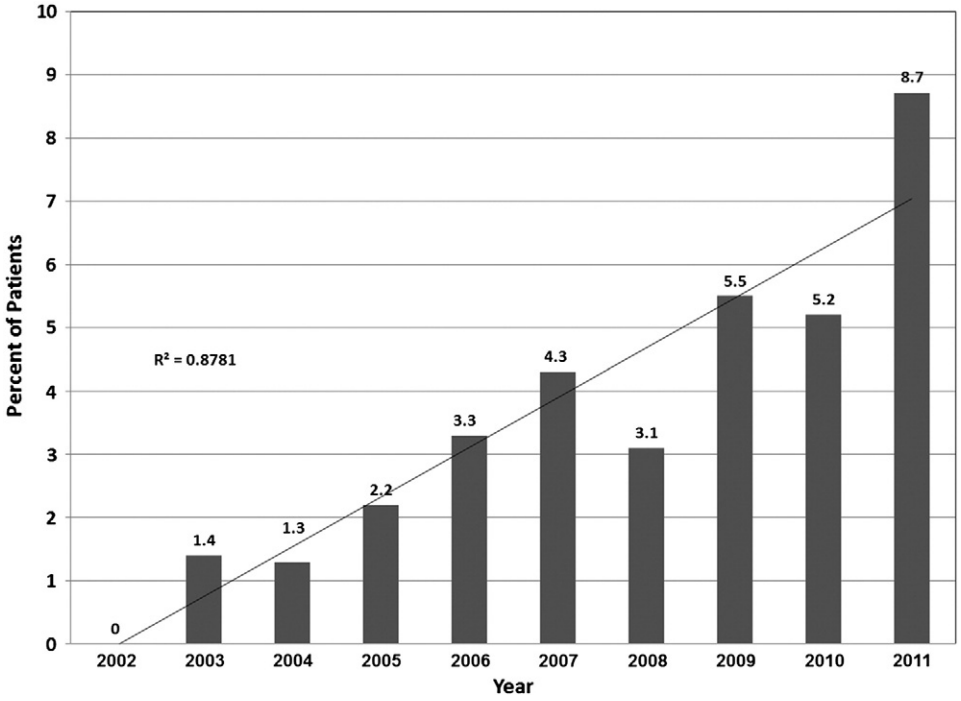

Fig. 1. Increasing incidence rate (% newly infected patients of total clinic patients each year) of new NTM infection over time, at the Graub CF center, SCMCI, from 2002 until 2011.

6.2. Patient demographics, genetic and clinical correlates

The total number of CF patients at the Graub CF center, SCMCI, increased from 70 in 2002 to 110 in 2011. Demographic and clinical characteristics of NTM-positive versus NTM-negative patients for 2008, the year when cytokine studies were done, are summarized in Table 1. At this time there were 90 CF patients, 54% males, age 16.9 (0–59) years, median (range).

All 90 patients in the 2008 cohort were genotyped: 50 had 2 class I or II mutations ('severe' genotype), 25 had at least 1 class

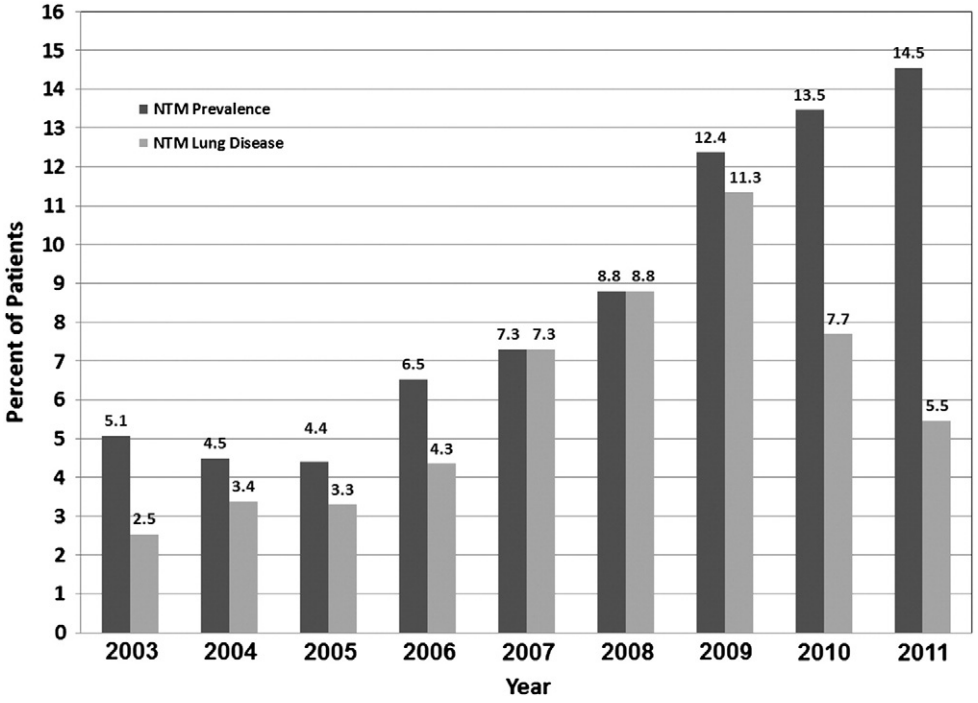

Fig. 2. Increasing NTM prevalence over time at the Graub CF center, SCMCI is depicted from 2003, once uniform management of NTM in clinic was instituted, until 2011.

IV or V mutation ('mild' genotype) and 15 had unknown genotype severity (Table 2). The most frequent CFTR mutations in our cohort were W1282X, ΔF508, G542X, D1152H, 3849 + 10kbC→T. No CFTR allele was more prevalent in patients with *M. abscessus* or who were NTM positive (E-Table 2, on-line data supplement). When considering the 75 patients with known CFTR genotype severity, 15/17 (88.2%) NTM positive patients had a severe genotype, compared with 35/58 (60.3%) NTM negative (p = 0.04). Of the nine patients with *M. abscessus* infection 8 (88.9%) had a severe genotype compared to 42/66 (66.7%) patients without *M. abscessus* (p = 0.26).

Patients with presence of NTM infection were more likely to be pancreatic insufficient compared to those without NTM infection (p = 0.02) and had more *P. aeruginosa* chronic infection (p = 0.06). Despite this, BMI-SDS, and FEV_1 were similar in both groups. There was no correlation with duration of azithromycin therapy or number of days of intravenous antibiotic therapy. A strong correlation was found between the presence of NTM infection and airway infection with *Aspergillus* (p = 0.003), as well as with the presence of allergic bronchopulmonary aspergillosis (ABPA) (p = 0.01).

6.3. NTM species distribution

Relative frequency of infection with the various NTM species is shown in Fig. 3. Rapidly growing mycobacteria (RGM) included *M. abscessus*, *Mycobacterium chelonae* and *Mycobacterium fortuitum*. Slow growing mycobacteria (SGM) included *M. avium*, *Mycobacterium simiae* and *Mycobacterium intracellulare*. RGM, and in particular the *M. abscessus* complex, make up the majority of cases throughout the decade. *M. abscessus* comprised an average of 46% of all mycobacteria isolates annually. *M. avium* and *M. intracellulare*, together comprising the *M. avium* complex, were isolated in approximately 24% of cases. In 2002 only *M. abscessus* and *M. avium* were identified, but institution of PCR species identification permitted more precise identification of NTM species in subsequent years. *Mycobacterium mucogenicum*, *Mycobacterium interjectum*, *Mycobacterium peregrinum*, *Mycobacterium smegmatis* and *M. kansasii*, each appeared only once during the entire observation period and are not shown in Fig. 3.

Table 1
CF patient demographic and clinical characteristics.[a]

	NTM positive[b]	NTM negative[b]	p-Value
Patients, n (% total)	17 (18.9%)	73 (81.1%)	
Age (years), median (range)	17.8 (4.3–55.3)	15.2 (0.2–59.3)	0.42
Male, n (%)	11 (64.7%)	38 (52.1%)	0.42
Sweat chloride (mmol/L), median (range)	101.0 (59.0–145.0)	91.0 (10.0–150.0)	0.46
BMI-SDS, mean ± SD	−0.02 ± 0.55	0.2 ± 0.76	0.18
CF related diabetes, n (%)	4 (23.5%)	7 (9.6%)	0.21
Pancreatic insufficiency, n (%)	15 (88.2%)	43 (58.9%)	0.02
FEV_1 (%predicted), median (range)	94.1 (40.0–125.0)	92.9 (32.0–120.0)	0.92
25-OH vitamin D (ng/ml), median (range)	24.1 (15.7–41.8)	22.8 (7.1–45.6)	0.21
Pseudomonas aeruginosa, n (%)	12 (70.6%)	32 (43.8%)	0.06
Aspergillus, n (%)	12 (70.6%)	18 (24.6%)	0.003
ABPA, n (%)	4 (23.5%)	2 (2.7%)	0.01
IV antibiotics days, median (range)	0 (0–161)	0 (0–84)	0.25
Azithromycin[c] years, median (range)	0 (0–4)	2 (0–4)	0.14

ABPA = allergic bronchopulmonary aspergillosis.
[a] In 2008.
[b] ≥1 sputum culture positive for NTM from 2006 to 2009.
[c] In 2006–2009.

Table 2
CFTR mutation severity for NTM positive and negative patients.

CFTR mutation severity	All subjects	*M. abscessus* positive	*M. abscessus* negative	NTM positive *	NTM negative *
n	90	9	81	17	73
Severe, n (%)	50 (55.6%)	8 (88.9%)	42 (51.9%)	15 (88.2%)	35 (47.9%)
Mild, n (%)	25 (27.8%)	1 (11.1%)	24 (29.6%)	2 (11.8%)	23 (31.5%)
Unknown, n (%)	15 (16.7%)	0 (0%)	15 (18.5%)	0 (0%)	15 (20.5%)

CFTR mutation severity was classified as 'severe', with minimal residual function, if patients had 2 mutations belonging to class I or II, 'mild', with residual CFTR function, if patients had at least one mutation from class IV or V and unknown if both mutations were unknown or 1 was class I or II and 1 unknown.
* p = 0.04 comparing NTM positive and NTM negative subjects and p = 0.25 comparing *M. abscessus* positive and negative subjects for mutation severity; subjects with unknown mutation severity were excluded from the analysis.

6.4. Duration of NTM infection and lung disease

M. abscessus was most frequently associated with NTM lung disease, whereas other NTM species were more likely to be associated with transient infection (Table 3) (p = 0.0004). Duration of infection for *M. abscessus* compared to all other NTM species was 10.5 (1–118) months compared to 1 (1–70) month, respectively, median (range) (p = 0.004).

As the timeline in Fig. 4 demonstrates, of the 16 patients with sputum cultures positive for *M. abscessus*, many had lung disease persisting for months or years (Fig. 4A), in contrast to non-*abscessus* NTM where transient infection was most common (Fig. 4B) (p = 0.004). This must be qualified, with regard to new NTM infection in 2011, where the duration is as yet unknown.

Of 11 patients who developed *M. abscessus* lung disease (Fig. 5A), 2 converted spontaneously after only 2 positive cultures, and 9 had sputum cultures persistently positive for several years. One was observed without treatment as there was no apparent clinical impact, but eight received triple therapy (oral and/or IV), according to susceptibility results for months to years continuously. Only two converted to negative sputum. In one patient, persistent *M. abscessus* led to respiratory failure and lung transplantation, with subsequent sputum conversion (total 5 years of continuous IV triple therapy). In contrast, for

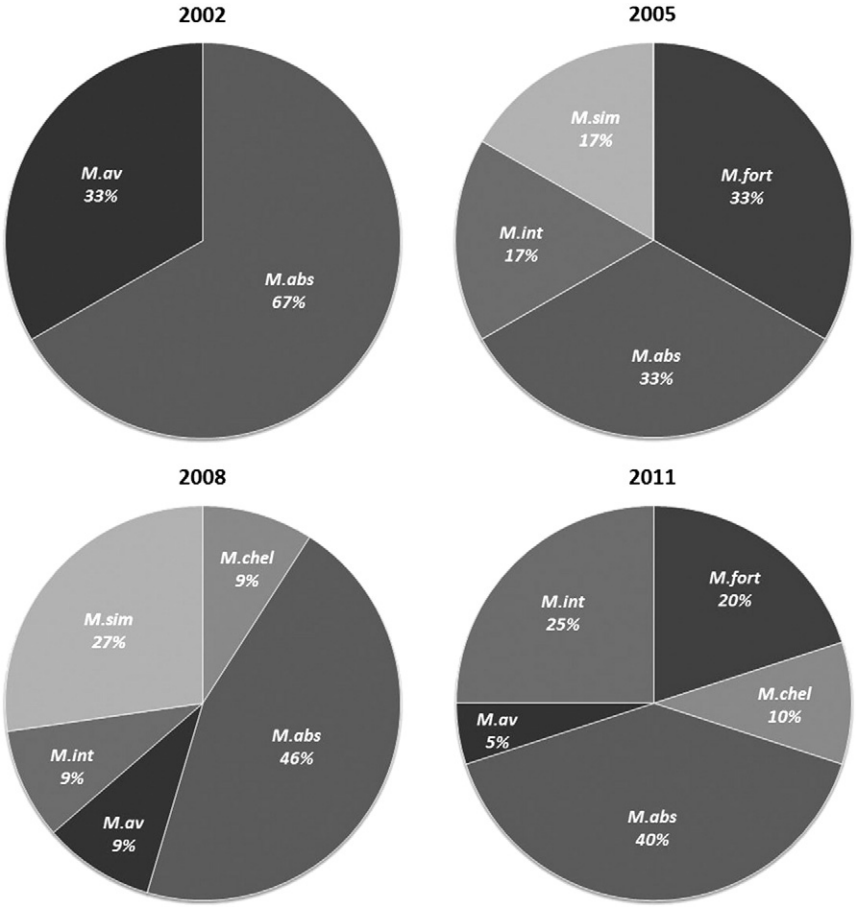

Fig. 3. Relative frequency of mycobacteria species during the observation period: Four representative years are demonstrated: A. 2002; B. 2005; C. 2008; D. 2011; M.av = *Mycobacterium avium*, M.abs = *Mycobacterium abscessus*, M.int = *Mycobacterium intracellulare*, M.sim = *Mycobacterium simiae*, M.fort = *Mycobacterium fortuitum*, M.chel = *Mycobacterium chelonae*.

the 6 patients with non-*abscessus* NTM lung disease, sputum conversion occurred spontaneously in 2 and following triple therapy in 3. Only 1 patient had persistent disease despite prolonged therapy. Thus, 4/11 (36%) with *M. abscessus* compared to 5/6 (83%) with other NTM lung disease achieved sputum conversion (which did not reach significance, possibly due to small numbers) (p = 0.13).

Table 3
NTM infection versus lung disease, by NTM species, 2002–2011.

NTM species		Duration (months)	NTM infection	NTM lung disease
		Median (range)	n (%)	n (%)
M. abscessus		10.5 (1–118) *	5 (31%)	11 (69%) **
All other NTM (non-*abscessus*)		1 (1–70) *	30 (83%)	6 (17%) **
NTM species	*M. intracellulare/ avium*	1 (1–70)	10	3
	M. simiae	5 (1–22)	4	2
	M. chelonae	1 (1–1)	3	0
	M. fortuitum	1 (1–17)	9	1
	M. kansasii	1 (1–1)	1	0
	M. gordonae	1 (1–1)	2	0
	M. mucogenicum	1 (1–1)	1	0

* p = 0.004, comparing duration of *M. abscessus* infection to infection by all other NTM.
** p = 0.0004, infection versus lung disease for *M. abscessus* compared to all other NTM.

6.5. Serum cytokines in NTM positive compared to NTM negative patients

There was a wide range in values for all serum cytokines measured in 2008. TGF-β, TNF-α, IL-1β, IL-2, IL-4 and IL-5 levels were increased significantly in the 9 patients positive for *M. abscessus* compared to *M. abscessus* negative patients and significance was maintained when looking only at the 6 patients with *M. abscessus* lung disease (Table 4). No association was found with NTM infection or lung disease between 2006 and 2009, for IL-4, IL-5, IL-10, IL-12, IL-17, TGF-β, TNF-α or INF-γ and leptin. GMCSF levels tended to be lower in patients with NTM infection compared to those without, 8.4 (2.7–35.3) pg/ml versus 15.4 (0.5–57.9) pg/ml, median (range), respectively (p = 0.06), E-Table 3, on-line data supplement.

7. Discussion

Over the past decade we have found that the incidence and prevalence of NTM infections within our CF clinic population

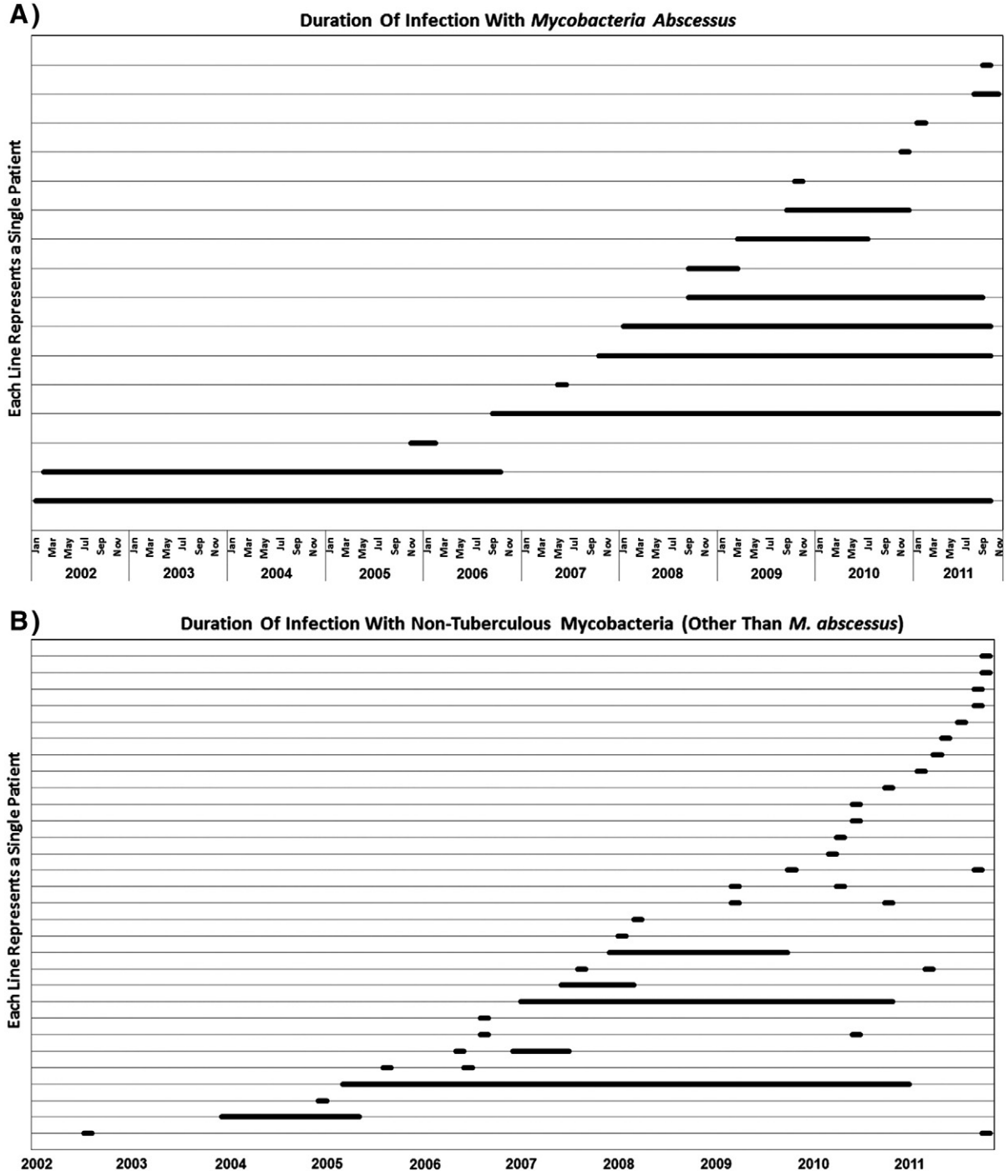

Fig. 4. Timeline representing duration of nontuberculous mycobacteria (NTM) infection or lung disease. 4A. *M. abscessus*, and 4B. Mycobacteria other than *M. abscessus*. Each horizontal level represents a single patient. Interrupted thick lines represent discrete episodes of infection, at least 12 months apart, not necessarily with the same NTM species. Shortest lines represent a single positive culture.

have risen dramatically. A majority of these patients have *M. abscessus* infection. Many have persistent NTM lung disease and sputum conversion often fails, despite prolonged multi-drug intravenous antibiotic therapy. This can be associated with inexorable clinical deterioration over many years and in one case led to the need for lung transplantation. This is in contrast with infection by other NTM species which is often transient and may not require treatment. Although new cases with a presence of NTM infection are increasing, NTM lung disease peaked in our population in 2009 and has decreased since then. Possibly this is due to isolation of patients with NTM lung disease to prevent cross infection, as well as aggressive triple therapy where indicated clinically. Previous reports from different CF centers around the world describe a wide range of NTM prevalence, from 3.3% to 24%, probably depending on geographic region [4,6]. NTM prevalence reported by US and

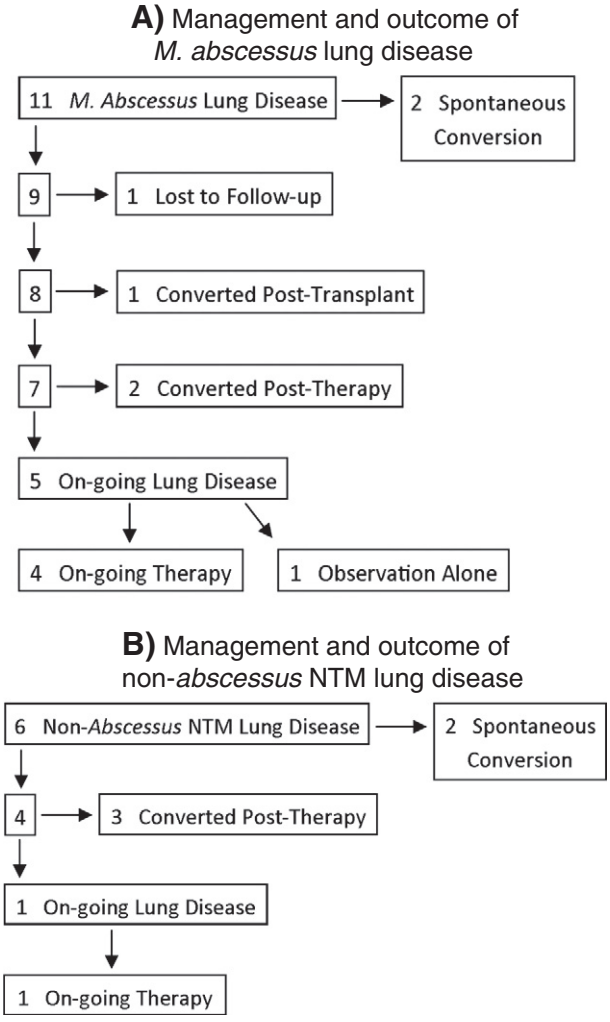

Fig. 5. Management of patients and outcome of nontuberculous mycobacteria (NTM) lung disease. 5A. 11 patients with *M. abscessus* lung disease, and 5B. 6 patients with lung disease associated with mycobacteria other than *M. abscessus*.

European CF registries is lower [24,25], but this may be due to under-reporting and lack of definitions for many aspects of NTM infection and disease. Fortunately, international guidelines for CF-related NTM disease are now in preparation.

NTM were previously considered commensal organisms, ubiquitous in the environment, e.g. soil and water, and nosocomial transmission was not thought to occur [4,12] However, a recent publication [14] demonstrated a high rate of transmission of *M. abscessus* between CF patients despite stringent segregation, and suggested that transmission occurred indirectly, through fomite contamination or aerosols during physiotherapy and spirometry testing. The study also demonstrated that a low inoculum is sufficient for infection from smear-negative, infected patients. This has provoked great concern regarding the need for extreme preventative measures. We did not perform whole genome sequencing of the mycobacteria isolated from our clinic patients, and therefore do not know whether cross-infection within our clinic explains some of the rising NTM incidence. We now segregate NTM-infected CF patients and are aiming for single-use rooms and whole genome sequencing for our entire patient cohort.

Table 4
Serum cytokines in *M. abscessus* positive and negative patients.

	M. abscessus positive n = 9		*M. abscessus* negative n = 81		
	Median	Range	Median	Range	p-Value
GM-CSF	16.2	2.7–35.5	12.4	0.5–57.9	0.99
TGF-β	2273	1214–3731	1314	0.1–5348	0.02 *
INF-γ	5	1.2–30.2	2.8	0.8–55.9	0.30
TNF-α	1.6	0.3–17.8	0.3	0.3–17.3	0.01 *
IL-1β	6.8	0.7–30.7	2.3	0.4–97.4	0.02 *
IL-2	1.9	1.0–6.6	1.0	1.0–10.4	0.04 *
IL-4	2	0.4–8.8	0.7	0.1–10.3	0.03 *
IL-5	62.7	12.4–232.1	22.1	0.2–203.5	0.01 *
IL-6	31.4	1.0–97.6	7.0	0.4–805.4	0.08
IL-8	4.4	1.0–13.2	2.2	0.5–762.8	0.11
IL-10	0.5	0.3–4.6	0.3	0.3–4.1	0.25
IL-12 p40	10.9	1.0–22.5	13.2	0.4–69.7	0.53
IL-12 p70	12.7	4.8–77.5	6.6	0.6–101.4	0.08
IL-17A	0.8	0.5–6.0	0.5	0.5–11.6	0.50
IL-17F	25.5	2.7–34.1	14.0	0.0–156.9	0.27
IL-22	134.8	0.4–455.6	53.5	0.4–532.8	0.36

Serum cytokine levels taken in 2008 and compared for patients with >positive sputum culture for *M. abscessus* compared to patients negative for *M. abscessus* between 2006 and 2009.

* p-Value of <0.05 which is considered significant.

In our clinic, NTM infection was associated with a 'severe' CFTR genotype and pancreatic insufficiency and thus, classic CF. This differs from the findings of Olivier [4] who described NTM as mainly affecting milder and older patients. However, as MAC was the main infecting organism in the USA study, the profile of disease was likely to be different [26].

We found a strong association between ABPA and NTM infection which we previously described in a smaller cohort of our patients [11] and which has been shown by others [27]. We suspect a specific immune dysregulation is involved in this subgroup, as compared to the rest of the CF population possibly associated with an increased TH2 response and this might increase susceptibility to NTM infection.

As cytokines were only measured at a single time point, it is difficult to say which predisposed to the *M. abscessus* infection and which reflect an inflammatory response following infection. Interestingly we found that patients with *M. abscessus* infection had significantly increased levels of IL-4, IL-5, associated with a TH2 response, as well as increased levels of pro-inflammatory cytokines including TNF-α and IL-1β. In a study of immune response to *M. abscessus* infection in a murine model, analysis of lung homogenates showed that both Th1 and Th2 cytokines increased simultaneously following high dose infection [28]. As an intracellular pathogen, *M. abscessus* elicits an innate immune response and production of pro-inflammatory cytokines, including TNF-α and IL-1β via TLR2 and MAPK pathways. Indeed, TNF-α production has been shown to be increased further following *M. abscessus* infection than following *M. avium* infection [29]. When comparing all NTM positive to NTM negative patients in our study, cytokine differences were not found, possibly reflecting the fact that lung disease was

associated mainly with *M. abscessus* infection whereas transient infection was more common for other NTM species.

There was also an association between NTM infection and aspergillus infection, even in patients without ABPA. Possibly both these infections were a marker for a more severe CF phenotype or certain features of the host-lung morphology or long-term cumulative antibiotic therapy, although these features were not assessed in this study.

As in this study, *M. abscessus* was the most prevalent species in reports from the UK and Greece [30] but in contrast to reports from other CF centers internationally [26]. In particular, MAC is the predominant mycobacteria isolated from the respiratory tract of patients in North America, although *M. abscessus* has been rising and now represents 20% of NTM. This may be explained by regional variation of the distribution of mycobacteria within the environment. It could also explain the differences in pathogenicity described for NTM in CF worldwide.

This study has several limitations. Firstly, as a retrospective single center study, it cannot accurately determine the importance of various associations observed within the patient cohort or what the true risk factors are for the rise in NTM incidence observed. In view of the major pathogenetic potential, in particular for *M. abscessus*, we support the need for a multi-center multinational prospective study of NTM infection in CF. A second limitation is that we measured cytokine levels at a single time point, not necessarily of most significance in relation to onset of NTM infection, and variability was great within our CF cohort. Furthermore, serum cytokine levels may not reflect the local milieu within the lung, and future BAL or induced sputum studies may shed further light on this question.

In conclusion, we describe a persistent increase in the incidence of NTM infection over the past decade, with a peak in NTM lung disease in 2009 and some decrease since then in our CF population. This was associated with pancreatic insufficiency, chronic *P. aeruginosa*, aspergillus and ABPA. In particular, *M. abscessus* sputum conversion often failed, even with prolonged triple therapy, and NTM lung disease could be associated with inexorable pulmonary decline. The possible role of transmission between patients is of concern.

Take home message

NTM infection, with prolonged *M. abscessus* lung disease, increased dramatically, over the past decade at our CF center.

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